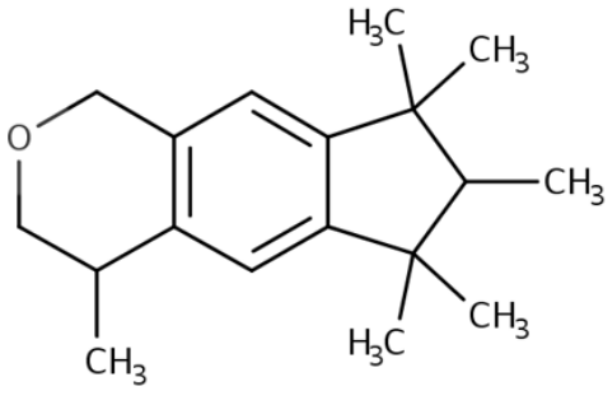
United States
Environmental Protection Agency

March 2026
Office of Chemical Safety and
Pollution Prevention

Draft Data Quality Evaluation Information for Environmental Hazard for 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[γ]-2-benzopyran (HHCB)

Systematic Review Support Document for the Draft Risk Evaluation

CASRN: 1222-05-5



March 2026

This supplemental file contains information regarding the data quality evaluation results relevant to the analysis of environmental hazard for the *Draft Human Health and Environmental Hazard Assessment for 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta [γ]-2-benzopyran (HHCB)*. EPA conducted data quality evaluation based on author-reported descriptions and results; additional analyses (*e.g.*, statistical analyses performed during data integration into the risk evaluation) potentially conducted by EPA are not contained in this supplemental file. EPA used the TSCA systematic review process described in the *Draft Systematic Review Protocol Supporting TSCA Risk Evaluations for Chemical Substances* (also referred to as '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 Risk Evaluation for 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta [γ]-2-benzopyran (HHCB)*.

Different data quality evaluation forms were used depending on the organism as described in the PECO statement in Appendix H.5.12 of the 2021 Draft Systematic Review Protocol. Each health outcome was evaluated independently within a given reference; therefore, each reference may have more than one overall quality determination (OQD) to more appropriately reflect the quality of each health outcome and the respective hazard endpoints as described by the study authors. Some data evaluation forms have general additional comments presented adjacent to the OQD to add further context. No OQD is determined for each reference as a whole, if it contains data from more than one evidence stream. The study details and respective endpoints are organized by first the relevant habitat (*i.e.*, aquatic vs. terrestrial), then taxa categories (*e.g.*, vertebrates, invertebrates, vegetation) followed by taxonomic groups (*e.g.*, fish, amphibian, mammalian, avian, worms, vascular plants), individual species, and finally exposure duration and health outcome (*e.g.*, mortality) categories relevant to the endpoint being evaluated.

Table of Contents

Study Citation:	Chen, F., Gao, J., Zhou, Q. (2012). Toxicity assessment of simulated urban runoff containing polycyclic musks and cadmium in Carassius auratus using oxidative stress biomarkers. Environmental Pollution 162:91-97.
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; *Carassius auratus*; Adult
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)
Chemical:	HHCB
HERO ID:	4648141

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as HHCB (galaxolide).
	Metric 2:	Test Substance Source	High	The test substance was acquired from Tianjin Zhongkejian Chemical Col., Ltd (Tianjin, China).
	Metric 3:	Test Substance Purity	High	The test substance is 77.45% pure (analytical grade), and impurities are not likely to substantially impact study results.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	The study included a blank control, solvent and runoff controls.
	Metric 5:	Negative Control Response	High	There was no significant difference between the blank and controls for all biomarker activity.
	Metric 6:	Randomized Allocation	Low	Researchers did not report randomized allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Semi-static exposures with renewal every 48 hours by adding test substance to 80 L glass aquariums.
	Metric 8:	Consistency of Exposure Administration	High	Test substances were added after 20 hour of equilibrium across replicates and test concentrations.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Initial exposure concentrations were measured, but not reported, however its measured concentrations are expected to be close to nominal based on its p-chem properties and semi-static renewal.
	Metric 10:	Exposure Duration and Frequency	High	The 7-day exposure with renewal every 48 hours was compared to 14 and 21 day exposures to study effects over time.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	In addition to the blank and controls, there were five exposure groups that provided a range of responses."0.15, 1.5, 15, 75 and 150 mg/L for HHCB"
	Metric 12:	Testing at or Below Solubility Limit	High	Testing was conducted below and approaching the water solubility limit of the test substance; a solvent was used."The highest concentrations of the solvent and emulsifier in our experiment were lower than the maximum level allowed by the test guidelines (OECD, 1984). A solvent control group containing acetone and Tween-80 was added to the experiment simultaneously. Concentrations of the solvent and emulsifier in the solvent control group were equal to their highest concentrations used in our experiment." – no adverse effects seen in solvent control group
Domain 4: Test Organism				

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Study Citation: Chen, F., Gao, J., Zhou, Q. (2012). Toxicity assessment of simulated urban runoff containing polycyclic musks and cadmium in Carassius auratus using oxidative stress biomarkers. Environmental Pollution 162:91-97.
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; *Carassius auratus*; Adult
Health Outcome: Mechanistic-Oxidative stress (including redox biology)
Chemical: HHCB
HERO ID: 4648141

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	Low	While Carassius auratus are well studied, they are not recommended for acute fish tests and were obtained from an aquatic store, with very few details regarding the test organisms provided.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test fish were acclimated for 14 days in dechlorinated water, at room temperature prior to testing.
	Metric 15:	Number of Organisms and Replicates per Group	Low	Test guidance for acute fish toxicity test (OECD TG 203) recommends seven fish per test group. The authors replicated the test three times, with five fish per test group.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions appeared adequate for the test organisms.
	Metric 17:	Outcome Assessment Methodology	High	The study measured biomarkers of oxidative stress.
	Metric 18:	Consistency of Outcome Assessment	High	The researchers assessed results for all test groups and controls.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported treatment differences among study groups outside test substance exposure concentrations.
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no mortalities for any exposure or controls, and there were no significant differences in biomarker outcomes amongst the controls and blank.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The researchers used one-way ANOVA of test exposure means and standard deviation. The least significant differences test was used to compare the blank to treatment groups.
	Metric 22:	Reporting of Data	High	Data were reported as means and their SD for each control and treatment group.
	Metric 23:	Explanation of Unexpected Outcomes	High	All outcomes were adequately discussed in the study.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Chen, F., Gao, J., Zhou, Q. (2012). Toxicity assessment of simulated urban runoff containing polycyclic musks and cadmium in Carassius auratus using oxidative stress biomarkers. Environmental Pollution 162:91-97.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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; *Carassius auratus*; Adult
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)
Chemical:	HHCB
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Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The test substance was identified as HHCB (galaxolide).
	Metric 2:	Test Substance Source	High	The test substance was acquired from Tianjin Zhongkejian Chemical Col., Ltd (Tianjin, China).
	Metric 3:	Test Substance Purity	High	The test substance is 77.45% pure (analytical grade), and impurities are not likely to substantially impact study results.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	The study included a blank control, solvent and runoff controls.
	Metric 5:	Negative Control Response	High	There was no significant difference between the blank and controls for all biomarker activity.
	Metric 6:	Randomized Allocation	Low	Researchers did not report randomized allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Semi-static exposures with renewal every 48 hours by adding test substance to 80 L glass aquariums.
	Metric 8:	Consistency of Exposure Administration	High	Test substances were added after 20 hour of equilibrium across replicates and test concentrations.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Initial exposure concentrations were measured, but not reported, however its measured concentrations are expected to be close to nominal based on its p-chem properties and semi-static renewal.
	Metric 10:	Exposure Duration and Frequency	High	The 7-day exposure with renewal every 48 hours was compared to 14 and 21 day exposures to study effects over time.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	In addition to the blank and controls, there were five exposure groups that provided a range of responses."0.15, 1.5, 15, 75 and 150 mg/L for HHCB"
	Metric 12:	Testing at or Below Solubility Limit	High	Testing was conducted below and approaching the water solubility limit of the test substance; a solvent was used."The highest concentrations of the solvent and emulsifier in our experiment were lower than the maximum level allowed by the test guidelines (OECD, 1984). A solvent control group containing acetone and Tween-80 was added to the experiment simultaneously. Concentrations of the solvent and emulsifier in the solvent control group were equal to their highest concentrations used in our experiment." – no adverse effects seen in solvent control group
Domain 4: Test Organism				

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Taxa, Species, Age: Vertebrate; Fish; *Carassius auratus*; Adult
Health Outcome: Mechanistic-Oxidative stress (including redox biology)
Chemical: HHCB
HERO ID: 4648141

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	Low	While Carassius auratus are well studied, they are not recommended for acute fish tests and were obtained from an aquatic store, with very few details regarding the test organisms provided.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test fish were acclimated for 14 days in dechlorinated water, at room temperature prior to testing.
	Metric 15:	Number of Organisms and Replicates per Group	Low	Test guidance for acute fish toxicity test (OECD TG 203) recommends seven fish per test group. The authors replicated the test three times, with five fish per test group.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions appeared adequate for the test organisms.
	Metric 17:	Outcome Assessment Methodology	High	The study measured biomarkers of oxidative stress.
	Metric 18:	Consistency of Outcome Assessment	High	The researchers assessed results for all test groups and controls.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported treatment differences among study groups outside test substance exposure concentrations.
	Metric 20:	Outcomes Unrelated to Exposure	High	There were no mortalities for any exposure or controls, and there were no significant differences in biomarker outcomes amongst the controls and blank.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The researchers used one-way ANOVA of test exposure means and standard deviation. The least significant differences test was used to compare the blank to treatment groups.
	Metric 22:	Reporting of Data	High	Data were reported as means and their SD for each control and treatment group.
	Metric 23:	Explanation of Unexpected Outcomes	High	All outcomes were adequately discussed in the study.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Chae, H., Kwon, B. R., Lee, S., Moon, H. B., Choi, K. (2023). Adverse thyroid hormone and behavioral alterations induced by three frequently used synthetic musk compounds in embryo-larval zebrafish (Danio rerio). Chemosphere 324:138273.
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; *Danio rerio*; wild type; Larvae
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	11791368

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	High	The HHCB was reported to be from Sigma-Aldrich in St. Louis, MO, USA. CAS no. 1222-05-5, purity: 99%. GC/MS used for identification and quantification of test samples.
	Metric 3:	Test Substance Purity	High	The purity was reported to be 99%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control in which only a solvent (DMSO 0.01% v/v) was used.
	Metric 5:	Negative Control Response	High	The negative control response for behavior was reported in Fig. 3 and was appropriate.
	Metric 6:	Randomized Allocation	Low	It was not reported how the embryos were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The preparation of the test concentrations and/or stock solution was unclear and not reported in great detail. The test system was not described in detail either. Zebrafish were either exposed in 500mL beakers with 300mL of test solution or in 50mL of test solution. It is unclear which system was used for behavioral outcomes.The behavioral test system was described as being in 96 well plate wells.
	Metric 8:	Consistency of Exposure Administration	Low	Details regarding the exposure administration were limited. Test substance preparation as not reported, so it is unclear if the preparation was consistent. Different size beakers were used for different portions of this study, and it is unclear which size beakers were used for the behavioral portion of the study.
	Metric 9:	Measurement of Test Substance Concentration	High	Test concentrations were measured using GC-MS/MS. Three samples were taken from exposure media for analysis. Because the nominal and measured concentrations deviated by more the 20%, measured concentrations were used for the interpretation of the results.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be five days. This was adequate to observe the outcomes of interest.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were four exposure concentrations. For the behavioral portion of this study, only the second highest test concentration was significantly different from the control. More concentrations or different spacing may have clarified this response.
	Metric 12:	Testing at or Below Solubility Limit	High	All tests concentrations were below the water solubility limit.

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Study Citation:	Chae, H., Kwon, B. R., Lee, S., Moon, H. B., Choi, K. (2023). Adverse thyroid hormone and behavioral alterations induced by three frequently used synthetic musk compounds in embryo-larval zebrafish (Danio rerio). Chemosphere 324:138273.
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Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type; Larvae
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	11791368

Domain	Metric		Rating	Comments
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The zebrafish were from two different sources for different portions of the study. Zebrafish were cultured in-house at the Environmental Toxicology Laboratory at Seoul National University, Seoul, Korea for the neurobehavioral observations. For the thyroid disruption portion of the study, freshly fertilized zebrafish eggs were obtained from a commercial hatchery, Gangnam Aquarium in Suwon, Korea. All embryos used in the study were 4 hpf.All was reported. These differences between embryo sources is not concerning.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the test organisms were acclimated at any point. It was unclear if the culture conditions were similar to test conditions. It was also unclear if any acclimation occurred for the portion of the study where the embryos were obtained from a commercial hatchery.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of test organisms and the number of replicates was unclear for the behavioral outcome. It is unclear which portion of the study behavior outcomes were assessed (thyroid hormone assessment or transcriptional changes). behavior was separate from the other parts.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	The test conditions were described adequately. Organisms were kept at 26C under a 14L:10D photoperiod. Basic water quality parameters were monitored. However, culture/ pre-test conditions were not reported.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-behavioral changes in terms of distance moved after changes in light/dark. Changes in behavior were assessed using a Daniovision video tracking system and Ethovision XT 8.5 software.
	Metric 18:	Consistency of Outcome Assessment	High	The outcomes were assessed consistently across study groups After 120h of exposure, the larva were moved to 96 well plates with the same test concentration as the exposure. Movements were recorded for 60min under different light conditions. Total distance moved, mean velocity, and thigmotaxis were measured.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The study did not provide enough information to allow a comparison of environmental conditions or other non-treatment-related factors across study groups. It was unclear if the organisms were acclimated, and pre-test conditions were unclear. they describe no differences in environmental conditions among treatment groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.

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Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type; Larvae
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	11791368

Domain	Metric		Rating	Comments
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Dunnett's analysis of variance was conducted to compare with the control. Spearman's rank correlation was applied for trend determination.
	Metric 22:	Reporting of Data	High	Results for the control and exposure responses were reported in Figure 3 for distance moved. Effects on thigmotaxis were reported in Figure S1.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.

Additional Comments: This evaluation was for the behavioral effects on zebrafish larvae after 120h of exposure to HHCB. Distance moved after changes in light/dark, velocity, and thigmotaxis were assessed.

Overall Quality Determination High

Study Citation:	Chae, H., Kwon, B. R., Lee, S., Moon, H. B., Choi, K. (2023). Adverse thyroid hormone and behavioral alterations induced by three frequently used synthetic musk compounds in embryo-larval zebrafish (Danio rerio). Chemosphere 324:138273.
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Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type; Larvae
Health Outcome:	Mechanistic-Endocrine toxicity
Chemical:	HHCB
HERO ID:	11791368

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	High	The HHCB was reported to be from Sigma-Aldrich in St. Louis, MO, USA.CAS no. 1222-05-5, purity: 99%. GC/MS used for identification and quantification of the target compound in thetest media.
	Metric 3:	Test Substance Purity	High	The purity was reported to be 99%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control in which only a solvent (DMSO 0.01% v/v) was used.
	Metric 5:	Negative Control Response	High	The negative control response for whole body T4 levels was reported in Fig. 1 and is acceptable.
	Metric 6:	Randomized Allocation	Low	It was not reported how the embryos were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the test concentrations and/or stock solution was clear. The test system was not described in detail either. Zebrafish were exposed in 500mL beakers with 300mL of test solution for the thyroid hormone assessment. Exposure was conductedat 26 ± 1 °C, under a photoperiod of 14:10 h light:dark in theEnvironmental Toxicology Laboratory, Seoul National University. Basicwater parameters such as water temperature, pH, dissolved oxygen, andconductivity were recorded before renewal of exposure media.
	Metric 8:	Consistency of Exposure Administration	Low	Details regarding the exposure administration were limited. Test substance preparation was not reported, so it is unclear if the preparation was consistent.
	Metric 9:	Measurement of Test Substance Concentration	High	Test concentrations were measured using GC-MS/MS. Three samples were taken from exposure media for analysis. Because the nominal and measured concentrations deviated by more the 20%, measured concentrations were used for the interpretation of the results.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be five days. This was adequate to observe the outcomes of interest.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were four exposure concentrations. This was adequate for the outcome of interest.
	Metric 12:	Testing at or Below Solubility Limit	High	All tests concentrations were below the water solubility limit.
Domain 4: Test Organism				

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Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type; Larvae
Health Outcome:	Mechanistic-Endocrine toxicity
Chemical:	HHCB
HERO ID:	11791368

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	The zebrafish were from two different sources for different portions of the study. Zebrafish were cultured in-house at the Environmental Toxicology Laboratory at Seoul National University, Seoul, Korea for the neurobehavioral observations. For the thyroid disruption portion of the study, freshly fertilized zebrafish eggs were obtained from a commercial hatchery, Gangnam Aquarium in Suwon, Korea. All embryos used in the study were 4 hpf.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the test organisms were acclimated at any point. It was unclear if the culture conditions were similar to test conditions. It was also unclear if any acclimation occurred for the portion of the study where the embryos were obtained from a commercial hatchery.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	For the thyroid hormone portion of the study, there were 250 fertilized embryos per replicate, and there were three replicates per treatment and per control.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	The test conditions were described adequately. Organisms were kept at 26C under a 14L:10D photoperiod. Basic water quality parameters were monitored. However, culture/ pre-test conditions were not reported.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-changes in T4 levels due to exposure to HHCB.
	Metric 18:	Consistency of Outcome Assessment	High	The outcomes were assessed consistently across study groups After 120h of exposure, 200 larval fish were chosen for for each replicate. They were homogenized, and an ELISA kit was used to measure T4.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variables were reported
	Metric 20:	Outcomes Unrelated to Exposure	High	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	Dunnett's analysis of variance was conducted to compare with the control. Spearman's rank correlation was applied for trend determination.
	Metric 22:	Reporting of Data	High	Results for the control and exposure responses were reported in Figure 1 for whole body T4 levels.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.

Additional Comments: This evaluation was for the effect of HHCB on whole-body T4 levels.

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Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type; Larvae
Health Outcome:	Mechanistic-Endocrine toxicity
Chemical:	HHCB
HERO ID:	11791368

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

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Taxa, Species, Age:	Vertebrate; Fish; *Danio rerio*; wild type; Larvae
Health Outcome:	Mechanistic-Cell signaling/function
Chemical:	HHCB
HERO ID:	11791368

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	High	The HHCB was reported to be from Sigma-Aldrich in St. Louis, MO, USA.CAS no. 1222-05-5, purity: 99%. GC/MS was used for identification and quantification of the target compounds
	Metric 3:	Test Substance Purity	High	The purity was reported to be 99%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported the use of a negative control in which only a solvent (DMSO 0.01% v/v) was used.
	Metric 5:	Negative Control Response	High	The negative control response for transcriptional changes of thyroid hormone regulating genes is reported in Fig. 2, and the negative control response for transcriptional changes in neuron-related genes is reported in Fig. 4.
	Metric 6:	Randomized Allocation	Low	It was not reported how the embryos were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The preparation of the test concentrations and/or stock solution was described. Zebrafish were exposed in 50mL of test solution with 20 fertilized eggs for the assessment of transcriptional changes of thyroid- related and neurodevelopment-related genes.
	Metric 8:	Consistency of Exposure Administration	High	Nothing about the description includes inconsistencies.
	Metric 9:	Measurement of Test Substance Concentration	High	Test concentrations were measured using GC-MS/MS. Three samples were taken from exposure media for analysis. Because the nominal and measured concentrations deviated by more the 20%, measured concentrations were used for the interpretation of the results.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be five days. This was adequate to observe the outcomes of interest.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were four exposure concentrations. This was adequate for the outcome of interest.
	Metric 12:	Testing at or Below Solubility Limit	High	All tests concentrations were below the water solubility limit.
Domain 4: Test Organism				

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Study Citation:	Chae, H., Kwon, B. R., Lee, S., Moon, H. B., Choi, K. (2023). Adverse thyroid hormone and behavioral alterations induced by three frequently used synthetic musk compounds in embryo-larval zebrafish (Danio rerio). Chemosphere 324:138273.
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; *Danio rerio*; wild type; Larvae
Health Outcome:	Mechanistic-Cell signaling/function
Chemical:	HHCB
HERO ID:	11791368

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	Medium	The zebrafish were from two different sources for different portions of the study. Zebrafish were cultured in-house at the Environmental Toxicology Laboratory at Seoul National University, Seoul, Korea for the neurobehavioral observations. For the thyroid disruption portion of the study, freshly fertilized zebrafish eggs were obtained from a commercial hatchery, Gangnam Aquarium in Suwon, Korea. All embryos used in the study were 4 hpf.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Nothing in the study indicates that organisms were not acclimated.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	For the transcriptional assessment, there were 20 fertilized eggs per replicate and four replicates per test concentration.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The test conditions were described adequately. Organisms were kept at 26C under a 14L:10D photoperiod. Basic water quality parameters were monitored. However, culture/ pre-test conditions were not reported.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest-transcriptional changes in thyroid-related genes and in development-related genes.
	Metric 18:	Consistency of Outcome Assessment	High	The outcomes were assessed consistently across study groups After 120h of exposure, mRNA was extracted with an RNeasy mini kit, and cDNAs were synthesized using a cDNA synthesis kit. Quantitative real-time PCR was used.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Nothin in the study indicates confounding variables were used or encountered.
	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	Dunnett's analysis of variance was conducted to compare with the control. Spearman's rank correlation was applied for trend determination.
	Metric 22:	Reporting of Data	High	Results for the control and exposure responses were reported in Fig 2 and Fig 4.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes.

Additional Comments: This evaluation was for the effect of HHCB on transcription of thyroid-related genes and development-related genes.

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Study Citation:	Chae, H., Kwon, B. R., Lee, S., Moon, H. B., Choi, K. (2023). Adverse thyroid hormone and behavioral alterations induced by three frequently used synthetic musk compounds in embryo-larval zebrafish (Danio rerio). Chemosphere 324:138273.
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; *Danio rerio*; wild type; Larvae
Health Outcome:	Mechanistic-Cell signaling/function
Chemical:	HHCB
HERO ID:	11791368

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

Study Citation:	Zhang, L., An, J., Zhou, Q. (2012). Single and joint effects of HHCB and cadmium on zebrafish (Danio rerio) in feculent water containing bedloads. Frontiers of Environmental Science & Engineering 6(3):360-372.
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; *Danio rerio*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	3405953

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCB identified as test substance
	Metric 2:	Test Substance Source	High	The test substance source was reported - Huiyu Musk Company in China. The test substance was analytically verified by the study authors
	Metric 3:	Test Substance Purity	Low	HHCB was described as having a purity of 75%. The product was also described as containing 25%diethylphthalate (DEP) from the Huiyu Musk Companyin Tianjin, China. The presence of 25% DEP could affect the study outcome. However, DEP was added to the controls.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls were used - bothacetone and DEP were used as the solvent control in theexperiments with HHCB.
	Metric 5:	Negative Control Response	Low	Controls were used, but the mortality of control groups was not reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not report if allocation was random
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Concentrations were validated at one time point, and open test systems were used. HHCB is negligibly volatile so an open system for an acute exposure is acceptable. Some uncertainty surrounding the use of soil from a river, which may contain other contaminants.
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration was administered consistently across groups
	Metric 9:	Measurement of Test Substance Concentration	Low	GC-MS analysis was conducted to validate exposure concentrations in water, however only at one time point, 12 hours. Measured concentrations were significantly different than nominal, but nominal were used for analyses.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was appropriate to capture acute mortality (120 hours - 5 days)
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	7 exposure concentrations were used.
	Metric 12:	Testing at or Below Solubility Limit	Medium	A subset of the nominal concentrations used may exceed the water solubility estimate for HHCB. Measured concentrations are provided - but not used in the analysis.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	It is unclear what life-stage of zebrafish were used for the experiment
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Fish were acclimatized prior to exposure

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Study Citation:	Zhang, L., An, J., Zhou, Q. (2012). Single and joint effects of HHCB and cadmium on zebrafish (Danio rerio) in feculent water containing bedloads. Frontiers of Environmental Science & Engineering 6(3):360-372.
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; *Danio rerio*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	3405953

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 fish per tank and three replicate tanks per exposure concentration
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism housing is acceptable
	Metric 17:	Outcome Assessment Methodology	High	Mortality was adequately assessed in the 120 hour experiment
	Metric 18:	Consistency of Outcome Assessment	High	Mortality in every tank was recorded on hours 12, 24, 48, 96, and 120
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Soil from a river that could contain other contaminants was used in tanks and mixed with HHCB. Thus, exposures likely included contaminants. The authors also detected Cd and Pb in the soil. All tanks had sediment from the same source so the exposure was consistent.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information suggested outcomes unrelated to exposure
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed adequately, but used nominal concentrations rather than actual.
	Metric 22:	Reporting of Data	Medium	Summary LC50 values and measures of variation were reported, but means and std error for each treatment and control were not reported. However the regression equation and correlation estimates were fully reported in Table 2.
	Metric 23:	Explanation of Unexpected Outcomes	High	Outcomes were explained

Additional Comments: This review is for the acute exposure to HHCB only, examining mortality.

Overall Quality Determination Medium

Study Citation:	Zhang, L., An, J., Zhou, Q. (2012). Single and joint effects of HHCB and cadmium on zebrafish (Danio rerio) in feculent water containing bedloads. Frontiers of Environmental Science & Engineering 6(3):360-372.
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; *Danio rerio*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)
Chemical:	HHCB
HERO ID:	3405953

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCB identified as test substance
	Metric 2:	Test Substance Source	High	The test substance source was reported - Huiyu Musk Company in China. The test substance was analytically verified by the study authors
	Metric 3:	Test Substance Purity	Low	HHCB was described as having a purity of 75%. The product was also described as containing 25%diethylphthalate (DEP) from the Huiyu Musk Companyin Tianjin, China. The presence of 25% DEP could affect the study outcome. However, DEP was added to the controls.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls were used - bothacetone and DEP were used as the solvent control in theexperiments with HHCB.
	Metric 5:	Negative Control Response	High	Controls were used, and enzyme activity of the controls was reported in the figures
	Metric 6:	Randomized Allocation	Low	Researchers did not report if allocation was random
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Concentrations were validated at one time point, and open test systems were used. HHCB is negligibly volatile so an open system for an acute exposure is acceptable. Some uncertainty surrounding the use of soil from a river, which may contain other contaminants.
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration was administered consistently across groups
	Metric 9:	Measurement of Test Substance Concentration	Low	GC-MS analysis was conducted to validate exposure concentrations in water, however only at one time point, 12 hours. Measured concentrations were significantly different than nominal, but nominal were used for analyses.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was appropriate to capture enzyme activity (samples taken at 1st 2nd 4th and 7th days)
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	4-5 concentrations were used for the enzyme activity experiments. Dose response was not conducted for the enzyme/oxidative stress markers - a separate exposure in this study.
	Metric 12:	Testing at or Below Solubility Limit	Medium	A subset of the nominal concentrations used may exceed the water solubility estimate for HHCB. Measured concentrations are provided for the first experiment - but not used in the analysis.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	It is unclear what life-stage of zebrafish were used for the experiment

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Study Citation:	Zhang, L., An, J., Zhou, Q. (2012). Single and joint effects of HHCB and cadmium on zebrafish (Danio rerio) in feculent water containing bedloads. Frontiers of Environmental Science & Engineering 6(3):360-372.
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; *Danio rerio*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)
Chemical:	HHCB
HERO ID:	3405953

Domain	Metric		Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Fish were acclimatized prior to exposure
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Enzyme activities and contents of MDA and SP were done with three parallel samples for all the treatments concentration
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism housing is acceptable
	Metric 17:	Outcome Assessment Methodology	Low	Outcome assessments in the second experiment were for enzymes related to oxidative stress: SOD, POD, and MDA content. Some methods were cited to another publication.
	Metric 18:	Consistency of Outcome Assessment	High	Mortality in every tank was recorded on hours 12, 24, 48, 96, and 120
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Soil from a river that could contain other contaminants was used in tanks and mixed with HHCB. Thus, exposures likely included contaminants. The authors also detected Cd and Pb in the soil. All tanks had sediment from the same source so the exposure was consistent.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information suggested outcomes unrelated to exposure
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis was performed adequately, but used nominal concentrations rather than actual.
	Metric 22:	Reporting of Data	Medium	Summary LC50 values and measures of variation were reported, but means and std error for each treatment and control were not reported. However the regression equation and correlation estimates were fully reported in Table 2.
	Metric 23:	Explanation of Unexpected Outcomes	High	Outcomes were explained

Additional Comments: This review is for the second experiment, exposure to HHCB only, examining enzymes associated with oxidative stress.

Overall Quality Determination Medium

Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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:	Vertebrate; Fish; *Gobiocypris rarus*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Correct nomenclature was used. There was a typo in the CASRN (1222-05 reported vs 1222-05-5 correct); given the correct chemical name, correct abbreviation, and correct trade name, it is likely that the missing digit for the CASRN was a genuine typo.
	Metric 2:	Test Substance Source	High	The source was reported (Klamar-reagent company, Shanghai).
	Metric 3:	Test Substance Purity	Medium	Purity of the substance was reported at 75%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	The authors reported that mortality rates of test organisms in all control groups were <10% at end of the tests and that toxicity tests followed ASTM standard guidelines. Moreover, they reported that test containers were set with blank control and solvent control (acetone). However, supplemental Table S1, which reports the nominal HHCB concentrations used in the toxicity test, does not list the control group as 0 ug/L.
	Metric 5:	Negative Control Response	Low	The authors reported in the methods that mortality rates of test organisms in all control groups were <10% at end of the tests. However, they did not report the raw data in the results (they only provided LC50 values for each test).
	Metric 6:	Randomized Allocation	Low	The authors did not report if the organisms were randomly allocated to study groups. They included a general statement saying that toxicity tests followed ASTM standard guidelines without mention of random allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	All tests were static-renewal and test solutions were totally replaced at 24 h intervals. Due to the physical-chemical properties of the HHCB, a solvent control (acetone) was used.
	Metric 8:	Consistency of Exposure Administration	High	There was no evidence to suggest that exposure administration was not administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured (HPLC, Agilent 1200 Series, USA). The recoveries of HHCB in water samples were 98.42%–106.90%, and the limit of detection (LOD)was 10.70 ng/L for water samples. The variability of HHCB concentration was <20% (3.08%–9.12%).
	Metric 10:	Exposure Duration and Frequency	High	This data evaluation form is for the acute toxicity studies. The reported acute toxicity exposure durations were 48 h for Daphnia magna and Chironomus plumosus and 96 h for the other aquatic animals. This exposure durations were appropriate for the study type.

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Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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:	Vertebrate; Fish; *Gobiocypris rarus*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were appropriate (5 to 7 groups plus control (solvent control).
	Metric 12:	Testing at or Below Solubility Limit	High	For all tests the nominal HHCB concentrations were below the HHCB water solubility limit of 1750 ug/L expect for the acute Daphnia magna test. However, it likely was not a concern because a solvent control (acetone) was used and the recoveries of measured HHCB in water samples were 98.42%–106.90%.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described (e.g., source, size, age, healthy organism).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The authors reported that, for example, cultures of Gobiocypris rarus and Daphnia magna were from their biological culture laboratory and thus acclimated. For organisms not reared in the laboratory, the authors reported that larvae were domesticated for at least 7 days in biological culture laboratory and the mortality rates were lower than 5% before toxicity tests started.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were appropriate: Three replicates for test containers each containing 10 organisms (except for D. magna) were set with blank control and solvent control (acetone), while four replicates for test containers each containing 5 organisms were set for D. magna.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The reporting of housing and environmental conditions were sufficient: The tests were conducted at 21 $\pm$ 2 °C with a 12:12 h light/dark cycle. Test organisms were not fed in acute toxicity tests. During the test period, the temperature, pH, and dissolved oxygen (DO) were measured daily.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (mortality) addressed or reported the intended outcome (LC50 values to generate SSDs to derive the ALC).
	Metric 18:	Consistency of Outcome Assessment	High	Reported details suggest that the outcomes were assessed consistently across study groups.
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 that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				

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Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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:	Vertebrate; Fish; *Gobiocypris rarus*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	High	The 48 h-EC50 and 96 h-LC50 values for acute toxicity tests and corresponding 95% confidence intervals were calculated by probit methodology. The ALC derivation was based on USEPA guidelines. The joint probability curves (JPC) were based on probability distributions of concentrations in water (exposure) and response (SSD) of HHCB.
	Metric 22:	Reporting of Data	Medium	The goal of the study was to derive the ALC based on SSDs. To generate the SSDs, the authors generated LC50 values, they also used LC50 values from the literature. So, the goal was not to determine the toxicity across treatment groups; keeping this is mind, this metric received a medium (instead of low) because mortality data was not reported for all treatment groups per species.
	Metric 23:	Explanation of Unexpected Outcomes	High	The study results provide variability (range).

Additional Comments: The goal of the study was to derive an ALC (aquatic life criteria) for HHCB based on aquatic organisms in China. The study included 8 Chinese resident aquatic species for acute and chronic toxicity tests. Lognormal species sensitivity distribution (SSD) and the log-logistic SSD methods were used to develop the ALC. The authors did not provide raw data of the toxicity studies. They also used LC50 values from the literature. Because the goal to run acute and chromic toxicity studies was to generate LC50 values that were ultimately used to generate SSDs, there are two forms included in the evaluation: acute data and chronic data for all species. This form is the data evaluation for the acute data for all species combined.

Overall Quality Determination High

Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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; *Gobiocypris rarus*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Correct nomenclature was used. There was a typo in the CASRN (1222-05 reported vs 1222-05-5 correct); given the correct chemical name, correct abbreviation, and correct trade name, it is likely that the missing digit for the CASRN was a genuine typo.
	Metric 2:	Test Substance Source	High	The source was reported (Klamar-reagent company, Shanghai).
	Metric 3:	Test Substance Purity	Medium	Purity of the substance was reported at 75%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	The authors reported that mortality rates of test organisms in all control groups were <10% at end of the tests and that toxicity tests followed ASTM standard guidelines. Moreover, they reported that test containers were set with blank control and solvent control (acetone). However, supplemental Table S1, which reports the nominal HHCB concentrations used in the toxicity test, does not list the control group as 0 ug/L.
	Metric 5:	Negative Control Response	Low	The authors reported in the methods that mortality rates of test organisms in all control groups were <10% at end of the tests. However, they did not report the raw data in the results (they only provided LC50 values for each test).
	Metric 6:	Randomized Allocation	Low	The authors did not report if the organisms were randomly allocated to study groups. They included a general statement saying that toxicity tests followed ASTM standard guidelines without mention of random allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	All tests were static-renewal and test solutions were totally replaced at 24 h intervals. Due to the physical-chemical properties of the HHCB, a solvent control (acetone) was used.
	Metric 8:	Consistency of Exposure Administration	High	There was no evidence to suggest that exposure administration was not administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured (HPLC, Agilent 1200 Series, USA). The recoveries of HHCB in water samples were 98.42%–106.90%, and the limit of detection (LOD)was 10.70 ng/L for water samples. The variability of HHCB concentration was <20% (3.08%–9.12%).
	Metric 10:	Exposure Duration and Frequency	High	This data evaluation form is for the chronic toxicity studies with Gobiocypris rarus and Macrobrachium nipponense. The exposure duration was 28 days; this exposure durations was appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were appropriate (5 groups plus control (solvent control).

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Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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; *Gobiocypris rarus*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
	Metric 12:	Testing at or Below Solubility Limit	High	For two chronic tests, the nominal HHCB concentrations were below the HHCB water solubility limit of 1750 ug/L.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described (e.g., source, size, age, healthy organism).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The authors reported that organisms not reared in their laboratory were domesticated for at least 7 days in biological culture laboratory and the mortality rates were lower than 5% before toxicity tests started.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were appropriate: Three replicates for test containers each containing 10 organisms were set with blank control and solvent control (acetone).
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The reporting of housing and environmental conditions were sufficient: The tests were conducted at 21 $\pm$ 2 °C with a 12:12 h light/dark cycle. Test organisms were fed twice daily in chronic toxicity tests andthe total food intake reached 0.1% of bodyweight of test organisms. During the test period, the temperature, pH, and dissolved oxygen (DO) were measured daily.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (mortality) addressed or reported the intended outcome (LC50 values to generate SSDs to derive the ALC).
	Metric 18:	Consistency of Outcome Assessment	High	Reported details suggest that the outcomes were assessed consistently across study groups.
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 that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The 28 day-EC10 values and corresponding 95% confidence intervals were calculated by probit methodology. The ALC derivation was based on USEPA guidelines. The joint probability curves (JPC) were based on probability distributions of concentrations in water (exposure) and response (SSD) of HHCB.

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Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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; *Gobiocypris rarus*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
	Metric 22:	Reporting of Data	Medium	The goal of the study was to derive the ALC based on SSDs. To generate the SSDs, the authors generated LC50 values, they also used LC50 values from the literature. So, the goal was not to determine the toxicity across treatment groups; keeping this is mind, this metric received a medium (instead of low) because mortality data was not reported for all treatment groups per species.
	Metric 23:	Explanation of Unexpected Outcomes	High	The study results provide variability (range).

Additional Comments: The goal of the study was to derive an ALC (aquatic life criteria) for HHCB based on aquatic organisms in China. The study included 8 Chinese resident aquatic species for acute and chronic toxicity tests. Lognormal species sensitivity distribution (SSD) and the log-logistic SSD methods were used to develop the ALC. The authors did not provide raw data of the toxicity studies. They also used LC50 values from the literature. Because the goal to run acute and chromic toxicity studies was to generate LC50 values that were ultimately used to generate SSDs, there are two forms included in the evaluation: acute data and chronic data for all species combined. This form is the data evaluation for the two chronic studies. Finally, the authors reported that the chronic toxicity endpoint was 28 d-EC10 (survival,SGR and body weight). However, only mortality/survival data is shown in the results; therefore, this data evaluation form is for mortality.

Overall Quality Determination High

Study Citation:	Dijk, Van, A. (1996). Accumulation and elimination of 14C-HHCB by Bluegill Sunfish in a dynamic flow-through system.
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:	Vertebrate; Fish; *Lepomis macrochirus*; Juvenile
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5352386

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Correct nomenclature and CASRN (1222-05-5) was provided.
	Metric 2:	Test Substance Source	High	The report indicates that the the chemical was supplied by the sponsor RIFM.
	Metric 3:	Test Substance Purity	High	The reported purity of unlabeled HHCB was 99.15% and that of total radiochemically labeled HHCB was 99.5%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A control group was used in the study.
	Metric 5:	Negative Control Response	High	There was no mortality in the control tank.
	Metric 6:	Randomized Allocation	Low	The study did not specifically state that they used random allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	There were limited details for this pre-test exposure.
	Metric 8:	Consistency of Exposure Administration	Low	Since there were limited details it is hard to say whether the exposure administration was consistent.
	Metric 9:	Measurement of Test Substance Concentration	Low	It was not mentioned if test concentrations were measured for this test.
	Metric 10:	Exposure Duration and Frequency	High	Duration was acceptable at 96 hours.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Three exposure concentrations were used. Mortality was low which authors stated justified the selected concentrations.
	Metric 12:	Testing at or Below Solubility Limit	High	HHCB concentrations were below the water solubility limit; radiolabeled solutions were prepared in the presence of Tween 80 and DMF.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms (bluegill sunfish) were appropriately described and adequate for the study. The species is listed as an acceptable test species by EPA OCSPP 850.1730 and OECD TG 305. The age should have been stated (and seems to not be) but authors did provide average weight of the individual fish.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not mentioned if fish were acclimated for the 96-hour test.
	Metric 15:	Number of Organisms and Replicates per Group	Low	It was not mentioned how many total fish were used for this test. No replicates were mentioned.
Domain 5: Outcome Assessment				

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Study Citation:	Dijk, Van, A. (1996). Accumulation and elimination of 14C-HHCB by Bluegill Sunfish in a dynamic flow-through system.
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:	Vertebrate; Fish; *Lepomis macrochirus*; Juvenile
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5352386

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	Low	No information was provided on test conditions.
	Metric 17:	Outcome Assessment Methodology	High	Mortality was the intended outcome.
	Metric 18:	Consistency of Outcome Assessment	Low	Limited details were provided.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Not enough information was provided to allow a comparison of environmental conditions or other non-treatment-related factors across study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	No information provided on statistics for this test.
	Metric 22:	Reporting of Data	Medium	Some data was shown in the table in section 3.2, along with some results in the text. However, no raw data was provided.
	Metric 23:	Explanation of Unexpected Outcomes	Low	No measures of variability shown.

Additional Comments: In addition to the bioaccumulation and depuration study, the authors conducted a toxicity pre-test by exposing fish to 10, 20, 50 ug/L un-labelled (parent compound) HHCB for 96 hr in order to determine dosage for the bioaccumulation portion of the study; the author reported no mortalities or health symptoms were observed in the toxicity pre-test.

Overall Quality Determination Low

Study Citation:	Wüthrich, V. (1996). HHCB: 21-Day prolonged toxicity study in the bluegill sunfish under flow-through conditions.
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:	Vertebrate; Fish; *Lepomis macrochirus*; Juvenile
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	7607846

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCB was identified by appropriate nomenclature and CASRN.
	Metric 2:	Test Substance Source	High	Test substance was supplied by the sponsor: RIFM, Two University Plaza, Suite 406, Hackensack, NJ 07601-6209, U.S.A. There was no information on the manufacturer of the substance, but a lot number was identified as ES-6302-H and expiration date as April 1996.This was an industry submitted test study by test facility: RCC UMWELTCHEMIE AG, CH-4452 Itingen, Switzerland. The experiment was conducted in October-November 1994 and the study was completed in March 1996.
	Metric 3:	Test Substance Purity	High	Test substance purity was listed as 99.15% on April 5, 1994. The experiment was conducted in October-November 1994 and the study was completed in March 1996. The test facility: RCC UMWELTCHEMIE AG, CH-4452 Itingen, Switzerland. This was an industry submitted test study under the EPA 2019 initiated REs; industry volunteered the study.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	An appropriate negative control was reported.
	Metric 5:	Negative Control Response	Low	The negative control response was not reported.
	Metric 6:	Randomized Allocation	Low	The range finding study did not report how the fish were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The test system for the range finding test and the preparation of the test media were not described in great detail.
	Metric 8:	Consistency of Exposure Administration	Low	Little information was provided about the exposure administration for the range finding test.
	Metric 9:	Measurement of Test Substance Concentration	Low	It was not reported if the test concentrations were analyze in the range finding test.
	Metric 10:	Exposure Duration and Frequency	Medium	The range finding test was for 96h, which was appropriate.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were five test concentrations in the range finding test. It may have been beneficial to test more test concentrations between 10000 and 2000ug/L
	Metric 12:	Testing at or Below Solubility Limit	Medium	The highest test concentration was 10000ug/L, which was well above the solubility limit. The test concentrations were only reported as nominal for the range finding test, so it is unclear what the actual test concentration was. The other test concentrations used were within the solubility limit. DMF was used as a solvent.
Domain 4: Test Organism				

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Study Citation:	Wüthrich, V. (1996). HHCB: 21-Day prolonged toxicity study in the bluegill sunfish under flow-through conditions.
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:	Vertebrate; Fish; *Lepomis macrochirus*; Juvenile
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	7607846

Domain		Metric	Rating	Comments
	Metric 13:	Test Organism Characteristics	High	Bluegill sunfish is a recognized species for fish testing (e.g., OECD Test No. 203). This study followed OECD Test No 204, which was later deleted in 2014.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Fish were acclimatized prior to start of the experiment (7 days of holding).
	Metric 15:	Number of Organisms and Replicates per Group	Low	There were five fish per test concentration in the range finding study. The number of replicates was not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism housing was appropriate. Fish were fed daily: 4% body weight in week 1 followed by 25% increase from week 1 in week 2, and 50% increase from week 1 in week 3. Water temperature, dissolved oxygen and pH values were recorded 3X a week and were appropriate (see Tables 1, 2, 3). Biomass loading was appropriate (0.12-0.16 g/l).
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (fish tanks were checked for dead fish daily) addressed the intended outcome of interest and was appropriate. (Mortality after 96h.)
	Metric 18:	Consistency of Outcome Assessment	High	Little information was provided on the consistency of the outcome assessment and the study protocol for the range finding test.
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.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Uninformative	Statistical analysis was not reported for the range finding test, nor was data reported for each test concentration.
	Metric 22:	Reporting of Data	Low	Data was not reported for each exposure group or control.
	Metric 23:	Explanation of Unexpected Outcomes	Low	The range finding study did not report any variability.

Additional Comments: This evaluation is for the range finding test. It received an unacceptable rating due to lack of information on statistical analysis and lack of reporting on results for an independent statistical analysis.

Overall Quality Determination Uninformative

Study Citation:	Wüthrich, V. (1996). HHCB: 21-Day prolonged toxicity study in the bluegill sunfish under flow-through conditions.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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; *Lepomis macrochirus*; Juvenile
Health Outcome:	Respiratory
Chemical:	HHCB
HERO ID:	7607846

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCB was identified by appropriate nomenclature and CASRN.
	Metric 2:	Test Substance Source	High	Test substance was supplied by the sponsor: RIFM, Two University Plaza, Suite 406, Hackensack, NJ 07601-6209, U.S.A. There was no information on the manufacturer of the substance, but a lot number was identified as ES-6302-H and expiration date as April 1996.This was an industry submitted test study by test facility: RCC UMWELTCHEMIE AG, CH-4452 Itingen, Switzerland. The experiment was conducted in October-November 1994 and the study was completed in March 1996.
	Metric 3:	Test Substance Purity	High	Test substance purity was listed as 99.15% on April 5, 1994. The experiment was conducted in October-November 1994 and the study was completed in March 1996. The test facility: RCC UMWELTCHEMIE AG, CH-4452 Itingen, Switzerland. This was an industry submitted test study under the EPA 2019 initiated REs; industry volunteered the study.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A solvent control and negative controls were used. Briefly, the flow-through system comprised of five vessels for the test concentrations, one vessel for the solvent control containing Tween 80 (0.005%) and dimethylformamide (DMF, 0.005%), and one vessel for the control without solvents.
	Metric 5:	Negative Control Response	High	Clinical signs found in the control were reported in Table 10 and were appropriate.
	Metric 6:	Randomized Allocation	Medium	The study reported that prior to treatment 10 randomly netted fish were weighed and their body length was measured.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Test media preparations were described in detail in section 2.7. The exposure was a flow-through exposure to yield test article concentrations of 2000, 1000, 500, 250, and 125 ug/L, each containing 0.005% Tween 80 and 0.005% DMF. The stock solutions were freshly prepared every 24 hours. In addition, a stock solution containing 5 ml of DMF and 5 g of Tween 80 per 1000 ml of bidistilled water was prepared for connection to the dilutor system to prepare a control tank containing the same content of solvents as the test solutions with test article. A seventh tank was filled with tap water, only for the control without solvents.
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration was consistent across treatment groups. Treatment was administered via a dilutor system that was monitored every 24 hours.

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Study Citation: Wüthrich, V. (1996). HHCB: 21-Day prolonged toxicity study in the bluegill sunfish under flow-through conditions.
Duration: Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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; *Lepomis macrochirus*; Juvenile
Health Outcome: Respiratory
Chemical: HHCB
HERO ID: 7607846

Domain	Metric		Rating	Comments
	Metric 9:	Measurement of Test Substance Concentration	High	Nominal test concentrations were analytically verified. Briefly, samples were taken on Day 0, 9, and 21. Duplicate samples of the solvent control, 125, 250, 500, 1000, and 2000 ug/L nominal test concentrations were analyzed by HPLC on the day of sampling.
	Metric 10:	Exposure Duration and Frequency	Medium	This was an industry submitted test study. The experiment was conducted in October-November 1994 and the study was completed in March 1996. It followed OECD Test No. 204: Fish, Prolonged Toxicity Test: 14-Day Study Information. The author extended the study test to 21 days. Following the OECD Council decision, the Test Guideline 204 'Fish, Prolonged Toxicity Test: 14-Day Study' was deleted on 2nd April 2014 (two decades after the experiment was conducted). Current OECD Test No. 215 'Fish, Juvenile Growth Test' is the test guideline that determines mortality and growth in juvenile fish. Note that in OECD Test No. 215, bluegill sunfish are not listed among the recommended species and recommended the test duration is 28 days.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The experiment included 5 exposure groups (measured concentration of 92.5, 181.8, 393, 830, and 1566 ug/L), a solvent control group, and a negative control group. This experiment was conducted following OECD Test No. 204, which was deleted in 2014, but for example, OECD Test No. 215 (similar currently active test guideline) asks for five exposure groups.
	Metric 12:	Testing at or Below Solubility Limit	High	HHCB solubility in water is 1.65-1.99 mg/L (1650-1990 ug/L) at 25 °C and pH 5-9. Nominal test concentrations were 125, 250, 500, 1000, and 2000 ug/L. Solutions were prepared in 0.005% Tween 80 and 0.005% DMF to facilitate solubility and produce homogenous solutions. Treatment was administered in a flow-through system and solutions were prepared freshly every 24 hours. Reported measured concentrations were 92.5, 181.8, 393, 830, and 1566 ug/L, respectively.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Bluegill sunfish is a recognized species for fish testing (e.g., OECD Test No. 203). This study followed OECD Test No 204, which was later deleted in 2014.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Fish were acclimatized prior to start of the experiment (7 days of holding).
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 fish/group were used in the study and no replicate tanks. For example, OECD Test No. 215 indicates that between-tank variability is very small compared with within-tank (i.e. between-fish); thus, replicate tanks are recommended but not required.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism housing was appropriate. Fish were fed daily: 4% body weight in week 1 followed by 25% increase from week 1 in week 2, and 50% increase from week 1 in week 3. Water temperature, dissolved oxygen and pH values were recorded 3X a week and were appropriate (see Tables 1, 2, 3). Biomass loading was appropriate (0.12-0.16 g/l).

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Study Citation:	Wüthrich, V. (1996). HHCB: 21-Day prolonged toxicity study in the bluegill sunfish under flow-through conditions.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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; *Lepomis macrochirus*; Juvenile
Health Outcome:	Respiratory
Chemical:	HHCB
HERO ID:	7607846

Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest– Respiratory rate due to exposure and other clinical signs of intoxication.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported (Table 10), and outcomes were assessed consistently across study groups.
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.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Possible statistical analysis could be conducted on the behavioral and clinical outcomes.
	Metric 22:	Reporting of Data	High	Respiratory rate changes due to intoxication are reported for all exposure groups and the controls in Table 10.
	Metric 23:	Explanation of Unexpected Outcomes	High	Variability was explained as S.D.

Additional Comments: This evaluation is for the changes in respiratory rate reported in Table 10 due to HHCB toxicity.

Overall Quality Determination High

Study Citation:	Wüthrich, V. (1996). HHCB: 21-Day prolonged toxicity study in the bluegill sunfish under flow-through conditions.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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; *Lepomis macrochirus*; Juvenile
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	7607846

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCB was identified by appropriate nomenclature and CASRN.
	Metric 2:	Test Substance Source	High	Test substance was supplied by the sponsor: RIFM, Two University Plaza, Suite 406, Hackensack, NJ 07601-6209, U.S.A. There was no information on the manufacturer of the substance, but a lot number was identified as ES-6302-H and expiration date as April 1996.This was an industry submitted test study by test facility: RCC UMWELTCHEMIE AG, CH-4452 Itingen, Switzerland. The experiment was conducted in October-November 1994 and the study was completed in March 1996.
	Metric 3:	Test Substance Purity	High	Test substance purity was listed as 99.15% on April 5, 1994. The experiment was conducted in October-November 1994 and the study was completed in March 1996. The test facility: RCC UMWELTCHEMIE AG, CH-4452 Itingen, Switzerland. This was an industry submitted test study under the EPA 2019 initiated REs; industry volunteered the study.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A solvent control and negative controls were used. Briefly, the flow-through system comprised of five vessels for the test concentrations, one vessel for the solvent control containing Tween 80 (0.005%) and dimethylformamide (DMF, 0.005%), and one vessel for the control without solvents.
	Metric 5:	Negative Control Response	High	Clinical signs found in the control were reported in Table 10 and were appropriate.
	Metric 6:	Randomized Allocation	Medium	The study reported that prior to treatment 10 randomly netted fish were weighed and their body length was measured.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Test media preparations were described in detail in section 2.7. The exposure was a flow-through exposure to yield test article concentrations of 2000, 1000, 500, 250, and 125 ug/L, each containing 0.005% Tween 80 and 0.005% DMF. The stock solutions were freshly prepared every 24 hours. In addition, a stock solution containing 5 ml of DMF and 5 g of Tween 80 per 1000 ml of bidistilled water was prepared for connection to the dilutor system to prepare a control tank containing the same content of solvents as the test solutions with test article. A seventh tank was filled with tap water, only for the control without solvents.
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration was consistent across treatment groups. Treatment was administered via a dilutor system that was monitored every 24 hours.
	Metric 9:	Measurement of Test Substance Concentration	High	Nominal test concentrations were analytically verified. Briefly, samples were taken on Day 0, 9, and 21. Duplicate samples of the solvent control, 125, 250, 500, 1000, and 2000 ug/L nominal test concentrations were analyzed by HPLC on the day of sampling.

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Study Citation:	Wüthrich, V. (1996). HHCB: 21-Day prolonged toxicity study in the bluegill sunfish under flow-through conditions.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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; *Lepomis macrochirus*; Juvenile
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	7607846

Domain	Metric		Rating	Comments
	Metric 10:	Exposure Duration and Frequency	Medium	This was an industry submitted test study. The experiment was conducted in October-November 1994 and the study was completed in March 1996. It followed OECD Test No. 204: Fish, Prolonged Toxicity Test: 14-Day Study Information. The author extended the study test to 21 days. Following the OECD Council decision, the Test Guideline 204 'Fish, Prolonged Toxicity Test: 14-Day Study' was deleted on 2nd April 2014 (two decades after the experiment was conducted). Current OECD Test No. 215 'Fish, Juvenile Growth Test' is the test guideline that determines mortality and growth in juvenile fish. Note that in OECD Test No. 215, bluegill sunfish are not listed among the recommended species and recommended the test duration is 28 days.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The experiment included 5 exposure groups (measured concentration of 92.5, 181.8, 393, 830, and 1566 ug/L), a solvent control group, and a negative control group. This experiment was conducted following OECD Test No. 204, which was deleted in 2014, but for example, OECD Test No. 215 (similar currently active test guideline) asks for five exposure groups.
	Metric 12:	Testing at or Below Solubility Limit	High	HHCB solubility in water is 1.65-1.99 mg/L (1650-1990 ug/L) at 25 °C and pH 5-9. Nominal test concentrations were 125, 250, 500, 1000, and 2000 ug/L. Solutions were prepared in 0.005% Tween 80 and 0.005% DMF to facilitate solubility and produce homogenous solutions. Treatment was administered in a flow-through system and solutions were prepared freshly every 24 hours. Reported measured concentrations were 92.5, 181.8, 393, 830, and 1566 ug/L, respectively.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Bluegill sunfish is a recognized species for fish testing (e.g., OECD Test No. 203). This study followed OECD Test No 204, which was later deleted in 2014.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Fish were acclimatized prior to start of the experiment (7 days of holding).
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 fish/group were used in the study and no replicate tanks. For example, OECD Test No. 215 indicates that between-tank variability is very small compared with within-tank (i.e. between-fish); thus, replicate tanks are recommended but not required.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism housing was appropriate. Fish were fed daily: 4% body weight in week 1 followed by 25% increase from week 1 in week 2, and 50% increase from week 1 in week 3. Water temperature, dissolved oxygen and pH values were recorded 3X a week and were appropriate (see Tables 1, 2, 3). Biomass loading was appropriate (0.12-0.16 g/l).
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed the intended outcome of interest– behavioral changes due to exposure and other clinical signs of intoxication.

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Study Citation:	Wüthrich, V. (1996). HHCB: 21-Day prolonged toxicity study in the bluegill sunfish under flow-through conditions.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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; *Lepomis macrochirus*; Juvenile
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	7607846

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported (Table 10), and outcomes were assessed consistently across study groups.
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.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Possible statistical analysis could be conducted on the behavioral and clinical outcomes.
	Metric 22:	Reporting of Data	High	Behavioral changes due to intoxication are reported for all exposure groups and the controls in Table 10.
	Metric 23:	Explanation of Unexpected Outcomes	High	Variability was explained as S.D.

Additional Comments: This evaluation is for the changes in behavior reported in Table 10 due to HHCB toxicity.

Overall Quality Determination High

Study Citation:	Wüthrich, V. (1996). HHCB: 21-Day prolonged toxicity study in the bluegill sunfish under flow-through conditions.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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; *Lepomis macrochirus*; Juvenile
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	7607846

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCB was identified by appropriate nomenclature and CASRN.
	Metric 2:	Test Substance Source	High	Test substance was supplied by the sponsor: RIFM, Two University Plaza, Suite 406, Hackensack, NJ 07601-6209, U.S.A. There was no information on the manufacturer of the substance, but a lot number was identified as ES-6302-H and expiration date as April 1996.This was an industry submitted test study by test facility: RCC UMWELTCHEMIE AG, CH-4452 Itingen, Switzerland. The experiment was conducted in October-November 1994 and the study was completed in March 1996.
	Metric 3:	Test Substance Purity	High	Test substance purity was listed as 99.15% on April 5, 1994. The experiment was conducted in October-November 1994 and the study was completed in March 1996. The test facility: RCC UMWELTCHEMIE AG, CH-4452 Itingen, Switzerland. This was an industry submitted test study under the EPA 2019 initiated REs; industry volunteered the study.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A solvent control and negative controls were used. Briefly, the flow-through system comprised of five vessels for the test concentrations, one vessel for the solvent control containing Tween 80 (0.005%) and dimethylformamide (DMF, 0.005%), and one vessel for the control without solvents.
	Metric 5:	Negative Control Response	High	Mortality in the control groups was 0% (see Tables 4 and 5 for details).
	Metric 6:	Randomized Allocation	Medium	The study reported that prior to treatment 10 randomly netted fish were weighed and their body length was measured.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Test media preparations were described in detail in section 2.7. The exposure was a flow-through exposure to yield test article concentrations of 2000, 1000, 500, 250, and 125 ug/L, each containing 0.005% Tween 80 and 0.005% DMF. The stock solutions were freshly prepared every 24 hours. In addition, a stock solution containing 5 ml of DMF and 5 g of Tween 80 per 1000 ml of bidistilled water was prepared for connection to the dilutor system to prepare a control tank containing the same content of solvents as the test solutions with test article. A seventh tank was filled with tap water, only for the control without solvents.
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration was consistent across treatment groups. Treatment was administered via a dilutor system that was monitored every 24 hours.
	Metric 9:	Measurement of Test Substance Concentration	High	Nominal test concentrations were analytically verified. Briefly, samples were taken on Day 0, 9, and 21. Duplicate samples of the solvent control, 125, 250, 500, 1000, and 2000 ug/L nominal test concentrations were analyzed by HPLC on the day of sampling.

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Study Citation:	Wüthrich, V. (1996). HHCB: 21-Day prolonged toxicity study in the bluegill sunfish under flow-through conditions.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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; *Lepomis macrochirus*; Juvenile
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	7607846

Domain	Metric		Rating	Comments
	Metric 10:	Exposure Duration and Frequency	Medium	This was an industry submitted test study. The experiment was conducted in October-November 1994 and the study was completed in March 1996. It followed OECD Test No. 204: Fish, Prolonged Toxicity Test: 14-Day Study Information. The author extended the study test to 21 days. Following the OECD Council decision, the Test Guideline 204 'Fish, Prolonged Toxicity Test: 14-Day Study' was deleted on 2nd April 2014 (two decades after the experiment was conducted). Current OECD Test No. 215 'Fish, Juvenile Growth Test' is the test guideline that determines mortality and growth in juvenile fish. Note that in OECD Test No. 215, bluegill sunfish are not listed among the recommended species and recommended the test duration is 28 days.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The experiment included 5 exposure groups (measured concentration of 92.5, 181.8, 393, 830, and 1566 ug/L), a solvent control group, and a negative control group. This experiment was conducted following OECD Test No. 204, which was deleted in 2014, but for example, OECD Test No. 215 (similar currently active test guideline) asks for five exposure groups.
	Metric 12:	Testing at or Below Solubility Limit	High	HHCB solubility in water is 1.65-1.99 mg/L (1650-1990 ug/L) at 25 °C and pH 5-9. Nominal test concentrations were 125, 250, 500, 1000, and 2000 ug/L. Solutions were prepared in 0.005% Tween 80 and 0.005% DMF to facilitate solubility and produce homogenous solutions. Treatment was administered in a flow-through system and solutions were prepared freshly every 24 hours. Reported measured concentrations were 92.5, 181.8, 393, 830, and 1566 ug/L, respectively.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Bluegill sunfish is a recognized species for fish testing (e.g., OECD Test No. 203). This study followed OECD Test No 204, which was later deleted in 2014.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Fish were acclimatized prior to start of the experiment (7 days of holding).
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 fish/group were used in the study and no replicate tanks. For example, OECD Test No. 215 indicates that between-tank variability is very small compared with within-tank (i.e. between-fish); thus, replicate tanks are recommended but not required.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism housing was appropriate. Fish were fed daily: 4% body weight in week 1 followed by 25% increase from week 1 in week 2, and 50% increase from week 1 in week 3. Water temperature, dissolved oxygen and pH values were recorded 3X a week and were appropriate (see Tables 1, 2, 3). Biomass loading was appropriate (0.12-0.16 g/l).
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (fish tanks were checked for dead fish daily) addressed the intended outcome of interest and was appropriate. 21-day LC50 = 452 ug/L (95% confidence limits 316-911 ug/L); mortality (without growth effects) LOEC = 393 ug/L.

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Study Citation:	Wüthrich, V. (1996). HHCB: 21-Day prolonged toxicity study in the bluegill sunfish under flow-through conditions.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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; *Lepomis macrochirus*; Juvenile
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	7607846

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported (Tables 4, 5, 6), and outcomes were assessed consistently across study groups.
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.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The LC50 value was estimated by Logit model. 21-day LC50 = 452 ug/L (95% confidence limits 316-911 ug/L); mortality (without growth effects) LOEC = 393 ug/L.
	Metric 22:	Reporting of Data	High	Mortality was reported for all treatment groups. Tables 4 and 5.
	Metric 23:	Explanation of Unexpected Outcomes	High	Variability was explained as 95% confidence limits 316-911 ug/L.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Wüthrich, V. (1996). HHCB: 21-Day prolonged toxicity study in the bluegill sunfish under flow-through conditions.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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; *Lepomis macrochirus*; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	7607846

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCB was identified by appropriate nomenclature and CASRN.
	Metric 2:	Test Substance Source	High	Test substance was supplied by the sponsor: RIFM, Two University Plaza, Suite 406, Hackensack, NJ 07601-6209, U.S.A. There was no information on the manufacturer of the substance, but a lot number was identified as ES-6302-H and expiration date as April 1996.This was an industry submitted test study by test facility: RCC UMWELTCHEMIE AG, CH-4452 Itingen, Switzerland. The experiment was conducted in October-November 1994 and the study was completed in March 1996.
	Metric 3:	Test Substance Purity	High	Test substance purity was listed as 99.15% on April 5, 1994. The experiment was conducted in October-November 1994 and the study was completed in March 1996. The test facility: RCC UMWELTCHEMIE AG, CH-4452 Itingen, Switzerland. This was an industry submitted test study under the EPA 2019 initiated REs; industry volunteered the study.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A solvent control and negative controls were used. Briefly, the flow-through system comprised of five vessels for the test concentrations, one vessel for the solvent control containing Tween 80 (0.005%) and dimethylformamide (DMF, 0.005%), and one vessel for the control without solvents.
	Metric 5:	Negative Control Response	High	Weight and length in the solvent and negative control groups increased as expected after 21-days. (see Tables 8 and 9 for details).
	Metric 6:	Randomized Allocation	Medium	The study reported that prior to treatment 10 randomly netted fish were weighed and their body length was measured.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Test media preparations were described in detail in section 2.7. The exposure was a flow-through exposure to yield test article concentrations of 2000, 1000, 500, 250, and 125 ug/L, each containing 0.005% Tween 80 and 0.005% DMF. The stock solutions were freshly prepared every 24 hours. In addition, a stock solution containing 5 ml of DMF and 5 g of Tween 80 per 1000 ml of bidistilled water was prepared for connection to the dilutor system to prepare a control tank containing the same content of solvents as the test solutions with test article. A seventh tank was filled with tap water, only for the control without solvents.
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration was consistent across treatment groups. Treatment was administered via a dilutor system that was monitored every 24 hours.
	Metric 9:	Measurement of Test Substance Concentration	High	Nominal test concentrations were analytically verified. Briefly, samples were taken on Day 0, 9, and 21. Duplicate samples of the solvent control, 125, 250, 500, 1000, and 2000 ug/L nominal test concentrations were analyzed by HPLC on the day of sampling.

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Study Citation:	Wüthrich, V. (1996). HHCB: 21-Day prolonged toxicity study in the bluegill sunfish under flow-through conditions.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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; *Lepomis macrochirus*; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	7607846

Domain	Metric		Rating	Comments
	Metric 10:	Exposure Duration and Frequency	Medium	This was an industry submitted test study. The experiment was conducted in October-November 1994 and the study was completed in March 1996. It followed OECD Test No. 204: Fish, Prolonged Toxicity Test: 14-Day Study Information. The author extended the study test to 21 days. Following the OECD Council decision, the Test Guideline 204 'Fish, Prolonged Toxicity Test: 14-Day Study' was deleted on 2nd April 2014 (two decades after the experiment was conducted). Current OECD Test No. 215 'Fish, Juvenile Growth Test' is the test guideline that determines mortality and growth in juvenile fish. Note that in OECD Test No. 215, bluegill sunfish are not listed among the recommended species and recommended the test duration is 28 days.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The experiment included 5 exposure groups (measured concentration of 92.5, 181.8, 393, 830, and 1566 ug/L), a solvent control group, and a negative control group. This experiment was conducted following OECD Test No. 204, which was deleted in 2014, but for example, OECD Test No. 215 (similar currently active test guideline) asks for five exposure groups.
	Metric 12:	Testing at or Below Solubility Limit	High	HHCB solubility in water is 1.65-1.99 mg/L (1650-1990 ug/L) at 25 °C and pH 5-9. Nominal test concentrations were 125, 250, 500, 1000, and 2000 ug/L. Solutions were prepared in 0.005% Tween 80 and 0.005% DMF to facilitate solubility and produce homogenous solutions. Treatment was administered in a flow-through system and solutions were prepared freshly every 24 hours. Reported measured concentrations were 92.5, 181.8, 393, 830, and 1566 ug/L, respectively.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Bluegill sunfish is a recognized species for fish testing (e.g., OECD Test No. 203). This study followed OECD Test No 204, which was later deleted in 2014.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Fish were acclimatized prior to start of the experiment (7 days of holding).
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 fish/group were used in the study and no replicate tanks. For example, OECD Test No. 215 indicates that between-tank variability is very small compared with within-tank (i.e. between-fish); thus, replicate tanks are recommended but not required.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism housing was appropriate. Fish were fed daily: 4% body weight in week 1 followed by 25% increase from week 1 in week 2, and 50% increase from week 1 in week 3. Water temperature, dissolved oxygen and pH values were recorded 3X a week and were appropriate (see Tables 1, 2, 3). Biomass loading was appropriate (0.12-0.16 g/l).

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Study Citation:	Wüthrich, V. (1996). HHCB: 21-Day prolonged toxicity study in the bluegill sunfish under flow-through conditions.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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; *Lepomis macrochirus*; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	7607846

Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (fish tanks were measured and weighed at the end of the study) addressed the intended outcome of interest. No statistically significant effect on fish growth (weight and length) was observed after 21 days of exposure at test concentrations 92.5 and 181.8 ug/L. A statistical influence on length and weight was observed at 393 ug/L. Fish died after 14 days at 830 ug/L and after 6 days at 1566 ug/L (so, no growth effects could not be calculated for these two highest concentrations).
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported (Tables 7, 8, 9), and outcomes were assessed consistently across study groups.
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.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Body length and weight were evaluated statistically by Dunnett test. No statistically significant effect on fish growth (weight and length) was observed after 21 days of exposure at test concentrations 92.5 and 181.8 ug/L. A statistical influence on length and weight was observed at 393 ug/L. Fish died after 14 days at 830 ug/L and after 6 days at 1566 ug/L (so, no growth effects could not be calculated for these two highest concentrations).
	Metric 22:	Reporting of Data	High	Growth (weight and length) was reported for all treatment groups. Tables 8 and 9.
	Metric 23:	Explanation of Unexpected Outcomes	High	Variability was explained as S.D.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Dijk, Van, A. (1996). Accumulation and elimination of 14C-HHCB by Bluegill Sunfish in a dynamic flow-through system.
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; *Lepomis macrochirus*; Juvenile
Health Outcome:	ADME (biotransformation)
Chemical:	HHCB
HERO ID:	5352386

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Correct nomenclature and CASRN (1222-05-5) was provided.
	Metric 2:	Test Substance Source	High	The report indicates that the the chemical was supplied by the sponsor RIFM.
	Metric 3:	Test Substance Purity	High	The reported purity of unlabeled HHCB was 99.15% and that of total radiochemically labeled HHCB was 99.5%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A control group (0 ug/L labelled HHCB) was used in the study.
	Metric 5:	Negative Control Response	N/A	This was a bioaccumulation study and thus, there was no biological response per se that was measured. Authors measured uptake of HHCB; in the control group, HHCB was not detected. That said, the study would be deemed unacceptable if mortality in the control group exceeded 10%; but, mortality in the control group was 0% throughout study as reported by the author.
	Metric 6:	Randomized Allocation	Low	The study did not specifically state that they used random allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	This was a flow-through system, and test media preparation was described.
	Metric 8:	Consistency of Exposure Administration	High	HHCB administration was consistent throughout the study (28 days exposure + 28 days depuration) as per the measured values of HHCB in water and fish tissue.
	Metric 9:	Measurement of Test Substance Concentration	High	The goal of the study was to examine bioaccumulation and depuration of HHCB in fish. For this, the author measured HHCB concentrations in water and fish on exposure days 0 (only water), 3, 7, 14, 21, and 28 & on depuration days 1, 3, 7, 10, 14, and 28 using thin-layer chromatography (fish samples) and high performance liquid chromatography (water samples and select fish samples).
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration for bioaccumulation studies was appropriate and followed standard guidelines: exposure phase of 28 days and depuration phase of 28 days for a total study duration of 56 days.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	This was a bioaccumulation study, a dose-dependent health effect was not the goal of the study. The use of two concentrations for a biaccumulation test was appropriate because of HHCB's intermediate polarity (i.e., HHCB is not a very polar compound although one of its metabolites is known to be polar). [For non-polar organic substances, the exposure of fish to a single concentration is sufficient; although two concentrations may be required for the relevant regulatory framework. For polar substances, the test should be run with at least two concentrations.]

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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; *Lepomis macrochirus*; Juvenile
Health Outcome:	ADME (biotransformation)
Chemical:	HHCB
HERO ID:	5352386

Domain	Metric		Rating	Comments
	Metric 12:	Testing at or Below Solubility Limit	High	HHCB concentrations were below the water solubility limit; radiolabeled solutions were prepared in the presence of Tween 80 and DMF.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms (bluegill sunfish) were appropriately described and adequate for the bioaccumulation study. The species is listed as an acceptable test species by EPA OCSPP 850.1730 and OECD TG 305. The age should have been stated (and seems to not be) but authors did provide average weight of the individual fish.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimation period for at least a week was reported by the author (section 2.2).
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of fish per group was appropriate for the bioaccumulation study. At least six fish per concentration per sampling time were collected during five accumulation sampling points (day 3, 7, 14, 21, & 28) and seven depuration sampling points (day 1, 3, 7, 10, 14, & 28). [OCSPP 850.1730 indicates that a minimum of four fish per sample are available at each sampling interval per test concentration. The fish may be distributed among two or more replicates at each treatment.]
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Housing conditions were appropriate.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (bioaccumulation and depuration of HHCB in fish) was appropriate to calculate the BCF and thus determine if HHCB is bioaccumulative. BCF values were 498, 2175 and 1635 for edibles, non-edibles and whole fish, respectively.
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment methods were consistently across study groups.
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.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Calculations to determine the accumulation rate constant, depuration rate constant, and BCFs were appropriate and followed standardized guidelines.
	Metric 22:	Reporting of Data	High	The reporting of data (e.g., BCFs) for the bioaccumulation test was appropriate.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

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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; *Lepomis macrochirus*; Juvenile
Health Outcome:	ADME (biotransformation)
Chemical:	HHCB
HERO ID:	5352386

Domain	Metric	Rating	Comments
Additional Comments:	The goal of the study was to examine HHCB bioaccumulation and depuration using radioactively labelled 1 ug/L and 10 ug/L of HHCB in bluegill sunfish. BCFs were calculated from the analyses. In addition to the bioaccumulation and depuration study, the authors conducted a toxicity pre-test by exposing fish to 10, 20, 50 ug/L un-labelled (parent compound) HHCB for 96 hr in order to determine dosage for the bioaccumulation portion of the study; the author reported no mortalities or health symptoms were observed in the toxicity pre-test (i.e., no actual data was shown in graph or table form on the pre-test in section 3.1). Therefore, only one health outcome (child form 2b; ADME) was added for evaluation of bioaccumulation and depuration. Also, in the allocation table in section 2.2, the author reported using bluegill sunfish with an average fish weight of 1.2-1.4g , which is indicative of juvenile size.		

Overall Quality Determination High

Study Citation:	Schneider, S. Z., Zhang, L., Martin, K. H., Aufderheide, J. A. (2021). HHCB: A dietary exposure bioaccumulation test with the bluegill sunfish (Lepomis macrochirus). Final report.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Fish; *Lepomis macrochirus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	7607948

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Correct nomenclature was used.
	Metric 2:	Test Substance Source	High	Authors indicated that the radio-labelled HHCB was received from Moravek and identified as HHCB, [3-14C], Lot number 252-186-0587-A-20060515-JI (testing facility identification number 12213). The non-radio-labelled test substance was received from International Flavors and Fragrances, Inc. and identified as HHCB, Lot number 148-114 (testing facility identification number 12161).
	Metric 3:	Test Substance Purity	Medium	The certificate of analysis indicated that the radio-labelled HHCB was 99.3% pure (page 78) and that of the reference substance (HCB, as recommended by OECD Test No. 305) was >99% (page 79). However, it is not clear what purity of the the non-radio-labelled HHCB was.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A negative control (0 ug HHCB/g of diet) was included in the study.
	Metric 5:	Negative Control Response	High	This was a bioaccumulation study. Uptake and depuration results showed that HHCB in the control was below the level of quantitation (LOQ).
	Metric 6:	Randomized Allocation	Low	The study did not state/describe random allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The study was a dietary bioaccumulation with uptake and depuration phases. The experimental methods for preparation of diet for exposure were adequate and described in detail.
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration details were reported and administered consistently across groups. Fish were fed daily 2% body weight. During uptake, nominal concentrations were 50 or 500 ug HHCB/g of diet; measured concentrations 41 or 548 ug HHCB/g of diet.
	Metric 9:	Measurement of Test Substance Concentration	High	Tissue samples were collected on uptake day 14 and on depuration days 1, 3, 7, 14 and 28. Radiolabeled analysis of HHCB (concentrations of the test substance in fish food and fish tissue) was done using liquid scintillation counting (LSC). In addition, analysis of HCB (the reference substance recommended by OECD Test No. 305) in fish diet was measured by gas chromatography with mass selective detection (GC/MSD).
	Metric 10:	Exposure Duration and Frequency	High	The bioaccumulation study had an uptake phase and depuration phase. The total length of the study was 42 Days (14 Days Uptake; 28 Days Depuration). Uptake duration and depuration duration followed dietary exposure test guideline (OECD Test No. 305 Bioaccumulation in Fish: Aqueous and Dietary Exposure).

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Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Fish; *Lepomis macrochirus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	7607948

Domain	Metric		Rating	Comments
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Nominal test concentrations: 0, 50, 500 ug/L; measured test concentrations: <LOQ, 41, 548 ug/L. The two exposure concentration groups satisfied OECD Test No. 305, which states that one test group is sufficient in dietary bioaccumulation exposures.
	Metric 12:	Testing at or Below Solubility Limit	High	The water solubility of HHCB is 1750 ug/L (also stated on page 57 of the study). The dietary exposure was at 50 and 500 ug/g of diet, which was below the solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organism was bluegill (Lepomis macrochirus). Source of test organisms was Osage Catfisheries, Inc. Bluegill are listed as recommended test species in Annex 3 of OECD Test No. 305.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Test organisms were acclimatized prior to the uptake phase for 14 days.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Each treatment group consisted of one test chamber with 90 fish in each chamber. Replicate tanks are not required in the dietary bioaccumulation study.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Housing conditions (temperature, pH, dissolved oxyden, hardness, alkalinity, conductivity) were adequate (see Appendix 9 through 12).
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (dietary exposure of feed spiked with radiolabeled HHCB) was appropriate for determining the bioaccumulation potential of HHCB (BMFs of HHCB) in fish.
	Metric 18:	Consistency of Outcome Assessment	High	Study details pointed to outcome assessment protocol being assessed consistently across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups that could influence the outcome.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was performed to calculate BMFs; methods followed standardized guidelines (OECD Test No. 305). HHCB Lipid Corrected Biomagnification Factor (BMF KL) was 0.082.
	Metric 22:	Reporting of Data	High	Reporting of data was adequate and authors followed standardized guidelines (OECD Test No. 305).

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Exposure Route, Media, Path:	Aquatic (freshwater); Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Fish; *Lepomis macrochirus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	7607948

Domain	Metric	Rating	Comments
	Metric 23: Explanation of Unexpected Outcomes	High	SD and CV were calculated for measured concentrations of total radioactivity in HHCB treatment diet (e.g., Tables 2 and 3).

Additional Comments: Study was a laboratory bioaccumulation test [HHCB: A DIETARY EXPOSURE BIOACCUMULATION TESTWITH THE BLUEGILL SUNFISH (Lepomis macrochirus); STUDY NUMBER: 558A-125]. Submitted to Research Institute for Fragrance Materials, Inc. by testing facility Eurofins EAG Agroscience, LLC. The study was initiated on 13 March 2015, completed on 23 October 2020, and amended on 1 April 2021. The objective of this study was to obtain laboratory data to determine the bioaccumulation potential of HHCB in the bluegill sunfish, Lepomis macrochirus, through dietary exposure. The test substance used in the test was HHCB. The reference substance used in the test was hexachlorobenzene (HCB). This evaluation form is for the mortality outcome reported in Appendix 13.

Overall Quality Determination High

Study Citation:	Schneider, S. Z., Zhang, L., Martin, K. H., Aufderheide, J. A. (2021). HHCB: A dietary exposure bioaccumulation test with the bluegill sunfish (Lepomis macrochirus). Final report.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Fish; *Lepomis macrochirus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Nutritional & Metabolic
Chemical:	HHCB
HERO ID:	7607948

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Correct nomenclature was used.
	Metric 2:	Test Substance Source	High	Authors indicated that the radio-labelled HHCB was received from Moravek and identified as HHCB, [3-14C], Lot number 252-186-0587-A-20060515-JI (testing facility identification number 12213). The non-radio-labelled test substance was received from International Flavors and Fragrances, Inc. and identified as HHCB, Lot number 148-114 (testing facility identification number 12161).
	Metric 3:	Test Substance Purity	Medium	The certificate of analysis indicated that the radio-labelled HHCB was 99.3% pure (page 78) and that of the reference substance (HCB, as recommended by OECD Test No. 305) was >99% (page 79). However, it is not clear what purity of the the non-radio-labelled HHCB was.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A negative control (0 ug HHCB/g of diet) was included in the study.
	Metric 5:	Negative Control Response	High	This was a bioaccumulation study. Uptake and depuration results showed that HHCB in the control was below the level of quantitation (LOQ).
	Metric 6:	Randomized Allocation	Low	The study did not state/describe random allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The study was a dietary bioaccumulation with uptake and depuration phases. The experimental methods for preparation of diet for exposure were adequate and described in detail.
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration details were reported and administered consistently across groups. Fish were fed daily 2% body weight. During uptake, nominal concentrations were 50 or 500 ug HHCB/g of diet; measured concentrations 41 or 548 ug HHCB/g of diet.
	Metric 9:	Measurement of Test Substance Concentration	High	Tissue samples were collected on uptake day 14 and on depuration days 1, 3, 7, 14 and 28. Radiolabeled analysis of HHCB (concentrations of the test substance in fish food and fish tissue) was done using liquid scintillation counting (LSC). In addition, analysis of HCB (the reference substance recommended by OECD Test No. 305) in fish diet was measured by gas chromatography with mass selective detection (GC/MSD).
	Metric 10:	Exposure Duration and Frequency	High	The bioaccumulation study had an uptake phase and depuration phase. The total length of the study was 42 Days (14 Days Uptake; 28 Days Depuration). Uptake duration and depuration duration followed dietary exposure test guideline (OECD Test No. 305 Bioaccumulation in Fish: Aqueous and Dietary Exposure).

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Study Citation:	Schneider, S. Z., Zhang, L., Martin, K. H., Aufderheide, J. A. (2021). HHCB: A dietary exposure bioaccumulation test with the bluegill sunfish (Lepomis macrochirus). Final report.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Fish; *Lepomis macrochirus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Nutritional & Metabolic
Chemical:	HHCB
HERO ID:	7607948

Domain	Metric		Rating	Comments
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Nominal test concentrations: 0, 50, 500 ug/L; measured test concentrations: <LOQ, 41, 548 ug/L. The two exposure concentration groups satisfied OECD Test No. 305, which states that one test group is sufficient in dietary bioaccumulation exposures.
	Metric 12:	Testing at or Below Solubility Limit	High	The water solubility of HHCB is 1750 ug/L (also stated on page 57 of the study). The dietary exposure was at 50 and 500 ug/g of diet, which was below the solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organism was bluegill (Lepomis macrochirus). Source of test organisms was Osage Catfisheries, Inc. Bluegill are listed as recommended test species in Annex 3 of OECD Test No. 305.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Test organisms were acclimatized prior to the uptake phase for 14 days.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Each treatment group consisted of one test chamber with 90 fish in each chamber. Replicate tanks are not required in the dietary bioaccumulation study.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Housing conditions (temperature, pH, dissolved oxyden, hardness, alkalinity, conductivity) were adequate (see Appendix 9 through 12).
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (dietary exposure of feed spiked with radiolabeled HHCB) was appropriate for determining the bioaccumulation potential of HHCB (BMFs of HHCB) in fish.
	Metric 18:	Consistency of Outcome Assessment	High	Study details pointed to outcome assessment protocol being assessed consistently across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups that could influence the outcome.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was performed to calculate BMFs; methods followed standardized guidelines (OECD Test No. 305). HHCB Lipid Corrected Biomagnification Factor (BMF KL) was 0.082.
	Metric 22:	Reporting of Data	High	Reporting of data was adequate and authors followed standardized guidelines (OECD Test No. 305).

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Study Citation:	Schneider, S. Z., Zhang, L., Martin, K. H., Aufderheide, J. A. (2021). HHCB: A dietary exposure bioaccumulation test with the bluegill sunfish (Lepomis macrochirus). Final report.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Fish; *Lepomis macrochirus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Nutritional & Metabolic
Chemical:	HHCB
HERO ID:	7607948

Domain	Metric	Rating	Comments
	Metric 23: Explanation of Unexpected Outcomes	High	SD and CV were calculated for measured concentrations of total radioactivity in HHCB treatment diet (e.g., Tables 2 and 3).

Additional Comments: Study was a laboratory bioaccumulation test [HHCB: A DIETARY EXPOSURE BIOACCUMULATION TESTWITH THE BLUEGILL SUNFISH (Lepomis macrochirus); STUDY NUMBER: 558A-125]. Submitted to Research Institute for Fragrance Materials, Inc. by testing facility Eurofins EAG Agroscience, LLC. The study was initiated on 13 March 2015, completed on 23 October 2020, and amended on 1 April 2021. The objective of this study was to obtain laboratory data to determine the bioaccumulation potential of HHCB in the bluegill sunfish, Lepomis macrochirus, through dietary exposure. The test substance used in the test was HHCB. The reference substance used in the test was hexachlorobenzene (HCB). This evaluation form is for percent lipid outcome reported in Table 16 and in Appendices 7.31 and 7.32.

Overall Quality Determination High

Study Citation:	Schneider, S. Z., Zhang, L., Martin, K. H., Aufderheide, J. A. (2021). HHCB: A dietary exposure bioaccumulation test with the bluegill sunfish (Lepomis macrochirus). Final report.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Fish; *Lepomis macrochirus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	7607948

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Correct nomenclature was used.
	Metric 2:	Test Substance Source	High	Authors indicated that the radio-labelled HHCB was received from Moravek and identified as HHCB, [3-14C], Lot number 252-186-0587-A-20060515-JI (testing facility identification number 12213). The non-radio-labelled test substance was received from International Flavors and Fragrances, Inc. and identified as HHCB, Lot number 148-114 (testing facility identification number 12161).
	Metric 3:	Test Substance Purity	Medium	The certificate of analysis indicated that the radio-labelled HHCB was 99.3% pure (page 78) and that of the reference substance (HCB, as recommended by OECD Test No. 305) was >99% (page 79). However, it is not clear what purity of the the non-radio-labelled HHCB was.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A negative control (0 ug HHCB/g of diet) was included in the study.
	Metric 5:	Negative Control Response	High	This was a bioaccumulation study. Uptake and depuration results showed that HHCB in the control was below the level of quantitation (LOQ).
	Metric 6:	Randomized Allocation	Low	The study did not state/describe random allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The study was a dietary bioaccumulation with uptake and depuration phases. The experimental methods for preparation of diet for exposure were adequate and described in detail.
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration details were reported and administered consistently across groups. Fish were fed daily 2% body weight. During uptake, nominal concentrations were 50 or 500 ug HHCB/g of diet; measured concentrations 41 or 548 ug HHCB/g of diet.
	Metric 9:	Measurement of Test Substance Concentration	High	Tissue samples were collected on uptake day 14 and on depuration days 1, 3, 7, 14 and 28. Radiolabeled analysis of HHCB (concentrations of the test substance in fish food and fish tissue) was done using liquid scintillation counting (LSC). In addition, analysis of HCB (the reference substance recommended by OECD Test No. 305) in fish diet was measured by gas chromatography with mass selective detection (GC/MSD).
	Metric 10:	Exposure Duration and Frequency	High	The bioaccumulation study had an uptake phase and depuration phase. The total length of the study was 42 Days (14 Days Uptake; 28 Days Depuration). Uptake duration and depuration duration followed dietary exposure test guideline (OECD Test No. 305 Bioaccumulation in Fish: Aqueous and Dietary Exposure).

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Study Citation:	Schneider, S. Z., Zhang, L., Martin, K. H., Aufderheide, J. A. (2021). HHCB: A dietary exposure bioaccumulation test with the bluegill sunfish (Lepomis macrochirus). Final report.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Fish; *Lepomis macrochirus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	7607948

Domain	Metric		Rating	Comments
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Nominal test concentrations: 0, 50, 500 ug/L; measured test concentrations: <LOQ, 41, 548 ug/L. The two exposure concentration groups satisfied OECD Test No. 305, which states that one test group is sufficient in dietary bioaccumulation exposures.
	Metric 12:	Testing at or Below Solubility Limit	High	The water solubility of HHCB is 1750 ug/L (also stated on page 57 of the study). The dietary exposure was at 50 and 500 ug/g of diet, which was below the solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organism was bluegill (Lepomis macrochirus). Source of test organisms was Osage Catfisheries, Inc. Bluegill are listed as recommended test species in Annex 3 of OECD Test No. 305.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Test organisms were acclimatized prior to the uptake phase for 14 days.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Each treatment group consisted of one test chamber with 90 fish in each chamber. Replicate tanks are not required in the dietary bioaccumulation study.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Housing conditions (temperature, pH, dissolved oxyden, hardness, alkalinity, conductivity) were adequate (see Appendix 9 through 12).
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (dietary exposure of feed spiked with radiolabeled HHCB) was appropriate for determining the bioaccumulation potential of HHCB (BMFs of HHCB) in fish.
	Metric 18:	Consistency of Outcome Assessment	High	Study details pointed to outcome assessment protocol being assessed consistently across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups that could influence the outcome.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was performed to calculate BMFs; methods followed standardized guidelines (OECD Test No. 305). HHCB Lipid Corrected Biomagnification Factor (BMF KL) was 0.082.
	Metric 22:	Reporting of Data	High	Reporting of data was adequate and authors followed standardized guidelines (OECD Test No. 305).

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Study Citation:	Schneider, S. Z., Zhang, L., Martin, K. H., Aufderheide, J. A. (2021). HHCB: A dietary exposure bioaccumulation test with the bluegill sunfish (Lepomis macrochirus). Final report.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Fish; *Lepomis macrochirus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	7607948

Domain	Metric	Rating	Comments
	Metric 23: Explanation of Unexpected Outcomes	High	SD and CV were calculated for measured concentrations of total radioactivity in HHCB treatment diet (e.g., Tables 2 and 3).

Additional Comments: Study was a laboratory bioaccumulation test [HHCB: A DIETARY EXPOSURE BIOACCUMULATION TESTWITH THE BLUEGILL SUNFISH (Lepomis macrochirus); STUDY NUMBER: 558A-125]. Submitted to Research Institute for Fragrance Materials, Inc. by testing facility Eurofins EAG Agroscience, LLC. The study was initiated on 13 March 2015, completed on 23 October 2020, and amended on 1 April 2021. The objective of this study was to obtain laboratory data to determine the bioaccumulation potential of HHCB in the bluegill sunfish, Lepomis macrochirus, through dietary exposure. The test substance used in the test was HHCB. The reference substance used in the test was hexachlorobenzene (HCB). This evaluation form is for the growth outcomes reported in Appendices 14 and 15 for growth during uptake and depuration phases respectively.

Overall Quality Determination High

Study Citation:	Schneider, S. Z., Zhang, L., Martin, K. H., Aufderheide, J. A. (2021). HHCB: A dietary exposure bioaccumulation test with the bluegill sunfish (Lepomis macrochirus). Final report.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Fish; *Lepomis macrochirus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	HHCB
HERO ID:	7607948

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Correct nomenclature was used.
	Metric 2:	Test Substance Source	High	Authors indicated that the radio-labelled HHCB was received from Moravek and identified as HHCB, [3-14C], Lot number 252-186-0587-A-20060515-JI (testing facility identification number 12213). The non-radio-labelled test substance was received from International Flavors and Fragrances, Inc. and identified as HHCB, Lot number 148-114 (testing facility identification number 12161).
	Metric 3:	Test Substance Purity	Medium	The certificate of analysis indicated that the radio-labelled HHCB was 99.3% pure (page 78) and that of the reference substance (HCB, as recommended by OECD Test No. 305) was >99% (page 79). However, it is not clear what purity of the the non-radio-labelled HHCB was.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A negative control (0 ug HHCB/g of diet) was included in the study.
	Metric 5:	Negative Control Response	High	This was a bioaccumulation study. Uptake and depuration results showed that HHCB in the control was below the level of quantitation (LOQ).
	Metric 6:	Randomized Allocation	Low	The study did not state/describe random allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The study was a dietary bioaccumulation with uptake and depuration phases. The experimental methods for preparation of diet for exposure were adequate and described in detail.
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration details were reported and administered consistently across groups. Fish were fed daily 2% body weight. During uptake, nominal concentrations were 50 or 500 ug HHCB/g of diet; measured concentrations 41 or 548 ug HHCB/g of diet.
	Metric 9:	Measurement of Test Substance Concentration	High	Tissue samples were collected on uptake day 14 and on depuration days 1, 3, 7, 14 and 28. Radiolabeled analysis of HHCB (concentrations of the test substance in fish food and fish tissue) was done using liquid scintillation counting (LSC). In addition, analysis of HCB (the reference substance recommended by OECD Test No. 305) in fish diet was measured by gas chromatography with mass selective detection (GC/MSD).
	Metric 10:	Exposure Duration and Frequency	High	The bioaccumulation study had an uptake phase and depuration phase. The total length of the study was 42 Days (14 Days Uptake; 28 Days Depuration). Uptake duration and depuration duration followed dietary exposure test guideline (OECD Test No. 305 Bioaccumulation in Fish: Aqueous and Dietary Exposure).

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Study Citation:	Schneider, S. Z., Zhang, L., Martin, K. H., Aufderheide, J. A. (2021). HHCB: A dietary exposure bioaccumulation test with the bluegill sunfish (Lepomis macrochirus). Final report.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Fish; *Lepomis macrochirus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	HHCB
HERO ID:	7607948

Domain	Metric		Rating	Comments
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Nominal test concentrations: 0, 50, 500 ug/L; measured test concentrations: <LOQ, 41, 548 ug/L. The two exposure concentration groups satisfied OECD Test No. 305, which states that one test group is sufficient in dietary bioaccumulation exposures.
	Metric 12:	Testing at or Below Solubility Limit	High	The water solubility of HHCB is 1750 ug/L (also stated on page 57 of the study). The dietary exposure was at 50 and 500 ug/g of diet, which was below the solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organism was bluegill (Lepomis macrochirus). Source of test organisms was Osage Catfisheries, Inc. Bluegill are listed as recommended test species in Annex 3 of OECD Test No. 305.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Test organisms were acclimatized prior to the uptake phase for 14 days.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Each treatment group consisted of one test chamber with 90 fish in each chamber. Replicate tanks are not required in the dietary bioaccumulation study.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Housing conditions (temperature, pH, dissolved oxyden, hardness, alkalinity, conductivity) were adequate (see Appendix 9 through 12).
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (dietary exposure of feed spiked with radiolabeled HHCB) was appropriate for determining the bioaccumulation potential of HHCB (BMFs of HHCB) in fish.
	Metric 18:	Consistency of Outcome Assessment	High	Study details pointed to outcome assessment protocol being assessed consistently across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups that could influence the outcome.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was performed to calculate BMFs; methods followed standardized guidelines (OECD Test No. 305). HHCB Lipid Corrected Biomagnification Factor (BMF KL) was 0.082.
	Metric 22:	Reporting of Data	High	Reporting of data was adequate and authors followed standardized guidelines (OECD Test No. 305).

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Study Citation:	Schneider, S. Z., Zhang, L., Martin, K. H., Aufderheide, J. A. (2021). HHCB: A dietary exposure bioaccumulation test with the bluegill sunfish (Lepomis macrochirus). Final report.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Fish; *Lepomis macrochirus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	HHCB
HERO ID:	7607948

Domain	Metric	Rating	Comments
	Metric 23: Explanation of Unexpected Outcomes	High	SD and CV were calculated for measured concentrations of total radioactivity in HHCB treatment diet (e.g., Tables 2 and 3).

Additional Comments: Study was a laboratory bioaccumulation test [HHCB: A DIETARY EXPOSURE BIOACCUMULATION TESTWITH THE BLUEGILL SUNFISH (Lepomis macrochirus); STUDY NUMBER: 558A-125]. Submitted to Research Institute for Fragrance Materials, Inc. by testing facility Eurofins EAG Agroscience, LLC. The study was initiated on 13 March 2015, completed on 23 October 2020, and amended on 1 April 2021. The objective of this study was to obtain laboratory data to determine the bioaccumulation potential of HHCB in the bluegill sunfish, Lepomis macrochirus, through dietary exposure. The test substance used in the test was HHCB. The reference substance used in the test was hexachlorobenzene (HCB).

Overall Quality Determination High

Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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:	Vertebrate; Fish; *Misgurnus anguillicaudatus*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Correct nomenclature was used. There was a typo in the CASRN (1222-05 reported vs 1222-05-5 correct); given the correct chemical name, correct abbreviation, and correct trade name, it is likely that the missing digit for the CASRN was a genuine typo.
	Metric 2:	Test Substance Source	High	The source was reported (Klamar-reagent company, Shanghai).
	Metric 3:	Test Substance Purity	Medium	Purity of the substance was reported at 75%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	The authors reported that mortality rates of test organisms in all control groups were <10% at end of the tests and that toxicity tests followed ASTM standard guidelines. Moreover, they reported that test containers were set with blank control and solvent control (acetone). However, supplemental Table S1, which reports the nominal HHCB concentrations used in the toxicity test, does not list the control group as 0 ug/L.
	Metric 5:	Negative Control Response	Low	The authors reported in the methods that mortality rates of test organisms in all control groups were <10% at end of the tests. However, they did not report the raw data in the results (they only provided LC50 values for each test).
	Metric 6:	Randomized Allocation	Low	The authors did not report if the organisms were randomly allocated to study groups. They included a general statement saying that toxicity tests followed ASTM standard guidelines without mention of random allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	All tests were static-renewal and test solutions were totally replaced at 24 h intervals. Due to the physical-chemical properties of the HHCB, a solvent control (acetone) was used.
	Metric 8:	Consistency of Exposure Administration	High	There was no evidence to suggest that exposure administration was not administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured (HPLC, Agilent 1200 Series, USA). The recoveries of HHCB in water samples were 98.42%–106.90%, and the limit of detection (LOD)was 10.70 ng/L for water samples. The variability of HHCB concentration was <20% (3.08%–9.12%).
	Metric 10:	Exposure Duration and Frequency	High	This data evaluation form is for the acute toxicity studies. The reported acute toxicity exposure durations were 48 h for Daphnia magna and Chironomus plumosus and 96 h for the other aquatic animals. This exposure durations were appropriate for the study type.

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Study Citation: Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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: Vertebrate; Fish; *Misgurnus anguillicaudatus*; Larvae
Health Outcome: Mortality
Chemical: HHCB
HERO ID: 5428397

Domain	Metric		Rating	Comments
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were appropriate (5 to 7 groups plus control (solvent control).
	Metric 12:	Testing at or Below Solubility Limit	High	For all tests the nominal HHCB concentrations were below the HHCB water solubility limit of 1750 ug/L expect for the acute Daphnia magna test. However, it likely was not a concern because a solvent control (acetone) was used and the recoveries of measured HHCB in water samples were 98.42%–106.90%.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described (e.g., source, size, age, healthy organism).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The authors reported that, for example, cultures of Gobiocypris rarus and Daphnia magna were from their biological culture laboratory and thus acclimated. For organisms not reared in the laboratory, the authors reported that larvae were domesticated for at least 7 days in biological culture laboratory and the mortality rates were lower than 5% before toxicity tests started.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were appropriate: Three replicates for test containers each containing 10 organisms (except for D. magna) were set with blank control and solvent control (acetone), while four replicates for test containers each containing 5 organisms were set for D. magna.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The reporting of housing and environmental conditions were sufficient: The tests were conducted at 21 $\pm$ 2 °C with a 12:12 h light/dark cycle. Test organisms were not fed in acute toxicity tests. During the test period, the temperature, pH, and dissolved oxygen (DO) were measured daily.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (mortality) addressed or reported the intended outcome (LC50 values to generate SSDs to derive the ALC).
	Metric 18:	Consistency of Outcome Assessment	High	Reported details suggest that the outcomes were assessed consistently across study groups.
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 that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				

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Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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:	Vertebrate; Fish; *Misgurnus anguillicaudatus*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	High	The 48 h-EC50 and 96 h-LC50 values for acute toxicity tests and corresponding 95% confidence intervals were calculated by probit methodology. The ALC derivation was based on USEPA guidelines. The joint probability curves (JPC) were based on probability distributions of concentrations in water (exposure) and response (SSD) of HHCB.
	Metric 22:	Reporting of Data	Medium	The goal of the study was to derive the ALC based on SSDs. To generate the SSDs, the authors generated LC50 values, they also used LC50 values from the literature. So, the goal was not to determine the toxicity across treatment groups; keeping this is mind, this metric received a medium (instead of low) because mortality data was not reported for all treatment groups per species.
	Metric 23:	Explanation of Unexpected Outcomes	High	The study results provide variability (range).

Additional Comments: The goal of the study was to derive an ALC (aquatic life criteria) for HHCB based on aquatic organisms in China. The study included 8 Chinese resident aquatic species for acute and chronic toxicity tests. Lognormal species sensitivity distribution (SSD) and the log-logistic SSD methods were used to develop the ALC. The authors did not provide raw data of the toxicity studies. They also used LC50 values from the literature. Because the goal to run acute and chromic toxicity studies was to generate LC50 values that were ultimately used to generate SSDs, there are two forms included in the evaluation: acute data and chronic data for all species. This form is the data evaluation for the acute data for all species combined.

Overall Quality Determination High

Study Citation:	Yamauchi, R., Ishibashi, H., Hirano, M., Mori, T., Kim, J.W., Arizono, K. (2008). Effects of synthetic polycyclic musks on estrogen receptor, vitellogenin, pregnane X receptor, and cytochrome P450 3A gene expression in the livers of male medaka (Oryzias latipes). Aquatic Toxicology 90(4):261-268.
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:	Vertebrate; Fish; *Oryzias latipes*; Adult
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Receptor binding/ regulation of receptor activity
Chemical:	HHCB
HERO ID:	5352387

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Authors used correct nomenclature: 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[gamma]-2-benzopyran (HHCB, Galaxolide).
	Metric 2:	Test Substance Source	Low	HHCB source was identified (Promochem), but test substance was not analytically verified by study authors, batch/lot # was not given.
	Metric 3:	Test Substance Purity	Low	Purity of HHCB was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Authors reported using a solvent control (0.01% DMSO for the male medaka HHCB exposure).
	Metric 5:	Negative Control Response	High	The biological responses (hepatic VTG protein, Fig. 1B; expression levels of ER-alpha and ER-beta mRNA, Fig. 2B; expression levels of VTG I and VTG II mRNA, Fig. 3B; expression levels of PXR mRNA, Fig. 5; and expression levels of CYP3A38 and CYP3A40 mRNA, Fig. 6B) of the solvent control group were adequate.
	Metric 6:	Randomized Allocation	Low	Study did not specifically state 'random allocation'.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The authors did not report details on test media preparations. The cited references do not provide details on test media preparations for HHCB. The results for the measured concentrations and the figures showed the nominal and measured concentrations. The authors reported changing solutions daily which was appropriate given that their analytical chemistry results indicated loss of HHCB at 24 h (section 3.2 in the results).
	Metric 8:	Consistency of Exposure Administration	Medium	There was no evidence that test exposures were not administered consistently, but authors did not offer details.
	Metric 9:	Measurement of Test Substance Concentration	High	The authors reported measuring the test media daily during the exposure and water samples were analyzed using GC-MS. The results for the HHCB measured concentrations were at 0 h 4.8±0.16, 49±1.3, and 434±18 ug/L HHCB; and at 24 h <0.020, 0.14±0.025, and 0.46±0.10ug/L HHCB (section 3.2 in the results). The figures in the results show that the nominal concentrations were 0, 5, 50, and 500 ug/L HHCB.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure (72h) was reported and appropriate to determine the mechanistic endpoints (estrogen receptor, vitellogenin, pregnane X receptor, and cytochrome P450 3A gene expression).

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Study Citation:	Yamauchi, R., Ishibashi, H., Hirano, M., Mori, T., Kim, J.W., Arizono, K. (2008). Effects of synthetic polycyclic musks on estrogen receptor, vitellogenin, pregnane X receptor, and cytochrome P450 3A gene expression in the livers of male medaka (Oryzias latipes). Aquatic Toxicology 90(4):261-268.
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:	Vertebrate; Fish; *Oryzias latipes*; Adult
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Receptor binding/ regulation of receptor activity
Chemical:	HHCB
HERO ID:	5352387

Domain	Metric		Rating	Comments
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing were appropriate. Nominal: 0, 5, 50, and 500 ug/L HHCB. Measured: 0 h 4.8±0.16, 49±1.3, and 434±18 ug/L HHCB; and at 24 h <0.020, 0.14±0.025, and 0.46±0.10ug/L HHCB. Solutions were changed in the test containers daily.
	Metric 12:	Testing at or Below Solubility Limit	High	HHCB's water solubility is 1750 ug/L. Authors used a solvent carrier (0.01% DMSO). Nominal concentrations were: 0, 5, 50, and 500 ug/L HHCB. Measured concentrations were: at 0 h 4.8±0.16, 49±1.3, and 434±18 ug/L HHCB; and at 24 h <0.020, 0.14±0.025, and 0.46±0.10ug/L HHCB. Solutions were changed daily. Based on professional judgement, exposure concentrations were below the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Medaka is a well-recognized fish model organism for toxicity testing. Fish were from a fish culture maintained in the laboratory for several years.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The authors clearly stated a pre-exposure period of 2 weeks.
	Metric 15:	Number of Organisms and Replicates per Group	Low	Three (for vitellogenin protein analysis) or six (for gene expression analysis) adult male medaka were exposed to HHCB, but the authors did not mention any replicates per tank.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism housing and environmental conditions were appropriate.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (ELISA and PCR) reported the intended outcome of interest (levels of vitellogenin protein and fold induction of ER-alpha and ER-beta mRNA, vitellogenin VTG I and VTG II mRNA, PXR mRNA, and CYP3A38 and CYP3A40 mRNA).
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups .
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.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups in animal attrition.
Domain 7: Data Presentation and Analysis				

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Study Citation:	Yamauchi, R., Ishibashi, H., Hirano, M., Mori, T., Kim, J.W., Arizono, K. (2008). Effects of synthetic polycyclic musks on estrogen receptor, vitellogenin, pregnane X receptor, and cytochrome P450 3A gene expression in the livers of male medaka (Oryzias latipes). Aquatic Toxicology 90(4):261-268.
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:	Vertebrate; Fish; *Oryzias latipes*; Adult
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Receptor binding/ regulation of receptor activity
Chemical:	HHCB
HERO ID:	5352387

Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	High	Differences in mRNA/protein levels were analyzed by a one-way analysis of variance (ANOVA), followed by Dunnett's post hoc test if normally distributed; or, by the non-parametric Kruskal–Wallis test followed by a Mann–Whitney U test with Bonferroni's adjustment. The differences were considered significant at $P < 0.05$ and $P < 0.01$, respectively.
	Metric 22:	Reporting of Data	High	mRNA/protein levels were reported for all exposure groups (Figures 1, 2, 3, 5, and 6).
	Metric 23:	Explanation of Unexpected Outcomes	High	The authors reported expression values ± S.D.

Additional Comments: This form includes the sublethal test with adult medaka to examine the effects of HHCB on estrogen receptor, vitellogenin, pregnane X receptor, and cytochrome P450 3A gene expression. All of these assays (ELISA for protein levels and PCR for gene expression) are evaluated in this one form under the mechanistic- receptor binding classification. In essence, while they are mechanistic endpoints, estrogenic effects fall under reproductive effects; however, there were no apical reproductive effects included in the test, so reproductive effects were not selected as an endpoint..

Overall Quality Determination High

Study Citation:	Yamauchi, R., Ishibashi, H., Hirano, M., Mori, T., Kim, J.W., Arizono, K. (2008). Effects of synthetic polycyclic musks on estrogen receptor, vitellogenin, pregnane X receptor, and cytochrome P450 3A gene expression in the livers of male medaka (Oryzias latipes). Aquatic Toxicology 90(4):261-268.
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:	Vertebrate; Fish; *Oryzias latipes*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5352387

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Authors used correct nomenclature: 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[gamma]-2-benzopyran (HHCB, Galaxolide).
	Metric 2:	Test Substance Source	Low	HHCB source was identified (Promochem), but test substance was not analytically verified by study authors, batch/lot # was not given.
	Metric 3:	Test Substance Purity	Low	Purity of HHCB was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Authors reported using a solvent control (0.1% DMSO for the larval HHCB exposure).
	Metric 5:	Negative Control Response	Low	The mortality of the solvent control group was not reported. Table 1 reported the LC50 value for medaka larvae exposed to HHCB for 96 h. This study was used as a range-finding test prior to the sublethal study with the male medaka.
	Metric 6:	Randomized Allocation	Low	Study did not specifically state 'random allocation'.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The authors did not report details on test media preparations. They reported measuring the test media daily during the exposure, but the test media was analytically measured (GC-MS) only for the adult medaka exposure and not the larval exposure. This larval exposure was like a range-finding test for the sublethal study with the male medaka.
	Metric 8:	Consistency of Exposure Administration	Medium	There was not evidence that test exposures were not administered consistently, but authors did not offer details. Unfortunately, they did not indicate test concentrations per treatment group, they only indicated a range: Each group of larvae was exposed to 0.60–1.5 mg/L HHCB and the control group was exposed to solvent carrier only (0.1% DMSO).
	Metric 9:	Measurement of Test Substance Concentration	Low	The authors did not verify exposure concentrations in the larval study (i.e., LC50 study). They only measured the test media concentrations (GC-MS) for the sublethal adult medaka exposure.
	Metric 10:	Exposure Duration and Frequency	High	The duration of exposure (96h) was reported and appropriate to determine the 96h-LC50 value. Solutions in the test containers were changed daily.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Uninformative	They did not indicate test concentrations or spacing per treatment group, they only indicated a range: Each group of larvae was exposed to 0.60–1.5 mg/L HHCB and the control group was exposed to solvent carrier only (0.1% DMSO).

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Study Citation:	Yamauchi, R., Ishibashi, H., Hirano, M., Mori, T., Kim, J.W., Arizono, K. (2008). Effects of synthetic polycyclic musks on estrogen receptor, vitellogenin, pregnane X receptor, and cytochrome P450 3A gene expression in the livers of male medaka (Oryzias latipes). Aquatic Toxicology 90(4):261-268.
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:	Vertebrate; Fish; *Oryzias latipes*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5352387

Domain	Metric		Rating	Comments
	Metric 12:	Testing at or Below Solubility Limit	High	HHCB water solubility is 1.75 mg/L, but the authors used a carrier/solvent (0.1% DMSO). The range of test concentration was 0.60–1.5 mg/L HHCB + 0.1% DMSO with the control group being 0.1% DMSO alone. Solutions in the test containers were changed daily. The decision to change solutions daily was appropriate given that their analytical chemistry results in the sublethal study indicated loss of HHCB at 24 h (section 3.2 in the results).
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Medaka is a well-recognized fish model organism for toxicity testing. Fish were produced as part of a fish culture maintained in the laboratory for several years.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The organisms are 24h larvae; so they were collected the day before. The authors cited Ishibashi et al 2005., which in turn described in more detail how embryos immediately post-fertilization were handled by the authors.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of organisms was reported (fifteen 24-h-old larvae for each treatment) but the number of replicates was not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism housing and environmental conditions were appropriate.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (mortality) reported the intended outcome of interest (96hLC50 = 0.95).
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across study groups .
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.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups in animal attrition.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	The study was a range-finding test for the sublethal exposure with male medaka. Authors reported that median LC50 value was calculated using probit analysis.
	Metric 22:	Reporting of Data	Low	The authors only reported the LC50. They did not report mortality data per treatment group. But it was not deemed unacceptable, because this test was used as a range-finding test for the adult sublethal exposure that looked at the effects of HHCB on estrogen receptor, vitellogenin, pregnane X receptor, and cytochrome P450 3A gene expression.
	Metric 23:	Explanation of Unexpected Outcomes	High	The study reported median LC50 value and variability = 0.95 (0.91–1.01) mg/L.

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Study Citation:	Yamauchi, R., Ishibashi, H., Hirano, M., Mori, T., Kim, J.W., Arizono, K. (2008). Effects of synthetic polycyclic musks on estrogen receptor, vitellogenin, pregnane X receptor, and cytochrome P450 3A gene expression in the livers of male medaka (Oryzias latipes). Aquatic Toxicology 90(4):261-268.
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:	Vertebrate; Fish; *Oryzias latipes*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5352387

Domain	Metric	Rating	Comments

Additional Comments: This laval test was used as a range-finding test to determine the LC50 values and to then conduct a sublethal test with adult medaka to examine the effects of HHCB on estrogen receptor, vitellogenin, pregnane X receptor, and cytochrome P450 3A gene expression. The authors did not offer a rationale for why they they used larvae to determine an LC50 value that was used to then conduct a sublethal study with male fish (this is a completely different developmental stage).

Overall Quality Determination Uninformative

Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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:	Vertebrate; Fish; *Oryzias latipes sinensis*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Correct nomenclature was used. There was a typo in the CASRN (1222-05 reported vs 1222-05-5 correct); given the correct chemical name, correct abbreviation, and correct trade name, it is likely that the missing digit for the CASRN was a genuine typo.
	Metric 2:	Test Substance Source	High	The source was reported (Klamar-reagent company, Shanghai).
	Metric 3:	Test Substance Purity	Medium	Purity of the substance was reported at 75%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	The authors reported that mortality rates of test organisms in all control groups were <10% at end of the tests and that toxicity tests followed ASTM standard guidelines. Moreover, they reported that test containers were set with blank control and solvent control (acetone). However, supplemental Table S1, which reports the nominal HHCB concentrations used in the toxicity test, does not list the control group as 0 ug/L.
	Metric 5:	Negative Control Response	Low	The authors reported in the methods that mortality rates of test organisms in all control groups were <10% at end of the tests. However, they did not report the raw data in the results (they only provided LC50 values for each test).
	Metric 6:	Randomized Allocation	Low	The authors did not report if the organisms were randomly allocated to study groups. They included a general statement saying that toxicity tests followed ASTM standard guidelines without mention of random allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	All tests were static-renewal and test solutions were totally replaced at 24 h intervals. Due to the physical-chemical properties of the HHCB, a solvent control (acetone) was used.
	Metric 8:	Consistency of Exposure Administration	High	There was no evidence to suggest that exposure administration was not administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured (HPLC, Agilent 1200 Series, USA). The recoveries of HHCB in water samples were 98.42%–106.90%, and the limit of detection (LOD)was 10.70 ng/L for water samples. The variability of HHCB concentration was <20% (3.08%–9.12%).
	Metric 10:	Exposure Duration and Frequency	High	This data evaluation form is for the acute toxicity studies. The reported acute toxicity exposure durations were 48 h for Daphnia magna and Chironomus plumosus and 96 h for the other aquatic animals. This exposure durations were appropriate for the study type.

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Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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:	Vertebrate; Fish; *Oryzias latipes sinensis*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were appropriate (5 to 7 groups plus control (solvent control).
	Metric 12:	Testing at or Below Solubility Limit	High	For all tests the nominal HHCB concentrations were below the HHCB water solubility limit of 1750 ug/L expect for the acute Daphnia magna test. However, it likely was not a concern because a solvent control (acetone) was used and the recoveries of measured HHCB in water samples were 98.42%–106.90%.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described (e.g., source, size, age, healthy organism).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The authors reported that, for example, cultures of Gobiocypris rarus and Daphnia magna were from their biological culture laboratory and thus acclimated. For organisms not reared in the laboratory, the authors reported that larvae were domesticated for at least 7 days in biological culture laboratory and the mortality rates were lower than 5% before toxicity tests started.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were appropriate: Three replicates for test containers each containing 10 organisms (except for D. magna) were set with blank control and solvent control (acetone), while four replicates for test containers each containing 5 organisms were set for D. magna.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The reporting of housing and environmental conditions were sufficient: The tests were conducted at 21 $\pm$ 2 °C with a 12:12 h light/dark cycle. Test organisms were not fed in acute toxicity tests. During the test period, the temperature, pH, and dissolved oxygen (DO) were measured daily.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (mortality) addressed or reported the intended outcome (LC50 values to generate SSDs to derive the ALC).
	Metric 18:	Consistency of Outcome Assessment	High	Reported details suggest that the outcomes were assessed consistently across study groups.
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 that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				

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Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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:	Vertebrate; Fish; *Oryzias latipes sinensis*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	High	The 48 h-EC50 and 96 h-LC50 values for acute toxicity tests and corresponding 95% confidence intervals were calculated by probit methodology. The ALC derivation was based on USEPA guidelines. The joint probability curves (JPC) were based on probability distributions of concentrations in water (exposure) and response (SSD) of HHCB.
	Metric 22:	Reporting of Data	Medium	The goal of the study was to derive the ALC based on SSDs. To generate the SSDs, the authors generated LC50 values, they also used LC50 values from the literature. So, the goal was not to determine the toxicity across treatment groups; keeping this is mind, this metric received a medium (instead of low) because mortality data was not reported for all treatment groups per species.
	Metric 23:	Explanation of Unexpected Outcomes	High	The study results provide variability (range).

Additional Comments: The goal of the study was to derive an ALC (aquatic life criteria) for HHCB based on aquatic organisms in China. The study included 8 Chinese resident aquatic species for acute and chronic toxicity tests. Lognormal species sensitivity distribution (SSD) and the log-logistic SSD methods were used to develop the ALC. The authors did not provide raw data of the toxicity studies. They also used LC50 values from the literature. Because the goal to run acute and chromic toxicity studies was to generate LC50 values that were ultimately used to generate SSDs, there are two forms included in the evaluation: acute data and chronic data for all species. This form is the data evaluation for the acute data for all species combined.

Overall Quality Determination High

Study Citation:	Croudace, C. P., Caunter, J. E., Johnson, P. A. (1997). HHCB: Chronic toxicity to fathead minnow (Pimephales promelas) embryos and larvae.
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; *Pimephales promelas*; Larvae
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	7607847

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Correct nomenclature of common name and chemical name was used.
	Metric 2:	Test Substance Source	High	The test material, HHCB (lot no 7953-I), was supplied by International Flavors and Fragances (IFF), 1515 Highway 36, Union Beach, NJ 07735, USA, for, and on behalf of, the sponsor RIFM (Research Institute for Fragance Materials, Two University Plaza Drive, Suite 406, Hackensack, NJ 07601, USA). The test performing lab was Brixham Environmental Laboratory, ZENECA Limited, Brixham Devon TQ5 8BA, UK.
	Metric 3:	Test Substance Purity	Low	The purity of HHCB was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Appropriate solvent (triethylene glycol) and negative controls were used.
	Metric 5:	Negative Control Response	High	Hatching and weights and length of negative control and solvent control larvae at 32 days post-hatch was as expected.
	Metric 6:	Randomized Allocation	Medium	As reported by authors, sets of five fish eggs were randomly selected, microscopically examined for viability and placed in incubating cups by stratified random assignment. This process was repeated until each cup contained 20 randomly selected eggs.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental flow-through system and methods for preparation of test media (section 4.2) were described in adequate detail and were appropriate. Ethylene glycol was used as solvent control.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups. The nominal flow rate of the flow-through system was 250 ml/min. Each chamber received the required amounts of HHCB by a Watson-Marlow peristaltic pump, and the mixing chambers were fitted with magnetic stirrers to ensure adequate mixing. The dosing system was designed so that each tank received 40 ml/min of required test solution.
	Metric 9:	Measurement of Test Substance Concentration	High	Gas chromatography was used to measured HHCB concentrations prior to the start of the experiment and throughout the experiment (for details, see sections 4.3 and 5.1 and Tables 1 and 2).
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration was 32 days post-hatch (36 days overall) and followed standardized EPA guideline (Environmental Effects Test Guideline Part 2, 1982, EPA 560/6-82-002).

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Study Citation:	Croudace, C. P., Caunter, J. E., Johnson, P. A. (1997). HHCB: Chronic toxicity to fathead minnow (Pimephales promelas) embryos and larvae.
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; *Pimephales promelas*; Larvae
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	7607847

Domain	Metric		Rating	Comments
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Authors reported five treatment concentrations, solvent control, and negative control. Five treatment concentrations are appropriate as per EPA guideline (Environmental Effects Test Guideline Part 2, 1982, EPA 560/6-82-002). Quadruplicate treatment concentrations used were as follows: 12.5, 24, 50, 100, and 200 ug/L (measured 9.1, 19, 37, 68, 140 ug/L, respectively).
	Metric 12:	Testing at or Below Solubility Limit	High	The limit of solubility of HHCB in water is 1750 ug/L. Treatment concentrations used were as follows: 12.5, 24, 50, 100, and 200 ug/L (measured 9.1, 19, 37, 68, 140 ug/L, respectively). So, study was conducted with the limit of solubility.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organism (fathead minnow) was adequately described and appropriate as per EPA guideline (Environmental Effects Test Guideline Part 2, 1982, EPA 560/6-82-002).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The pretreatment conditions were the same for control and exposed organisms and culture was adequately described in the summary (section 1) and section 3.1.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of organisms and replicates (20 organisms in quadruplicate for a total of 80 organisms) exceeded the minimum number of organisms and replicates (30 organisms in duplicate for a total of 60 organisms) as per EPA guideline (Environmental Effects Test Guideline Part 2, 1982, EPA 560/6-82-002).
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism housing conditions (pH, dissolved oxygen, temperature, light, and feeding regimen) were appropriate and adequately described in the summary and sections 4.4, 4.5, and 4.6.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (counting hatched organisms and measuring weights and lengths) was appropriate for the outcomes of interest to determine chronic toxicity at early life stages.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, appropriate, and assessed consistently across study groups.
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.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				

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Study Citation:	Croudace, C. P., Caunter, J. E., Johnson, P. A. (1997). HHCB: Chronic toxicity to fathead minnow (Pimephales promelas) embryos and larvae.
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; *Pimephales promelas*; Larvae
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	7607847

Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	High	The percent hath data were analyzed by 2x2 contingency table tests to compare the treatments against the controls (differences at 5% significance). The larval weight and length data sets were tested for differences (P=0.05) using the non-parametric Wilcoxon rank sum test.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate (for details, see summary, sections 5.5, 5.7, Tables 3, 4, 5, 7, 8, 9).
	Metric 23:	Explanation of Unexpected Outcomes	High	Variability as relative standard deviation was reported for lengths and weights.

Additional Comments: This study was voluntarily submitted by industry. The test performing lab was Brixham Environmental Laboratory, ZENECA Limited, Brixham Devon TQ5 8BA, UK. The study sponsor was Research Institute for Fragance Materials, Two University Plaza Drive, Suite 406, Hackensack, NJ 07601, USA. The goal of the study was to test the chronic toxicity of HHCB to fathead minnow embryos and larvae and the study was performed in 1997. Authors reported following EPA guideline: Environmental Effects Test Guideline Part 2, 1982, EPA 560/6-82-002. The authors reported percent survival, percent hatch, mean larval length and weight. The mortality form represents evaluation of the percent survival data. This development/growth form represents the evaluation of the percent hatch and mean larval length and weight.

Overall Quality Determination High

Study Citation:	Croudace, C. P., Caunter, J. E., Johnson, P. A. (1997). HHCB: Chronic toxicity to fathead minnow (Pimephales promelas) embryos and larvae.
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; *Pimephales promelas*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	7607847

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Correct nomenclature of common name and chemical name was used.
	Metric 2:	Test Substance Source	High	The test material, HHCB (lot no 7953-I), was supplied by International Flavors and Fragances (IFF), 1515 Highway 36, Union Beach, NJ 07735, USA, for, and on behalf of, the sponsor RIFM (Research Institute for Fragance Materials, Two University Plaza Drive, Suite 406, Hackensack, NJ 07601, USA). The test performing lab was Brixham Environmental Laboratory, ZENECA Limited, Brixham Devon TQ5 8BA, UK.
	Metric 3:	Test Substance Purity	Low	The purity of HHCB was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Appropriate solvent (triethylene glycol) and negative controls were used.
	Metric 5:	Negative Control Response	High	Mortality of negative control and solvent control larvae at 32 days post-hatch was 8% and 7%, respectively, and thus, it was <10%, which the acceptable limit.
	Metric 6:	Randomized Allocation	Medium	As reported by authors, sets of five fish eggs were randomly selected, microscopically examined for viability and placed in incubating cups by stratified random assignment. This process was repeated until each cup contained 20 randomly selected eggs.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental flow-through system and methods for preparation of test media (section 4.2) were described in adequate detail and were appropriate. Ethylene glycol was used as solvent control.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups. The nominal flow rate of the flow-through system was 250 ml/min. Each chamber received the required amounts of HHCB by a Watson-Marlow peristaltic pump, and the mixing chambers were fitted with magnetic stirrers to ensure adequate mixing. The dosing system was designed so that each tank received 40 ml/min of required test solution.
	Metric 9:	Measurement of Test Substance Concentration	High	Gas chromatography was used to measured HHCB concentrations prior to the start of the experiment and throughout the experiment (for details, see sections 4.3 and 5.1 and Tables 1 and 2).
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration was 32 days post-hatch (36 days overall) and followed standardized EPA guideline (Environmental Effects Test Guideline Part 2, 1982, EPA 560/6-82-002).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Authors reported five treatment concentrations, solvent control, and negative control. Five treatment concentrations are appropriate as per EPA guideline (Environmental Effects Test Guideline Part 2, 1982, EPA 560/6-82-002). Quadruplicate treatment concentrations used were as follows: 12.5, 24, 50, 100, and 200 ug/L (measured 9.1, 19, 37, 68, 140 ug/L, respectively).

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Study Citation:	Croudace, C. P., Caunter, J. E., Johnson, P. A. (1997). HHCB: Chronic toxicity to fathead minnow (Pimephales promelas) embryos and larvae.
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; *Pimephales promelas*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	7607847

Domain	Metric		Rating	Comments
	Metric 12:	Testing at or Below Solubility Limit	High	The limit of solubility of HHCB in water is 1750 ug/L. Treatment concentrations used were as follows: 12.5, 24, 50, 100, and 200 ug/L (measured 9.1, 19, 37, 68, 140 ug/L, respectively). So, study was conducted with the limit of solubility.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organism was adequately described and appropriate as per EPA guideline (Environmental Effects Test Guideline Part 2, 1982, EPA 560/6-82-002).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The pretreatment conditions were the same for control and exposed organisms and culture was adequately described in the summary (section 1) and section 3.1.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of organisms and replicates (20 organisms in quadruplicate for a total of 80 organisms) exceeded the minimum number of organisms and replicates (30 organisms in duplicate for a total of 60 organisms) as per EPA guideline (Environmental Effects Test Guideline Part 2, 1982, EPA 560/6-82-002).
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism housing conditions (pH, dissolved oxygen, temperature, light, and feeding regimen) were appropriate and adequately described in the summary and sections 4.4, 4.5, and 4.6.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (counting dead organisms) was appropriate for the outcomes of interest (chronic mortality assessment and deriving the LC50 value)).
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, appropriate, and assessed consistently across study groups.
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.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The percent survival data were analyzed by 2x2 contingency table tests to compare the treatments against the controls (differences at 5% significance).
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate (for details, see summary, sections 5.5, 5.7, Tables 3, 4, 5, 7, 8).
	Metric 23:	Explanation of Unexpected Outcomes	High	Variability as relative standard deviation was reported for lengths and weights.

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Study Citation:	Croudace, C. P., Caunter, J. E., Johnson, P. A. (1997). HHCB: Chronic toxicity to fathead minnow (Pimephales promelas) embryos and larvae.
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; *Pimephales promelas*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	7607847

Domain	Metric	Rating	Comments
Additional Comments:			This study was voluntarily submitted by industry. The test performing lab was Brixham Environmental Laboratory, ZENECA Limited, Brixham Devon TQ5 8BA, UK. The study sponsor was Research Institute for Fragance Materials, Two University Plaza Drive, Suite 406, Hackensack, NJ 07601, USA. The goal of the study was to test the chronic toxicity of HHCB to fathead minnow embryos and larvae and the study was performed in 1997. Authors reported following EPA guideline: Environmental Effects Test Guideline Part 2, 1982, EPA 560/6-82-002. The authors reported percent survival, percent hatch, mean larval length and weight. This mortality form represents evaluation of the percent survival data. The development/growth form represents the evaluation of the percent hatch and mean larval length and weight.

Overall Quality Determination High

Study Citation:	Croudace, C. P., Caunter, J. E., Johnson, P. A. (1997). HHCB: Chronic toxicity to fathead minnow (Pimephales promelas) embryos and larvae.
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; *Pimephales promelas*; Larvae
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	7607847

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Correct nomenclature of common name and chemical name was used.
	Metric 2:	Test Substance Source	High	The test material, HHCB (lot no 7953-I), was supplied by International Flavors and Fragances (IFF), 1515 Highway 36, Union Beach, NJ 07735, USA, for, and on behalf of, the sponsor RIFM (Research Institute for Fragance Materials, Two University Plaza Drive, Suite 406, Hackensack, NJ 07601, USA). The test performing lab was Brixham Environmental Laboratory, ZENECA Limited, Brixham Devon TQ5 8BA, UK.
	Metric 3:	Test Substance Purity	Low	The purity of HHCB was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Appropriate solvent (triethylene glycol) and negative controls were used.
	Metric 5:	Negative Control Response	Low	The control response for behavioral changes due to toxicity was not reported.
	Metric 6:	Randomized Allocation	Medium	As reported by authors, sets of five fish eggs were randomly selected, microscopically examined for viability and placed in incubating cups by stratified random assignment. This process was repeated until each cup contained 20 randomly selected eggs.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experimental flow-through system and methods for preparation of test media (section 4.2) were described in adequate detail and were appropriate. Ethylene glycol was used as solvent control.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups. The nominal flow rate of the flow-through system was 250 ml/min. Each chamber received the required amounts of HHCB by a Watson-Marlow peristaltic pump, and the mixing chambers were fitted with magnetic stirrers to ensure adequate mixing. The dosing system was designed so that each tank received 40 ml/min of required test solution.
	Metric 9:	Measurement of Test Substance Concentration	High	Gas chromatography was used to measured HHCB concentrations prior to the start of the experiment and throughout the experiment (for details, see sections 4.3 and 5.1 and Tables 1 and 2).
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration was 32 days post-hatch (36 days overall) and followed standardized EPA guideline (Environmental Effects Test Guideline Part 2, 1982, EPA 560/6-82-002).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Authors reported five treatment concentrations, solvent control, and negative control. Five treatment concentrations are appropriate as per EPA guideline (Environmental Effects Test Guideline Part 2, 1982, EPA 560/6-82-002). Quadruplicate treatment concentrations used were as follows: 12.5, 24, 50, 100, and 200 ug/L (measured 9.1, 19, 37, 68, 140 ug/L, respectively).

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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; *Pimephales promelas*; Larvae
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	7607847

Domain	Metric		Rating	Comments
	Metric 12:	Testing at or Below Solubility Limit	High	The limit of solubility of HHCB in water is 1750 ug/L. Treatment concentrations used were as follows: 12.5, 24, 50, 100, and 200 ug/L (measured 9.1, 19, 37, 68, 140 ug/L, respectively). So, study was conducted with the limit of solubility.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organism (fathead minnow) was adequately described and appropriate as per EPA guideline (Environmental Effects Test Guideline Part 2, 1982, EPA 560/6-82-002).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The pretreatment conditions were the same for control and exposed organisms and culture was adequately described in the summary (section 1) and section 3.1.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of organisms and replicates (20 organisms in quadruplicate for a total of 80 organisms) exceeded the minimum number of organisms and replicates (30 organisms in duplicate for a total of 60 organisms) as per EPA guideline (Environmental Effects Test Guideline Part 2, 1982, EPA 560/6-82-002).
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Organism housing conditions (pH, dissolved oxygen, temperature, light, and feeding regimen) were appropriate and adequately described in the summary and sections 4.4, 4.5, and 4.6.
	Metric 17:	Outcome Assessment Methodology	High	Daily observations in behavior were made.
	Metric 18:	Consistency of Outcome Assessment	High	Daily observations in behavior were made.
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.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Uninformative	It was simply stated that treated fish experienced changes in activity, swimming behavior, and loss of balance. None of these changes were quantified, so statistics could not be conducted.
	Metric 22:	Reporting of Data	Low	Data for exposure related findings for each treatment group and control were not reported.
	Metric 23:	Explanation of Unexpected Outcomes	Low	No measures of variability were reported for behavioral outcomes.

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Study Citation:	Croudace, C. P., Caunter, J. E., Johnson, P. A. (1997). HHCB: Chronic toxicity to fathead minnow (Pimephales promelas) embryos and larvae.
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; *Pimephales promelas*; Larvae
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	7607847

Domain	Metric	Rating	Comments
Additional Comments:	This study was voluntarily submitted by industry. The test performing lab was Brixham Environmental Laboratory, ZENECA Limited, Brixham Devon TQ5 8BA, UK. The study sponsor was Research Institute for Fragance Materials, Two University Plaza Drive, Suite 406, Hackensack, NJ 07601, USA. The goal of the study was to test the chronic toxicity of HHCB to fathead minnow embryos and larvae and the study was performed in 1997. Authors reported following EPA guideline: Environmental Effects Test Guideline Part 2, 1982, EPA 560/6-82-002. The authors reported percent survival, percent hatch, mean larval length and weight. The mortality form represents evaluation of the percent survival data. This evaluation form is for the behavioral outcome pertaining to general activity, erratic swimming behavior, and loss of balance. This portion received an unacceptable rating due to lack of statistical analysis due to the lack of quantification of the outcomes.		

Overall Quality Determination — Uninformative

Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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:	Vertebrate; Amphibian; *Rana nigromaculata*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Correct nomenclature was used. There was a typo in the CASRN (1222-05 reported vs 1222-05-5 correct); given the correct chemical name, correct abbreviation, and correct trade name, it is likely that the missing digit for the CASRN was a genuine typo.
	Metric 2:	Test Substance Source	High	The source was reported (Klamar-reagent company, Shanghai).
	Metric 3:	Test Substance Purity	Medium	Purity of the substance was reported at 75%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	The authors reported that mortality rates of test organisms in all control groups were <10% at end of the tests and that toxicity tests followed ASTM standard guidelines. Moreover, they reported that test containers were set with blank control and solvent control (acetone). However, supplemental Table S1, which reports the nominal HHCB concentrations used in the toxicity test, does not list the control group as 0 ug/L.
	Metric 5:	Negative Control Response	Low	The authors reported in the methods that mortality rates of test organisms in all control groups were <10% at end of the tests. However, they did not report the raw data in the results (they only provided LC50 values for each test).
	Metric 6:	Randomized Allocation	Low	The authors did not report if the organisms were randomly allocated to study groups. They included a general statement saying that toxicity tests followed ASTM standard guidelines without mention of random allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	All tests were static-renewal and test solutions were totally replaced at 24 h intervals. Due to the physical-chemical properties of the HHCB, a solvent control (acetone) was used.
	Metric 8:	Consistency of Exposure Administration	High	There was no evidence to suggest that exposure administration was not administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured (HPLC, Agilent 1200 Series, USA). The recoveries of HHCB in water samples were 98.42%–106.90%, and the limit of detection (LOD)was 10.70 ng/L for water samples. The variability of HHCB concentration was <20% (3.08%–9.12%).
	Metric 10:	Exposure Duration and Frequency	High	This data evaluation form is for the acute toxicity studies. The reported acute toxicity exposure durations were 48 h for Daphnia magna and Chironomus plumosus and 96 h for the other aquatic animals. This exposure durations were appropriate for the study type.

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Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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:	Vertebrate; Amphibian; *Rana nigromaculata*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were appropriate (5 to 7 groups plus control (solvent control).
	Metric 12:	Testing at or Below Solubility Limit	High	For all tests the nominal HHCB concentrations were below the HHCB water solubility limit of 1750 ug/L expect for the acute Daphnia magna test. However, it likely was not a concern because a solvent control (acetone) was used and the recoveries of measured HHCB in water samples were 98.42%–106.90%.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described (e.g., source, size, age, healthy organism).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The authors reported that, for example, cultures of Gobiocypris rarus and Daphnia magna were from their biological culture laboratory and thus acclimated. For organisms not reared in the laboratory, the authors reported that larvae were domesticated for at least 7 days in biological culture laboratory and the mortality rates were lower than 5% before toxicity tests started.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were appropriate: Three replicates for test containers each containing 10 organisms (except for D. magna) were set with blank control and solvent control (acetone), while four replicates for test containers each containing 5 organisms were set for D. magna.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The reporting of housing and environmental conditions were sufficient: The tests were conducted at 21 $\pm$ 2 °C with a 12:12 h light/dark cycle. Test organisms were not fed in acute toxicity tests. During the test period, the temperature, pH, and dissolved oxygen (DO) were measured daily.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (mortality) addressed or reported the intended outcome (LC50 values to generate SSDs to derive the ALC).
	Metric 18:	Consistency of Outcome Assessment	High	Reported details suggest that the outcomes were assessed consistently across study groups.
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 that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				

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Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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:	Vertebrate; Amphibian; *Rana nigromaculata*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	High	The 48 h-EC50 and 96 h-LC50 values for acute toxicity tests and corresponding 95% confidence intervals were calculated by probit methodology. The ALC derivation was based on USEPA guidelines. The joint probability curves (JPC) were based on probability distributions of concentrations in water (exposure) and response (SSD) of HHCB.
	Metric 22:	Reporting of Data	Medium	The goal of the study was to derive the ALC based on SSDs. To generate the SSDs, the authors generated LC50 values, they also used LC50 values from the literature. So, the goal was not to determine the toxicity across treatment groups; keeping this is mind, this metric received a medium (instead of low) because mortality data was not reported for all treatment groups per species.
	Metric 23:	Explanation of Unexpected Outcomes	High	The study results provide variability (range).

Additional Comments: The goal of the study was to derive an ALC (aquatic life criteria) for HHCB based on aquatic organisms in China. The study included 8 Chinese resident aquatic species for acute and chronic toxicity tests. Lognormal species sensitivity distribution (SSD) and the log-logistic SSD methods were used to develop the ALC. The authors did not provide raw data of the toxicity studies. They also used LC50 values from the literature. Because the goal to run acute and chromic toxicity studies was to generate LC50 values that were ultimately used to generate SSDs, there are two forms included in the evaluation: acute data and chronic data for all species. This form is the data evaluation for the acute data for all species combined.

Overall Quality Determination High

Study Citation:	Carlsson, G., Norrgren, L. (2004). Synthetic musk toxicity to early life stages of zebrafish (Danio rerio). Archives of Environmental Contamination and Toxicology 46(1):102-105.
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:	Vertebrate; Fish; *Zebrafish (Danio rerio)*; Embryo
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5185657

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	HHCB was only identified by name.
	Metric 2:	Test Substance Source	Low	No analytical verification was conducted.
	Metric 3:	Test Substance Purity	Low	The test substance purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Results were compared against a negative control group, but it is unclear if there was a DMSO control in addition to a water-only control.
	Metric 5:	Negative Control Response	Low	The biological response of the control groups was not reported - results were compared to controls, and reported as NOEC and LOECs only for significant treatment groups.
	Metric 6:	Randomized Allocation	Low	It was not reported how test organisms were allocated to treatment groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The exposure system was described adequately. Preparation of the test substance was also reported.
	Metric 8:	Consistency of Exposure Administration	High	No indication to the contrary.
	Metric 9:	Measurement of Test Substance Concentration	Low	It was not reported if exposure concentrations were measured.
	Metric 10:	Exposure Duration and Frequency	High	The duration was appropriate for an acute toxicity test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The range of test concentrations (1-1000 ug/L) used was reported, but the specific test concentrations used for HHCB were not given.
	Metric 12:	Testing at or Below Solubility Limit	High	A solvent was used (DMSO).
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	Not much information was given on the test organisms.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	There was no indication of an acclimation period.
	Metric 15:	Number of Organisms and Replicates per Group	Low	There was no mention of replicates. It was reported that at least 20 eggs were used per exposure group.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Housing conditions were not sufficiently reported. Only the exposure temperature was reported.

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Study Citation:	Carlsson, G., Norrgren, L. (2004). Synthetic musk toxicity to early life stages of zebrafish (Danio rerio). Archives of Environmental Contamination and Toxicology 46(1):102-105.
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:	Vertebrate; Fish; *Zebrafish (Danio rerio)*; Embryo
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5185657

Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	Low	Methods were not explained for the mortality endpoint (coagulated egg).
	Metric 18:	Consistency of Outcome Assessment	Low	Details were lacking.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Not enough information was provided to allow for comparison of environmental conditions and non-treatment-related factors.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences in test organisms between treatment groups that could affect the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Endpoints in the embryo toxicity test were analysed using Fisher's exact test, where each group was compared with respective control group.
	Metric 22:	Reporting of Data	Low	Only NOEC and LOECs for significant effects were reported in the table. HHCB was not significant and so the LOEC and NOECs were not reported. Table 1 shows the yes/no results (24 and 48 hours) for the coagulated egg endpoint.
	Metric 23:	Explanation of Unexpected Outcomes	Low	No measures of variability were reported.

Additional Comments: This evaluation is for assessment of the mortality endpoint, which is coagulated egg.

Overall Quality Determination Low

Study Citation:	Carlsson, G., Norrgren, L. (2004). Synthetic musk toxicity to early life stages of zebrafish (Danio rerio). Archives of Environmental Contamination and Toxicology 46(1):102-105.
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:	Vertebrate; Fish; *Zebrafish (Danio rerio)*; Embryo
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	5185657

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	HHCB was only identified by name.
	Metric 2:	Test Substance Source	Low	No analytical verification was conducted.
	Metric 3:	Test Substance Purity	Low	The test substance purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Results were compared against a negative control group, but it is unclear if there was a DMSO control in addition to a water-only control.
	Metric 5:	Negative Control Response	Low	The biological response of the control groups was not reported - results were compared to controls, and reported as NOEC and LOECs only for significant treatment groups.
	Metric 6:	Randomized Allocation	Low	It was not reported how test organisms were allocated to treatment groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The exposure system was described adequately. Preparation of the test substance was also reported.
	Metric 8:	Consistency of Exposure Administration	High	No indication to the contrary.
	Metric 9:	Measurement of Test Substance Concentration	Low	It was not reported if exposure concentrations were measured.
	Metric 10:	Exposure Duration and Frequency	High	The duration was appropriate for an acute toxicity test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The range of test concentrations (1-1000 ug/L) used was reported, but the specific test concentrations used for HHCB were not given.
	Metric 12:	Testing at or Below Solubility Limit	High	A solvent was used (DMSO).
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	Not much information was given on the test organisms.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	There was no indication of an acclimation period.
	Metric 15:	Number of Organisms and Replicates per Group	Low	There was no mention of replicates. It was reported that at least 20 eggs were used per exposure group.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Housing conditions were not sufficiently reported. Only the exposure temperature was reported.
	Metric 17:	Outcome Assessment Methodology	Low	Methods were not explained for the behavioral endpoints assessed.

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Study Citation:	Carlsson, G., Norrgren, L. (2004). Synthetic musk toxicity to early life stages of zebrafish (Danio rerio). Archives of Environmental Contamination and Toxicology 46(1):102-105.
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:	Vertebrate; Fish; *Zebrafish (Danio rerio)*; Embryo
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	5185657

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	Low	Details were lacking.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Not enough information was provided to allow for comparison of environmental conditions and non-treatment-related factors.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences in test organisms between treatment groups that could affect the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Endpoints in the embryo toxicity test were analysed using Fisher's exact test, where each group was compared with respective control group.
	Metric 22:	Reporting of Data	Low	Only NOEC and LOECs for significant effects were reported in the table. HHCB was not significant and so the LOEC and NOECs were not reported. Table 1 shows the yes/no results (24 and 48 hours) for the spontaneous movement endpoint.
	Metric 23:	Explanation of Unexpected Outcomes	Low	No measures of variability were reported.

Additional Comments: This evaluation is for assessment of the behavioral endpoints.

Overall Quality Determination Low

Study Citation:	Carlsson, G., Norrgren, L. (2004). Synthetic musk toxicity to early life stages of zebrafish (Danio rerio). Archives of Environmental Contamination and Toxicology 46(1):102-105.
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:	Vertebrate; Fish; *Zebrafish (Danio rerio)*; Embryo
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5185657

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	HHCB was only identified by name.
	Metric 2:	Test Substance Source	Low	No analytical verification was conducted.
	Metric 3:	Test Substance Purity	Low	The test substance purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Results were compared against a negative control group, but it is unclear if there was a DMSO control in addition to a water-only control.
	Metric 5:	Negative Control Response	Low	The biological response of the control groups was not reported - results were compared to controls, and reported as NOEC and LOECs only for significant treatment groups.
	Metric 6:	Randomized Allocation	Low	It was not reported how test organisms were allocated to treatment groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The exposure system was described adequately. Preparation of the test substance was also reported.
	Metric 8:	Consistency of Exposure Administration	High	No indication to the contrary.
	Metric 9:	Measurement of Test Substance Concentration	Low	It was not reported if exposure concentrations were measured.
	Metric 10:	Exposure Duration and Frequency	High	The duration was appropriate for an acute toxicity test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The range of test concentrations (1-1000 ug/L) used was reported, but the specific test concentrations used for HHCB were not given.
	Metric 12:	Testing at or Below Solubility Limit	High	A solvent was used (DMSO).
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	Not much information was given on the test organisms.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	There was no indication of an acclimation period.
	Metric 15:	Number of Organisms and Replicates per Group	Low	There was no mention of replicates. It was reported that at least 20 eggs were used per exposure group.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Housing conditions were not sufficiently reported. Only the exposure temperature was reported.
	Metric 17:	Outcome Assessment Methodology	Low	Methods were not explained for the development/growth endpoints assessed.

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Study Citation:	Carlsson, G., Norrgren, L. (2004). Synthetic musk toxicity to early life stages of zebrafish (Danio rerio). Archives of Environmental Contamination and Toxicology 46(1):102-105.
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:	Vertebrate; Fish; *Zebrafish (Danio rerio)*; Embryo
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5185657

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	Low	Details were lacking.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Not enough information was provided to allow for comparison of environmental conditions and non-treatment-related factors.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences in test organisms between treatment groups that could affect the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Endpoints in the embryo toxicity test were analysed using Fisher's exact test, where each group was compared with respective control group.
	Metric 22:	Reporting of Data	Low	Only NOEC and LOECs for significant effects were reported in the table. HHCB was not significant and so the LOEC and NOECs were not reported. Table 1 shows the yes/no results (24 and 48 hours) for the extension of tail and oedema endpoints.
	Metric 23:	Explanation of Unexpected Outcomes	Low	No measures of variability were reported.

Additional Comments: This evaluation is for assessment of the development/growth endpoints.

Overall Quality Determination Low

Study Citation:	Carlsson, G., Norrgren, L. (2004). Synthetic musk toxicity to early life stages of zebrafish (Danio rerio). Archives of Environmental Contamination and Toxicology 46(1):102-105.
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:	Vertebrate; Fish; *Zebrafish (Danio rerio)*; Embryo
Health Outcome:	Cardiovascular
Chemical:	HHCB
HERO ID:	5185657

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	HHCB was only identified by name.
	Metric 2:	Test Substance Source	Low	No analytical verification was conducted.
	Metric 3:	Test Substance Purity	Low	The test substance purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Results were compared against a negative control group, but it is unclear if there was a DMSO control in addition to a water-only control.
	Metric 5:	Negative Control Response	Low	The biological response of the control groups was not reported - results were compared to controls, and reported as NOEC and LOECs only for significant treatment groups.
	Metric 6:	Randomized Allocation	Low	It was not reported how test organisms were allocated to treatment groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The exposure system was described adequately. Preparation of the test substance was also reported.
	Metric 8:	Consistency of Exposure Administration	High	No indication to the contrary.
	Metric 9:	Measurement of Test Substance Concentration	Low	It was not reported if exposure concentrations were measured.
	Metric 10:	Exposure Duration and Frequency	High	The duration was appropriate for an acute toxicity test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The range of test concentrations (1-1000 ug/L) used was reported, but the specific test concentrations used for HHCB were not given. The embryos exposed to HHCB didn't have different heart rates than controls even at the highest tested concentration, 1000 ug/L.
	Metric 12:	Testing at or Below Solubility Limit	High	A solvent was used (DMSO).
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	Not much information was given on the test organisms.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	There was no indication of an acclimation period.
	Metric 15:	Number of Organisms and Replicates per Group	Low	There was no mention of replicates. It was reported that at least 20 eggs were used per exposure group.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Housing conditions were not sufficiently reported. Only the exposure temperature was reported.

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Study Citation:	Carlsson, G., Norrgren, L. (2004). Synthetic musk toxicity to early life stages of zebrafish (Danio rerio). Archives of Environmental Contamination and Toxicology 46(1):102-105.
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:	Vertebrate; Fish; *Zebrafish (Danio rerio)*; Embryo
Health Outcome:	Cardiovascular
Chemical:	HHCB
HERO ID:	5185657

Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	Low	Methods were not explained for the cardiovascular endpoints assessed.
	Metric 18:	Consistency of Outcome Assessment	Low	Details were lacking.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Not enough information was provided to allow for comparison of environmental conditions and non-treatment-related factors.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported differences in test organisms between treatment groups that could affect the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Endpoints in the embryo toxicity test were analysed using Fisher's exact test, where each group was compared with respective control group.
	Metric 22:	Reporting of Data	Low	Only NOEC and LOECs for significant effects were reported in the table. HHCB was not significant and so the LOEC and NOECs were not reported. Table 1 shows the yes/no results (24 and 48 hours) for the heartbeat, circulation, and heart rate endpoints.
	Metric 23:	Explanation of Unexpected Outcomes	Low	No measures of variability were reported.

Additional Comments: This evaluation is for assessment of the cardiovascular endpoints.

Overall Quality Determination Low

Study Citation:	Peng, F.J., Kiggen, F., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Brink, P.J. (2019). Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms. Ecotoxicology and Environmental Safety 169:902-910.
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:	Invertebrate; Mollusks; *Bellamya sp.*; Not reported; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428151

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Chemical scientific name and source are reported.
	Metric 2:	Test Substance Source	High	Source was reported.
	Metric 3:	Test Substance Purity	Medium	Galaxolide was used as a standard.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Both a solvent and negative control used.
	Metric 5:	Negative Control Response	High	Survival in control and treatments, low treatments not significant or near 100%.
	Metric 6:	Randomized Allocation	Low	Authors discuss random sampling, but not random allocation to experimental chambers.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Brief description in this paper. Extended description of the Microcosms set-up, Test sediment and chemical spiking described in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across all treatments.
	Metric 9:	Measurement of Test Substance Concentration	High	Used GC-MS analysis of HHCB in overlying water.
	Metric 10:	Exposure Duration and Frequency	High	28 day exposure period.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Authors used 4 exposure groups and a negative and solvent controls.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent effects were similar to the negative control.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	Purchased from an aquatic market in Guangzhou, South China.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimated for 2-14 days prior to placing in the microcosm, as described in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	6 replicates, 6 organisms per replicate.

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Study Citation:	Peng, F.J., Kiggen, F., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Brink, P.J. (2019). Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms. Ecotoxicology and Environmental Safety 169:902-910.
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:	Invertebrate; Mollusks; *Bellamya sp.*; Not reported; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428151

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Housing, environmental conditions, food, water, and nutrients described briefly in this paper and in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 17:	Outcome Assessment Methodology	High	Table S14 provides data.
	Metric 18:	Consistency of Outcome Assessment	High	Measured at the same durations.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among study grooups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Authors do not discuss differences in outcomes related to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The Williams test (Williams, 1972) which assumes a concentration-effect relationship and is, therefore, is used extensively to analyse the results of eco-toxicity tests. The Williams tests were performed using the Community Analysis computer program, version 4.3.05 (Hommenet al., 1994).
	Metric 22:	Reporting of Data	High	Data reported on Table S14.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexplained outcomes were reported by the authors.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Peng, F.J., Hu, L.X., Pan, C.G., Ying, G.G., Brink, P.J. (2019). Insights into the sediment toxicity of personal care products to freshwater oligochaete worms using Fourier transform infrared spectroscopy. Ecotoxicology and Environmental Safety 172:296-302.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Branchiura sowerbyi*; Not reported.; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428154

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Commercial name, CAS Number, structure and molecular formula provided Table S1
	Metric 2:	Test Substance Source	Low	Source was not reported and chemical was not analyzed.
	Metric 3:	Test Substance Purity	Low	Purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Solvent control reported and solvent control mortality was <10% (29.5 of 30 surviving)
	Metric 5:	Negative Control Response	High	Solvent control mortality was <10% (29.5 of 30 surviving)
	Metric 6:	Randomized Allocation	Low	Did not report random allocation but did note random sampling.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicology and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 8:	Consistency of Exposure Administration	High	Same volume of solvent and volume of exposure water similar across exposures.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Nominal concentrations were reported and used.
	Metric 10:	Exposure Duration and Frequency	High	28 day exposure.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	4 concentrations and a solvent control, 4 replicates.
	Metric 12:	Testing at or Below Solubility Limit	High	<10% Control mortality.
Domain 4: Test Organism				

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Study Citation:	Peng, F.J., Hu, L.X., Pan, C.G., Ying, G.G., Brink, P.J. (2019). Insights into the sediment toxicity of personal care products to freshwater oligochaete worms using Fourier transform infrared spectroscopy. Ecotoxicology and Environmental Safety 172:296-302.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Branchiura sowerbyi*; Not reported.; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428154

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	Medium	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicol-ogy and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicol-ogy and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 15:	Number of Organisms and Replicates per Group	Medium	4 replicates, 24 organisms per replicate.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicol-ogy and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 17:	Outcome Assessment Methodology	High	Mortality data in Table S2.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes measured at the same durations.
Domain 6: Confounding / Variable Control				

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Study Citation:	Peng, F.J., Hu, L.X., Pan, C.G., Ying, G.G., Brink, P.J. (2019). Insights into the sediment toxicity of personal care products to freshwater oligochaete worms using Fourier transform infrared spectroscopy. Ecotoxicology and Environmental Safety 172:296-302.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Branchiura sowerbyi*; Not reported.; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428154

Domain	Metric		Rating	Comments
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Authors did not report any differences among the study groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	Authors did not report any unexpected outcomes.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Standard (Williams test, $p < 0.05$) and nonstandard (Monte Carlo permutation test, $p < 0.05$) were used.
	Metric 22:	Reporting of Data	High	Mortality NOEC and LOECs reported and data for all concentration were reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes reported.

Additional Comments: Multiple species, microcosm study

Overall Quality Determination High

Study Citation:	Peng, F.J., Hu, L.X., Pan, C.G., Ying, G.G., Brink, P.J. (2019). Insights into the sediment toxicity of personal care products to freshwater oligochaete worms using Fourier transform infrared spectroscopy. Ecotoxicology and Environmental Safety 172:296-302.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Branchiura sowerbyi*; Not reported.; Adult
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5428154

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Commercial name, CAS Number, structure and molecular formula provided Table S1
	Metric 2:	Test Substance Source	Low	Source was not reported and chemical was not analyzed.
	Metric 3:	Test Substance Purity	Low	Purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Solvent control reported and solvent control mortality was <10% (232.5 of 240 surviving)
	Metric 5:	Negative Control Response	High	Solvent control mortality was <10% (232.5 of 240 surviving)
	Metric 6:	Randomized Allocation	Low	Did not report random allocation but did note random sampling.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicology and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 8:	Consistency of Exposure Administration	High	Same volume of solvent and volume of exposure water similar across exposures.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Nominal concentrations were reported and used.
	Metric 10:	Exposure Duration and Frequency	High	28 day exposure.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	4 concentrations and a solvent control, 4 replicates.
	Metric 12:	Testing at or Below Solubility Limit	High	<10% Control mortality.
Domain 4: Test Organism				

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Study Citation:	Peng, F.J., Hu, L.X., Pan, C.G., Ying, G.G., Brink, P.J. (2019). Insights into the sediment toxicity of personal care products to freshwater oligochaete worms using Fourier transform infrared spectroscopy. Ecotoxicology and Environmental Safety 172:296-302.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Branchiura sowerbyi*; Not reported.; Adult
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5428154

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	Medium	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicol-ogy and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicol-ogy and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 15:	Number of Organisms and Replicates per Group	Medium	4 replicates, 240 organisms per replicate.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicol-ogy and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 17:	Outcome Assessment Methodology	High	Growth data in Table S3.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes measured at the same durations.
Domain 6: Confounding / Variable Control				

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Study Citation:	Peng, F.J., Hu, L.X., Pan, C.G., Ying, G.G., Brink, P.J. (2019). Insights into the sediment toxicity of personal care products to freshwater oligochaete worms using Fourier transform infrared spectroscopy. Ecotoxicology and Environmental Safety 172:296-302.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Branchiura sowerbyi*; Not reported.; Adult
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5428154

Domain	Metric		Rating	Comments
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Authors did not report any differences among the study groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	Authors did not report any unexpected outcomes.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Standard (Williams test, $p < 0.05$) and nonstandard (Monte Carlo permutation test, $p < 0.05$) were used.
	Metric 22:	Reporting of Data	High	Growth NOECs were reported in Table S3.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes reported.

Additional Comments: Multiple species, microcosm study. This evaluation was for the growth outcome reported in Table S3.

Overall Quality Determination High

Study Citation:	Peng, F.J., Hu, L.X., Pan, C.G., Ying, G.G., Brink, P.J. (2019). Insights into the sediment toxicity of personal care products to freshwater oligochaete worms using Fourier transform infrared spectroscopy. Ecotoxicology and Environmental Safety 172:296-302.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Branchiura sowerbyi*; Not reported.; Adult
Health Outcome:	Mechanistic-Genotox (including DNA repair)
Chemical:	HHCB
HERO ID:	5428154

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Commercial name, CAS Number, structure and molecular formula provided Table S1
	Metric 2:	Test Substance Source	Low	Source was not reported and chemical was not analyzed.
	Metric 3:	Test Substance Purity	Low	Purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Solvent control reported and solvent control mortality was <10% (29.5 of 30 surviving)
	Metric 5:	Negative Control Response	High	Solvent control mortality was <10% (29.5 of 30 surviving)
	Metric 6:	Randomized Allocation	Low	Did not report random allocation but did note random sampling.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicology and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 8:	Consistency of Exposure Administration	High	Same volume of solvent and volume of exposure water similar across exposures.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Nominal concentrations were reported and used.
	Metric 10:	Exposure Duration and Frequency	High	28 day exposure.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	2 concentrations sampled of 4 concentrations and a solvent control used. Four (4) replicates.
	Metric 12:	Testing at or Below Solubility Limit	High	<10% Control mortality.
Domain 4: Test Organism				

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Study Citation:	Peng, F.J., Hu, L.X., Pan, C.G., Ying, G.G., Brink, P.J. (2019). Insights into the sediment toxicity of personal care products to freshwater oligochaete worms using Fourier transform infrared spectroscopy. Ecotoxicology and Environmental Safety 172:296-302.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Branchiura sowerbyi*; Not reported.; Adult
Health Outcome:	Mechanistic-Genotox (including DNA repair)
Chemical:	HHCB
HERO ID:	5428154

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	Medium	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicol-ogy and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicol-ogy and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 15:	Number of Organisms and Replicates per Group	Medium	4 replicates, 30 organisms per replicate.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicol-ogy and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 17:	Outcome Assessment Methodology	High	Data in text and Table S3.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes measured at the same durations.
Domain 6: Confounding / Variable Control				

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Study Citation:	Peng, F.J., Hu, L.X., Pan, C.G., Ying, G.G., Brink, P.J. (2019). Insights into the sediment toxicity of personal care products to freshwater oligochaete worms using Fourier transform infrared spectroscopy. Ecotoxicology and Environmental Safety 172:296-302.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Branchiura sowerbyi*; Not reported.; Adult
Health Outcome:	Mechanistic-Genotox (including DNA repair)
Chemical:	HHCB
HERO ID:	5428154

Domain	Metric		Rating	Comments
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Authors did not report any differences among the study groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	Authors did not report any unexpected outcomes.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical significance of the differences in biochemical measurements between treatments were tested using Monte Carlo permutation tests under redundancy analysis (RDA) option. 5% significance level was defined for statistical tests.
	Metric 22:	Reporting of Data	Medium	Mortality NOECs reported and data for lowest two concentrations were reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes reported.

Additional Comments: Multiple species, microcosm study

Overall Quality Determination High

Study Citation:	Peng, F.J., Kiggen, F., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Brink, P.J. (2019). Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms. Ecotoxicology and Environmental Safety 169:902-910.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Branchiura sowerbyi*; Not reported; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	HHCB
HERO ID:	5428151

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Chemical scientific name and source are reported.
	Metric 2:	Test Substance Source	High	Source was reported.
	Metric 3:	Test Substance Purity	Medium	Galaxolide was used as a standard.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Both a solvent and negative control used.
	Metric 5:	Negative Control Response	Low	No control data was shown.
	Metric 6:	Randomized Allocation	Low	Authors discuss random sampling, but not random allocation to experimental chambers.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Brief description in this paper. Extended description of the Microcosms set-up, Test sediment and chemical spiking described in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across all treatments.
	Metric 9:	Measurement of Test Substance Concentration	High	Used GC-MS analysis of HHCB in overlying water.
	Metric 10:	Exposure Duration and Frequency	High	28 day exposure period.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Authors used 4 exposure groups and a negative and solvent controls.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent effects were similar to the negative control.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	Purchased from an aquatic market in Guangzhou, South China.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimated for 2-14 days prior to placing in the microcosm, as described in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	6 replicates, 30 organisms per replicate.

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Study Citation:	Peng, F.J., Kiggen, F., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Brink, P.J. (2019). Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms. Ecotoxicology and Environmental Safety 169:902-910.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Branchiura sowerbyi*; Not reported; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	HHCB
HERO ID:	5428151

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Housing, environmental conditions, food, water, and nutrients described briefly in this paper and in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 17:	Outcome Assessment Methodology	High	Figure 4 shows the HHCB concentrations.
	Metric 18:	Consistency of Outcome Assessment	High	Measured at the same durations.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among study grooups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Authors do not discuss differences in outcomes related to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The Williams test (Williams, 1972) which assumes a concentration-effect relationship and is, therefore, is used extensively to analyse the results of eco-toxicity tests. The Williams tests were performed using the Community Analysis computer program, version 4.3.05 (Hommenet al., 1994).
	Metric 22:	Reporting of Data	High	Figure 4 shows the HHCB concentrations.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexplained outcomes were reported by the authors.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Peng, F.J., Kiggen, F., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Brink, P.J. (2019). Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms. Ecotoxicology and Environmental Safety 169:902-910.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Branchiura sowerbyi*; Not reported; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428151

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Chemical scientific name and source are reported.
	Metric 2:	Test Substance Source	High	Source was reported.
	Metric 3:	Test Substance Purity	Medium	Galaxolide was used as a standard.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Both a solvent and negative control used.
	Metric 5:	Negative Control Response	High	Survival in control and treatments, low treatments not significant or near 100%.
	Metric 6:	Randomized Allocation	Low	Authors discuss random sampling, but not random allocation to experimental chambers.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Brief description in this paper. Extended description of the Microcosms set-up, Test sediment and chemical spiking described in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across all treatments.
	Metric 9:	Measurement of Test Substance Concentration	High	Used GC-MS analysis of HHCB in overlying water.
	Metric 10:	Exposure Duration and Frequency	High	28 day exposure period.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Authors used 4 exposure groups and a negative and solvent controls.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent effects were similar to the negative control.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	Purchased from an aquatic market in Guangzhou, South China.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimated for 2-14 days prior to placing in the microcosm, as described in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	6 replicates, 30 organisms per replicate.
Domain 5: Outcome Assessment				

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Study Citation:	Peng, F.J., Kiggen, F., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Brink, P.J. (2019). Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms. Ecotoxicology and Environmental Safety 169:902-910.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Branchiura sowerbyi*; Not reported; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428151

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Housing, environmental conditions, food, water, and nutrients described briefly in this paper and in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 17:	Outcome Assessment Methodology	High	Table S14 provides data.
	Metric 18:	Consistency of Outcome Assessment	High	Measured at the same durations.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among study grooups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Authors do not discuss differences in outcomes related to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The Williams test (Williams, 1972) which assumes a concentration-effect relationship and is, therefore, is used extensively to analyse the results of eco-toxicity tests. The Williams tests were performed using the Community Analysis computer program, version 4.3.05 (Hommenet al., 1994).
	Metric 22:	Reporting of Data	High	Data reported on Table S14.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexplained outcomes were reported by the authors.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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; *Chironomus plumosus*; Larvae
Health Outcome:	Immobilization
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Correct nomenclature was used. There was a typo in the CASRN (1222-05 reported vs 1222-05-5 correct); given the correct chemical name, correct abbreviation, and correct trade name, it is likely that the missing digit for the CASRN was a genuine typo.
	Metric 2:	Test Substance Source	High	The source was reported (Klamar-reagent company, Shanghai).
	Metric 3:	Test Substance Purity	Medium	Purity of the substance was reported at 75%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	The authors reported that mortality rates of test organisms in all control groups were <10% at end of the tests and that toxicity tests followed ASTM standard guidelines. Moreover, they reported that test containers were set with blank control and solvent control (acetone). However, supplemental Table S1, which reports the nominal HHCB concentrations used in the toxicity test, does not list the control group as 0 ug/L.
	Metric 5:	Negative Control Response	Low	The authors reported in the methods that mortality rates of test organisms in all control groups were <10% at end of the tests. However, they did not report the raw data in the results (they only provided LC50 values for each test).
	Metric 6:	Randomized Allocation	Low	The authors did not report if the organisms were randomly allocated to study groups. They included a general statement saying that toxicity tests followed ASTM standard guidelines without mention of random allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	All tests were static-renewal and test solutions were totally replaced at 24 h intervals. Due to the physical-chemical properties of the HHCB, a solvent control (acetone) was used.
	Metric 8:	Consistency of Exposure Administration	High	There was no evidence to suggest that exposure administration was not administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured (HPLC, Agilent 1200 Series, USA). The recoveries of HHCB in water samples were 98.42%–106.90%, and the limit of detection (LOD)was 10.70 ng/L for water samples. The variability of HHCB concentration was <20% (3.08%–9.12%).
	Metric 10:	Exposure Duration and Frequency	High	This data evaluation form is for the acute toxicity studies. The reported acute toxicity exposure durations were 48 h for Daphnia magna and Chironomus plumosus and 96 h for the other aquatic animals. This exposure durations were appropriate for the study type.

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Study Citation: Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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; *Chironomus plumosus*; Larvae
Health Outcome: Immobilization
Chemical: HHCB
HERO ID: 5428397

Domain	Metric		Rating	Comments
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were appropriate (5 to 7 groups plus control (solvent control).
	Metric 12:	Testing at or Below Solubility Limit	High	For all tests the nominal HHCB concentrations were below the HHCB water solubility limit of 1750 ug/L expect for the acute Daphnia magna test. However, it likely was not a concern because a solvent control (acetone) was used and the recoveries of measured HHCB in water samples were 98.42%–106.90%.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described (e.g., source, size, age, healthy organism).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The authors reported that, for example, cultures of Gobiocypris rarus and Daphnia magna were from their biological culture laboratory and thus acclimated. For organisms not reared in the laboratory, the authors reported that larvae were domesticated for at least 7 days in biological culture laboratory and the mortality rates were lower than 5% before toxicity tests started.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were appropriate: Three replicates for test containers each containing 10 organisms (except for D. magna) were set with blank control and solvent control (acetone), while four replicates for test containers each containing 5 organisms were set for D. magna.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The reporting of housing and environmental conditions were sufficient: The tests were conducted at 21 $\pm$ 2 °C with a 12:12 h light/dark cycle. Test organisms were not fed in acute toxicity tests. During the test period, the temperature, pH, and dissolved oxygen (DO) were measured daily.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (mortality) addressed or reported the intended outcome (LC50 values to generate SSDs to derive the ALC).
	Metric 18:	Consistency of Outcome Assessment	High	Reported details suggest that the outcomes were assessed consistently across study groups.
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 that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				

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Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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; *Chironomus plumosus*; Larvae
Health Outcome:	Immobilization
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	High	The 48 h-EC50 and 96 h-LC50 values for acute toxicity tests and corresponding 95% confidence intervals were calculated by probit methodology. The ALC derivation was based on USEPA guidelines. The joint probability curves (JPC) were based on probability distributions of concentrations in water (exposure) and response (SSD) of HHCB.
	Metric 22:	Reporting of Data	Medium	The goal of the study was to derive the ALC based on SSDs. To generate the SSDs, the authors generated LC50 values, they also used LC50 values from the literature. So, the goal was not to determine the toxicity across treatment groups; keeping this is mind, this metric received a medium (instead of low) because mortality data was not reported for all treatment groups per species.
	Metric 23:	Explanation of Unexpected Outcomes	High	The study results provide variability (range).

Additional Comments: The goal of the study was to derive an ALC (aquatic life criteria) for HHCB based on aquatic organisms in China. The study included 8 Chinese resident aquatic species for acute and chronic toxicity tests. Lognormal species sensitivity distribution (SSD) and the log-logistic SSD methods were used to develop the ALC. The authors did not provide raw data of the toxicity studies. They also used LC50 values from the literature. Because the goal to run acute and chromic toxicity studies was to generate LC50 values that were ultimately used to generate SSDs, there are two forms included in the evaluation: acute data and chronic data for all species. This form is the data evaluation for the acute data for all species combined.

Overall Quality Determination High

Study Citation:	Artola-Garicano, E., Sinnige, T.L., Holsteijn, I.V., Vaes, W.H., Hermens, J.L. (2003). Bioconcentration and acute toxicity of polycyclic musks in two benthic organisms (Chironomus riparius and Lumbriculus variegatus). Environmental Toxicology and Chemistry 22(5):1086-1092.
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; *Chironomus riparius*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5352378

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCB was identified as the test substance
	Metric 2:	Test Substance Source	High	Source of HHCB clearly identified (International Flavors andFragrances, Hilversum, The Netherlands).
	Metric 3:	Test Substance Purity	High	Purity of chemical provided (98%)
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls reported (copper free water + highest isopropanol concentration used in treatment groups)
	Metric 5:	Negative Control Response	Low	Results from each individual study group are not reported. The only reported result from the acute toxicity test is the final LC50 determination.
	Metric 6:	Randomized Allocation	Low	Source of organisms reported with limited details on allocation of organisms in experiments provided
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	System and methods provided, including details of monitoring pH, temperature, and other experimental conditions throughout the study period
	Metric 8:	Consistency of Exposure Administration	Medium	Exposure via water
	Metric 9:	Measurement of Test Substance Concentration	High	GCMS utilized to measure concentrations of HHCB throughout experiment
	Metric 10:	Exposure Duration and Frequency	High	Appropriate exposure lengths
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	”A dilution series resulting in six different concentrations was prepared. Nominal test concentrations ranged from... 0 to 6.6mM” for HHCB.
	Metric 12:	Testing at or Below Solubility Limit	High	Concentrations were below the limit of solubility
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	”Fourth-instar midge larvae (C. riparius) were kindly providedby the Department of Aquatic Ecology and Ecotoxicologyat the University of Amsterdam (Amsterdam, The Netherlands).”
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Acclimation periods reported for other analyses in study, not stated in the methods for the acute toxicity
	Metric 15:	Number of Organisms and Replicates per Group	Medium	”10 midge larvae wereindividually exposed in 40-ml vials containing 10 ml of testsolution (24-h renewal).”

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Study Citation:	Artola-Garicano, E., Sinnige, T.L., Holsteijn, I.V., Vaes, W.H., Hermens, J.L. (2003). Bioconcentration and acute toxicity of polycyclic musks in two benthic organisms (Chironomus riparius and Lumbriculus variegatus). Environmental Toxicology and Chemistry 22(5):1086-1092.
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; *Chironomus riparius*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5352378

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions conducive and monitored throughout study
	Metric 17:	Outcome Assessment Methodology	Low	Large confidence intervals reported
	Metric 18:	Consistency of Outcome Assessment	Medium	Residue analysis of bodies of organisms was not reported
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Differences not reported
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis performed but not described in detail
	Metric 22:	Reporting of Data	Medium	Data for each individual treatment group was not reported
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Most uncertainties were reported

Additional Comments: None

Overall Quality Determination High

Study Citation:	Artola-Garicano, E., Sinnige, T.L., Holsteijn, I.V., Vaes, W.H., Hermens, J.L. (2003). Bioconcentration and acute toxicity of polycyclic musks in two benthic organisms (Chironomus riparius and Lumbriculus variegatus). Environmental Toxicology and Chemistry 22(5):1086-1092.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	Invertebrate; Arthropods; *Chironomus riparius*; Larvae
Health Outcome:	ADME (biotransformation)
Chemical:	HHCB
HERO ID:	5352378

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCB was identified as the test substance
	Metric 2:	Test Substance Source	High	Source of HHCB clearly identified (International Flavors andFragrances, Hilversum, The Netherlands).
	Metric 3:	Test Substance Purity	High	Purity of chemical provided (98%)
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls reported (copper free water + highest isopropanol concentration used in treatment groups)
	Metric 5:	Negative Control Response	Low	Results from each individual study group are not reported. Control responses were not always reported.
	Metric 6:	Randomized Allocation	Low	Source of organisms reported with limited details on allocation of organisms in experiments provided
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	System and methods provided, including details of monitoring pH, temperature, and other experimental conditions throughout the study period
	Metric 8:	Consistency of Exposure Administration	Medium	Exposure via water
	Metric 9:	Measurement of Test Substance Concentration	High	GCMS utilized to measure concentrations of HHCB throughout experiment
	Metric 10:	Exposure Duration and Frequency	High	Appropriate exposure lengths
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	”A dilution series resulting in six different concentrations was prepared. Nominal test concentrations ranged from... 0 to 6.6mM” for HHCB.
	Metric 12:	Testing at or Below Solubility Limit	High	Concentrations were below the limit of solubility
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	”Lumbriculus variegatus were cultured at room temperaturein our laboratory on uncontaminated paper pulp andwere fed a mixture of Tetraphyllt (Trouw, Fontaine-les-Vervins,France) and Trouvitt (Tetra Werke, Melle, Germany) ona daily basis prior to testing.”
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Acclimation periods reported for other analyses in study, not stated in the methods for the acute toxicity

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Study Citation:	Artola-Garicano, E., Sinnige, T.L., Holsteijn, I.V., Vaes, W.H., Hermens, J.L. (2003). Bioconcentration and acute toxicity of polycyclic musks in two benthic organisms (Chironomus riparius and Lumbriculus variegatus). Environmental Toxicology and Chemistry 22(5):1086-1092.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	Invertebrate; Arthropods; *Chironomus riparius*; Larvae
Health Outcome:	ADME (biotransformation)
Chemical:	HHCB
HERO ID:	5352378

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 organisms per treatment completed in triplicate
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions conducive and monitored throughout study
	Metric 17:	Outcome Assessment Methodology	Low	Large confidence intervals reported
	Metric 18:	Consistency of Outcome Assessment	Medium	Residue analysis of bodies of organisms was not reported
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Differences not reported
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis performed but not described in detail
	Metric 22:	Reporting of Data	Low	Data for each individual treatment group was not reported
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Most uncertainties were reported

Additional Comments: This evaluation form is for the BCF values reported in the paper.

Overall Quality Determination High

Study Citation:	Artola-Garicano, E., Sinnige, T.L., Holsteijn, I.V., Vaes, W.H., Hermens, J.L. (2003). Bioconcentration and acute toxicity of polycyclic musks in two benthic organisms (Chironomus riparius and Lumbriculus variegatus). Environmental Toxicology and Chemistry 22(5):1086-1092.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	Invertebrate; Arthropods; *Chironomus riparius*; Larvae
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)
Chemical:	HHCB
HERO ID:	5352378

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCB was identified as the test substance
	Metric 2:	Test Substance Source	High	Source of HHCB clearly identified (International Flavors andFragrances, Hilversum, The Netherlands).
	Metric 3:	Test Substance Purity	High	Purity of chemical provided (98%)
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls reported (copper free water + highest isopropanol concentration used in treatment groups)
	Metric 5:	Negative Control Response	High	The control responses were reported in Table 3.
	Metric 6:	Randomized Allocation	Low	Source of organisms reported with limited details on allocation of organisms in experiments provided
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	System and methods provided, including details of monitoring pH, temperature, and other experimental conditions throughout the study period
	Metric 8:	Consistency of Exposure Administration	Medium	Exposure via water
	Metric 9:	Measurement of Test Substance Concentration	High	GCMS utilized to measure concentrations of HHCB throughout experiment
	Metric 10:	Exposure Duration and Frequency	High	Appropriate exposure lengths
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	”A dilution series resulting in six different concentrations was prepared. Nominal test concentrations ranged from... 0 to 6.6mM” for HHCB.
	Metric 12:	Testing at or Below Solubility Limit	High	Concentrations were below the limit of solubility
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	”Fourth-instar midge larvae (C. riparius) were kindly providedby the Department of Aquatic Ecology and Ecotoxicologyat the University of Amsterdam (Amsterdam, The Netherlands).”
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Acclimation periods reported for other analyses in study, not stated in the methods for the acute toxicity
	Metric 15:	Number of Organisms and Replicates per Group	Medium	”10 midge larvae wereindividually exposed in 40-ml vials containing 10 ml of testsolution (24-h renewal).”
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions conducive and monitored throughout study

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Study Citation:	Artola-Garicano, E., Sinnige, T.L., Holsteijn, I.V., Vaes, W.H., Hermens, J.L. (2003). Bioconcentration and acute toxicity of polycyclic musks in two benthic organisms (Chironomus riparius and Lumbriculus variegatus). Environmental Toxicology and Chemistry 22(5):1086-1092.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	Invertebrate; Arthropods; *Chironomus riparius*; Larvae
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)
Chemical:	HHCB
HERO ID:	5352378

Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	Low	Large confidence intervals reported
	Metric 18:	Consistency of Outcome Assessment	Medium	Residue analysis of bodies of organisms was not reported
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Differences not reported
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis performed but not described in detail
	Metric 22:	Reporting of Data	High	Data for the treatment and control groups were reported in Table 3.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Most uncertainties were reported

Additional Comments: This evaluation was for the outcomes pertaining to MROD, EROD, and PROD enzyme activities. This would fall under mechanistic outcomes.

Overall Quality Determination High

Study Citation:	IFF, (2004). HHCB/galaxolide: A study on the toxicity to the sediment dweller chironomus riparius (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Chironomus riparius*; Larvae
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	8784980

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The CAS number and batch number of the test substance was given.
	Metric 2:	Test Substance Source	Low	A certificate of analysis for the test item was provided but it is very hard to read and unclear what the source is.
	Metric 3:	Test Substance Purity	High	The purity of the test substance used was 98.5 percent.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A control with unspiked sediment and a solvent control (acetone) were included in the test.
	Metric 5:	Negative Control Response	High	There were zero ratite individuals in the sediment control and 2.6% (emerged midges in all replicates) in the solvent control.
	Metric 6:	Randomized Allocation	Medium	Chironomus riparius larvae were randomly allocated to the test vessels on day zero of the study.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The preparation of the formulated sediment, overlying water and test substance stock solution was described in great detail and were adequate for the study. The composition of the formulated sediment was according to OECD Guideline 218. Samples were taken for test concentration analysis (performed by another lab offsite) but that data was not shown so it is unclear if there was any loss or gain of the test substance throughout the study exposure.
	Metric 8:	Consistency of Exposure Administration	Medium	The test exposure system was the same for the control and treatment groups with the chironomids. Samples taken for chemical analysis were from separate vessels prepared with the same overlying water and sediment (just sediment for control) with test item at appropriate concentrations. So the samples for test concentration analysis were not taken from the actual vessels where the organisms were being exposed to determine biological results. There were changes in ammonium concentrations over the course of the test and they were presumably due to the presence and the varying quantity of nitrifying microflora in the test system (organic components of the sediment). Study authors mentioned this was not assumed to have a negative influence on the performance of the test organisms and this was supported by the biological results of the study.

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Study Citation:	IFF, (2004). HHCB/galaxolide: A study on the toxicity to the sediment dweller chironomus riparius (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Chironomus riparius*; Larvae
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	8784980

Domain		Metric	Rating	Comments
	Metric 9:	Measurement of Test Substance Concentration	Low	Study authors indicated that test concentrations were measured in the sediment, pore water, and overlying water at four different times throughout the test. Those samples were processed and shipped to a specialized laboratory for analysis. None of that data was given in the report. The authors mentioned the analytical investigations were not part of the study and therefore were not included in the report. The biological results were based on nominal test concentrations. These nominal concentration levels were chosen based on a range-finding test performed prior to the start of the definitive test.
	Metric 10:	Exposure Duration and Frequency	High	The study was performed according to the OECD Guideline for the Testing of Chemical; Revised Draft Guideline No. 218: Sediment-water chironomid toxicity test using spiked sediment (Dec 2002).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Basing results off of nominal concentrations, the desired NOEC, LOEC, and EC endpoints were determined for the emergence ratio parameter, meaning adequate test concentrations were used. For the endpoint development rate there was no dose response relationship, therefore no EC values could be calculated. The NOEC and LOEC for development rate were determined to be greater than or equal to the highest test concentration (1000 mg/kg).
	Metric 12:	Testing at or Below Solubility Limit	N/A	For this study the sediment is spiked with the HHCB.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The Chironomus riparius used in this study were from in-house cultures. The original supplier information in the report is redacted as CBI. The 1st instar larvae used at the initiation of the test was appropriate to determine the desired endpoints of emergence ratio and development rate.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	In preparation of the test system, the spiked sediment will be topped with overlying water and equilibrated under a light and temperature regime comparable to the test conditions for at least seven days. At least two weeks prior to the start of the test the larvae were held at a temperature and photo period that would be used during the test exposure.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were four replicates per treatment concentration level as well as the controls. Twenty chironomid larvae were placed in each test vessel at the initiation.
Domain 5: Outcome Assessment				

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Study Citation:	IFF, (2004). HHCB/galaxolide: A study on the toxicity to the sediment dweller chironomus riparius (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Chironomus riparius*; Larvae
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	8784980

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate for the chironomids. Urtica powder (food) was mixed with the sediment. The overlying water was aerated during the test. The following water quality parameters were measured in the overlying water throughout the exposure: temperature, pH, dissolved oxygen, total hardness, and total ammonia. TOC was measured in the sediment. Light intensity was measured once during the test and room temperature was monitored continuously throughout the test. Water quality conditions included in the validity criteria were adequate for the test. The TOC value of the sediment slightly exceeded the range given in the Guideline (2.59% instead of 2.50%) but it was reported this deviation did not impact the integrity of the study. The data for these measurements is shown in Appendix l.
	Metric 17:	Outcome Assessment Methodology	High	At least three times a week and daily from day 11 the test vessels were observed in order to assess visually any behavioral differences in the treatments compared with the control. The specific behavior indicated in the observed results was looking for ratite midges (live midges floating on the surface, not able to fly).
	Metric 18:	Consistency of Outcome Assessment	High	Behavior was observed the same way in both the control and treatment groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	As mentioned in metric 8 there was inconsistency in the ammonium concentration throughout the study but study authors assumed the source of the varying concentration and indicated that it did not have a negative effect on the endpoints of interest based on the results. As mentioned in metric 16 the TOC value measured slightly higher than the guideline requirement but again study authors mentioned this deviation did not impact the integrity of the study.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among treatment groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Data was shown for the percentage of ratite midges in all replicates but no statistics were given. It was specifically mentioned in the report that they did not statistically evaluate these observations.
	Metric 22:	Reporting of Data	High	Ratite midge data was recorded and it is shown in Table 15 in the report. It was indicated that at concentrations greater than or equal to 250 mg/kg a higher percentage of fully emerged midges were not able to fly.
	Metric 23:	Explanation of Unexpected Outcomes	High	Results were described adequately with data shown. Measures of variability were shown with the data.

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Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Chironomus riparius*; Larvae
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	8784980

Domain	Metric	Rating	Comments
Additional Comments:	This evaluation is for the Behavioral outcome. The sediment was the main exposure route for the Chironomus riparius because it was spiked with HHCB. Because food items were included in the sediment there was potential for exposure from food. Additionally, they could of been exposed through the overlying water if any of the test item leached into it.		

Overall Quality Determination High

Study Citation:	IFF, (2004). HHCB/galaxolide: A study on the toxicity to the sediment dweller chironomus riparius (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Chironomus riparius*; Larvae
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784980

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The CAS number and batch number of the test substance was given.
	Metric 2:	Test Substance Source	Low	A certificate of analysis for the test item was provided but it is very hard to read and unclear what the source is.
	Metric 3:	Test Substance Purity	High	The purity of the test substance used was 98.5 percent.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A control with unspiked sediment and a solvent control (acetone) were included in the test.
	Metric 5:	Negative Control Response	High	The mean emergence in the controls was 96.3 % (OECD guidelines require at least 70%) and the emergence of adults in the control occurred between day 12 and 23 (OECD guidelines require emergence between day 12 and 23 after start of exposure). For the development rate parameter there was a significant difference between the means of the control and the solvent control (Welch t-test). Therefore, an influence of the solvent (acetone) used to apply the test item to the sediment on the development rate of the midges could not be excluded. For further analyses the solvent control was used as the control group.
	Metric 6:	Randomized Allocation	Medium	Chironomus riparius larvae were randomly allocated to the test vessels on day zero of the study.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The preparation of the formulated sediment, overlying water and test substance stock solution was described in great detail and were adequate for the study. The composition of the formulated sediment was according to OECD Guideline 218. Samples were taken for test concentration analysis (performed by another lab offsite) but that data was not shown so it is unclear if there was any loss or gain of the test substance throughout the study exposure.
	Metric 8:	Consistency of Exposure Administration	Medium	The test exposure system was the same for the control and treatment groups with the chironomids. Samples taken for chemical analysis were from separate vessels prepared with the same overlying water and sediment (just sediment for control) with test item at appropriate concentrations. So the samples for test concentration analysis were not taken from the actual vessels where the organisms were being exposed to determine biological results. There were changes in ammonium concentrations over the course of the test and they were presumably due to the presence and the varying quantity of nitrifying microflora in the test system (organic components of the sediment). Study authors mentioned this was not assumed to have a negative influence on the performance of the test organisms and this was supported by the biological results of the study.

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Taxa, Species, Age:	Invertebrate; Arthropods; *Chironomus riparius*; Larvae
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784980

Domain		Metric	Rating	Comments
	Metric 9:	Measurement of Test Substance Concentration	Low	Study authors indicated that test concentrations were measured in the sediment, pore water, and overlying water at four different times throughout the test. Those samples were processed and shipped to a specialized laboratory for analysis. None of that data was given in the report. The authors mentioned the analytical investigations were not part of the study and therefore were not included in the report. The biological results were based on nominal test concentrations. These nominal concentration levels were chosen based on a range-finding test performed prior to the start of the definitive test.
	Metric 10:	Exposure Duration and Frequency	High	The study was performed according to the OECD Guideline for the Testing of Chemical; Revised Draft Guideline No. 218: Sediment-water chironomid toxicity test using spiked sediment (Dec 2002).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Basing results off of nominal concentrations, the desired NOEC, LOEC, and EC endpoints were determined for the emergence ratio parameter, meaning adequate test concentrations were used. For the endpoint development rate there was no dose response relationship, therefore no EC values could be calculated. The NOEC and LOEC for development rate were determined to be greater than or equal to the highest test concentration (1000 mg/kg).
	Metric 12:	Testing at or Below Solubility Limit	N/A	For this study the sediment is spiked with the HHCB.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The Chironomus riparius used in this study were from in-house cultures. The original supplier information in the report is redacted as CBI. The 1st instar larvae used at the initiation of the test was appropriate to determine the desired endpoints of emergence ratio and development rate.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	In preparation of the test system, the spiked sediment will be topped with overlying water and equilibrated under a light and temperature regime comparable to the test conditions for at least seven days. At least two weeks prior to the start of the test the larvae were held at a temperature and photo period that would be used during the test exposure.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were four replicates per treatment concentration level as well as the controls. Twenty chironomid larvae were placed in each test vessel at the initiation.
Domain 5: Outcome Assessment				

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Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Chironomus riparius*; Larvae
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784980

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate for the chironomids. Urtica powder (food) was mixed with the sediment. The overlying water was aerated during the test. The following water quality parameters were measured in the overlying water throughout the exposure: temperature, pH, dissolved oxygen, total hardness, and total ammonia. TOC was measured in the sediment. Light intensity was measured once during the test and room temperature was monitored continuously throughout the test. Water quality conditions included in the validity criteria were adequate for the test. The TOC value of the sediment slightly exceeded the range given in the Guideline (2.59% instead of 2.50%) but it was reported this deviation did not impact the integrity of the study. The data for these measurements is shown in Appendix l.
	Metric 17:	Outcome Assessment Methodology	High	The methods of determining emergence ratio and development rate (to assess the Development/Growth health outcome) were thoroughly described in the report. These were determined at the end of the study exposure. Using these data the desired NOEC, LOEC, and EC endpoints were calculated. This information was adequately described in the report.
	Metric 18:	Consistency of Outcome Assessment	High	Emergence ratio and development rate were measured at the end of the study the same way in both the control and treatment groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	As mentioned in metric 8 there was inconsistency in the ammonium concentration throughout the study but study authors assumed the source of the varying concentration and indicated that it did not have a negative effect on the endpoints of interest based on the results. As mentioned in metric 16 the TOC value measured slightly higher than the guideline requirement but again study authors mentioned this deviation did not impact the integrity of the study.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among treatment groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analyses were adequately described and were appropriate for analyzing the growth and development data. In the report statistical results are shown in section 17.3 and in the Appendix l Table 38-45.
	Metric 22:	Reporting of Data	High	Growth/development data is given and all analyses shown with it to determine specific endpoints (ECx, NOEC, LOEC). Summarized emergence ratio data is shown in Table 11 and Figures 1 and 2 of the report. Raw data is shown in Table 36 of Appendix l. Summarized development data is shown in Tables 12-14 and Figure 3 of the report.
	Metric 23:	Explanation of Unexpected Outcomes	High	Results were described adequately with data shown. Measures of variability were shown with the data where appropriate.

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Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Chironomus riparius*; Larvae
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784980

Domain	Metric	Rating	Comments
Additional Comments:	This evaluation is for the Development/Growth outcome. Study results for emergence ratio and development rate were considered for this outcome evaluation. The sediment was the main exposure route for the Chironomus riparius because it was spiked with HHCB. Because food items were included in the sediment there was potential for exposure from food. Additionally, they could of been exposed through the overlying water if any of the test item leached into it.		

Overall Quality Determination High

Study Citation:	IFF, (2004). HHCB/galaxolide: A study on the toxicity to the sediment dweller chironomus riparius (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Chironomus riparius*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8784980

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The CAS number and batch number of the test substance was given.
	Metric 2:	Test Substance Source	Low	A certificate of analysis for the test item was provided but it is very hard to read and unclear what the source is.
	Metric 3:	Test Substance Purity	High	The purity of the test substance used was 98.5 percent.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A control with unspiked sediment and a solvent control (acetone) were included in the test.
	Metric 5:	Negative Control Response	High	Mortality was checked daily from day 11 on. Dead emerged midges and dead pupae were recorded. There were zero mortalities in the sediment control and 1.3% (in all replicates) dead pupae in the solvent control.
	Metric 6:	Randomized Allocation	Medium	Chironomus riparius larvae were randomly allocated to the test vessels on day zero of the study.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The preparation of the formulated sediment, overlying water and test substance stock solution was described in great detail and were adequate for the study. The composition of the formulated sediment was according to OECD Guideline 218. Samples were taken for test concentration analysis (performed by another lab offsite) but that data was not shown so it is unclear if there was any loss or gain of the test substance throughout the study exposure.
	Metric 8:	Consistency of Exposure Administration	Medium	The test exposure system was the same for the control and treatment groups with the chironomids. Samples taken for chemical analysis were from separate vessels prepared with the same overlying water and sediment (just sediment for control) with test item at appropriate concentrations. So the samples for test concentration analysis were not taken from the actual vessels where the organisms were being exposed to determine biological results. There were changes in ammonium concentrations over the course of the test and they were presumably due to the presence and the varying quantity of nitrifying microflora in the test system (organic components of the sediment). Study authors mentioned this was not assumed to have a negative influence on the performance of the test organisms and this was supported by the biological results of the study.

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Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Chironomus riparius*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8784980

Domain		Metric	Rating	Comments
	Metric 9:	Measurement of Test Substance Concentration	Low	Study authors indicated that test concentrations were measured in the sediment, pore water, and overlying water at four different times throughout the test. Those samples were processed and shipped to a specialized laboratory for analysis. None of that data was given in the report. The authors mentioned the analytical investigations were not part of the study and therefore were not included in the report. The biological results were based on nominal test concentrations. These nominal concentration levels were chosen based on a range-finding test performed prior to the start of the definitive test.
	Metric 10:	Exposure Duration and Frequency	High	The study was performed according to the OECD Guideline for the Testing of Chemical; Revised Draft Guideline No. 218: Sediment-water chironomid toxicity test using spiked sediment (Dec 2002).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Basing results off of nominal concentrations, the desired NOEC, LOEC, and EC endpoints were determined for the emergence ratio parameter, meaning adequate test concentrations were used. For the endpoint development rate there was no dose response relationship, therefore no EC values could be calculated. The NOEC and LOEC for development rate were determined to be greater than or equal to the highest test concentration (1000 mg/kg).
	Metric 12:	Testing at or Below Solubility Limit	N/A	For this study the sediment is spiked with the HHCB.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The Chironomus riparius used in this study were from in-house cultures. The original supplier information in the report is redacted as CBI. The 1st instar larvae used at the initiation of the test was appropriate to determine the desired endpoints of emergence ratio and development rate.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	In preparation of the test system, the spiked sediment will be topped with overlying water and equilibrated under a light and temperature regime comparable to the test conditions for at least seven days. At least two weeks prior to the start of the test the larvae were held at a temperature and photo period that would be used during the test exposure.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were four replicates per treatment concentration level as well as the controls. Twenty chironomid larvae were placed in each test vessel at the initiation.
Domain 5: Outcome Assessment				

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Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Chironomus riparius*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8784980

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate for the chironomids. Urtica powder (food) was mixed with the sediment. The overlying water was aerated during the test. The following water quality parameters were measured in the overlying water throughout the exposure: temperature, pH, dissolved oxygen, total hardness, and total ammonia. TOC was measured in the sediment. Light intensity was measured once during the test and room temperature was monitored continuously throughout the test. Water quality conditions included in the validity criteria were adequate for the test. The TOC value of the sediment slightly exceeded the range given in the Guideline (2.59% instead of 2.50%) but it was reported this deviation did not impact the integrity of the study. The data for these measurements is shown in Appendix l.
	Metric 17:	Outcome Assessment Methodology	High	The methods for observing and recording surviving and dead chironomids was adequately described in the report. Observations were expressed as percentage of emerged midges in all replicates. These results were not evaluated statistically.
	Metric 18:	Consistency of Outcome Assessment	High	Survival/mortality was determined the same way in both the control and treatment groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	As mentioned in metric 8 there was inconsistency in the ammonium concentration throughout the study but study authors assumed the source of the varying concentration and indicated that it did not have a negative effect on the endpoints of interest based on the results. As mentioned in metric 16 the TOC value measured slightly higher than the guideline requirement but again study authors mentioned this deviation did not impact the integrity of the study.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among treatment groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Data was given for number of dead emerged midges and dead incompletely hatched midges but no statistical analyses were performed on that data. It was specifically mentioned in the report that they did not statistically evaluate these observations.
	Metric 22:	Reporting of Data	High	Dead emerged and dead incompletely emerged midge data was recorded and it is shown in Table 15 in the report.
	Metric 23:	Explanation of Unexpected Outcomes	High	Results were described adequately with data shown. Measures of variability were shown with the data where appropriate.

Additional Comments: This evaluation is for the Mortality outcome. The sediment was the main exposure route for the Chironomus riparius because it was spiked with HHCB. Because food items were included in the sediment there was potential for exposure from food. Additionally, they could of been exposed through the overlying water if any of the test item leached into it.

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Taxa, Species, Age:	Invertebrate; Arthropods; *Chironomus riparius*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8784980

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

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Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Chironomus riparius*; Larvae
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	8784980

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The CAS number and batch number of the test substance was given.
	Metric 2:	Test Substance Source	Low	A certificate of analysis for the test item was provided but it is very hard to read and unclear what the source is.
	Metric 3:	Test Substance Purity	High	The purity of the test substance used was 98.5 percent.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A control with unspiked sediment and a solvent control (acetone) were included in the test.
	Metric 5:	Negative Control Response	High	The mean emergence in the controls was 96.3 % (OECD guidelines require at least 70%) and the emergence of adults in the control occurred between day 12 and 23 (OECD guidelines require emergence between day 12 and 23 after start of exposure). For the development rate parameter there was a significant difference between the means of the control and the solvent control (Welch t-test). Therefore, an influence of the solvent (acetone) used to apply the test item to the sediment on the development rate of the midges could not be excluded. For further analyses the solvent control was used as the control group.
	Metric 6:	Randomized Allocation	Medium	Chironomus riparius larvae were randomly allocated to the test vessels on day zero of the study.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The preparation of the formulated sediment, overlying water and test substance stock solution was described in great detail and were adequate for the study. The composition of the formulated sediment was according to OECD Guideline 218. Samples were taken for test concentration analysis (performed by another lab offsite) but that data was not shown so it is unclear if there was any loss or gain of the test substance throughout the study exposure.
	Metric 8:	Consistency of Exposure Administration	Medium	The test exposure system was the same for the control and treatment groups with the chironomids. Samples taken for chemical analysis were from separate vessels prepared with the same overlying water and sediment (just sediment for control) with test item at appropriate concentrations. So the samples for test concentration analysis were not taken from the actual vessels where the organisms were being exposed to determine biological results. There were changes in ammonium concentrations over the course of the test and they were presumably due to the presence and the varying quantity of nitrifying microflora in the test system (organic components of the sediment). Study authors mentioned this was not assumed to have a negative influence on the performance of the test organisms and this was supported by the biological results of the study.

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Taxa, Species, Age:	Invertebrate; Arthropods; *Chironomus riparius*; Larvae
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	8784980

Domain		Metric	Rating	Comments
	Metric 9:	Measurement of Test Substance Concentration	Low	Study authors indicated that test concentrations were measured in the sediment, pore water, and overlying water at four different times throughout the test. Those samples were processed and shipped to a specialized laboratory for analysis. None of that data was given in the report. The authors mentioned the analytical investigations were not part of the study and therefore were not included in the report. The biological results were based on nominal test concentrations. These nominal concentration levels were chosen based on a range-finding test performed prior to the start of the definitive test.
	Metric 10:	Exposure Duration and Frequency	High	The study was performed according to the OECD Guideline for the Testing of Chemical; Revised Draft Guideline No. 218: Sediment-water chironomid toxicity test using spiked sediment (Dec 2002).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Basing results off of nominal concentrations, the desired NOEC, LOEC, and EC endpoints were determined for the emergence ratio parameter, meaning adequate test concentrations were used. For the endpoint development rate there was no dose response relationship, therefore no EC values could be calculated. The NOEC and LOEC for development rate were determined to be greater than or equal to the highest test concentration (1000 mg/kg).
	Metric 12:	Testing at or Below Solubility Limit	N/A	For this study the sediment is spiked with the HHCB.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The Chironomus riparius used in this study were from in-house cultures. The original supplier information in the report is redacted as CBI. The 1st instar larvae used at the initiation of the test was appropriate to determine the desired endpoints of emergence ratio and development rate.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	In preparation of the test system, the spiked sediment will be topped with overlying water and equilibrated under a light and temperature regime comparable to the test conditions for at least seven days. At least two weeks prior to the start of the test the larvae were held at a temperature and photo period that would be used during the test exposure.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were four replicates per treatment concentration level as well as the controls. Twenty chironomid larvae were placed in each test vessel at the initiation.
Domain 5: Outcome Assessment				

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Taxa, Species, Age:	Invertebrate; Arthropods; *Chironomus riparius*; Larvae
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	8784980

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were adequate for the chironomids. Urtica powder (food) was mixed with the sediment. The overlying water was aerated during the test. The following water quality parameters were measured in the overlying water throughout the exposure: temperature, pH, dissolved oxygen, total hardness, and total ammonia. TOC was measured in the sediment. Light intensity was measured once during the test and room temperature was monitored continuously throughout the test. Water quality conditions included in the validity criteria were adequate for the test. The TOC value of the sediment slightly exceeded the range given in the Guideline (2.59% instead of 2.50%) but it was reported this deviation did not impact the integrity of the study. The data for these measurements is shown in Appendix l.
	Metric 17:	Outcome Assessment Methodology	High	The methods of determining emergence ratio and development rate (to assess the Development/Growth health outcome) were thoroughly described in the report. These were determined at the end of the study exposure. Using these data the desired NOEC, LOEC, and EC endpoints were calculated. This information was adequately described in the report.
	Metric 18:	Consistency of Outcome Assessment	High	Emergence ratio and development rate were measured at the end of the study the same way in both the control and treatment groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	As mentioned in metric 8 there was inconsistency in the ammonium concentration throughout the study but study authors assumed the source of the varying concentration and indicated that it did not have a negative effect on the endpoints of interest based on the results. As mentioned in metric 16 the TOC value measured slightly higher than the guideline requirement but again study authors mentioned this deviation did not impact the integrity of the study.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among treatment groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analyses were adequately described and were appropriate for analyzing the growth and development data. In the report statistical results are shown in section 17.3 and in the Appendix l Table 38-45.
	Metric 22:	Reporting of Data	High	Growth/development data is given and all analyses shown with it to determine specific endpoints (ECx, NOEC, LOEC). Summarized emergence ratio data is shown in Table 11 and Figures 1 and 2 of the report. Raw data is shown in Table 36 of Appendix l. Summarized development data is shown in Tables 12-14 and Figure 3 of the report.
	Metric 23:	Explanation of Unexpected Outcomes	High	Results were described adequately with data shown. Measures of variability were shown with the data where appropriate.

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Study Citation:	IFF, (2004). HHCB/galaxolide: A study on the toxicity to the sediment dweller chironomus riparius (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Chironomus riparius*; Larvae
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	8784980

Domain	Metric	Rating	Comments
Additional Comments:	This evaluation is for the Reproductive/Teratogenic outcome. Study results for sex ratio were considered for this outcome evaluation. The sediment was the main exposure route for the Chironomus riparius because it was spiked with HHCB. Because food items were included in the sediment there was potential for exposure from food. Additionally, they could of been exposed through the overlying water if any of the test item leached into it.		

Overall Quality Determination High

Study Citation:	Peng, F.J., Kiggen, F., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Brink, P.J. (2019). Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms. Ecotoxicology and Environmental Safety 169:902-910.
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:	Invertebrate; Arthropods; *Chironomus riparius*; Larvae
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5428151

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Chemical scientific name and source are reported.
	Metric 2:	Test Substance Source	High	Source was reported.
	Metric 3:	Test Substance Purity	Medium	Galaxolide was used as a standard.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Both a solvent and negative control used.
	Metric 5:	Negative Control Response	Low	No midges were found in the controls at the end of the experiment.
	Metric 6:	Randomized Allocation	Low	Authors discuss random sampling, but not random allocation to experimental chambers.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Brief description in this paper. Extended description of the Microcosms set-up, Test sediment and chemical spiking described in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across all treatments.
	Metric 9:	Measurement of Test Substance Concentration	High	Used GC-MS analysis of HHCB in overlying water.
	Metric 10:	Exposure Duration and Frequency	High	28 day exposure period.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Authors used 4 exposure groups and a negative and solvent controls.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent effects were similar to the negative control.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	The midges were obtained from an aquatic market inGuangzhou (South China).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimated for 2-14 days prior to placing in the microcosm, as described in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of midges used was not reported.

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Study Citation:	Peng, F.J., Kiggen, F., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Brink, P.J. (2019). Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms. Ecotoxicology and Environmental Safety 169:902-910.
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:	Invertebrate; Arthropods; *Chironomus riparius*; Larvae
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5428151

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Housing, environmental conditions, food, water, and nutrients described briefly in this paper and in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 17:	Outcome Assessment Methodology	High	Table S14 provides data.
	Metric 18:	Consistency of Outcome Assessment	High	Measured at the same durations.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among study grooups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Authors do not discuss differences in outcomes related to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The Williams test (Williams, 1972) which assumes a concentration-effect relationship and is, therefore, is used extensively to analyse the results of eco-toxicity tests. The Williams tests were performed using the Community Analysis computer program, version 4.3.05 (Hommenet al., 1994).
	Metric 22:	Reporting of Data	High	Data reported in Table S14.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexplained outcomes were reported by the authors.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Peng, F.J., Kiggen, F., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Brink, P.J. (2019). Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms. Ecotoxicology and Environmental Safety 169:902-910.
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:	Invertebrate; Mollusks; *Corbicula fluminea*; Not reported; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428151

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Chemical scientific name and source are reported.
	Metric 2:	Test Substance Source	High	Source was reported.
	Metric 3:	Test Substance Purity	Medium	Galaxolide was used as a standard.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Both a solvent and negative control used.
	Metric 5:	Negative Control Response	High	Survival in control and treatments, low treatments not significant or near 100%.
	Metric 6:	Randomized Allocation	Low	Authors discuss random sampling, but not random allocation to experimental chambers.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Brief description in this paper. Extended description of the Microcosms set-up, Test sediment and chemical spiking described in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across all treatments.
	Metric 9:	Measurement of Test Substance Concentration	High	Used GC-MS analysis of HHCB in overlying water.
	Metric 10:	Exposure Duration and Frequency	High	28 day exposure period.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Authors used 4 exposure groups and a negative and solvent controls.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent effects were similar to the negative control.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	Purchased from an aquatic market in Guangzhou, South China.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimated for 2-14 days prior to placing in the microcosm, as described in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	6 replicates, 6 organisms per replicate.

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Study Citation:	Peng, F.J., Kiggen, F., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Brink, P.J. (2019). Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms. Ecotoxicology and Environmental Safety 169:902-910.
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:	Invertebrate; Mollusks; *Corbicula fluminea*; Not reported; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428151

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Housing, environmental conditions, food, water, and nutrients described briefly in this paper and in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 17:	Outcome Assessment Methodology	High	Table S14 provides data.
	Metric 18:	Consistency of Outcome Assessment	High	Measured at the same durations.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among study grooups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Authors do not discuss differences in outcomes related to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The Williams test (Williams, 1972) which assumes a concentration-effect relationship and is, therefore, is used extensively to analyse the results of eco-toxicity tests. The Williams tests were performed using the Community Analysis computer program, version 4.3.05 (Hommenet al., 1994).
	Metric 22:	Reporting of Data	High	Data reported on Table S14.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexplained outcomes were reported by the authors.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Chen, F., Yao, Q., Zhou, X. (2015). The influence of suspended solids on the combined toxicity of galaxolide and lead to Daphnia magna. Bulletin of Environmental Contamination and Toxicology 95(1):73-79.
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*; Adult
Health Outcome:	Immobilization
Chemical:	HHCB
HERO ID:	4690050

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Definitively identified by nomenclature.
	Metric 2:	Test Substance Source	High	Tianjin Flavors and Fragrance Corporation.
	Metric 3:	Test Substance Purity	High	All of the chemicals used were ofanalytical grade.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	appropriate negative control group
	Metric 5:	Negative Control Response	High	No observable toxic effects in blank or solvent controls.
	Metric 6:	Randomized Allocation	Low	No report of how randomization was conducted.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Experimental system (beakers with solution & soil) were well-described and appropriate for this short-term exposure.
	Metric 8:	Consistency of Exposure Administration	High	The same solvents and soils were used across all groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Actual concentrations were measured by GC/MS at the start of the experiment and were close to nominal in the no-suspended-solids treatment. The loss of detected HHCB in the suspended solids treatment is consistent with the predicted partitioning behavior of HHCB.
	Metric 10:	Exposure Duration and Frequency	High	Tests conducted according to OECD 202. 48h acute toxicity with immobilization checked at 24 and 48h.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Number & spacing of exposure groups acceptable (0.1 to 6 mg/L, 8 groups for no soil, 5 groups for soil).
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent (acetone and Tween-80) did not cause any measurable acute toxic effect in solvent control groups.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Organisms maintained from culture & maintained according to OECD 202.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Pretreatment conditions were the same for all groups, and water conditions used in tests were the same as those used in maintaining source culture.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 individuals per group.
Domain 5: Outcome Assessment				

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Study Citation:	Chen, F., Yao, Q., Zhou, X. (2015). The influence of suspended solids on the combined toxicity of galaxolide and lead to Daphnia magna. Bulletin of Environmental Contamination and Toxicology 95(1):73-79.
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*; Adult
Health Outcome:	Immobilization
Chemical:	HHCB
HERO ID:	4690050

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Conditions were as standard for culture of D. magna.
	Metric 17:	Outcome Assessment Methodology	High	Visual inspection of immobilization at 24 and 48h.
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessment was conducted the same for all groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	In soil-added tests, actual concentration was between 65-73% of nominal due to adsorption to soil particles. Variation between groups may have minor impact on results.
	Metric 20:	Outcomes Unrelated to Exposure	High	No unexpected outcomes such as attrition were noted.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	EC50 calculated by regression.
	Metric 22:	Reporting of Data	High	Data reported for all groups.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: None

Overall Quality Determination **High**

Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Correct nomenclature was used. There was a typo in the CASRN (1222-05 reported vs 1222-05-5 correct); given the correct chemical name, correct abbreviation, and correct trade name, it is likely that the missing digit for the CASRN was a genuine typo.
	Metric 2:	Test Substance Source	High	The source was reported (Klamar-reagent company, Shanghai).
	Metric 3:	Test Substance Purity	Medium	Purity of the substance was reported at 75%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	The authors reported that mortality rates of test organisms in all control groups were <10% at end of the tests and that toxicity tests followed ASTM standard guidelines. Moreover, they reported that test containers were set with blank control and solvent control (acetone). However, supplemental Table S1, which reports the nominal HHCB concentrations used in the toxicity test, does not list the control group as 0 ug/L.
	Metric 5:	Negative Control Response	Low	The authors reported in the methods that mortality rates of test organisms in all control groups were <10% at end of the tests. However, they did not report the raw data in the results (they only provided LC50 values for each test).
	Metric 6:	Randomized Allocation	Low	The authors did not report if the organisms were randomly allocated to study groups. They included a general statement saying that toxicity tests followed ASTM standard guidelines without mention of random allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	All tests were static-renewal and test solutions were totally replaced at 24 h intervals. Due to the physical-chemical properties of the HHCB, a solvent control (acetone) was used.
	Metric 8:	Consistency of Exposure Administration	High	There was no evidence to suggest that exposure administration was not administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured (HPLC, Agilent 1200 Series, USA). The recoveries of HHCB in water samples were 98.42%–106.90%, and the limit of detection (LOD)was 10.70 ng/L for water samples. The variability of HHCB concentration was <20% (3.08%–9.12%).
	Metric 10:	Exposure Duration and Frequency	High	This data evaluation form is for the acute toxicity studies. The reported acute toxicity exposure durations were 48 h for Daphnia magna and Chironomus plumosus and 96 h for the other aquatic animals. This exposure durations were appropriate for the study type.

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Study Citation: Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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: HHCB
HERO ID: 5428397

Domain	Metric		Rating	Comments
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were appropriate (5 to 7 groups plus control (solvent control).
	Metric 12:	Testing at or Below Solubility Limit	High	For all tests the nominal HHCB concentrations were below the HHCB water solubility limit of 1750 ug/L expect for the acute Daphnia magna test. However, it likely was not a concern because a solvent control (acetone) was used and the recoveries of measured HHCB in water samples were 98.42%–106.90%.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described (e.g., source, size, age, healthy organism).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The authors reported that, for example, cultures of Gobiocypris rarus and Daphnia magna were from their biological culture laboratory and thus acclimated. For organisms not reared in the laboratory, the authors reported that larvae were domesticated for at least 7 days in biological culture laboratory and the mortality rates were lower than 5% before toxicity tests started.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were appropriate: Three replicates for test containers each containing 10 organisms (except for D. magna) were set with blank control and solvent control (acetone), while four replicates for test containers each containing 5 organisms were set for D. magna.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The reporting of housing and environmental conditions were sufficient: The tests were conducted at 21 $\pm$ 2 °C with a 12:12 h light/dark cycle. Test organisms were not fed in acute toxicity tests. During the test period, the temperature, pH, and dissolved oxygen (DO) were measured daily.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (mortality) addressed or reported the intended outcome (LC50 values to generate SSDs to derive the ALC).
	Metric 18:	Consistency of Outcome Assessment	High	Reported details suggest that the outcomes were assessed consistently across study groups.
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 that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				

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Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	High	The 48 h-EC50 and 96 h-LC50 values for acute toxicity tests and corresponding 95% confidence intervals were calculated by probit methodology. The ALC derivation was based on USEPA guidelines. The joint probability curves (JPC) were based on probability distributions of concentrations in water (exposure) and response (SSD) of HHCB.
	Metric 22:	Reporting of Data	Medium	The goal of the study was to derive the ALC based on SSDs. To generate the SSDs, the authors generated LC50 values, they also used LC50 values from the literature. So, the goal was not to determine the toxicity across treatment groups; keeping this is mind, this metric received a medium (instead of low) because mortality data was not reported for all treatment groups per species.
	Metric 23:	Explanation of Unexpected Outcomes	High	The study results provide variability (range).

Additional Comments: The goal of the study was to derive an ALC (aquatic life criteria) for HHCB based on aquatic organisms in China. The study included 8 Chinese resident aquatic species for acute and chronic toxicity tests. Lognormal species sensitivity distribution (SSD) and the log-logistic SSD methods were used to develop the ALC. The authors did not provide raw data of the toxicity studies. They also used LC50 values from the literature. Because the goal to run acute and chromic toxicity studies was to generate LC50 values that were ultimately used to generate SSDs, there are two forms included in the evaluation: acute data and chronic data for all species. This form is the data evaluation for the acute data for all species combined.

Overall Quality Determination High

Study Citation:	Wüthrich, V. (1996). Influence of HHCB on the reproduction of Daphnia magna.
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:	Invertebrate; Arthropods; *Daphnia magna*; Juvenile
Health Outcome:	Immobilization
Chemical:	HHCB
HERO ID:	7607958

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test material described using all appropriate identifiers and descriptions of form.
	Metric 2:	Test Substance Source	Low	Test material was described as being "supplied by the sponsor"
	Metric 3:	Test Substance Purity	High	99.15% Purity
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Concurrent negative and solvent controls included.
	Metric 5:	Negative Control Response	High	No unexplained effects in the control
	Metric 6:	Randomized Allocation	Low	Randomization procedure not described- minimal effect on the outcome expected
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Static renewal procedure acceptable for this type of testing. Total of 8 renewals were done throughout the course of the test. Volatilization of the test material was minimized by covering the test vessels.
	Metric 8:	Consistency of Exposure Administration	High	exposure administration was consistent among all test concentrations and replications.
	Metric 9:	Measurement of Test Substance Concentration	High	Mean measured test concentrations were quantified using HPLC
	Metric 10:	Exposure Duration and Frequency	High	21 day exposure duration was appropriate and consistent with guideline recommendations.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	10 Daphnia per test vessel is appropriate, dose spacing is also appropriate to quantify an EC50/ NOAEC/LOAEC. Test concentrations were spaced by a geometric progression factor of 2 and determined by a rage -finding test conducted prior to this test.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent was used (DMF and Tween 80) to account for the low solubility of the test material. This was appropriate, as no significant effects were observed in the solvent controls.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Daphnia magna used in the experiment were adequately sourced and at an appropriate age/life stage for the experiment.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Acclimatization procedure was not described, but as no effects were reported in the controls, this was not expected to affect the outcome of the experiment.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 individuals/test vessel is consistent with 850.1300 test guideline.

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Study Citation:	Wüthrich, V. (1996). Influence of HHCB on the reproduction of Daphnia magna.
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:	Invertebrate; Arthropods; *Daphnia magna*; Juvenile
Health Outcome:	Immobilization
Chemical:	HHCB
HERO ID:	7607958

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions appeared to be consistent with test guideline recommendations.
	Metric 17:	Outcome Assessment Methodology	High	Adult immobility following 21 days of exposure is an appropriate endpoint for this type of testing.
	Metric 18:	Consistency of Outcome Assessment	High	Protocol was applied consistently among groups and outcome was consistent among replicates within a test concentration.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No cofounding variables were observed or reported
	Metric 20:	Outcomes Unrelated to Exposure	Low	Coefficient of variation (%CV) around the mean of the number of living offspring around the control was 27% and no explanation was given. %CV around the mean of the number of offspring in the solvent control was significantly lower (3.4%). No explanation was given.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	EC50 value of the immobility of the parent animals was calculated based on the mean of the test concentrations. This is not typical, but given the outcome, where half the test concentrations showed no immobility and half reported 100% immobility, this was appropriate.
	Metric 22:	Reporting of Data	Low	Data for the range finding test was reported in the text only.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Measures of variability were not reported for the range finder.

Additional Comments: This evaluation form was for the immobilization outcome reported in section 3.1 for the range finding test.

Overall Quality Determination High

Study Citation:	Wüthrich, V. (1996). Influence of HHCB on the reproduction of Daphnia magna.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	Invertebrate; Arthropods; *Daphnia magna*; Juvenile
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	7607958

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test material described using all appropriate identifiers and descriptions of form.
	Metric 2:	Test Substance Source	Low	Test material was described as being "supplied by the sponsor"
	Metric 3:	Test Substance Purity	High	99.15% Purity
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Concurrent negative and solvent controls included.
	Metric 5:	Negative Control Response	High	No unexplained effects in the control
	Metric 6:	Randomized Allocation	Low	Randomization procedure not described- minimal effect on the outcome expected
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Static renewal procedure acceptable for this type of testing. Total of 8 renewals were done throughout the course of the test. Volatilization of the test material was minimized by covering the test vessels.
	Metric 8:	Consistency of Exposure Administration	High	exposure administration was consistent among all test concentrations and replications.
	Metric 9:	Measurement of Test Substance Concentration	High	Mean measured test concentrations were quantified using HPLC
	Metric 10:	Exposure Duration and Frequency	High	21 day exposure duration was appropriate and consistent with guideline recommendations.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	10 Daphnia per test vessel is appropriate, dose spacing is also appropriate to quantify an EC50/ NOAEC/LOAEC. Test concentrations were spaced by a geometric progression factor of 2 and determined by a rage -finding test conducted prior to this test.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent was used (DMF and Tween 80) to account for the low solubility of the test material. This was appropriate, as no significant effects were observed in the solvent controls.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Daphnia magna used in the experiment were adequately sourced and at an appropriate age/life stage for the experiment.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Acclimatization procedure was not described, but as no effects were reported in the controls, this was not expected to affect the outcome of the experiment.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 individuals/test vessel is consistent with 850.1300 test guideline.

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Study Citation:	Wüthrich, V. (1996). Influence of HHCB on the reproduction of Daphnia magna.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	Invertebrate; Arthropods; *Daphnia magna*; Juvenile
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	7607958

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions appeared to be consistent with test guideline recommendations.
	Metric 17:	Outcome Assessment Methodology	High	Adult immobility following 21 days of exposure is an appropriate endpoint for this type of testing.
	Metric 18:	Consistency of Outcome Assessment	High	Protocol was applied consistently among groups and outcome was consistent among replicates within a test concentration.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No cofounding variables were observed or reported
	Metric 20:	Outcomes Unrelated to Exposure	Low	Coefficient of variation (%CV) around the mean of the number of living offspring around the control was 27% and no explanation was given. %CV around the mean of the number of offspring in the solvent control was significantly lower (3.4%). No explanation was given.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	EC50 value of the immobility of the parent animals was calculated based on the mean of the test concentrations. This is not typical, but given the outcome, where half the test concentrations showed no immobility and half reported 100% immobility, this was appropriate.
	Metric 22:	Reporting of Data	High	All necessary data were reported to calculate the desired endpoints.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Study reported slightly higher than typical coefficient of variation (27%) in the reproductive effects observed in the controls but did not provide explanation.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Wüthrich, V. (1996). Influence of HHCB on the reproduction of Daphnia magna.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	Invertebrate; Arthropods; *Daphnia magna*; Juvenile
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	7607958

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test material described using all appropriate identifiers and descriptions of form.
	Metric 2:	Test Substance Source	Low	Test material was described as being "supplied by the sponsor"
	Metric 3:	Test Substance Purity	High	99.15% Purity
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Concurrent negative and solvent controls included.
	Metric 5:	Negative Control Response	High	No unexplained effects in the control
	Metric 6:	Randomized Allocation	Low	Randomization procedure not described- minimal effect on the outcome expected
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Static renewal procedure acceptable for this type of testing. Total of 8 renewals were done throughout the course of the test. Volatilization of the test material was minimized by covering the test vessels.
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration was consistent among all test concentrations and replications.
	Metric 9:	Measurement of Test Substance Concentration	High	Mean measured test concentrations were quantified using HPLC.
	Metric 10:	Exposure Duration and Frequency	High	21 day exposure duration was appropriate and consistent with guideline recommendations.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	10 Daphnia per test vessel is appropriate, dose spacing is also appropriate to quantify an EC50/ NOAEC/LOAEC. Test concentrations were spaced by a geometric progression factor of 2 and determined by a rage -finding test conducted prior to this test.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent was used (DMF and Tween 80) to account for the low solubility of the test material. This was appropriate, as no significant effects were observed in the solvent controls.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Daphnia magna used in the experiment were adequately sourced and at an appropriate age/life stage for the experiment.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Acclimatization procedure was not described, but as no effects were reported in the controls, this was not expected to affect the outcome of the experiment.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 individuals/test vessel is consistent with 850.1300 test guideline.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions appeared to be consistent with test guideline recommendations.

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Study Citation:	Wüthrich, V. (1996). Influence of HHCB on the reproduction of Daphnia magna.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	Invertebrate; Arthropods; *Daphnia magna*; Juvenile
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	7607958

Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	Reproductive output of daphnids following 21 days of exposure is an appropriate end-point for this type of testing.
	Metric 18:	Consistency of Outcome Assessment	High	Protocol was applied consistently among groups and outcome was consistent among replicates within a test concentration.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No cofounding variables were observed or reported
	Metric 20:	Outcomes Unrelated to Exposure	Low	Coefficient of variation (%CV) around the mean of the number of living offspring around the control was 27% and no explanation was given. %CV around the mean of the number of offspring in the solvent control was significantly lower (3.4%). No explanation was given.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Reproductive output of the surviving and reproducing animals was calculated using Dunnett's test, as is appropriate. EC50 of reproductive output was calculated using the Logit model.
	Metric 22:	Reporting of Data	High	All necessary data were reported to calculate the desired endpoints.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Study reported slightly higher than typical coefficient of variation (27%) in the reproductive effects observed in the controls but did not provide explanation.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Wüthrich, V. (1996). Influence of HHCB on the reproduction of Daphnia magna.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	Invertebrate; Arthropods; *Daphnia magna*; Juvenile
Health Outcome:	Immobilization
Chemical:	HHCB
HERO ID:	7607958

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test material described using all appropriate identifiers and descriptions of form.
	Metric 2:	Test Substance Source	Low	Test material was described as being "supplied by the sponsor"
	Metric 3:	Test Substance Purity	High	99.15% Purity
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Concurrent negative and solvent controls included.
	Metric 5:	Negative Control Response	High	No unexplained effects in the control
	Metric 6:	Randomized Allocation	Low	Randomization procedure not described- minimal effect on the outcome expected
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Static renewal procedure acceptable for this type of testing. Total of 8 renewals were done throughout the course of the test. Volatilization of the test material was minimized by covering the test vessels.
	Metric 8:	Consistency of Exposure Administration	High	exposure administration was consistent among all test concentrations and replications.
	Metric 9:	Measurement of Test Substance Concentration	High	Mean measured test concentrations were quantified using HPLC
	Metric 10:	Exposure Duration and Frequency	High	21 day exposure duration was appropriate and consistent with guideline recommendations.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	10 Daphnia per test vessel is appropriate, dose spacing is also appropriate to quantify an EC50/ NOAEC/LOAEC. Test concentrations were spaced by a geometric progression factor of 2 and determined by a rage -finding test conducted prior to this test.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent was used (DMF and Tween 80) to account for the low solubility of the test material. This was appropriate, as no significant effects were observed in the solvent controls.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Daphnia magna used in the experiment were adequately sourced and at an appropriate age/life stage for the experiment.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	Acclimatization procedure was not described, but as no effects were reported in the controls, this was not expected to affect the outcome of the experiment.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 individuals/test vessel is consistent with 850.1300 test guideline.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions appeared to be consistent with test guideline recommendations.

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Study Citation:	Wüthrich, V. (1996). Influence of HHCB on the reproduction of Daphnia magna.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	Invertebrate; Arthropods; *Daphnia magna*; Juvenile
Health Outcome:	Immobilization
Chemical:	HHCB
HERO ID:	7607958

Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	Adult immobility following 21 days of exposure is an appropriate endpoint for this type of testing.
	Metric 18:	Consistency of Outcome Assessment	High	Protocol was applied consistently among groups and outcome was consistent among replicates within a test concentration.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No cofounding variables were observed or reported
	Metric 20:	Outcomes Unrelated to Exposure	Low	Coefficient of variation (%CV) around the mean of the number of living offspring around the control was 27% and no explanation was given. %CV around the mean of the number of offspring in the solvent control was significantly lower (3.4%). No explanation was given.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	EC50 value of the immobility of the parent animals was calculated based on the mean of the test concentrations. This is not typical, but given the outcome, where half the test concentrations showed no immobility and half reported 100% immobility, this was appropriate.
	Metric 22:	Reporting of Data	High	All necessary data were reported to calculate the desired endpoints.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Study reported slightly higher than typical coefficient of variation (27%) in the reproductive effects observed in the controls but did not provide explanation.

Additional Comments: This evaluation form was for the immobilization outcome reported in section 3.2.

Overall Quality Determination High

Study Citation:	Peng, F.J., Kiggen, F., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Brink, P.J. (2019). Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms. Ecotoxicology and Environmental Safety 169:902-910.
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:	Invertebrate; Arthropods; *Daphnia magna*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5428151

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Chemical scientific name and source are reported.
	Metric 2:	Test Substance Source	High	Source was reported.
	Metric 3:	Test Substance Purity	Medium	Galaxolide was used as a standard.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Both a solvent and negative control used.
	Metric 5:	Negative Control Response	Low	No Daphnia were found in the controls at the end of the experiment.
	Metric 6:	Randomized Allocation	Low	Authors discuss random sampling, but not random allocation to experimental chambers.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Brief description in this paper. Extended description of the Microcosms set-up, Test sediment and chemical spiking described in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across all treatments.
	Metric 9:	Measurement of Test Substance Concentration	High	Used GC-MS analysis of HHCB in overlying water.
	Metric 10:	Exposure Duration and Frequency	High	28 day exposure period.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Authors used 4 exposure groups and a negative and solvent controls.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent effects were similar to the negative control.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	Daphnia were cultured in the laboratory.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimated for 2-14 days prior to placing in the microcosm, as described in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	6 replicates, 12 organisms per replicate.

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Study Citation:	Peng, F.J., Kiggen, F., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Brink, P.J. (2019). Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms. Ecotoxicology and Environmental Safety 169:902-910.
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:	Invertebrate; Arthropods; *Daphnia magna*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5428151

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Housing, environmental conditions, food, water, and nutrients described briefly in this paper and in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 17:	Outcome Assessment Methodology	High	Table S14 provides data.
	Metric 18:	Consistency of Outcome Assessment	High	Measured at the same durations.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among study grooups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Authors do not discuss differences in outcomes related to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The Williams test (Williams, 1972) which assumes a concentration-effect relationship and is, therefore, is used extensively to analyse the results of eco-toxicity tests. The Williams tests were performed using the Community Analysis computer program, version 4.3.05 (Hommenet al., 1994).
	Metric 22:	Reporting of Data	High	Data reported on Table S14.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexplained outcomes were reported by the authors.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	IFF, (2004). HHCB/galaxolide: A study on the toxicity to the sediment dweller hyalella azteca (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Hyalella azteca*; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784982

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The CAS number and batch number of the test substance was given.
	Metric 2:	Test Substance Source	Low	A certificate of analysis for the test item was provided but it is very hard to read and unclear what the source is.
	Metric 3:	Test Substance Purity	High	The purity of the test substance used was 98.5 percent.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A control with unspiked sediment and a solvent control (acetone) were included in the test.
	Metric 5:	Negative Control Response	High	Length and weight of the control test organisms were adequate.
	Metric 6:	Randomized Allocation	Medium	Hyallela azteca were randomly allocated to the test vessels on day zero of the study. However, the method of randomization was not stated.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The preparation of the formulated sediment, overlying water and test substance stock solution was described in great detail and were adequate for the study. The composition of the formulated sediment was according to OECD Guideline 218. Samples were taken for test concentration analysis (performed by another lab offsite) but that data was not shown.
	Metric 8:	Consistency of Exposure Administration	Medium	The test exposure system was the same for the control and treatment groups with Hyalella. Water quality conditions were consistent across exposure groups throughout the study. Samples taken for chemical analysis were from separate vessels prepared with the same overlying water and sediment (just sediment for control) with test item at appropriate concentrations. So the samples for test concentration analysis were not taken from the actual vessels where the organisms were being exposed to determine biological results. In addition, the vessels used for the chemical analysis were larger than the glass vessels used for the Hyalella (1 L versus 250 mL, respectively). And because of the larger size vessels they had more sediment and overlying water added.
	Metric 9:	Measurement of Test Substance Concentration	Low	Study authors indicated that test concentrations were measured in the sediment, pore water, and overlying water at four different times throughout the test. Those samples were processed and shipped to a specialized laboratory for analysis. None of that data was given in the report. The authors mentioned the analytical investigations were not part of the study and therefore were not included in the report. The biological results were based on nominal test concentrations. These nominal concentration levels were chosen based on a range-finding test performed prior to the start of the definitive test.

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Study Citation:	IFF, (2004). HHCB/galaxolide: A study on the toxicity to the sediment dweller hyalella azteca (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Hyalella azteca*; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784982

Domain	Metric		Rating	Comments
	Metric 10:	Exposure Duration and Frequency	High	The report mentioned that for the study there was no standard guideline available. The design of the study took into account information from both OECD Guideline 218 and USEPA (2000)-Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. The USEPA (2000) guidance mentions that 28-d sediment tests are run to measure the endpoints of growth and survival. Previous guidelines and papers were cited for using this method (ASTM, 1999a and Ingersoll et al. 1996). This most recent EPA guidance (2000) mentions the method for conducting a 42-d sediment test which can assess the endpoints of growth, survival and reproduction.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	As mentioned in metric 9, basing results off of nominal concentrations, the desired NOEC, LOEC, LC 15 and 50, and EC 15 and 50 values were determined, meaning adequate test concentrations were used.
	Metric 12:	Testing at or Below Solubility Limit	N/A	HHCB was spiked into sediment.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Originally the Hyalella azteca were obtained from Dresden University of Technology. They were cultured and prepared for use in the test according to USEPA (2000) methods and OECD Guideline 203 (OECD 1992). The age of the test organisms was appropriate for use in a 28-d study measuring survival and growth.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	At least 2 weeks before the start of the exposure period the hyalella were held in glass vessels at the appropriate temperature and photo period used in the test. Five days before adding test organisms to the test vessels, juvenile amphipods were sieved from the culture and transferred to glass vessels with test medium and fed twice until the start of the exposure. These vessels were incubated in the test room at temperature and light conditions used in the exposure phase and gently aerated. These conditions were the same for the control and treatment organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 4 replicates per treatment concentration and 6 replicates in the controls. Ten Hyalella were placed in each test replicate vessel at the start of the exposure.
Domain 5: Outcome Assessment				

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Study Citation:	IFF, (2004). HHCB/galaxolide: A study on the toxicity to the sediment dweller hyalella azteca (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Hyalella azteca*; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784982

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Conditions in test vessels were adequate for the Hyalella. Due to the low nutrient content of the artificial sediment used in the test, urtica powder and cellulose powder were added to the sediment to ensure survival and growth of the animals under control conditions. This was done the same way for both the control and treatment groups. The following water quality parameters were measured in the overlying water throughout the exposure: temperature, pH, dissolved oxygen, total hardness, and total ammonia. pH and TOC were measured in the sediment. Light intensity was measured once during the test and room temperature was monitored continuously throughout the test. Water quality conditions were adequate for the test. The data for these measurements is shown in Appendix l.
	Metric 17:	Outcome Assessment Methodology	High	Length of each surviving amphipod was determined at the end of the test duration using a dissecting microscope. After length measurements were completed, the total dry weight of the pooled amphipods of each replicate was measured. Individual dry weights were also determined. The recovered amphipods were placed in weigh pans (one per replicate) and dried overnight in an oven before being weighed. Raw data of lengths is shown in Table 33 of Appendix l. Raw data of total dry weight per replicate and mean individual dry weight are shown in Table 35.
	Metric 18:	Consistency of Outcome Assessment	High	Lengths and weights were measured at the end of the study the same way in both the control and treatment groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Hyalella were acclimated to test conditions prior to initiation and water quality conditions throughout the study were adequate across all exposure groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among treatment groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analyses was adequately described and were appropriate for analyzing the growth (length and weight) data. In the report statistical results for length are shown in section 17.3.2 and in the Appendix l Table 42-44. In the report statistical results for total biomass per replicate and individual biomass are shown in section 17.3.3 and 17.3.4 as well as in Appendix l Table 45-53.
	Metric 22:	Reporting of Data	High	Growth (length and weight) data was provided along with all analyses used to determine specific endpoints (ECx, NOEC, LOEC). Summarized length data is shown in Table 12 and Figure 2 of the report, total biomass per replicate data is shown in Table 13 and Figure 3, and individual biomass data is shown in Table 14.
	Metric 23:	Explanation of Unexpected Outcomes	High	Results were described adequately with data shown. Measures of variability were shown with the data.

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Study Citation:	IFF, (2004). HHCB/galaxolide: A study on the toxicity to the sediment dweller hyalella azteca (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Hyalella azteca*; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784982

Domain	Metric	Rating	Comments
Additional Comments:	This evaluation is for the development/growth (length and biomass data determined) endpoint. Biological results were based on nominal concentrations. With respect to the EC15 and EC50 values, total biomass was slightly more sensitive than survival. The EC50 for total biomass was 53.52 and for survival it was 62.47. The NOEC and LOEC for survival were, 34.7 and 83.3, respectively. The NOEC and LOEC for total biomass were 14.5 and 34.7, respectively. The sediment was the main exposure route for the Hyalella azteca because it was spiked with HHCB. Because food items were included in the sediment there was potential for exposure from their food. Additionally, they could of been exposed through the overlying water if any of the test item leached into it.		

Overall Quality Determination High

Study Citation:	IFF, (2004). HHCB/galaxolide: A study on the toxicity to the sediment dweller hyalella azteca (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Hyalella azteca*; Juvenile
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8784982

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The CAS number and batch number of the test substance was given.
	Metric 2:	Test Substance Source	Low	A certificate of analysis for the test item was provided but it is very hard to read and unclear what the source is.
	Metric 3:	Test Substance Purity	High	The purity of the test substance used was 98.5 percent.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A control with unspiked sediment and a solvent control (acetone) were included in the test.
	Metric 5:	Negative Control Response	High	Mortality in the control vessels was 1.7%. Guidelines require less than or equal to 30%.
	Metric 6:	Randomized Allocation	Medium	Hyallela azteca were stated to be randomly allocated to the test vessels on day zero of the study. However, the method of randomization was not stated.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The preparation of the formulated sediment, overlying water and test substance stock solution was described in great detail and were adequate for the study. The composition of the formulated sediment was according to OECD Guideline 218. Samples were taken for test concentration analysis (performed by another lab offsite) but that data was not shown.
	Metric 8:	Consistency of Exposure Administration	Medium	The test exposure system was the same for the control and treatment groups with Hyalella. Water quality conditions were consistent across exposure groups throughout the study. Samples taken for chemical analysis were from separate vessels prepared with the same overlying water and sediment (just sediment for control) with test item at appropriate concentrations. So the samples for test concentration analysis were not taken from the actual vessels where the organisms were being exposed to determine biological results. In addition, the vessels used for the chemical analysis were larger than the glass vessels used for the Hyalella (1 L versus 250 mL, respectively). And because of the larger size vessels they had more sediment and overlying water added.
	Metric 9:	Measurement of Test Substance Concentration	Low	Study authors indicated that test concentrations were measured in the sediment, pore water, and overlying water at four different times throughout the test. Those samples were processed and shipped to a specialized laboratory for analysis. None of that data was given in the report. The authors mentioned the analytical investigations were not part of the study and therefore were not included in the report. The biological results were based on nominal test concentrations. These nominal concentration levels were chosen based on a range-finding test performed prior to the start of the definitive test.

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Study Citation:	IFF, (2004). HHCB/galaxolide: A study on the toxicity to the sediment dweller hyalella azteca (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Hyalella azteca*; Juvenile
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8784982

Domain	Metric		Rating	Comments
	Metric 10:	Exposure Duration and Frequency	High	The report mentioned that for the study there was no standard guideline available. The design of the study took into account information from both OECD Guideline 218 and USEPA (2000)-Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. The USEPA (2000) guidance mentions that 28-d sediment tests are run to measure the endpoints of growth and survival. Previous guidelines and papers were cited for using this method (ASTM, 1999a and Ingersoll et al. 1996). This most recent EPA guidance (2000) mentions the method for conducting a 42-d sediment test which can assess the endpoints of growth, survival and reproduction.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	As mentioned in metric 9, basing results off of nominal concentrations, the desired NOEC, LOEC, LC 15 and 50, and EC 15 and 50 values were determined, meaning adequate test concentrations were used.
	Metric 12:	Testing at or Below Solubility Limit	N/A	HHCB was spiked into sediment.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Originally the Hyalella azteca were obtained from Dresden University of Technology. They were cultured and prepared for use in the test according to USEPA (2000) methods and OECD Guideline 203 (OECD 1992). The age of the test organisms was appropriate for use in a 28-d study measuring survival and growth.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	At least 2 weeks before the start of the exposure period the hyalella were held in glass vessels at the appropriate temperature and photo period used in the test. Five days before adding test organisms to the test vessels, juvenile amphipods were sieved from the culture and transferred to glass vessels with test medium and fed twice until the start of the exposure. These vessels were incubated in the test room at temperature and light conditions used in the exposure phase and gently aerated. These conditions were the same for the control and treatment organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 4 replicates per treatment concentration and 6 replicates in the controls. Ten Hyalella were placed in each test replicate vessel at the start of the exposure.
Domain 5: Outcome Assessment				

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Study Citation:	IFF, (2004). HHCB/galaxolide: A study on the toxicity to the sediment dweller hyalella azteca (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Hyalella azteca*; Juvenile
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8784982

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Conditions in test vessels were adequate for the Hyalella. Due to the low nutrient content of the artificial sediment used in the test, urtica powder and cellulose powder were added to the sediment to ensure survival and growth of the animals under control conditions. This was done the same way for both the control and treatment groups. The following water quality parameters were measured in the overlying water throughout the exposure: temperature, pH, dissolved oxygen, total hardness, and total ammonia. pH and TOC were measured in the sediment. Light intensity was measured once during the test and room temperature was monitored continuously throughout the test. Water quality conditions were adequate for the test. The data for these measurements is shown in Appendix l.
	Metric 17:	Outcome Assessment Methodology	High	Mortality was assessed at the end of the test duration. All replicates in both controls and treatments were examined for surviving Hyalella. Immobile and missing amphipods were recorded as dead. Raw mortality/survival data is shown in Table 32.
	Metric 18:	Consistency of Outcome Assessment	High	Mortality was measured at the end of the study the same way in both the control and treatment groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Hyalella were acclimated to test conditions prior to initiation and water quality conditions throughout the study were consistent across all exposure groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among treatment groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analyses was adequately described and were appropriate for analyzing the survival/mortality data. In the report statistical results are shown in section 17.3.1 and in the Appendix l Table 36-41.
	Metric 22:	Reporting of Data	High	Mortality data was provided along with all analyses used to determine specific endpoints (LCx, ECx, NOEC, LOEC). Summarized mortality/survival data is shown in Table 11 and Figure 1 of the report. Raw data is shown in Table 32 of Appendix l.
	Metric 23:	Explanation of Unexpected Outcomes	High	Results were described adequately with data shown. Measures of variability were shown with the data.

Additional Comments: This evaluation is for the mortality endpoint. Biological results were based on nominal concentrations. With respect to the EC15 and EC50 values, total biomass was slightly more sensitive than survival. The EC50 for total biomass was 53.52 and for survival it was 62.47. The NOEC and LOEC for survival were, 34.7 and 83.3, respectively. The NOEC and LOEC for total biomass were 14.5 and 34.7, respectively. The sediment was the main exposure route for the Hyalella azteca because it was spiked with HHCB. Because food items were included in the sediment there was potential for exposure from their food. Additionally, they could of been exposed through the overlying water if any of the test item leached into it.

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Study Citation:	IFF, (2004). HHCB/galaxolide: A study on the toxicity to the sediment dweller hyalella azteca (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Hyalella azteca*; Juvenile
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8784982

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

Study Citation:	IFF, (2004). HHCB/galaxolide: A study on the toxicity to the sediment dweller hyalella azteca (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Hyalella azteca*; Juvenile
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	8784982

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The CAS number and batch number of the test substance was given.
	Metric 2:	Test Substance Source	Low	A certificate of analysis for the test item was provided but it is very hard to read and unclear what the source is.
	Metric 3:	Test Substance Purity	High	The purity of the test substance used was 98.5 percent.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A control with unspiked sediment and a solvent control (acetone) were included in the test.
	Metric 5:	Negative Control Response	High	There were no behavior effects observed in the controls.
	Metric 6:	Randomized Allocation	Medium	Hyallela azteca were stated to be randomly allocated to the test vessels on day zero of the study. However, the method of randomization was not stated.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The preparation of the formulated sediment, overlying water and test substance stock solution was described in great detail and were adequate for the study. The composition of the formulated sediment was according to OECD Guideline 218. Samples were taken for test concentration analysis (performed by another lab offsite) but that data was not shown.
	Metric 8:	Consistency of Exposure Administration	Medium	The test exposure system was the same for the control and treatment groups with Hyalella. Water quality conditions were consistent across exposure groups throughout the study. Samples taken for chemical analysis were from separate vessels prepared with the same overlying water and sediment (just sediment for control) with test item at appropriate concentrations. So the samples for test concentration analysis were not taken from the actual vessels where the organisms were being exposed to determine biological results. In addition, the vessels used for the chemical analysis were larger than the glass vessels used for the Hyalella (1 L versus 250 mL, respectively). And because of the larger size vessels they had more sediment and overlying water added.
	Metric 9:	Measurement of Test Substance Concentration	Low	Study authors indicated that test concentrations were measured in the sediment, pore water, and overlying water at four different times throughout the test. Those samples were processed and shipped to a specialized laboratory for analysis. None of that data was given in the report. The authors mentioned the analytical investigations were not part of the study and therefore were not included in the report. The biological results were based on nominal test concentrations. These nominal concentration levels were chosen based on a range-finding test performed prior to the start of the definitive test.

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Study Citation:	IFF, (2004). HHCB/galaxolide: A study on the toxicity to the sediment dweller hyalella azteca (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Hyalella azteca*; Juvenile
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	8784982

Domain	Metric		Rating	Comments
	Metric 10:	Exposure Duration and Frequency	High	The report mentioned that for the study there was no standard guideline available. The design of the study took into account information from both OECD Guideline 218 and USEPA (2000)-Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. The USEPA (2000) guidance mentions that 28-d sediment tests are run to measure the endpoints of growth and survival. Previous guidelines and papers were cited for using this method (ASTM, 1999a and Ingersoll et al. 1996). This most recent EPA guidance (2000) mentions the method for conducting a 42-d sediment test which can assess the endpoints of growth, survival and reproduction.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	As mentioned in metric 9, basing results off of nominal concentrations, the desired NOEC, LOEC, LC 15 and 50, and EC 15 and 50 values were determined, meaning adequate test concentrations were used.
	Metric 12:	Testing at or Below Solubility Limit	N/A	HHCB was spiked in sediment.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Originally the Hyalella azteca were obtained from Dresden University of Technology. They were cultured and prepared for use in the test according to USEPA (2000) methods and OECD Guideline 203 (OECD 1992). The age of the test organisms was appropriate for use in a 28-d study measuring survival and growth.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	At least 2 weeks before the start of the exposure period the hyalella were held in glass vessels at the appropriate temperature and photo period used in the test. Five days before adding test organisms to the test vessels, juvenile amphipods were sieved from the culture and transferred to glass vessels with test medium and fed twice until the start of the exposure. These vessels were incubated in the test room at temperature and light conditions used in the exposure phase and gently aerated. These conditions were the same for the control and treatment organisms.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 4 replicates per treatment concentration and 6 replicates in the controls. Ten Hyalella were placed in each test replicate vessel at the start of the exposure.
Domain 5: Outcome Assessment				

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Study Citation:	IFF, (2004). HHCB/galaxolide: A study on the toxicity to the sediment dweller hyalella azteca (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; 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; *Hyalella azteca*; Juvenile
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	8784982

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Conditions in test vessels were adequate for the Hyalella. Due to the low nutrient content of the artificial sediment used in the test, urtica powder and cellulose powder were added to the sediment to ensure survival and growth of the animals under control conditions. This was done the same way for both the control and treatment groups. The following water quality parameters were measured in the overlying water throughout the exposure: temperature, pH, dissolved oxygen, total hardness, and total ammonia. pH and TOC were measured in the sediment. Light intensity was measured once during the test and room temperature was monitored continuously throughout the test. Water quality conditions were adequate for the test. The data for these measurements is shown in Appendix l.
	Metric 17:	Outcome Assessment Methodology	High	At least three times a week the test vessels were observed in order to assess visually if there were any behavioral effects in the treatment or controls.
	Metric 18:	Consistency of Outcome Assessment	High	Behavior effects were observed the same way in both the control and treatment groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Hyalella were acclimated to test conditions prior to initiation and water quality conditions throughout the study were adequate across all exposure groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There is no information to suggest differences among treatment groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	N/A	There were no reported effects on behavior for either the treatment or controls throughout the study so statistical analyses are not necessary.
	Metric 22:	Reporting of Data	High	In the results section 17.3.5 of the report it was mentioned in text that during the exposure, observations concerning behavior of the Hyalella did not reveal any concentration dependent differences between treatments and controls.
	Metric 23:	Explanation of Unexpected Outcomes	High	Results were described adequately with data shown. Measures of variability were shown with the data.

Additional Comments: This evaluation is for the behavioral endpoint. Biological results were based on nominal concentrations. With respect to the EC15 and EC50 values, total biomass was slightly more sensitive than survival. The EC50 for total biomass was 53.52 and for survival it was 62.47. The NOEC and LOEC for survival were, 34.7 and 83.3, respectively. The NOEC and LOEC for total biomass were 14.5 and 34.7, respectively. The sediment was the main exposure route for the Hyalella azteca because it was spiked with HHCB. Because food items were included in the sediment there was potential for exposure from their food. Additionally, they could of been exposed through the overlying water if any of the test item leached into it.

Overall Quality Determination High

Study Citation:	Gooding, M.P., Newton, T.J., Bartsch, M.R., Hornbuckle, K.C. (2006). Toxicity of synthetic musks to early life stages of the freshwater mussel Lampsilis cardium. Archives of Environmental Contamination and Toxicology 51(4):549-558.
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; Mollusks; *Lampsilis cardium*; Rafinesque, 1820; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	625761

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	"The HHCB technical product contains 26% diethylphthalate but this compound was not quantified in our samples."
	Metric 2:	Test Substance Source	High	Source of HHCB reported
	Metric 3:	Test Substance Purity	Low	HHCB technical grade reported to contain 26% diethyl phthalate; this was not confirmed by authors (no measurements were taken of diethyl phthalate). Purity of HHCB was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Appropriate controls identified, well-water control and solvent control
	Metric 5:	Negative Control Response	Low	Negative controls not represented in table form nor in text. Large span of values in Figure for presumably the control groups.
	Metric 6:	Randomized Allocation	Low	Random allocation was not stated specifically in this paper, larvae were obtained in the field and transported to the lab for experimental analysis.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Methods were described in detail
	Metric 8:	Consistency of Exposure Administration	High	Consistent exposure, solvent concentration did not exceed 0.005% of treatment
	Metric 9:	Measurement of Test Substance Concentration	High	Initial and final concentrations of HHCB were monitored by GC/MS
	Metric 10:	Exposure Duration and Frequency	High	Exposure frequency were reported and appropriate
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Experimental concentration ranges were appropriate, ranging from low to near-solubility limit, and completed in quadruplet reps per concentration with two duplicate experiments
	Metric 12:	Testing at or Below Solubility Limit	High	Highest concentration was below solubility limit
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Adults collected from the field and held in laboratory conditions (length of time not stated), mature glochidia (approximately 300 uM) collected from their gills and used to parasitize largemouth bass in laboratory settings until an excystment stage is achieved and juveniles collected.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Acclimation not specifically stated for juveniles.

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Study Citation:	Gooding, M.P., Newton, T.J., Bartsch, M.R., Hornbuckle, K.C. (2006). Toxicity of synthetic musks to early life stages of the freshwater mussel Lampsilis cardium. Archives of Environmental Contamination and Toxicology 51(4):549-558.
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; Mollusks; *Lampsilis cardium*; Rafinesque, 1820; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	625761

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Appropriate number of organisms and replicates per group
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Housing, conditions, feeding, etc. were appropriately reported
	Metric 17:	Outcome Assessment Methodology	High	Outcomes were clearly outlined in this study
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Diethyl phthalate not measured in water treatments, unclear if any observed biological effects was due to HHCB or DEP.
	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	Probit analysis for LC50. EC50 calculations described.
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were reported for most, but not all, outcomes by treatment and control group and/or data were not reported for outcomes with negative findings, but these minor uncertainties or limitations in outcome reporting are unlikely to have a substantial impact on results.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Minor uncertainties or limitations were identified in how the study characterized unexpected outcomes, including within-study variability and/or variation from historical measures, but those are not likely to have a substantial impact on results.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Gooding, M.P., Newton, T.J., Bartsch, M.R., Hornbuckle, K.C. (2006). Toxicity of synthetic musks to early life stages of the freshwater mussel Lampsilis cardium. Archives of Environmental Contamination and Toxicology 51(4):549-558.
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; Mollusks; *Lampsilis cardium*; Rafinesque, 1820; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	625761

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	"The HHCB technical product contains 26% diethylphthalate but this compound was not quantified in our samples."
	Metric 2:	Test Substance Source	High	Source of HHCB reported
	Metric 3:	Test Substance Purity	Low	HHCB technical grade reported to contain 26% diethyl phthalate; this was not confirmed by authors (no measurements were taken of diethyl phthalate). Purity of HHCB was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Appropriate controls identified, well-water control and solvent control
	Metric 5:	Negative Control Response	Medium	Negative controls not represented in table form nor in text. Less than 20% mortality for what is presumably the control groups.
	Metric 6:	Randomized Allocation	Low	Random allocation was not stated specifically in this paper, larvae were obtained in the field and transported to the lab for experimental analysis.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Methods were described in detail
	Metric 8:	Consistency of Exposure Administration	High	Consistent exposure, solvent concentration did not exceed 0.005% of treatment
	Metric 9:	Measurement of Test Substance Concentration	High	Initial and final concentrations of HHCB were monitored by GC/MS
	Metric 10:	Exposure Duration and Frequency	High	Exposure frequency were reported and appropriate
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Experimental concentration ranges were appropriate, ranging from low to near-solubility limit, and completed in quadruplet reps per concentration with two duplicate experiments
	Metric 12:	Testing at or Below Solubility Limit	High	Highest concentration was below solubility limit
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Adults collected from the field and held in laboratory conditions (length of time not stated), mature glochidia (approximately 300 uM) collected from their gills and utilized in experiments
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Not stated how long adults acclimated in lab before collecting glochidia.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Appropriate number of organisms and replicates per group

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Study Citation:	Gooding, M.P., Newton, T.J., Bartsch, M.R., Hornbuckle, K.C. (2006). Toxicity of synthetic musks to early life stages of the freshwater mussel Lampsilis cardium. Archives of Environmental Contamination and Toxicology 51(4):549-558.
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; Mollusks; *Lampsilis cardium*; Rafinesque, 1820; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	625761

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Housing, conditions, feeding, etc. were appropriately reported
	Metric 17:	Outcome Assessment Methodology	High	Outcomes were clearly outlined in this study
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Diethyl phthalate not measured in water treatments, unclear if any observed biological effects was due to HHCB or DEP.
	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	Probit analysis for LC50. EC50 calculations described.
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were reported for most, but not all, outcomes by treatment and control group and/or data were not reported for outcomes with negative findings, but these minor uncertainties or limitations in outcome reporting are unlikely to have a substantial impact on results.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Minor uncertainties or limitations were identified in how the study characterized unexpected outcomes, including within-study variability and/or variation from historical measures, but those are not likely to have a substantial impact on results.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Gooding, M.P., Newton, T.J., Bartsch, M.R., Hornbuckle, K.C. (2006). Toxicity of synthetic musks to early life stages of the freshwater mussel Lampsilis cardium. Archives of Environmental Contamination and Toxicology 51(4):549-558.
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; Mollusks; *Lampsilis cardium*; Rafinesque, 1820; Juvenile
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	625761

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	"The HHCB technical product contains 26% diethylphthalate but this compound was not quantified in our samples."
	Metric 2:	Test Substance Source	High	Source of HHCB reported
	Metric 3:	Test Substance Purity	Low	HHCB technical grade reported to contain 26% diethyl phthalate; this was not confirmed by authors (no measurements were taken of diethyl phthalate). Purity of HHCB was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Appropriate controls identified, well-water control and solvent control
	Metric 5:	Negative Control Response	Medium	Negative controls not represented in table form nor in text. Less than 20% mortality for what is presumably the control groups.
	Metric 6:	Randomized Allocation	Low	Random allocation was not stated specifically in this paper, larvae were obtained in the field and transported to the lab for experimental analysis.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Methods were described in detail
	Metric 8:	Consistency of Exposure Administration	High	Consistent exposure, solvent concentration did not exceed 0.005% of treatment
	Metric 9:	Measurement of Test Substance Concentration	High	Initial and final concentrations of HHCB were monitored by GC/MS
	Metric 10:	Exposure Duration and Frequency	High	Exposure frequency were reported and appropriate
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Experimental concentration ranges were appropriate, ranging from low to near-solubility limit, and completed in quadruplet reps per concentration with two duplicate experiments
	Metric 12:	Testing at or Below Solubility Limit	High	Highest concentration was below solubility limit
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Adults collected from the field and held in laboratory conditions (length of time not stated), mature glochidia (approximately 300 uM) collected from their gills and used to parasitize largemouth bass in laboratory settings until an excystment stage is achieved and juveniles collected.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Acclimation not specifically stated for juveniles.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Appropriate number of organisms and replicates per group

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Study Citation:	Gooding, M.P., Newton, T.J., Bartsch, M.R., Hornbuckle, K.C. (2006). Toxicity of synthetic musks to early life stages of the freshwater mussel Lampsilis cardium. Archives of Environmental Contamination and Toxicology 51(4):549-558.
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; Mollusks; *Lampsilis cardium*; Rafinesque, 1820; Juvenile
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	625761

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Housing, conditions, feeding, etc. were appropriately reported
	Metric 17:	Outcome Assessment Methodology	High	Outcomes were clearly outlined in this study
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Diethyl phthalate not measured in water treatments, unclear if any observed biological effects was due to HHCB or DEP.
	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	Probit analysis for LC50. EC50 calculations described.
	Metric 22:	Reporting of Data	Medium	Data for exposure-related findings were reported for most, but not all, outcomes by treatment and control group and/or data were not reported for outcomes with negative findings, but these minor uncertainties or limitations in outcome reporting are unlikely to have a substantial impact on results.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Minor uncertainties or limitations were identified in how the study characterized unexpected outcomes, including within-study variability and/or variation from historical measures, but those are not likely to have a substantial impact on results.

Additional Comments: None

Overall Quality Determination Medium

Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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; Worms (e.g., Annelids, Nematodes); *Limnodrilus hoffmeisteri*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Correct nomenclature was used. There was a typo in the CASRN (1222-05 reported vs 1222-05-5 correct); given the correct chemical name, correct abbreviation, and correct trade name, it is likely that the missing digit for the CASRN was a genuine typo.
	Metric 2:	Test Substance Source	High	The source was reported (Klamar-reagent company, Shanghai).
	Metric 3:	Test Substance Purity	Medium	Purity of the substance was reported at 75%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	The authors reported that mortality rates of test organisms in all control groups were <10% at end of the tests and that toxicity tests followed ASTM standard guidelines. Moreover, they reported that test containers were set with blank control and solvent control (acetone). However, supplemental Table S1, which reports the nominal HHCB concentrations used in the toxicity test, does not list the control group as 0 ug/L.
	Metric 5:	Negative Control Response	Low	The authors reported in the methods that mortality rates of test organisms in all control groups were <10% at end of the tests. However, they did not report the raw data in the results (they only provided LC50 values for each test).
	Metric 6:	Randomized Allocation	Low	The authors did not report if the organisms were randomly allocated to study groups. They included a general statement saying that toxicity tests followed ASTM standard guidelines without mention of random allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	All tests were static-renewal and test solutions were totally replaced at 24 h intervals. Due to the physical-chemical properties of the HHCB, a solvent control (acetone) was used.
	Metric 8:	Consistency of Exposure Administration	High	There was no evidence to suggest that exposure administration was not administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured (HPLC, Agilent 1200 Series, USA). The recoveries of HHCB in water samples were 98.42%–106.90%, and the limit of detection (LOD)was 10.70 ng/L for water samples. The variability of HHCB concentration was <20% (3.08%–9.12%).
	Metric 10:	Exposure Duration and Frequency	High	This data evaluation form is for the acute toxicity studies. The reported acute toxicity exposure durations were 48 h for Daphnia magna and Chironomus plumosus and 96 h for the other aquatic animals. This exposure durations were appropriate for the study type.

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Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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; Worms (e.g., Annelids, Nematodes); *Limnodrilus hoffmeisteri*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were appropriate (5 to 7 groups plus control (solvent control).
	Metric 12:	Testing at or Below Solubility Limit	High	For all tests the nominal HHCB concentrations were below the HHCB water solubility limit of 1750 ug/L expect for the acute Daphnia magna test. However, it likely was not a concern because a solvent control (acetone) was used and the recoveries of measured HHCB in water samples were 98.42%–106.90%.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described (e.g., source, size, age, healthy organism).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The authors reported that, for example, cultures of Gobiocypris rarus and Daphnia magna were from their biological culture laboratory and thus acclimated. For organisms not reared in the laboratory, the authors reported that larvae were domesticated for at least 7 days in biological culture laboratory and the mortality rates were lower than 5% before toxicity tests started.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were appropriate: Three replicates for test containers each containing 10 organisms (except for D. magna) were set with blank control and solvent control (acetone), while four replicates for test containers each containing 5 organisms were set for D. magna.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The reporting of housing and environmental conditions were sufficient: The tests were conducted at 21 $\pm$ 2 °C with a 12:12 h light/dark cycle. Test organisms were not fed in acute toxicity tests. During the test period, the temperature, pH, and dissolved oxygen (DO) were measured daily.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (mortality) addressed or reported the intended outcome (LC50 values to generate SSDs to derive the ALC).
	Metric 18:	Consistency of Outcome Assessment	High	Reported details suggest that the outcomes were assessed consistently across study groups.
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 that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				

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Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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; Worms (e.g., Annelids, Nematodes); *Limnodrilus hoffmeisteri*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	High	The 48 h-EC50 and 96 h-LC50 values for acute toxicity tests and corresponding 95% confidence intervals were calculated by probit methodology. The ALC derivation was based on USEPA guidelines. The joint probability curves (JPC) were based on probability distributions of concentrations in water (exposure) and response (SSD) of HHCB.
	Metric 22:	Reporting of Data	Medium	The goal of the study was to derive the ALC based on SSDs. To generate the SSDs, the authors generated LC50 values, they also used LC50 values from the literature. So, the goal was not to determine the toxicity across treatment groups; keeping this is mind, this metric received a medium (instead of low) because mortality data was not reported for all treatment groups per species.
	Metric 23:	Explanation of Unexpected Outcomes	High	The study results provide variability (range).

Additional Comments: The goal of the study was to derive an ALC (aquatic life criteria) for HHCB based on aquatic organisms in China. The study included 8 Chinese resident aquatic species for acute and chronic toxicity tests. Lognormal species sensitivity distribution (SSD) and the log-logistic SSD methods were used to develop the ALC. The authors did not provide raw data of the toxicity studies. They also used LC50 values from the literature. Because the goal to run acute and chromic toxicity studies was to generate LC50 values that were ultimately used to generate SSDs, there are two forms included in the evaluation: acute data and chronic data for all species. This form is the data evaluation for the acute data for all species combined.

Overall Quality Determination High

Study Citation:	Peng, F.J., Hu, L.X., Pan, C.G., Ying, G.G., Brink, P.J. (2019). Insights into the sediment toxicity of personal care products to freshwater oligochaete worms using Fourier transform infrared spectroscopy. Ecotoxicology and Environmental Safety 172:296-302.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Limnodrilus hoffmeisteri*; Not reported.; Adult
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5428154

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Commercial name, CAS Number, structure and molecular formula provided Table S1
	Metric 2:	Test Substance Source	Low	Source was not reported and chemical was not analyzed.
	Metric 3:	Test Substance Purity	Low	Purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Solvent control reported and solvent control mortality was <10% (232.5 of 240 surviving)
	Metric 5:	Negative Control Response	High	Solvent control mortality was <10% (232.5 of 240 surviving)
	Metric 6:	Randomized Allocation	Low	Did not report random allocation but did note random sampling.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicology and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 8:	Consistency of Exposure Administration	High	Same volume of solvent and volume of exposure water similar across exposures.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Nominal concentrations were reported and used.
	Metric 10:	Exposure Duration and Frequency	High	28 day exposure.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	4 concentrations and a solvent control, 4 replicates.
	Metric 12:	Testing at or Below Solubility Limit	High	<10% Control mortality.
Domain 4: Test Organism				

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Study Citation:	Peng, F.J., Hu, L.X., Pan, C.G., Ying, G.G., Brink, P.J. (2019). Insights into the sediment toxicity of personal care products to freshwater oligochaete worms using Fourier transform infrared spectroscopy. Ecotoxicology and Environmental Safety 172:296-302.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Limnodrilus hoffmeisteri*; Not reported.; Adult
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5428154

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	Medium	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicol-ogy and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicol-ogy and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 15:	Number of Organisms and Replicates per Group	Medium	4 replicates, 240 organisms per replicate.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicol-ogy and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 17:	Outcome Assessment Methodology	High	Growth data in Table S3.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes measured at the same durations.
Domain 6: Confounding / Variable Control				

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Study Citation:	Peng, F.J., Hu, L.X., Pan, C.G., Ying, G.G., Brink, P.J. (2019). Insights into the sediment toxicity of personal care products to freshwater oligochaete worms using Fourier transform infrared spectroscopy. Ecotoxicology and Environmental Safety 172:296-302.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Limnodrilus hoffmeisteri*; Not reported.; Adult
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5428154

Domain	Metric		Rating	Comments
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Authors did not report any differences among the study groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	Authors did not report any unexpected outcomes.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Standard (Williams test, $p < 0.05$) and nonstandard (Monte Carlo permutation test, $p < 0.05$) were used.
	Metric 22:	Reporting of Data	High	Growth NOECs were reported in Table S3.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes reported.

Additional Comments: Multiple species, microcosm study. This evaluation was for the growth outcome reported in Table S3.

Overall Quality Determination High

Study Citation:	Peng, F.J., Hu, L.X., Pan, C.G., Ying, G.G., Brink, P.J. (2019). Insights into the sediment toxicity of personal care products to freshwater oligochaete worms using Fourier transform infrared spectroscopy. Ecotoxicology and Environmental Safety 172:296-302.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Limnodrilus hoffmeisteri*; Not reported.; Adult
Health Outcome:	Mechanistic-Genotox (including DNA repair)
Chemical:	HHCB
HERO ID:	5428154

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Commercial name, CAS Number, structure and molecular formula provided Table S1
	Metric 2:	Test Substance Source	Low	Source was not reported and chemical was not analyzed.
	Metric 3:	Test Substance Purity	Low	Purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Solvent control reported and solvent control mortality was <10% (232.5 of 240 surviving)
	Metric 5:	Negative Control Response	High	Solvent control mortality was <10% (232.5 of 240 surviving)
	Metric 6:	Randomized Allocation	Low	Did not report random allocation but did note random sampling.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicology and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 8:	Consistency of Exposure Administration	High	Same volume of solvent and volume of exposure water similar across exposures.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Nominal concentrations were reported and used.
	Metric 10:	Exposure Duration and Frequency	High	28 day exposure.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	2 concentrations sampled of 4 concentrations and a solvent control used. Four (4) replicates.
	Metric 12:	Testing at or Below Solubility Limit	High	<10% Control mortality.
Domain 4: Test Organism				

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Study Citation:	Peng, F.J., Hu, L.X., Pan, C.G., Ying, G.G., Brink, P.J. (2019). Insights into the sediment toxicity of personal care products to freshwater oligochaete worms using Fourier transform infrared spectroscopy. Ecotoxicology and Environmental Safety 172:296-302.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Limnodrilus hoffmeisteri*; Not reported.; Adult
Health Outcome:	Mechanistic-Genotox (including DNA repair)
Chemical:	HHCB
HERO ID:	5428154

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	Medium	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicol-ogy and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicol-ogy and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 15:	Number of Organisms and Replicates per Group	Medium	4 replicates, 240 organisms per replicate.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicol-ogy and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 17:	Outcome Assessment Methodology	High	Data in text and Table S3.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes measured at the same durations.
Domain 6: Confounding / Variable Control				

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Study Citation:	Peng, F.J., Hu, L.X., Pan, C.G., Ying, G.G., Brink, P.J. (2019). Insights into the sediment toxicity of personal care products to freshwater oligochaete worms using Fourier transform infrared spectroscopy. Ecotoxicology and Environmental Safety 172:296-302.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Limnodrilus hoffmeisteri*; Not reported.; Adult
Health Outcome:	Mechanistic-Genotox (including DNA repair)
Chemical:	HHCB
HERO ID:	5428154

Domain	Metric		Rating	Comments
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Authors did not report any differences among the study groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	Authors did not report any unexpected outcomes.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical significance of the differences in biochemical measurements between treatments were tested using Monte Carlo permutation tests under redundancy analysis (RDA) option. 5% significance level was defined for statistical tests.
	Metric 22:	Reporting of Data	Medium	Mortality NOECs reported and data for lowest two concentrations were reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes reported.

Additional Comments: Multiple species, microcosm study

Overall Quality Determination High

Study Citation:	Peng, F.J., Hu, L.X., Pan, C.G., Ying, G.G., Brink, P.J. (2019). Insights into the sediment toxicity of personal care products to freshwater oligochaete worms using Fourier transform infrared spectroscopy. Ecotoxicology and Environmental Safety 172:296-302.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Limnodrilus hoffmeisteri*; Not reported.; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428154

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Commercial name, CAS Number, structure and molecular formula provided Table S1
	Metric 2:	Test Substance Source	Low	Source was not reported and chemical was not analyzed.
	Metric 3:	Test Substance Purity	Low	Purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Solvent control reported and solvent control mortality was <10% (232.5 of 240 surviving)
	Metric 5:	Negative Control Response	High	Solvent control mortality was <10% (232.5 of 240 surviving)
	Metric 6:	Randomized Allocation	Low	Did not report random allocation but did note random sampling.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicology and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 8:	Consistency of Exposure Administration	High	Same volume of solvent and volume of exposure water similar across exposures.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Nominal concentrations were reported and used.
	Metric 10:	Exposure Duration and Frequency	High	28 day exposure.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	4 concentrations and a solvent control, 4 replicates.
	Metric 12:	Testing at or Below Solubility Limit	High	<10% Control mortality.
Domain 4: Test Organism				

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Study Citation:	Peng, F.J., Hu, L.X., Pan, C.G., Ying, G.G., Brink, P.J. (2019). Insights into the sediment toxicity of personal care products to freshwater oligochaete worms using Fourier transform infrared spectroscopy. Ecotoxicology and Environmental Safety 172:296-302.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Limnodrilus hoffmeisteri*; Not reported.; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428154

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	Medium	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicol-ogy and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicol-ogy and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 15:	Number of Organisms and Replicates per Group	Medium	4 replicates, 240 organisms per replicate.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Described in Bioaccumulation and Biotransformation of Triclosan and Galaxolide in the Freshwater Oligochaete Limnodrilus hoffmeisteri in a Water/Sediment MicrocosmFeng-Jiao Peng, Guang-Guo Ying, Chang-Gui Pan, Henriette Selck, Daniel Salvito, and Paul J. Van den BrinkEnvironmental Science & Technology 2018 52 (15), 8390-8398DOI: 10.1021/acs.est.8b02637 and Feng-Jiao Peng, Fionne Kiggen, Chang-Gui Pan, Sally A. Bracewell, Guang-Guo Ying, Daniel Salvito, Henriette Selck, Paul J. Van den Brink,Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms, Ecotoxicol-ogy and Environmental Safety, Volume 169, 2019, Pages 902-910, ISSN 0147-6513, https://doi.org/10.1016/j.ecoenv.2018.11.092.(https://www.sciencedirect.com/science/article/pii/S0147651318312363
	Metric 17:	Outcome Assessment Methodology	High	Mortality data in Table S2.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes measured at the same durations.
Domain 6: Confounding / Variable Control				

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Study Citation:	Peng, F.J., Hu, L.X., Pan, C.G., Ying, G.G., Brink, P.J. (2019). Insights into the sediment toxicity of personal care products to freshwater oligochaete worms using Fourier transform infrared spectroscopy. Ecotoxicology and Environmental Safety 172:296-302.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Limnodrilus hoffmeisteri*; Not reported.; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428154

Domain	Metric		Rating	Comments
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Authors did not report any differences among the study groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	Authors did not report any unexpected outcomes.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Standard (Williams test, $p < 0.05$) and nonstandard (Monte Carlo permutation test, $p < 0.05$) were used.
	Metric 22:	Reporting of Data	High	Mortality NOEC and LOECs reported and data for all concentration were reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes reported.

Additional Comments: Multiple species, microcosm study

Overall Quality Determination High

Study Citation:	Peng, F.J., Kiggen, F., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Brink, P.J. (2019). Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms. Ecotoxicology and Environmental Safety 169:902-910.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Limnodrilus hoffmeisteri*; Not reported; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	HHCB
HERO ID:	5428151

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Chemical scientific name and source are reported.
	Metric 2:	Test Substance Source	High	Source was reported.
	Metric 3:	Test Substance Purity	Medium	Galaxolide was used as a standard.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Both a solvent and negative control used.
	Metric 5:	Negative Control Response	Low	Control data was not shown.
	Metric 6:	Randomized Allocation	Low	Authors discuss random sampling, but not random allocation to experimental chambers.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Brief description in this paper. Extended description of the Microcosms set-up, Test sediment and chemical spiking described in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across all treatments.
	Metric 9:	Measurement of Test Substance Concentration	High	Used GC-MS analysis of HHCB in overlying water.
	Metric 10:	Exposure Duration and Frequency	High	28 day exposure period.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Authors used 4 exposure groups and a negative and solvent controls.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent effects were similar to the negative control.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	Purchased from an aquatic market in Guangzhou, South China.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimated for 2-14 days prior to placing in the microcosm, as described in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	6 replicates, 240 organisms per replicate.

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Study Citation:	Peng, F.J., Kiggen, F., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Brink, P.J. (2019). Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms. Ecotoxicology and Environmental Safety 169:902-910.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Limnodrilus hoffmeisteri*; Not reported; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	HHCB
HERO ID:	5428151

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Housing, environmental conditions, food, water, and nutrients described briefly in this paper and in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 17:	Outcome Assessment Methodology	High	Figure 4 shows the HHCB concentrations.
	Metric 18:	Consistency of Outcome Assessment	High	Measured at the same durations.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among study grooups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Authors do not discuss differences in outcomes related to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The Williams test (Williams, 1972) which assumes a concentration-effect relationship and is, therefore, is used extensively to analyse the results of eco-toxicity tests. The Williams tests were performed using the Community Analysis computer program, version 4.3.05 (Hommenet al., 1994).
	Metric 22:	Reporting of Data	High	Figure 4 shows the HHCB concentrations.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexplained outcomes were reported by the authors.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Peng, F.J., Kiggen, F., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Brink, P.J. (2019). Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms. Ecotoxicology and Environmental Safety 169:902-910.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Limnodrilus hoffmeisteri*; Not reported; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428151

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Chemical scientific name and source are reported.
	Metric 2:	Test Substance Source	High	Source was reported.
	Metric 3:	Test Substance Purity	Medium	Galaxolide was used as a standard.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Both a solvent and negative control used.
	Metric 5:	Negative Control Response	High	Survival in control and treatments, low treatments not significant or near 100%.
	Metric 6:	Randomized Allocation	Low	Authors discuss random sampling, but not random allocation to experimental chambers.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Brief description in this paper. Extended description of the Microcosms set-up, Test sediment and chemical spiking described in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across all treatments.
	Metric 9:	Measurement of Test Substance Concentration	High	Used GC-MS analysis of HHCB in overlying water.
	Metric 10:	Exposure Duration and Frequency	High	28 day exposure period.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Authors used 4 exposure groups and a negative and solvent controls.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent effects were similar to the negative control.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	Purchased from an aquatic market in Guangzhou, South China.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimated for 2-14 days prior to placing in the microcosm, as described in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	6 replicates, 240 organisms per replicate.
Domain 5: Outcome Assessment				

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Study Citation:	Peng, F.J., Kiggen, F., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Brink, P.J. (2019). Fate and effects of sediment-associated polycyclic musk HHCB in subtropical freshwater microcosms. Ecotoxicology and Environmental Safety 169:902-910.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Limnodrilus hoffmeisteri*; Not reported; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428151

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Housing, environmental conditions, food, water, and nutrients described briefly in this paper and in Peng, F.J., Diepens, N.J., Pan, C.G., Bracewell, S.A., Ying, G.G., Salvito, D., Selck, H., Vanden Brink, P.J., 2018b. Fate and effects of sediment-associated triclosan in subtropical freshwater microcosms. Aquat. Toxicol. 202, 117–125.
	Metric 17:	Outcome Assessment Methodology	High	Table S14 provides data.
	Metric 18:	Consistency of Outcome Assessment	High	Measured at the same durations.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among study grooups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Authors do not discuss differences in outcomes related to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The Williams test (Williams, 1972) which assumes a concentration-effect relationship and is, therefore, is used extensively to analyse the results of eco-toxicity tests. The Williams tests were performed using the Community Analysis computer program, version 4.3.05 (Hommenet al., 1994).
	Metric 22:	Reporting of Data	High	Data reported on Table S14.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexplained outcomes were reported by the authors.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Artola-Garicano, E., Sinnige, T.L., Holsteijn, I.V., Vaes, W.H., Hermens, J.L. (2003). Bioconcentration and acute toxicity of polycyclic musks in two benthic organisms (Chironomus riparius and Lumbriculus variegatus). Environmental Toxicology and Chemistry 22(5):1086-1092.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Lumbriculus variegatus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Immobilization
Chemical:	HHCB
HERO ID:	5352378

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCB was identified as the test substance
	Metric 2:	Test Substance Source	High	Source of HHCB clearly identified (International Flavors andFragrances, Hilversum, The Netherlands).
	Metric 3:	Test Substance Purity	High	Purity of chemical provided (98%)
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls reported (copper free water + highest isopropanol concentration used in treatment groups)
	Metric 5:	Negative Control Response	Low	Results from each individual study group are not reported. The only reported result from the acute toxicity test is the final LC50 determination.
	Metric 6:	Randomized Allocation	Low	Source of organisms reported with limited details on allocation of organisms in experiments provided
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	System and methods provided, including details of monitoring pH, temperature, and other experimental conditions throughout the study period
	Metric 8:	Consistency of Exposure Administration	Medium	Exposure via water
	Metric 9:	Measurement of Test Substance Concentration	High	GCMS utilized to measure concentrations of HHCB throughout experiment
	Metric 10:	Exposure Duration and Frequency	High	Appropriate exposure lengths
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	”A dilution series resulting in six different concentrations was prepared. Nominal test concentrations ranged from... 0 to 6.6mM” for HHCB.
	Metric 12:	Testing at or Below Solubility Limit	High	Concentrations were below the limit of solubility
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	”Lumbriculus variegatus were cultured at room temperaturein our laboratory on uncontaminated paper pulp andwere fed a mixture of Tetraphyllt (Trouw, Fontaine-les-Vervins,France) and Trouvitt (Tetra Werke, Melle, Germany) ona daily basis prior to testing.”
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Acclimation periods reported for other analyses in study, not stated in the methods for the acute toxicity

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Study Citation:	Artola-Garicano, E., Sinnige, T.L., Holsteijn, I.V., Vaes, W.H., Hermens, J.L. (2003). Bioconcentration and acute toxicity of polycyclic musks in two benthic organisms (Chironomus riparius and Lumbriculus variegatus). Environmental Toxicology and Chemistry 22(5):1086-1092.
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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Lumbriculus variegatus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Immobilization
Chemical:	HHCB
HERO ID:	5352378

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 organisms per treatment completed in triplicate
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions conducive and monitored throughout study
	Metric 17:	Outcome Assessment Methodology	Low	Large confidence intervals reported
	Metric 18:	Consistency of Outcome Assessment	Medium	Residue analysis of bodies of organisms was not reported
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Differences not reported
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis performed but not described in detail
	Metric 22:	Reporting of Data	Low	Data for each individual treatment group was not reported
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Most uncertainties were reported

Additional Comments: None

Overall Quality Determination High

Study Citation:	Artola-Garicano, E., Sinnige, T.L., Holsteijn, I.V., Vaes, W.H., Hermens, J.L. (2003). Bioconcentration and acute toxicity of polycyclic musks in two benthic organisms (Chironomus riparius and Lumbriculus variegatus). Environmental Toxicology and Chemistry 22(5):1086-1092.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Lumbriculus variegatus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	HHCB
HERO ID:	5352378

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCB was identified as the test substance
	Metric 2:	Test Substance Source	High	Source of HHCB clearly identified (International Flavors andFragrances, Hilversum, The Netherlands).
	Metric 3:	Test Substance Purity	High	Purity of chemical provided (98%)
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls reported (copper free water + highest isopropanol concentration used in treatment groups)
	Metric 5:	Negative Control Response	Low	Results from each individual study group are not reported. Control responses were not always reported.
	Metric 6:	Randomized Allocation	Low	Source of organisms reported with limited details on allocation of organisms in experiments provided
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	System and methods provided, including details of monitoring pH, temperature, and other experimental conditions throughout the study period
	Metric 8:	Consistency of Exposure Administration	Medium	Exposure via water
	Metric 9:	Measurement of Test Substance Concentration	High	GCMS utilized to measure concentrations of HHCB throughout experiment
	Metric 10:	Exposure Duration and Frequency	High	Appropriate exposure lengths
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	”A dilution series resulting in six different concentrations was prepared. Nominal test concentrations ranged from... 0 to 6.6mM” for HHCB.
	Metric 12:	Testing at or Below Solubility Limit	High	Concentrations were below the limit of solubility
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	”Lumbriculus variegatus were cultured at room temperaturein our laboratory on uncontaminated paper pulp andwere fed a mixture of Tetraphyllt (Trouw, Fontaine-les-Vervins,France) and Trouvitt (Tetra Werke, Melle, Germany) ona daily basis prior to testing.”
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Acclimation periods reported for other analyses in study, not stated in the methods for the acute toxicity

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Study Citation:	Artola-Garicano, E., Sinnige, T.L., Holsteijn, I.V., Vaes, W.H., Hermens, J.L. (2003). Bioconcentration and acute toxicity of polycyclic musks in two benthic organisms (Chironomus riparius and Lumbriculus variegatus). Environmental Toxicology and Chemistry 22(5):1086-1092.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Lumbriculus variegatus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	ADME (biotransformation)
Chemical:	HHCB
HERO ID:	5352378

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 organisms per treatment completed in triplicate
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions conducive and monitored throughout study
	Metric 17:	Outcome Assessment Methodology	Low	Large confidence intervals reported
	Metric 18:	Consistency of Outcome Assessment	Medium	Residue analysis of bodies of organisms was not reported
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Differences not reported
	Metric 20:	Outcomes Unrelated to Exposure	High	Outcomes unrelated to exposure were not reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical analysis performed but not described in detail
	Metric 22:	Reporting of Data	Low	Data for each individual treatment group was not reported
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Most uncertainties were reported

Additional Comments: This evaluation form is for the BCF values reported in the paper.

Overall Quality Determination High

Study Citation:	IFF, (n.d.). HHCB/galaxolide: A study on the toxicity to the aquatic oligochaete lumbriculus variegatus (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Lumbriculus variegatus*; Adult
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	8784981

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the HHCB was not reported, though it is assumed to be from IFF. It was not reported to be analytically verified.
	Metric 3:	Test Substance Purity	High	The purity was reported to be 98.5%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported the use of an uncontaminated artificial sediment control as well as a solvent control.
	Metric 5:	Negative Control Response	High	The negative control responses were reported in the text under section 17.2.4 "Observations during exposure."
	Metric 6:	Randomized Allocation	Low	It was not reported how the worms were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The system was a static system with periodic compensation for evaporated water. Each test chamber was covered with a plastic lid. The test stock solution was prepared by dissolving 480.9mg HHCB in 100mL acetone. This was shaken for several seconds and then used directly. Test solutions for application were prepared by diluting the stock solution with acetone. These were then mixed with the appropriate amount of quartz sand for each treatment.
	Metric 8:	Consistency of Exposure Administration	Medium	All exposures occurred in 250mL glass vessels with approximately 2cm of test sediment. All vessels were gently aerated and had urtica powder and cellulose mixed into the sediment for food. However, vessels used for analytical samples were 1L instead of 250mL and contained a different amount of sediment and overlying water. Analytical measurements were not provided in this study as the were performed by an outside laboratory.
	Metric 9:	Measurement of Test Substance Concentration	Low	Analytical samples were reported to be collected, and measurements were reported to be performed by a third party laboratory. However, the results of these measurements were not reported, thus the low ranking of this metric.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 28d. This is typical for an Oligochaete toxicity test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure levels, and the spacing was adequate to see a dose response regarding behavior.
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via sediment.

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Study Citation:	IFF, (n.d.). HHCB/galaxolide: A study on the toxicity to the aquatic oligochaete lumbriculus variegatus (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Lumbriculus variegatus*; Adult
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	8784981

Domain	Metric		Rating	Comments
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The L. variegatus were reported to be cultured in the laboratory performing the study. The worms were synchronized prior to the study.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the Lumbriculus were acclimated to test conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were reported to be 10 worms per test chamber at the start of the test, and there were 4 replicates of each test concentration and 6 replicates for each control.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The worms were kept in reconstituted water according to OECD guideline 203 with a sediment to water ration of approx. 1:4. The temperature was kept at 20.5-22.6C, and the photoperiod was 16L:8D. The worms were reported to be fed urtica powder and cellulose that was mixed into the sediment.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–behavioral changes due to exposure. The worms were monitored for the duration of the study for differences in behavior between treatments and controls.
	Metric 18:	Consistency of Outcome Assessment	Medium	Worms were monitored for differences in behavior for the duration of the study. Worms in the highest concentrations were reported to not burry into the sediment as quickly as control worms, and there was a decrease or absence of fecal pellets indicating reduced feeding. Little detail was provided on how the behavior was assessed, and it was not quantified in any way.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	It was not reported if the worms were acclimated to study conditions.
	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	Uninformative	The statistics were not conducted for behavioral observations as they were not quantified in any way. They were simply noted.
	Metric 22:	Reporting of Data	Low	Differences in behavior were presented in the text in section 17.2.4 ”Observations during exposure.” They were not quantified.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Variability for this observation was not presented.

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Study Citation:	IFF, (n.d.). HHCB/galaxolide: A study on the toxicity to the aquatic oligochaete lumbriculus variegatus (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Lumbriculus variegatus*; Adult
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	8784981

Domain	Metric	Rating	Comments
Additional Comments:	The evaluation was on the effect of HHCB on L. variegatus behavior. Observations on worm behavior were made for the duration of the study. Differences were noted in burrowing behavior in higher concentrations as well as in feeding behavior. These differences were not quantified in anyway, and thus statistics were not conducted. This portion of the evaluation received an unacceptable ranking due to the lack of statistics.		

Overall Quality Determination Uninformative

Study Citation:	IFF, (n.d.). HHCB/galaxolide: A study on the toxicity to the aquatic oligochaete lumbriculus variegatus (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Lumbriculus variegatus*; Adult
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784981

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the HHCB was not reported, though it is assumed to be from IFF. It was not reported to be analytically verified.
	Metric 3:	Test Substance Purity	High	The purity was reported to be 98.5%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported the use of an uncontaminated artificial sediment control as well as a solvent control.
	Metric 5:	Negative Control Response	High	The negative control responses were reported in Table 26, as well as in Table 13 and Figure 3 and were adequate for the outcome of interest.
	Metric 6:	Randomized Allocation	Low	It was not reported how the worms were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The system was a static system with periodic compensation for evaporated water. Each test chamber was covered with a plastic lid. The test stock solution was prepared by dissolving 480.9mg HHCB in 100mL acetone. This was shaken for several seconds and then used directly. Test solutions for application were prepared by diluting the stock solution with acetone. These were then mixed with the appropriate amount of quartz sand for each treatment.
	Metric 8:	Consistency of Exposure Administration	Medium	All exposures occurred in 250mL glass vessels with approximately 2cm of test sediment. All vessels were gently aerated and had urtica powder and cellulose mixed into the sediment for food. However, vessels used for analytical samples were 1L instead of 250mL and contained a different amount of sediment and overlying water. Analytical measurements were not provided in this study as the were performed by an outside laboratory.
	Metric 9:	Measurement of Test Substance Concentration	Low	Analytical samples were reported to be collected, and measurements were reported to be performed by a third party laboratory. However, the results of these measurements were not reported, thus the low ranking of this metric.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 28d. This is typical for an Oligochaete toxicity test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure levels, and the spacing was adequate to see a dose response regarding biomass.
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via sediment.
Domain 4: Test Organism				

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Study Citation:	IFF, (n.d.). HHCB/galaxolide: A study on the toxicity to the aquatic oligochaete lumbriculus variegatus (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Lumbriculus variegatus*; Adult
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784981

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	The L. variegatus were reported to be cultured in the laboratory performing the study. The worms were synchronized prior to the study.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the Lumbriculus were acclimated to test conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were reported to be 10 worms per test chamber at the start of the test, and there were 4 replicates of each test concentration and 6 replicates for each control.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The worms were kept in reconstituted water according to OECD guideline 203 with a sediment to water ration of approx. 1:4. The temperature was kept at 20.5-22.6C, and the photoperiod was 16L:8D. The worms were reported to be fed urtica powder and cellulose that was mixed into the sediment.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–biomass. At the end of the study, the worms were placed in pre-weighted weigh boats and placed in a drying oven at 100C to dry overnight. They were then weighed and dry weights were determined.
	Metric 18:	Consistency of Outcome Assessment	High	At the end of the study, the worms were sieved through fine mesh to separate them from the sediment. They were transferred to pre-weighed weigh boats and dried overnight. Dry weights were then taken for each exposure level and compared to the control.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	It was not reported if the worms were acclimated to study conditions.
	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	The statistical methods used in this study were described in section 15.8 and were appropriate for the outcome of interest.
	Metric 22:	Reporting of Data	High	Data was reported or biomass in Table 26. Biomass for each treatment was also provided in Table 13 and Figure 3. The EC15 and EC50 were presented in Table 14 along with the 95% confidence intervals. The concentration-effect curve is presented in Fig 4.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in terms of the mean number of worms per treatment and 95% confidence limits.

Additional Comments: The evaluation was on the effect of HHCB on L. variegatus biomass. The worms were sieved at the end of the test to separate them from the sediment. Dry weights were taken at the end of the study. Development/growth was chosen as the outcome of interest.

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Study Citation:	IFF, (n.d.). HHCB/galaxolide: A study on the toxicity to the aquatic oligochaete lumbriculus variegatus (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Lumbriculus variegatus*; Adult
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784981

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

Study Citation:	IFF, (n.d.). HHCB/galaxolide: A study on the toxicity to the aquatic oligochaete lumbriculus variegatus (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Lumbriculus variegatus*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8784981

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the HHCB was not reported, though it is assumed to be from IFF. It was not reported to be analytically verified.
	Metric 3:	Test Substance Purity	High	The purity was reported to be 98.5%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported the use of an uncontaminated artificial sediment control as well as a solvent control.
	Metric 5:	Negative Control Response	High	The negative control responses were reported in Table 26, as well as in Table 11 and were adequate for the outcome of interest.
	Metric 6:	Randomized Allocation	Low	It was not reported how the worms were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The system was a static system with periodic compensation for evaporated water. Each test chamber was covered with a plastic lid. The test stock solution was prepared by dissolving 480.9mg HHCB in 100mL acetone. This was shaken for several seconds and then used directly. Test solutions for application were prepared by diluting the stock solution with acetone. These were then mixed with the appropriate amount of quartz sand for each treatment.
	Metric 8:	Consistency of Exposure Administration	Medium	All exposures occurred in 250mL glass vessels with approximately 2cm of test sediment. All vessels were gently aerated and had urtica powder and cellulose mixed into the sediment for food. However, vessels used for analytical samples were 1L instead of 250mL and contained a different amount of sediment and overlying water. Analytical measurements were not provided in this study as the were performed by an outside laboratory.
	Metric 9:	Measurement of Test Substance Concentration	Low	Analytical samples were reported to be collected, and measurements were reported to be performed by a third party laboratory. However, the results of these measurements were not reported, thus the low ranking of this metric.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 28d. This is typical for an Oligochaete toxicity test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were 5 exposure levels. However no dead worms were found in any of the test chambers for any of the treatment levels. Two were determined to be missing in one of the higher concentrations and therefore considered dead. Due to the lack of response in terms of mortality, different spacing may have yielded more data.
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via sediment.
Domain 4: Test Organism				

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Study Citation:	IFF, (n.d.). HHCB/galaxolide: A study on the toxicity to the aquatic oligochaete lumbriculus variegatus (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Lumbriculus variegatus*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8784981

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	The L. variegatus were reported to be cultured in the laboratory performing the study. The worms were synchronized prior to the study.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the Lumbriculus were acclimated to test conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were reported to be 10 worms per test chamber at the start of the test, and there were 4 replicates of each test concentration and 6 replicates for each control.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The worms were kept in reconstituted water according to OECD guideline 203 with a sediment to water ration of approx. 1:4. The temperature was kept at 20.5-22.6C, and the photoperiod was 16L:8D. The worms were reported to be fed urtica powder and cellulose that was mixed into the sediment.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology partially addressed or reported the intended outcomes of interest–mortality. Worms that were found to be decomposing or worms that did not respond to gentle stimulus were considered to be dead. Any missing worms were also considered to be mortalities.
	Metric 18:	Consistency of Outcome Assessment	High	At the end of the study, the worms were sieved through fine mesh to separate them from the sediment. Any worms that did not respond to gentle stimulus, that were decomposing, or that were missing were considered to be dead.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	It was not reported if the worms were acclimated to study conditions.
	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	The statistical methods used in this study were described in section 15.8 and were appropriate for the outcome of interest.
	Metric 22:	Reporting of Data	High	Mortality data for the controls and the exposure concentrations were presented in Table 26. The total number of worms per treatment was reported in Table 11 and Figure 1. Both the LOEC and the NOEC were reported to be >140.0mg/kg due to the low number of mortalities in the exposure levels.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in terms of the mean number of worms per treatment.

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Study Citation:	IFF, (n.d.). HHCB/galaxolide: A study on the toxicity to the aquatic oligochaete lumbriculus variegatus (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Lumbriculus variegatus*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8784981

Domain	Metric	Rating	Comments
Additional Comments:	The evaluation was on the effect of HHCB on L. variegatus survival. Mortalities were recorded at the end of the test by counting the number of worms that did not respond to stimulus, the number of worms that were found decomposing, or the number of worms missing from test chambers. The total number of worms per treatment was reported along with the number of mortalities. There were only 2 worms found to be missing in the highest concentration. This was not sufficient mortality to calculate and LC value. The NOEC and LOEC were both reported to be >140mg/kg.		

Overall Quality Determination High

Study Citation:	IFF, (n.d.). HHCB/galaxolide: A study on the toxicity to the aquatic oligochaete lumbriculus variegatus (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Lumbriculus variegatus*; Adult
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	8784981

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the HHCB was not reported, though it is assumed to be from IFF. It was not reported to be analytically verified.
	Metric 3:	Test Substance Purity	High	The purity was reported to be 98.5%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported the use of an uncontaminated artificial sediment control as well as a solvent control.
	Metric 5:	Negative Control Response	High	The negative control responses were reported in Table 26, as well as in Table 11 and Figure 1 and were adequate for the outcome of interest.
	Metric 6:	Randomized Allocation	Low	It was not reported how the worms were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The system was a static system with periodic compensation for evaporated water. Each test chamber was covered with a plastic lid. The test stock solution was prepared by dissolving 480.9mg HHCB in 100mL acetone. This was shaken for several seconds and then used directly. Test solutions for application were prepared by diluting the stock solution with acetone. These were then mixed with the appropriate amount of quartz sand for each treatment.
	Metric 8:	Consistency of Exposure Administration	Medium	All exposures occurred in 250mL glass vessels with approximately 2cm of test sediment. All vessels were gently aerated and had urtica powder and cellulose mixed into the sediment for food. However, vessels used for analytical samples were 1L instead of 250mL and contained a different amount of sediment and overlying water. Analytical measurements were not provided in this study as the were performed by an outside laboratory.
	Metric 9:	Measurement of Test Substance Concentration	Low	Analytical samples were reported to be collected, and measurements were reported to be performed by a third party laboratory. However, the results of these measurements were not reported, thus the low ranking of this metric.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was reported to be 28d. This is typical for an Oligochaete toxicity test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure levels, and the spacing was adequate to see a dose response regarding reproduction.
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via sediment.
Domain 4: Test Organism				

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Study Citation:	IFF, (n.d.). HHCB/galaxolide: A study on the toxicity to the aquatic oligochaete lumbriculus variegatus (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Lumbriculus variegatus*; Adult
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	8784981

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	The L. variegatus were reported to be cultured in the laboratory performing the study. The worms were synchronized prior to the study.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the Lumbriculus were acclimated to test conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were reported to be 10 worms per test chamber at the start of the test, and there were 4 replicates of each test concentration and 6 replicates for each control.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The worms were kept in reconstituted water according to OECD guideline 203 with a sediment to water ration of approx. 1:4. The temperature was kept at 20.5-22.6C, and the photoperiod was 16L:8D. The worms were reported to be fed urtica powder and cellulose that was mixed into the sediment.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology partially addressed or reported the intended outcomes of interest–reproduction. The number or worms was counted at the end of the study for each treatment. The number of full sized complete worms, small complete worms, and small incomplete worms were totaled for each treatment and control and then compared.
	Metric 18:	Consistency of Outcome Assessment	High	At the end of the study, the worms were sieved through fine mesh to separate them from the sediment. The number of adult complete worms, small complete worms, and small incomplete worms were totaled.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	It was not reported if the worms were acclimated to study conditions.
	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	The statistical methods used in this study were described in section 15.8 and were appropriate for the outcome of interest.
	Metric 22:	Reporting of Data	High	Reproduction data was provided in highest detail in Table 26. The total number of worms per treatment was reported in Table 11 and Figure 1. Table 12 reported EC values and 95% confidence limits. Figure 2 provided the concentration effect curve.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in terms of the mean number of worms per treatment and 95% confidence limits.

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Study Citation:	IFF, (n.d.). HHCB/galaxolide: A study on the toxicity to the aquatic oligochaete lumbriculus variegatus (sanitized).
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Lumbriculus variegatus*; Adult
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	8784981

Domain	Metric	Rating	Comments
Additional Comments:	The evaluation was on the effect of HHCB on L. variegatus reproduction. The worms were sieved at the end of the test to separate them from the sediment. The total number of full sized adult worms, small complete worms, and incomplete small worms were tallied and compared to control results. The EC15 and EC50 values were calculated to be 24.2 and 74.1 mg/kg respectively.		

Overall Quality Determination High

Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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; *Macrobrachium nipponense*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Correct nomenclature was used. There was a typo in the CASRN (1222-05 reported vs 1222-05-5 correct); given the correct chemical name, correct abbreviation, and correct trade name, it is likely that the missing digit for the CASRN was a genuine typo.
	Metric 2:	Test Substance Source	High	The source was reported (Klamar-reagent company, Shanghai).
	Metric 3:	Test Substance Purity	Medium	Purity of the substance was reported at 75%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	The authors reported that mortality rates of test organisms in all control groups were <10% at end of the tests and that toxicity tests followed ASTM standard guidelines. Moreover, they reported that test containers were set with blank control and solvent control (acetone). However, supplemental Table S1, which reports the nominal HHCB concentrations used in the toxicity test, does not list the control group as 0 ug/L.
	Metric 5:	Negative Control Response	Low	The authors reported in the methods that mortality rates of test organisms in all control groups were <10% at end of the tests. However, they did not report the raw data in the results (they only provided LC50 values for each test).
	Metric 6:	Randomized Allocation	Low	The authors did not report if the organisms were randomly allocated to study groups. They included a general statement saying that toxicity tests followed ASTM standard guidelines without mention of random allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	All tests were static-renewal and test solutions were totally replaced at 24 h intervals. Due to the physical-chemical properties of the HHCB, a solvent control (acetone) was used.
	Metric 8:	Consistency of Exposure Administration	High	There was no evidence to suggest that exposure administration was not administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured (HPLC, Agilent 1200 Series, USA). The recoveries of HHCB in water samples were 98.42%–106.90%, and the limit of detection (LOD)was 10.70 ng/L for water samples. The variability of HHCB concentration was <20% (3.08%–9.12%).
	Metric 10:	Exposure Duration and Frequency	High	This data evaluation form is for the acute toxicity studies. The reported acute toxicity exposure durations were 48 h for Daphnia magna and Chironomus plumosus and 96 h for the other aquatic animals. This exposure durations were appropriate for the study type.

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Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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; *Macrobrachium nipponense*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were appropriate (5 to 7 groups plus control (solvent control).
	Metric 12:	Testing at or Below Solubility Limit	High	For all tests the nominal HHCB concentrations were below the HHCB water solubility limit of 1750 ug/L expect for the acute Daphnia magna test. However, it likely was not a concern because a solvent control (acetone) was used and the recoveries of measured HHCB in water samples were 98.42%–106.90%.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described (e.g., source, size, age, healthy organism).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The authors reported that, for example, cultures of Gobiocypris rarus and Daphnia magna were from their biological culture laboratory and thus acclimated. For organisms not reared in the laboratory, the authors reported that larvae were domesticated for at least 7 days in biological culture laboratory and the mortality rates were lower than 5% before toxicity tests started.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were appropriate: Three replicates for test containers each containing 10 organisms (except for D. magna) were set with blank control and solvent control (acetone), while four replicates for test containers each containing 5 organisms were set for D. magna.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The reporting of housing and environmental conditions were sufficient: The tests were conducted at 21 $\pm$ 2 °C with a 12:12 h light/dark cycle. Test organisms were not fed in acute toxicity tests. During the test period, the temperature, pH, and dissolved oxygen (DO) were measured daily.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (mortality) addressed or reported the intended outcome (LC50 values to generate SSDs to derive the ALC).
	Metric 18:	Consistency of Outcome Assessment	High	Reported details suggest that the outcomes were assessed consistently across study groups.
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 that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				

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Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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; *Macrobrachium nipponense*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	High	The 48 h-EC50 and 96 h-LC50 values for acute toxicity tests and corresponding 95% confidence intervals were calculated by probit methodology. The ALC derivation was based on USEPA guidelines. The joint probability curves (JPC) were based on probability distributions of concentrations in water (exposure) and response (SSD) of HHCB.
	Metric 22:	Reporting of Data	Medium	The goal of the study was to derive the ALC based on SSDs. To generate the SSDs, the authors generated LC50 values, they also used LC50 values from the literature. So, the goal was not to determine the toxicity across treatment groups; keeping this is mind, this metric received a medium (instead of low) because mortality data was not reported for all treatment groups per species.
	Metric 23:	Explanation of Unexpected Outcomes	High	The study results provide variability (range).

Additional Comments: The goal of the study was to derive an ALC (aquatic life criteria) for HHCB based on aquatic organisms in China. The study included 8 Chinese resident aquatic species for acute and chronic toxicity tests. Lognormal species sensitivity distribution (SSD) and the log-logistic SSD methods were used to develop the ALC. The authors did not provide raw data of the toxicity studies. They also used LC50 values from the literature. Because the goal to run acute and chromic toxicity studies was to generate LC50 values that were ultimately used to generate SSDs, there are two forms included in the evaluation: acute data and chronic data for all species. This form is the data evaluation for the acute data for all species combined.

Overall Quality Determination High

Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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:	Invertebrate; Arthropods; *Macrobrachium nipponense*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Correct nomenclature was used. There was a typo in the CASRN (1222-05 reported vs 1222-05-5 correct); given the correct chemical name, correct abbreviation, and correct trade name, it is likely that the missing digit for the CASRN was a genuine typo.
	Metric 2:	Test Substance Source	High	The source was reported (Klamar-reagent company, Shanghai).
	Metric 3:	Test Substance Purity	Medium	Purity of the substance was reported at 75%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	The authors reported that mortality rates of test organisms in all control groups were <10% at end of the tests and that toxicity tests followed ASTM standard guidelines. Moreover, they reported that test containers were set with blank control and solvent control (acetone). However, supplemental Table S1, which reports the nominal HHCB concentrations used in the toxicity test, does not list the control group as 0 ug/L.
	Metric 5:	Negative Control Response	Low	The authors reported in the methods that mortality rates of test organisms in all control groups were <10% at end of the tests. However, they did not report the raw data in the results (they only provided LC50 values for each test).
	Metric 6:	Randomized Allocation	Low	The authors did not report if the organisms were randomly allocated to study groups. They included a general statement saying that toxicity tests followed ASTM standard guidelines without mention of random allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	All tests were static-renewal and test solutions were totally replaced at 24 h intervals. Due to the physical-chemical properties of the HHCB, a solvent control (acetone) was used.
	Metric 8:	Consistency of Exposure Administration	High	There was no evidence to suggest that exposure administration was not administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured (HPLC, Agilent 1200 Series, USA). The recoveries of HHCB in water samples were 98.42%–106.90%, and the limit of detection (LOD)was 10.70 ng/L for water samples. The variability of HHCB concentration was <20% (3.08%–9.12%).
	Metric 10:	Exposure Duration and Frequency	High	This data evaluation form is for the chronic toxicity studies with Gobiocypris rarus and Macrobrachium nipponense. The exposure duration was 28 days; this exposure durations was appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure levels were appropriate (5 groups plus control (solvent control).

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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:	Invertebrate; Arthropods; *Macrobrachium nipponense*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
	Metric 12:	Testing at or Below Solubility Limit	High	For two chronic tests, the nominal HHCB concentrations were below the HHCB water solubility limit of 1750 ug/L.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were adequately described (e.g., source, size, age, healthy organism).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The authors reported that organisms not reared in their laboratory were domesticated for at least 7 days in biological culture laboratory and the mortality rates were lower than 5% before toxicity tests started.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were appropriate: Three replicates for test containers each containing 10 organisms were set with blank control and solvent control (acetone).
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	The reporting of housing and environmental conditions were sufficient: The tests were conducted at 21 $\pm$ 2 °C with a 12:12 h light/dark cycle. Test organisms were fed twice daily in chronic toxicity tests andthe total food intake reached 0.1% of bodyweight of test organisms. During the test period, the temperature, pH, and dissolved oxygen (DO) were measured daily.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology (mortality) addressed or reported the intended outcome (LC50 values to generate SSDs to derive the ALC).
	Metric 18:	Consistency of Outcome Assessment	High	Reported details suggest that the outcomes were assessed consistently across study groups.
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 that could influence the outcome assessment.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The 28 day-EC10 values and corresponding 95% confidence intervals were calculated by probit methodology. The ALC derivation was based on USEPA guidelines. The joint probability curves (JPC) were based on probability distributions of concentrations in water (exposure) and response (SSD) of HHCB.

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Study Citation:	Fan, B., Wang, X., Li, J., Gao, X., Li, W., Huang, Y., Liu, Z. (2019). Deriving aquatic life criteria for galaxolide (HHCB) and ecological risk assessment. Science of the Total Environment 681:488-496.
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:	Invertebrate; Arthropods; *Macrobrachium nipponense*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428397

Domain	Metric		Rating	Comments
	Metric 22:	Reporting of Data	Medium	The goal of the study was to derive the ALC based on SSDs. To generate the SSDs, the authors generated LC50 values, they also used LC50 values from the literature. So, the goal was not to determine the toxicity across treatment groups; keeping this is mind, this metric received a medium (instead of low) because mortality data was not reported for all treatment groups per species.
	Metric 23:	Explanation of Unexpected Outcomes	High	The study results provide variability (range).

Additional Comments: The goal of the study was to derive an ALC (aquatic life criteria) for HHCB based on aquatic organisms in China. The study included 8 Chinese resident aquatic species for acute and chronic toxicity tests. Lognormal species sensitivity distribution (SSD) and the log-logistic SSD methods were used to develop the ALC. The authors did not provide raw data of the toxicity studies. They also used LC50 values from the literature. Because the goal to run acute and chromic toxicity studies was to generate LC50 values that were ultimately used to generate SSDs, there are two forms included in the evaluation: acute data and chronic data for all species combined. This form is the data evaluation for the two chronic studies. Finally, the authors reported that the chronic toxicity endpoint was 28 d-EC10 (survival,SGR and body weight). However, only mortality/survival data is shown in the results; therefore, this data evaluation form is for mortality.

Overall Quality Determination High

Study Citation:	Pedersen, S., Selck, H., Salvito, D., Forbes, V. (2009). Effects of the polycyclic musk HHCB on individual- and population-level endpoints in Potamopyrgus antipodarum. Ecotoxicology and Environmental Safety 72(4):1190-1199.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Mollusks; *Potamopyrgus antipodarum*; Juvenile
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428156

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The test substance was identified but there were uncertainties regarding test substance identification or characterization. The nominal concentrations of HHCB were 0.1, 1, 10, 30 and 100 microgram HHCB dw sediment to cover the range from environmentally realistic concentrations to those approaching toxic effect concentrations for growth of P. antipodarum determined in two week pilot experiment.
	Metric 2:	Test Substance Source	Low	Chemical source not reported.
	Metric 3:	Test Substance Purity	Low	Chemical purity was not reported
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls/negative control response was reported
	Metric 5:	Negative Control Response	High	100% survival at first reproduction.
	Metric 6:	Randomized Allocation	Medium	Organisms randomly distributed but distribution of test chambers not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Few details about the test system were provided
	Metric 8:	Consistency of Exposure Administration	Medium	Few details about the test system were provided
	Metric 9:	Measurement of Test Substance Concentration	Medium	Low recovery 64-86%, infrequent sampling made it difficult to determine accuracy
	Metric 10:	Exposure Duration and Frequency	High	Long test duration required to cover long lifespan of test organism
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Standard number of levels and dose spacing
	Metric 12:	Testing at or Below Solubility Limit	Medium	limited sampling and low recoveries left solubility concerns uncertain
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	Test organisms were adequately described. P. antipodarum were collected at Salvad Park, Roskilde Fjord, Denmark, in 2004 and subsequently reared in 10 L aquaria at 17 1C on natural pre-frozen sediment (o250 mm) and artificial freshwater (192 mg/L NaHCO, 8 mg/L KCl, 245 mg/L MgSO and 120 mg/L CaSO in de-ionized water, pH 7.2 (Jensen et al., 2001)). A food supplement of commercial fish food (Tetra Min, Tetra Werke, Melle, Germany), baby cereal (Milpo, Milupa, Hørsholm, Denmark) and dried spinach in equal ratios by weight was added to cultures twice a week.

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Study Citation:	Pedersen, S., Selck, H., Salvito, D., Forbes, V. (2009). Effects of the polycyclic musk HHCB on individual- and population-level endpoints in Potamopyrgus antipodarum. Ecotoxicology and Environmental Safety 72(4):1190-1199.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Mollusks; *Potamopyrgus antipodarum*; Juvenile
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428156

Domain	Metric		Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Limited details were provided. The overlying water was renewed every month, and every 4–6 weeks the cultures were given 3–4 spoonfuls of fresh pre-frozen sediment.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Acceptable and explained in 2.4.1 of the methods "Juvenile growth, survival, time to first reproduction and size at first reproduction"
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Although there is some description of the test system, not all environmental conditions of the were not reported
	Metric 17:	Outcome Assessment Methodology	High	The only mortality occurred at two the highest concs
	Metric 18:	Consistency of Outcome Assessment	High	Adult survival was not affected by HHCB, and all adultssurvived the entire exposure period. Juvenile survivaldecreased with increasing HHCB concentration. One hundred-percent of the juveniles exposed to 0,0.1,1and10 mg HHCB survived from birth to first reproduction. Juvenile survival frombirth to first reproduction was 90% at 30 mg HHCB and 80% at100 mgHHCB.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Simple but regular monitoring reported
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No details provided for unrelated outcomes
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Only reported as percent survival, no stats reported
	Metric 22:	Reporting of Data	Low	Statistical significance not reported
	Metric 23:	Explanation of Unexpected Outcomes	Low	Nothing unexpected, followed dose response but no stat analysis

Additional Comments: This sheet is for >21-d #1 mortality of HHCB.

Overall Quality Determination Medium

Study Citation:	Pedersen, S., Selck, H., Salvito, D., Forbes, V. (2009). Effects of the polycyclic musk HHCB on individual- and population-level endpoints in Potamopyrgus antipodarum. Ecotoxicology and Environmental Safety 72(4):1190-1199.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Mollusks; *Potamopyrgus antipodarum*; Adult
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5428156

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The test substance was identified but there were uncertainties regarding test substance identification or characterization. The nominal concentrations of HHCB were 0.1, 1, 10, 30 and 100 microgram HHCB dw sediment to cover the range from environmentally realistic concentrations to those approaching toxic effect concentrations for growth of P. antipodarum determined in two week pilot experiment.
	Metric 2:	Test Substance Source	Low	chemical source was not reported
	Metric 3:	Test Substance Purity	Low	chemical purity was not reported
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls/negative control response was reported
	Metric 5:	Negative Control Response	High	control response was adequate
	Metric 6:	Randomized Allocation	Medium	organisms randomly distributed but distribution of test chambers not reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	few details on test system were provided
	Metric 8:	Consistency of Exposure Administration	Medium	few details provided for dose administration
	Metric 9:	Measurement of Test Substance Concentration	Medium	low recovery, infrequent sampling so difficult to determine accuracy
	Metric 10:	Exposure Duration and Frequency	Medium	non standard duration seemed to be adequate
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	fairly standard number of levels and dose spacing
	Metric 12:	Testing at or Below Solubility Limit	Medium	low recovery, infrequent sampling led to uncertainty regarding solubility
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms were adequately described. P. antipodarum were collected at Salvad Park, Roskilde Fjord, Denmark, in 2004 and subsequently reared in 10 L aquaria at 17 1C on natural pre-frozen sediment (o250 mm) and artificial freshwater (192 mg/L NaHCO, 8 mg/L KCl, 245 mg/L MgSO and 120 mg/L CaSO in de-ionized water, pH 7.2 (Jensen et al., 2001)). A food supplement of commercial fish food (Tetra Min, Tetra Werke, Melle, Germany), baby cereal (Milpo, Milupa, Hørsholm, Denmark) and dried spinach in equal ratios by weight was added to cultures twice a week.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Limited details were provided. The overlying water was renewed every month, and every 4–6 weeks the cultures were given 3–4 spoonfuls of fresh pre-frozen sediment.

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Study Citation:	Pedersen, S., Selck, H., Salvito, D., Forbes, V. (2009). Effects of the polycyclic musk HHCB on individual- and population-level endpoints in Potamopyrgus antipodarum. Ecotoxicology and Environmental Safety 72(4):1190-1199.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Mollusks; *Potamopyrgus antipodarum*; Adult
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5428156

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Acceptable and explained in 2.4.1 of the methods "Juvenile growth, survival, time to first reproduction and size at first reproduction"
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Although there is some description of the test system, not all environmental conditions of the were not reported
	Metric 17:	Outcome Assessment Methodology	High	split outcome into a 4 week time frame and a six week time frame
	Metric 18:	Consistency of Outcome Assessment	High	Details were reported in Figure 1: Specific growth rates for juvenile and adult snails, Figure 2: Average adult weekly feeding rates, and Figure 5: Size at first reproduction vs HHCB concentration
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	details not reported on any variables that might effect the outcome
	Metric 20:	Outcomes Unrelated to Exposure	Medium	no information reported regarding unrelated outcomes
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	statistical analysis and explanation provided
	Metric 22:	Reporting of Data	High	reporting of data was adequate and sufficient to determine values of interest for the endpoint.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	uncertain why no dose response exhibited

Additional Comments: This sheet is for >21-d growth of snails exposed to HHCB

Overall Quality Determination Medium

Study Citation:	Pedersen, S., Selck, H., Salvito, D., Forbes, V. (2009). Effects of the polycyclic musk HHCB on individual- and population-level endpoints in Potamopyrgus antipodarum. Ecotoxicology and Environmental Safety 72(4):1190-1199.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Mollusks; *Potamopyrgus antipodarum*; Adult
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	5428156

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The test substance was identified but there were uncertainties regarding test substance identification or characterization. The nominal concentrations of HHCB were 0.1, 1, 10, 30 and 100 microgram HHCB dw sediment to cover the range from environmentally realistic concentrations to those approaching toxic effect concentrations for growth of P. antipodarum determined in two week pilot experiment.
	Metric 2:	Test Substance Source	Low	Chemical source was not reported
	Metric 3:	Test Substance Purity	Low	Chemical purity was not reported
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls/negative control response was reported
	Metric 5:	Negative Control Response	Medium	Control response was adequate to make comparisons to.
	Metric 6:	Randomized Allocation	Medium	Organisms randomly distributed but distribution of test chambers not reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Few details about the test system were provided
	Metric 8:	Consistency of Exposure Administration	Medium	Few details provided for dose administration
	Metric 9:	Measurement of Test Substance Concentration	Medium	Low recovery 64-86%, infrequent sampling made it difficult to determine accuracy
	Metric 10:	Exposure Duration and Frequency	Medium	Adequate duration to measure effects
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Standard number of levels and dose spacing
	Metric 12:	Testing at or Below Solubility Limit	Medium	low recovery, infrequent sampling led to uncertainty regarding solubility
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms were adequately described. P. antipodarum were collected at Salvad Park, Roskilde Fjord, Denmark, in 2004 and subsequently reared in 10 L aquaria at 17 1C on natural pre-frozen sediment (o250 mm) and artificial freshwater (192 mg/L NaHCO, 8 mg/L KCl, 245 mg/L MgSO and 120 mg/L CaSO in de-ionized water, pH 7.2 (Jensen et al., 2001)). A food supplement of commercial fish food (Tetra Min, Tetra Werke, Melle, Germany), baby cereal (Milpo, Milupa, Hørsholm, Denmark) and dried spinach in equal ratios by weight was added to cultures twice a week.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Limited details were provided. The overlying water was renewed every month, and every 4–6 weeks the cultures were given 3–4 spoonfuls of fresh pre-frozen sediment.

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Study Citation:	Pedersen, S., Selck, H., Salvito, D., Forbes, V. (2009). Effects of the polycyclic musk HHCB on individual- and population-level endpoints in Potamopyrgus antipodarum. Ecotoxicology and Environmental Safety 72(4):1190-1199.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Mollusks; *Potamopyrgus antipodarum*; Adult
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	5428156

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Acceptable and explained in 2.4.1 of the methods "Juvenile growth, survival, time to first reproduction and size at first reproduction"
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Although there is some description of the test system, not all environmental conditions of the were not reported
	Metric 17:	Outcome Assessment Methodology	Medium	split outcome into a 4 week time frame and a six week time frame
	Metric 18:	Consistency of Outcome Assessment	High	Details were reported in Figure 4: Time to first reproduction, Figure 6: Relationship between reproductive output and HHCB exposure over time, and figure 7: Reproductive output, averaged for weeks 5-10.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Details on variability were not reported. The study authors did make mention that laboratory condition in which snails are not subject to competition or predation which may impact wild snails.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	no information about unrelated outcomes were reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	statistical analysis and explanation provided
	Metric 22:	Reporting of Data	High	reporting of data was adequate and sufficient to determine values of interest for the endpoint.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	reproductive output varied over time without a clear explanation

Additional Comments: This sheet is for >21-d #2 reproductive effects of HHCB

Overall Quality Determination High

Study Citation:	Pedersen, S., Selck, H., Salvito, D., Forbes, V. (2009). Effects of the polycyclic musk HHCB on individual- and population-level endpoints in Potamopyrgus antipodarum. Ecotoxicology and Environmental Safety 72(4):1190-1199.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Mollusks; *Potamopyrgus antipodarum*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428156

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The test substance was identified but there were uncertainties regarding test substance identification or characterization. The nominal concentrations of HHCB were 0.1, 1, 10, 30 and 100 microgram HHCB dw sediment to cover the range from environmentally realistic concentrations to those approaching toxic effect concentrations for growth of P. antipodarum determined in two week pilot experiment.
	Metric 2:	Test Substance Source	Low	chemical source not reported
	Metric 3:	Test Substance Purity	Low	Chemical purity was not reported
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls/negative control response was reported
	Metric 5:	Negative Control Response	High	Control response was adequate to make comparisons to. No control mortality was reported.
	Metric 6:	Randomized Allocation	Medium	Organisms randomly distributed but distribution of test chambers not reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Few details about the test system were provided
	Metric 8:	Consistency of Exposure Administration	Medium	Few details provided for dose administration
	Metric 9:	Measurement of Test Substance Concentration	Medium	Low recovery 64-86%, infrequent sampling made it difficult to determine accuracy
	Metric 10:	Exposure Duration and Frequency	Medium	Adequate duration to measure effects
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Standard number of levels and dose spacing
	Metric 12:	Testing at or Below Solubility Limit	Medium	low recovery, infrequent sampling led to uncertainty regarding solubility
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms were adequately described. P. antipodarum were collected at Salvad Park, Roskilde Fjord, Denmark, in 2004 and subsequently reared in 10 L aquaria at 17 1C on natural pre-frozen sediment (o250 mm) and artificial freshwater (192 mg/L NaHCO, 8 mg/L KCl, 245 mg/L MgSO and 120 mg/L CaSO in de-ionized water, pH 7.2 (Jensen et al., 2001)). A food supplement of commercial fish food (Tetra Min, Tetra Werke, Melle, Germany), baby cereal (Milpo, Milupa, Hørsholm, Denmark) and dried spinach in equal ratios by weight was added to cultures twice a week.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Limited details were provided. The overlying water was renewed every month, and every 4–6 weeks the cultures were given 3–4 spoonfuls of fresh pre-frozen sediment.

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Study Citation:	Pedersen, S., Selck, H., Salvito, D., Forbes, V. (2009). Effects of the polycyclic musk HHCB on individual- and population-level endpoints in Potamopyrgus antipodarum. Ecotoxicology and Environmental Safety 72(4):1190-1199.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Mollusks; *Potamopyrgus antipodarum*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428156

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Acceptable and explained in 2.4.1 of the methods "Juvenile growth, survival, time to first reproduction and size at first reproduction"
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Although there is some description of the test system, not all environmental conditions of the were not reported
	Metric 17:	Outcome Assessment Methodology	High	split outcome into a 4 week time frame and a six week time frame
	Metric 18:	Consistency of Outcome Assessment	High	Adult survival was not affected by HHCB, and all adultssurvived the entire exposure period. Juvenile survivaldecreased with increasing HHCB concentration. One hundred-percent of the juveniles exposed to 0,0.1,1and10 mg HHCB survived from birth to first reproduction. Juvenile survival frombirth to first reproduction was 90% at 30 mg HHCB and 80% at100 mgHHCB.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Details on variability were not reported. The study authors did make mention that laboratory condition in which snails are not subject to competition or predation which may impact wild snails.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	no information on unrelated outcomes reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	statistical analysis not used, 100% survival
	Metric 22:	Reporting of Data	High	complete survival over entire exposure period
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes were reported
Additional Comments:	This sheet is for >21-d #2 mortality of HHCB.			

Overall Quality Determination Medium

Study Citation:	Pedersen, S., Selck, H., Salvito, D., Forbes, V. (2009). Effects of the polycyclic musk HHCB on individual- and population-level endpoints in Potamopyrgus antipodarum. Ecotoxicology and Environmental Safety 72(4):1190-1199.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Mollusks; *Potamopyrgus antipodarum*; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5428156

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The test substance was identified but there were uncertainties regarding test substance identification or characterization. The nominal concentrations of HHCB were 0.1, 1, 10, 30 and 100 microgram HHCB dw sediment to cover the range from environmentally realistic concentrations to those approaching toxic effect concentrations for growth of P. antipodarum determined in two week pilot experiment.
	Metric 2:	Test Substance Source	Low	chemical source not reported
	Metric 3:	Test Substance Purity	Low	percent purity not reported
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls/negative control response was reported
	Metric 5:	Negative Control Response	High	100% survival at time of first reproduction
	Metric 6:	Randomized Allocation	Medium	organisms randomly distributed but distribution of test chambers not reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	few details on test system were provided
	Metric 8:	Consistency of Exposure Administration	Medium	few details provided for dose administration
	Metric 9:	Measurement of Test Substance Concentration	Medium	low percent recovery, uncertain how often sediments were measured
	Metric 10:	Exposure Duration and Frequency	Medium	long test duration required to cover long lifespan of test organism
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	fairly standard number of levels and dose spacing
	Metric 12:	Testing at or Below Solubility Limit	Medium	limited sampling and low recoveries left solubility concerns uncertain
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	Test organisms were adequately described. P. antipodarum were collected at Salvad Park, Roskilde Fjord, Denmark, in 2004 and subsequently reared in 10 L aquaria at 17 1C on natural pre-frozen sediment (o250 mm) and artificial freshwater (192 mg/L NaHCO, 8 mg/L KCl, 245 mg/L MgSO and 120 mg/L CaSO in de-ionized water, pH 7.2 (Jensen et al., 2001)). A food supplement of commercial fish food (Tetra Min, Tetra Werke, Melle, Germany), baby cereal (Milpo, Milupa, Hørsholm, Denmark) and dried spinach in equal ratios by weight was added to cultures twice a week.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Limited details were provided. The overlying water was renewed every month, and every 4–6 weeks the cultures were given 3–4 spoonfuls of fresh pre-frozen sediment.

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Study Citation:	Pedersen, S., Selck, H., Salvito, D., Forbes, V. (2009). Effects of the polycyclic musk HHCB on individual- and population-level endpoints in Potamopyrgus antipodarum. Ecotoxicology and Environmental Safety 72(4):1190-1199.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Mollusks; *Potamopyrgus antipodarum*; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5428156

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Acceptable and explained in 2.4.1 of the methods "Juvenile growth, survival, time to first reproduction and size at first reproduction"
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Although there is some description of the test system, not all environmental conditions of the were not reported
	Metric 17:	Outcome Assessment Methodology	High	there was a reasonable dose response
	Metric 18:	Consistency of Outcome Assessment	Medium	reasonable growth rate variance but details were scant. Details were reported in Figure 1: Specific growth rates for juvenile and adult snails, Figure 2: Average adult weekly feeding rates, and Figure 5: Size at first reproduction vs HHCB concentration
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	utilized simple but regular monitoring of organisms
	Metric 20:	Outcomes Unrelated to Exposure	Medium	no details reported for unrelated outcomes
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	statistical analysis and explanation provided
	Metric 22:	Reporting of Data	High	reporting of data was adequate and sufficient to determine values of interest for the endpoint.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	SGR differed from adult test with a couple of possible explanations provided

Additional Comments: This sheet is for >21-d #1 growth of snails exposed to HHCB

Overall Quality Determination High

Study Citation:	Pedersen, S., Selck, H., Salvito, D., Forbes, V. (2009). Effects of the polycyclic musk HHCB on individual- and population-level endpoints in Potamopyrgus antipodarum. Ecotoxicology and Environmental Safety 72(4):1190-1199.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Mollusks; *Potamopyrgus antipodarum*; Juvenile
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	5428156

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The test substance was identified but there were uncertainties regarding test substance identification or characterization. The nominal concentrations of HHCB were 0.1, 1, 10, 30 and 100 microgram HHCB dw sediment to cover the range from environmentally realistic concentrations to those approaching toxic effect concentrations for growth of P. antipodarum determined in two week pilot experiment.
	Metric 2:	Test Substance Source	Low	Chemical source was not reported
	Metric 3:	Test Substance Purity	Low	Chemical purity was not reported
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls/negative control response was reported
	Metric 5:	Negative Control Response	High	100% survival at first reproduction
	Metric 6:	Randomized Allocation	Medium	Organisms randomly distributed but distribution of test chambers not reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Few details about the test system were provided
	Metric 8:	Consistency of Exposure Administration	Medium	Few details provided for dose administration
	Metric 9:	Measurement of Test Substance Concentration	Medium	Low recovery 64-86%, infrequent sampling made it difficult to determine accuracy
	Metric 10:	Exposure Duration and Frequency	High	long test duration required to cover long lifespan of test organism
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Standard number of levels and dose spacing
	Metric 12:	Testing at or Below Solubility Limit	Medium	limited sampling and low recoveries left solubility concerns uncertain
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms were adequately described. P. antipodarum were collected at Salvad Park, Roskilde Fjord, Denmark, in 2004 and subsequently reared in 10 L aquaria at 17 1C on natural pre-frozen sediment (o250 mm) and artificial freshwater (192 mg/L NaHCO, 8 mg/L KCl, 245 mg/L MgSO and 120 mg/L CaSO in de-ionized water, pH 7.2 (Jensen et al., 2001)). A food supplement of commercial fish food (Tetra Min, Tetra Werke, Melle, Germany), baby cereal (Milpo, Milupa, Hørsholm, Denmark) and dried spinach in equal ratios by weight was added to cultures twice a week.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Limited details were provided. The overlying water was renewed every month, and every 4–6 weeks the cultures were given 3–4 spoonfuls of fresh pre-frozen sediment.

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Study Citation:	Pedersen, S., Selck, H., Salvito, D., Forbes, V. (2009). Effects of the polycyclic musk HHCB on individual- and population-level endpoints in Potamopyrgus antipodarum. Ecotoxicology and Environmental Safety 72(4):1190-1199.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (freshwater); Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Mollusks; *Potamopyrgus antipodarum*; Juvenile
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	5428156

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Acceptable and explained in 2.4.1 of the methods "Juvenile growth, survival, time to first reproduction and size at first reproduction"
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Although there is some description of the test system, not all environmental conditions of the were not reported
	Metric 17:	Outcome Assessment Methodology	High	reasonable dose response for both time to first reproduction and size at first reproduction
	Metric 18:	Consistency of Outcome Assessment	Medium	Details were reported in Figure 4: Time to first reproduction, Figure 6: Relationship between reproductive output and HHCB exposure over time, and figure 7: Reproductive output, averaged for weeks 5-10.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Details on variability were not reported. The study authors did make mention that laboratory condition in which snails are not subject to competition or predation which may impact wild snails.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	no information about unrelated outcomes were reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	statistical analysis and explanation provided
	Metric 22:	Reporting of Data	High	reporting of data was adequate and sufficient to determine values of interest for the endpoint.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	an apparent dose response trend noted

Additional Comments: This sheet is for >21-d #1 reproductive effects of HHCB

Overall Quality Determination High

Study Citation:	Dijk, Van, A. (n.d.). Acute toxicity of HHCB to pseudokircheneriella subcapitata.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water;
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:	HHCB
HERO ID:	7607844

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test material was definitively characterized using all proper nomenclature and description of the form (colorless, viscous liquid).
	Metric 2:	Test Substance Source	Low	Test material was only described as being sourced "by the study sponsor". No additional details provided.
	Metric 3:	Test Substance Purity	High	Test material purity described as 99.15%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Concurrent negative and solvent controls were included and analytical verification was conducted on the controls.
	Metric 5:	Negative Control Response	Low	Comparison of the solvent and negative control indicates that the solvent used in the test inhibits a positive effect on growth of the test organisms. This is most apparent at the lowest concentrations, where non-linear, significant dose responses were reported but could not be explained. Additionally, the negative control did not reach a 100x cell count increase by the end of the test. The maximum cell count increase in the negative controls was 76.8x.
	Metric 6:	Randomized Allocation	Low	Reporting of random allocations was limited
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Significant degradation of the test material was observed throughout the test, but this was appropriately conducted as a static test, so renewal of the test material is not expected.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were administered consistently across study groups. Degradation of the test material throughout the test was reported across all test concentrations.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using appropriate analytical technologies and methods (HPLC).
	Metric 10:	Exposure Duration and Frequency	High	72 hours is an appropriate duration for algae toxicity studies.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	5 exposure groups spaced in a geometrically increasing sequence is appropriate for this test.
	Metric 12:	Testing at or Below Solubility Limit	Low	Solvent was used to account for the low water solubility of the test material- however the combination of solvents (Tween 80 and DMF) caused a positive growth response in the test organisms.
Domain 4: Test Organism				

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Study Citation:	Dijk, Van, A. (n.d.). Acute toxicity of HHCB to pseudokircheneriella subcapitata.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water;
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:	HHCB
HERO ID:	7607844

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	The test organisms are commonly used in these studies and were adequately described and were obtained from a reliable source.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Algae were acclimatized for 3 days prior to addition of test medium
	Metric 15:	Number of Organisms and Replicates per Group	Low	3 replicates is lower than the commonly accepted number of replicates for an algae acute study (4-minimum)
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	pH for all test concentrations was 8.1, above the recommended starting pH of 7.5. PH increased throughout the experiment beyond the recommended limit of 1.5 units (up to pH of 10 at the end of the experiment). As the negative control cell count did not reach the recommended 100x increase by the end of the test, it is possible that this had an effect on the outcome of the test.
	Metric 17:	Outcome Assessment Methodology	High	outcomes- growth, yield and AUC were appropriate to characterize the effects of acute exposure to the test material.
	Metric 18:	Consistency of Outcome Assessment	High	All replicates were treated concurrently
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Statistically significant growth reduction in lowest test concentration was reported but not explained. PH was reported to increase dramatically throughout the test (>1.5 units), but this was consistent across all concentrations and replications. Similarly, the impact of the growth response triggered by the use of an inappropriate solvent was presumably seen across all concentrations.
	Metric 20:	Outcomes Unrelated to Exposure	Low	Growth inhibition at the lowest concentration was not consistent with effects observed at other concentrations and did not appear to be dose-dependent.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Dunnett's test to determine significance is appropriate. Determination of %CV between reps in the controls were not provided.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoint(s) of interest.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Non-dose responsive effects on the organisms at the lowest test concentration were not explained.

Additional Comments: None

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Study Citation:	Dijk, Van, A. (n.d.). Acute toxicity of HHCB to pseudokircheneriella subcapitata.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water;
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:	HHCB
HERO ID:	7607844

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

Study Citation:	Sinkkonen, A., Myyrä, M., Penttinen, O.P., Rantalainen, A.L. (2011). Selective toxicity at low doses: experiments with three plant species and toxicants. Dose-Response 9(1):130-143.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Gypsophila elegans*; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	1294252

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified as HHCB
	Metric 2:	Test Substance Source	Low	”HHCB (LGC Promochem, Germany)” implies a source for HHCB, but no other details are provided.
	Metric 3:	Test Substance Purity	Low	Purity or grade was not provided.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Appropriate negative controls were used.
	Metric 5:	Negative Control Response	Medium	Response to control group was not significantly different than treatments, presumed do to low dose toxicity of experiment.
	Metric 6:	Randomized Allocation	Medium	Replicates were reported as randomized.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Closed static test conditions adequately described
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across groups and replicates.
	Metric 9:	Measurement of Test Substance Concentration	High	Concentrations of exposure reported. After plant removal, all samples were analyzed using a gas chromatograph after chemical extraction.
	Metric 10:	Exposure Duration and Frequency	High	Duration of exposure was 16 hours.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Number of exposure groups were acceptable. Exposure level was below EC50 for all groups.
	Metric 12:	Testing at or Below Solubility Limit	Low	exposure levels were below adverse effect levels.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Organisms adequately described and purchased from commercial horticulture company, Nelson Garden Oy,Turku, Finland.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Germination and acclimation conditions adequately described.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	6 replicates of n = 47 per treatment
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test condition were adequate and consistent across groups.

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Study Citation:	Sinkkonen, A., Myyrä, M., Penttinen, O.P., Rantalainen, A.L. (2011). Selective toxicity at low doses: experiments with three plant species and toxicants. Dose-Response 9(1):130-143.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Gypsophila elegans*; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	1294252

Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes were reported.
	Metric 18:	Consistency of Outcome Assessment	High	Assessment protocol reported and consistent across groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variables were reported.
	Metric 20:	Outcomes Unrelated to Exposure	High	No unrelated outcomes were reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were reported with sufficient detail.
	Metric 22:	Reporting of Data	High	Exposure data reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes reported.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Sinkkonen, A., Myyrä, M., Penttinen, O.P., Rantalainen, A.L. (2011). Selective toxicity at low doses: experiments with three plant species and toxicants. Dose-Response 9(1):130-143.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Portulaca oleracea*; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	1294252

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified as HHCB
	Metric 2:	Test Substance Source	Low	”HHCB (LGC Promochem, Germany)” implies a source for HHCB, but no other details are provided.
	Metric 3:	Test Substance Purity	Low	Purity or grade was not provided.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Appropriate negative controls were used.
	Metric 5:	Negative Control Response	Medium	Response to control group was not significantly different than treatments, presumed do to low dose toxicity of experiment.
	Metric 6:	Randomized Allocation	Medium	Replicates were reported as randomized.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Closed static test conditions adequately described
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across groups and replicates.
	Metric 9:	Measurement of Test Substance Concentration	High	Concentrations of exposure reported. After plant removal, all samples were analyzed using a gas chromatograph after chemical extraction.
	Metric 10:	Exposure Duration and Frequency	High	Duration of exposure was 16 hours.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	Number of exposure groups were acceptable. Exposure level was below EC50 for all groups.
	Metric 12:	Testing at or Below Solubility Limit	Low	exposure levels were below adverse effect levels.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Organisms adequately described and purchased from commercial horticulture company, Oy Schetelig Ab, Vantaa, Finland,).
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Germination and acclimation conditions adequately described.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	6 replicates of n = 50 per treatment
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test condition were adequate and consistent across groups.

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Study Citation:	Sinkkonen, A., Myyrä, M., Penttinen, O.P., Rantalainen, A.L. (2011). Selective toxicity at low doses: experiments with three plant species and toxicants. Dose-Response 9(1):130-143.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (freshwater); Water; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Portulaca oleracea*; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	1294252

Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes were reported.
	Metric 18:	Consistency of Outcome Assessment	High	Assessment protocol reported and consistent across groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No confounding variables were reported.
	Metric 20:	Outcomes Unrelated to Exposure	High	No unrelated outcomes were reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were reported with sufficient detail.
	Metric 22:	Reporting of Data	High	Exposure data reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes reported.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Wollenberger, L., Breitholtz, M., Kusk, K.O., Bengtsson, B.E. (2003). Inhibition of larval development of the marine copepod Acartia tonsa by four synthetic musk substances. Science of the Total Environment 305(1-3):53-64.
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:	Invertebrate; Arthropods; *Acartia tonsa*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	1942534

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCB identified as test substance.
	Metric 2:	Test Substance Source	High	Source of HHCB reported.
	Metric 3:	Test Substance Purity	Medium	technical product at 74% purity
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Both clean control and solvent controls used.
	Metric 5:	Negative Control Response	High	No control mortality.
	Metric 6:	Randomized Allocation	Low	Random allocation not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Test preparation was reported sufficiently.
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration was consistent.
	Metric 9:	Measurement of Test Substance Concentration	Low	Test concentrations not measured.
	Metric 10:	Exposure Duration and Frequency	High	Followed ISO guidelines.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Sufficient number of exposure groups.
	Metric 12:	Testing at or Below Solubility Limit	Medium	Salinity may be a factor in solubility.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Original source from Denmark.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Test organisms were acclimatized.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of organisms and replicated were reported and sufficient.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Followed ISO guidelines.
	Metric 17:	Outcome Assessment Methodology	High	Appropriate outcomes of interest.

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Study Citation:	Wollenberger, L., Breitholtz, M., Kusk, K.O., Bengtsson, B.E. (2003). Inhibition of larval development of the marine copepod Acartia tonsa by four synthetic musk substances. Science of the Total Environment 305(1-3):53-64.
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:	Invertebrate; Arthropods; *Acartia tonsa*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	1942534

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	Medium	Outcome assessment details not reported.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	Water quality parameters were in acceptable range.
	Metric 20:	Outcomes Unrelated to Exposure	High	No unrelated factors reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Used Probit analysis.
	Metric 22:	Reporting of Data	Medium	LC10 and LC50 values reported with no control mortality.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes reported.

Additional Comments: None

Overall Quality Determination High

Study Citation:	DHI, (2007). Acartia tonsa larval development test with "HHCB".
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
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:	Invertebrate; Arthropods; *Acartia tonsa*; Embryo
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8550022

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the test substance was reported, but it was redacted in the document.
	Metric 3:	Test Substance Purity	Low	The purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate negative control. There was a dilution water control as well as a vehicle control.
	Metric 5:	Negative Control Response	High	The negative control response for the dilution water control and the solvent control were presented in Annex 4.
	Metric 6:	Randomized Allocation	Low	It was not reported how the organisms were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The stock solution was prepared by weighing 125.88mg of non-labelled test substance and 0.103mg of 14C labelled test substance and mixing in ethanol. The test solutions were prepared by diluting the stock solution in test media. The test system was not described in great detail. Test flasks closed with screw caps, but it was unclear if the test system was flow through or static.
	Metric 8:	Consistency of Exposure Administration	Medium	Exposures were conducted in 110mL glass flasks with screw caps sealed with Teflon. There was 105mL of test solution in about 80 eggs per flask. Algae was added as feed. However, it was unclear if the test was static or flow-through.
	Metric 9:	Measurement of Test Substance Concentration	High	The test substance was measured using 14C and liquid scintillation counting. One test from each test concentration and one from the control were harvested each day for analytics.
	Metric 10:	Exposure Duration and Frequency	Medium	The exposure duration was reported to be 6 days. This was adequate to observe a response. This was reported to be only the last part of the test in the OECD Guideline.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure groups, and the spacing was adequate to see a response. The 5 exposure groups were chosen based off of preliminary testing.
	Metric 12:	Testing at or Below Solubility Limit	High	The test concentrations were below the water solubility limit and a vehicle solvent was used along with a solvent control.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The organisms were reported to be cultured in-house since 1987 and were originally from a population collected from the North Sea. Embryos were used at the start of the test.

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Study Citation:	DHI, (2007). Acartia tonsa larval development test with "HHCB".
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
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:	Invertebrate; Arthropods; *Acartia tonsa*; Embryo
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8550022

Domain	Metric		Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if any acclimation occurred.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were approximately 80 embryos per test chamber. There were 4 replicates per exposure concentration and 6 replicates for the control.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	The test was carried out at 20C with a 16L:8D photoperiod. Salinity was reported to be 30.2 0/00. The organisms were fed 7000 cells/ml of algae R. salina. Other water quality parameters were not reported.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–mortality in terms of hatch and larval survival.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. At the end of 6 days, the number of nauplii copepodites and non-hatched eggs were counted.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	It was not reported if the organisms were acclimated.
	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	Low	Statistical analysis was performed, but little information was provided on the methods used.
	Metric 22:	Reporting of Data	High	Mortality data for hatch and larval survival was reported in Annex 4 for the controls and for all exposure groups.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Variability was not reported.

Additional Comments: This portion of the evaluation was on the effect of HHCB on A. tonsa survival over a 6d exposure. Percent hatch and larval mortality were the endpoints assessed. Mortality was selected as the outcome of interest.

Overall Quality Determination Medium

Study Citation:	DHI, (2007). Acartia tonsa larval development test with "HHCB".
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
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:	Invertebrate; Arthropods; *Acartia tonsa*; Embryo
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8550022

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the test substance was reported, but it was redacted in the document.
	Metric 3:	Test Substance Purity	Low	The purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Uninformative	Study authors did not report the use of a negative control in the preliminary study.
	Metric 5:	Negative Control Response	Uninformative	No negative control response was reported for the preliminary study.
	Metric 6:	Randomized Allocation	Low	It was not reported how the organisms were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	Little information was provided on the preparation of the test concentrations or the test system for the preliminary study.
	Metric 8:	Consistency of Exposure Administration	Low	Little information was provided on the consistency of the exposure regarding the preliminary study.
	Metric 9:	Measurement of Test Substance Concentration	Low	It was not reported if the test concentrations were measured in the preliminary study.
	Metric 10:	Exposure Duration and Frequency	Uninformative	The exposure duration of the preliminary study was not reported.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were two exposure concentrations reported for the preliminary study of 10ug/L and 100ug/L.
	Metric 12:	Testing at or Below Solubility Limit	High	The test concentrations were below the water solubility limit.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The organisms were reported to be cultured in-house since 1987 and were originally from a population collected from the North Sea. Embryos were used at the start of the test.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if any acclimation occurred.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of replicates and the number of organisms for the preliminary study were not reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Little information regarding the test conditions of the preliminary study was presented.
	Metric 17:	Outcome Assessment Methodology	Low	The outcome assessment methodology of the preliminary study was not clearly reported. It was reported that there was a significant effect on growth in terms of length, but little other information was provided.

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Study Citation:	DHI, (2007). Acartia tonsa larval development test with "HHCB".
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
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:	Invertebrate; Arthropods; *Acartia tonsa*; Embryo
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8550022

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the outcome assessment for the preliminary study were limited or not reported.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	It was not reported if the organisms were acclimated.
	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	Uninformative	Statistical analysis did not appear to be performed for the preliminary study.
	Metric 22:	Reporting of Data	Low	It was simply reported that the 100ug/L test concentration experienced a "significant effect on larval growth (length)."
	Metric 23:	Explanation of Unexpected Outcomes	Low	Variability was not reported.

Additional Comments: This portion of the evaluation was on the preliminary HHCB study in which growth in length was assessed. This portion received an unacceptable ranking due to lack of negative control as well as lack of study duration.

Overall Quality Determination Uninformative

Study Citation:	DHI, (2007). Acartia tonsa larval development test with "HHCB".
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
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:	Invertebrate; Arthropods; *Acartia tonsa*; Embryo
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8550022

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the test substance was reported, but it was redacted in the document.
	Metric 3:	Test Substance Purity	Low	The purity was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported using an appropriate negative control. There was a dilution water control as well as a vehicle control.
	Metric 5:	Negative Control Response	High	The negative control response for the dilution water control and the solvent control were presented in Annex 4. Percent inhibition was presented in the first two tables. Larval growth test size is presented in um in the last two tables for both nauplii and copepods.
	Metric 6:	Randomized Allocation	Low	It was not reported how the organisms were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The stock solution was prepared by weighing 125.88mg of non-labelled test substance and 0.103mg of 14C labelled test substance and mixing in ethanol. The test solutions were prepared by diluting the stock solution in test media. The test system was not described in great detail. Test flasks closed with screw caps, but it was unclear if the test system was flow through or static.
	Metric 8:	Consistency of Exposure Administration	Medium	Exposures were conducted in 110mL glass flasks with screw caps sealed with Teflon. There was 105mL of test solution in about 80 eggs per flask. Algae was added as feed. However, it was unclear if the test was static or flow-through.
	Metric 9:	Measurement of Test Substance Concentration	High	The test substance was measured using 14C and liquid scintillation counting. One test from each test concentration and one from the control were harvested each day for analytics.
	Metric 10:	Exposure Duration and Frequency	Medium	The exposure duration was reported to be 6 days. This was adequate to observe a response. This was reported to be only the last part of the test in the OECD Guideline.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure groups, and the spacing was adequate to see a response. The 5 exposure groups were chosen based off of preliminary testing.
	Metric 12:	Testing at or Below Solubility Limit	High	The test concentrations were below the water solubility limit and a vehicle solvent was used along with a solvent control.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The organisms were reported to be cultured in-house since 1987 and were originally from a population collected from the North Sea. Embryos were used at the start of the test.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if any acclimation occurred.

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Study Citation:	DHI, (2007). Acartia tonsa larval development test with "HHCB".
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
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:	Invertebrate; Arthropods; *Acartia tonsa*; Embryo
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8550022

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were approximately 80 embryos per test chamber. There were 4 replicates per exposure concentration and 6 replicates for the control.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	The test was carried out at 20C with a 16L:8D photoperiod. Salinity was reported to be 30.2 0/00. The organisms were fed 7000 cells/ml of algae R. salina. Other water quality parameters were not reported.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–development/growth. The size in um, the number of organisms that developed to nauplii and copepods, and the larval growth rate were determined.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. At the end of 6 days, the number of nauplii copepodites and non-hatched eggs were counted. They were then measured.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	It was not reported if the organisms were acclimated.
	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	Low	Statistical analysis was performed, but little information was provided on the methods used.
	Metric 22:	Reporting of Data	High	Development/growth data was presented for the control and the exposure responses were presented in Annex 4. Data was presented for the larval growth rate as well as the number of nauplii and copepodites. The last tables in Annex 4 were labeled "Larval growth test" . It was unclear as to what most of those numbers indicated. However size in um was indicated at the bottom of the table for each exposure level.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Variability was not reported.

Additional Comments: This portion of the evaluation was on the effect of HHCB on A. tonsa growth and development over a 6d exposure. Larval growth rate was presented in the first two tables. The percent of embryos that developed as nauplii or copepodites were also presented in the next table. Several apparent measurements for both nauplii and copepodites were recorded in the last two tables. There were no units provided for these, but size in um was presented at the bottom of the tables.

Overall Quality Determination Medium

Study Citation:	Wollenberger, L., Breitholtz, M., Kusk, K.O., Bengtsson, B.E. (2003). Inhibition of larval development of the marine copepod Acartia tonsa by four synthetic musk substances. Science of the Total Environment 305(1-3):53-64.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
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:	Invertebrate; Arthropods; *Acartia tonsa*; Embryo
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	1942534

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCB identified as test substance.
	Metric 2:	Test Substance Source	High	Source of HHCB identified.
	Metric 3:	Test Substance Purity	Medium	Technical product at 74% purity.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls included in study.
	Metric 5:	Negative Control Response	High	Low mortality reported.
	Metric 6:	Randomized Allocation	Low	Random allocation not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Acetone used as solvent.
	Metric 8:	Consistency of Exposure Administration	High	Consistency was appropriate across exposures.
	Metric 9:	Measurement of Test Substance Concentration	Low	Test concentrations not measured.
	Metric 10:	Exposure Duration and Frequency	Medium	Modified non-standard procedure followed.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Adequate number of exposure groups.
	Metric 12:	Testing at or Below Solubility Limit	Medium	Salinity may influence solubility.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Original source from Denmark.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Unfamiliar procedure of holding organisms at cold temp prior to testing.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of test organisms were sufficient.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions adequate.
	Metric 17:	Outcome Assessment Methodology	High	Outcomes addressed.

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Study Citation:	Wollenberger, L., Breitholtz, M., Kusk, K.O., Bengtsson, B.E. (2003). Inhibition of larval development of the marine copepod Acartia tonsa by four synthetic musk substances. Science of the Total Environment 305(1-3):53-64.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
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:	Invertebrate; Arthropods; *Acartia tonsa*; Embryo
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	1942534

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	Medium	Details not reported so difficult to judge consistency.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Water quality parameters not reported.
	Metric 20:	Outcomes Unrelated to Exposure	High	No unrelated factors reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Probit analysis.
	Metric 22:	Reporting of Data	Medium	Percent hatch, mortality and acute to sub-chronic ratios reported with low control mortality.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes reported.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Wollenberger, L., Breitholtz, M., Kusk, K.O., Bengtsson, B.E. (2003). Inhibition of larval development of the marine copepod Acartia tonsa by four synthetic musk substances. Science of the Total Environment 305(1-3):53-64.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
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:	Invertebrate; Arthropods; *Acartia tonsa*; Embryo
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	1942534

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCB identified as test substance.
	Metric 2:	Test Substance Source	High	Source of HHCB reported.
	Metric 3:	Test Substance Purity	Medium	Technical product at 74% purity.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls reported.
	Metric 5:	Negative Control Response	High	Low control mortality reported.
	Metric 6:	Randomized Allocation	Low	Random allocation not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Acetone used as solvent.
	Metric 8:	Consistency of Exposure Administration	High	Exposures administered consistently.
	Metric 9:	Measurement of Test Substance Concentration	Low	Test solutions not measured.
	Metric 10:	Exposure Duration and Frequency	Medium	Modified non-standard procedure.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Appropriate number of exposure groups.
	Metric 12:	Testing at or Below Solubility Limit	Medium	Salinity may influence solubility.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Original source from Denmark.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Unfamiliar procedure of holding organisms at cold temp prior to testing.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Sufficient number of test organisms.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions adequate.
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes reported.
	Metric 18:	Consistency of Outcome Assessment	Medium	Details not reported for outcome assessment.
Domain 6: Confounding / Variable Control				

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Study Citation:	Wollenberger, L., Breitholtz, M., Kusk, K.O., Bengtsson, B.E. (2003). Inhibition of larval development of the marine copepod Acartia tonsa by four synthetic musk substances. Science of the Total Environment 305(1-3):53-64.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
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:	Invertebrate; Arthropods; *Acartia tonsa*; Embryo
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	1942534

Domain		Metric	Rating	Comments
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Water quality parameters were not reported.
	Metric 20:	Outcomes Unrelated to Exposure	High	No unrelated factors reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Probit analysis.
	Metric 22:	Reporting of Data	Medium	Larval development rates and acute to sub-chronic ratios reported with low control mortality.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes reported.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Ehiguese, F.O., Fernandez, M.C., Lara-Martin, P.A., Martin-Diaz, M.L., Araujo, C.V. (2019). Avoidance behaviour of the shrimp Palaemon varians regarding a contaminant gradient of galaxolide and tonalide in seawater. Chemosphere 232:113-129.
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:	Invertebrate; Arthropods; *Palaemon varians*; Adult
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	5400911

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by trade name (Galaxolide) and abbreviation (HHCB)
	Metric 2:	Test Substance Source	Low	HHCB identity not analytically verified; purchased from Sigma Aldrich Spain.
	Metric 3:	Test Substance Purity	Medium	Analytical grade HHCB (85.0% GC) purchased from Sigma Aldrich Spain.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	seawater control and solvent (DMSO) control were performed
	Metric 5:	Negative Control Response	High	Negative control response was normal:"The results of the control tests showed thatthe displacement of the shrimps inside the system in the absence of contaminants was non-preferential... This randomdistribution in the absence of contaminants validates the suitabilityof the experimental system to study the spatial avoidance behaviour of organ-isms exposed to contaminant gradients."
	Metric 6:	Randomized Allocation	Low	method for allocating test organisms was not reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	"The compartments were each filled with 1 L of seawater and then the shrimps (n = 3) were added to eachcompartment (18 shrimps in total per system) and the distributionsof the organisms were recorded at each 20 min for 3 h. The testswere performed in quadru-plicate.""The tests were conducted in the dark at 20 C"
	Metric 8:	Consistency of Exposure Administration	High	"stock solutions were prepared using dimethylsulfoxide (DMSO 0.001%v/v). The stock solutions were diluted using nanopure water to reach the desired concentrations""For each avoidance test with HHCB and AHTN, plasticine plugswere positioned at the junction of each compartment before fillingthem with seawater spiked with HHCB and AHTN. The gradients ofcontaminants were in the order of 0 (seawater), 0.005, 0.05, 0.5, 5and 50 mg/L. Three shrimps were added to each compartment andthen the plasticine plugs were removed."
	Metric 9:	Measurement of Test Substance Concentration	High	"Samples taken at the beginning and end of all the tests wereanalysed for HHCB and AHTN concentrations using stir bar sorptiveextraction (SBSE)" GC-MS used to identify and quantify the compounds
	Metric 10:	Exposure Duration and Frequency	High	3h exposure for avoidance tests
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Within test system, adjacent compartments contained 0.005, 0.05, 0.5, 5 and 50 mg/L HHCB. Each contained 1L of seawater and DMSO (0.001% v/v). Three shrimp were added in each compartment (18 shrimp total per test system). The tests were performed in quadruplicate.

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Study Citation:	Ehiguese, F.O., Fernandez, M.C., Lara-Martin, P.A., Martin-Diaz, M.L., Araujo, C.V. (2019). Avoidance behaviour of the shrimp Palaemon varians regarding a contaminant gradient of galaxolide and tonalide in seawater. Chemosphere 232:113-129.
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:	Invertebrate; Arthropods; *Palaemon varians*; Adult
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	5400911

Domain	Metric		Rating	Comments
	Metric 12:	Testing at or Below Solubility Limit	High	”No mortality was recorded in the non-forced [avoidance] experiments. The distribution of shrimps between the compartments over time was not significantly different ($p <$ 0.005) for both seawater (p ¼ 0.64,F(5,18) ¼ 0.693) and DMSO (p ¼ 0.99, F(5,18) ¼ 0.085) controls(Tables S3b and S4b e Supplementary Material) and the distributionof shrimps between the compartments was not significantlydifferent ($p < 0.005$) for the seawater control and the DMSO control(Tables S3c and S4c e Supplementary Material). In the absence ofcontaminants, the distribution of shrimps in both seawater andDMSO controls (Fig. 2) was random (showing no preference for anycompartment) during the 3 h exposure.”
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	”Shrimps (1.0-1.5 cm length) were obtained from the Salina ElPopulo aquaculture farm in San Fernando (Southwest Spain) andimmediately transported to the ecotoxicology laboratory of theInstitute of Marine Sciences of Andalusia (ICMAN-CSIC), in Puerto-Real (Spain).”
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Shrimp ”were acclimated for 2 weeks in two 250 L aquariasupplied with filtered (0.5 mm) seawater (deep-well seawater: pHof 7.5 and salinity of 35) in a flow-through system with continuousaeration. The organisms were monitored regularly and deadshrimps were removed immediately. No food was provided duringlaboratory acclimation. Laboratory conditions were: 20 ± 0.5 Ctemperature and a 12:12 h light/dark photoperiod.”
	Metric 15:	Number of Organisms and Replicates per Group	Medium	tests were carried out in quadruplicate, with each test system containing 18 shrimp. The two EPA testing guidelines I found that use shrimp as test organisms (850-1035 and 850-1045) recommend ”a minimum of 20 shrimp per concentration should be exposedto five or more concentrations” of the test chemical – but these are for acute tox tests. The avoidance tests showed conclusive results, with good statistical power using 18 shrimp x 4 replicates, so marking as medium.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Pretreatment conditions reported above. During 3hr test period: No mortality observed in test organisms
	Metric 17:	Outcome Assessment Methodology	High	outcome assessment methodology addressed the intended outcome of interest (avoidance behavior of shrimp exposed to HHCB)
	Metric 18:	Consistency of Outcome Assessment	High	Same protocol and pretreatment conditions used for all test replicates
Domain 6: Confounding / Variable Control				

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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:	Invertebrate; Arthropods; *Palaemon varians*; Adult
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	5400911

Domain	Metric		Rating	Comments
	Metric 19:	Confounding Variables in Test Design and Procedures	High	From details reported in the study, all test groups were subjected to the same environmental conditions, other than the HHCB concentration of the compartment they were initially placed in
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information in study to suggest differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistically analysis and full results are provided in the supplementary materials:"The statistical analysis of the shrimps' distribution in the contaminant gradients of HHCB (F(5,18) ¼ 7.388, $p < 0.001$) and AHTN (F(5,18) ¼ 6.127, $p < 0.002$) revealed that the organisms significantly ($p < 0.05$) preferred the uncontaminated compartments (Tables S5c and S6c e Supplementary Material)."
	Metric 22:	Reporting of Data	High	Data presented for each treatment & control.
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcome (no concentration-dependent response in mortality) explained adequately; HHCB not acutely toxic to test organisms at tested concentrations.

Additional Comments: Avoidance response

Overall Quality Determination High

Study Citation:	Ehiguese, F.O., Fernandez, M.C., Lara-Martin, P.A., Martin-Diaz, M.L., Araujo, C.V. (2019). Avoidance behaviour of the shrimp Palaemon varians regarding a contaminant gradient of galaxolide and tonalide in seawater. Chemosphere 232:113-129.
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:	Invertebrate; Arthropods; *Palaemon varians*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5400911

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by trade name (Galaxolide) and abbreviation (HHCB)
	Metric 2:	Test Substance Source	Low	HHCB identity not analytically verified; purchased from Sigma Aldrich Spain.
	Metric 3:	Test Substance Purity	Medium	Analytical grade HHCB (85.0% GC) purchased from Sigma Aldrich Spain.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	seawater control and solvent (DMSO) control were performed
	Metric 5:	Negative Control Response	High	Fig 4 shows zero mortality in control group.
	Metric 6:	Randomized Allocation	Low	method for allocating test organisms was not reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	”Each aquarium used in the tests was filled with 1 L of seawater spiked with the contaminants... No aeration was provided. The temperature was20 $\pm$ 0.1 C. Initial and final dissolved oxygen levels were 5.9 $\pm$ 0.1and 5.2 $\pm$ 0.2 mg/L, respectively”
	Metric 8:	Consistency of Exposure Administration	High	”stock solutions were prepared using dimethylsulfoxide (DMSO 0.001%v/v). The stock solutions were diluted using nanopure water to reach the desired concentrations””The concentrations used for each contaminant were0.005, 0.05, 0.5, 5 and 50 mg/L including seawater and solvent(DMSO) controls.”
	Metric 9:	Measurement of Test Substance Concentration	High	”Samples taken at the beginning and end of all the tests wereanalysed for HHCB and AHTN concentrations using stir bar sorptiveextraction (SBSE)” GC-MS used to identify and quantify the compounds
	Metric 10:	Exposure Duration and Frequency	High	24hr exposure for the acute toxicity tests
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Concentrations:0.005, 0.05, 0.5, 5 and 50 mg/LThe tests were carried out in quadruplicate: Three shrimps were added per aquariumtotaling 12 shrimps per treatment
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent (DSMO) concentration was reported, 0.001%v/v. Solvent control group experienced 0% mortality (see Supplementary information document, table S2).
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	”Shrimps (1.0-1.5 cm length) were obtained from the Salina ElPopulo aquaculture farm in San Fernando (Southwest Spain) andimmediately transported to the ecotoxicology laboratory of theInstitute of Marine Sciences of Andalusia (ICMAN-CSIC), in Puerto-Real (Spain).”

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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:	Invertebrate; Arthropods; *Palaemon varians*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5400911

Domain	Metric		Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Shrimp "were acclimated for 2 weeks in two 250 L aquariasupplied with filtered (0.5 mm) seawater (deep-well seawater: pHof 7.5 and salinity of 35) in a flow-through system with continuousaeration. The organisms were monitored regularly and deadshrimps were removed immediately. No food was provided duringlaboratory acclimation. Laboratory conditions were: 20 ± 0.5 Ctemperature and a 12:12 h light/dark photoperiod."
	Metric 15:	Number of Organisms and Replicates per Group	Low	"tests were carried out in quadruplicate. Eachaquarium used in the tests was filled with 1 L of seawater spikedwith the contaminants. Three shrimps were added per aquariumtotaling 12 shrimps per treatment"The two EPA testing guidelines I found that use shrimp as test organisms (850-1035 and 850-1045) recommend "a minimum of 20 shrimp per concentration should be exposedto five or more concentrations" of the test chemical, so marking as low since this study had fewer than 20 per exposure group
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Pretreatment conditions reported above. During 24hr test period: "Mortality was recorded at 1, 3, 7and 24 h. No aeration was provided. The temperature was20 ± 0.1 C. Initial and final dissolved oxygen levels were 5.9 ± 0.1and 5.2 ± 0.2 mg/L, respectively."
	Metric 17:	Outcome Assessment Methodology	Medium	Small sample size may have interfered with ability to see a dose-response relationship.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed identically among study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	From details reported in the study, all test groups were subjected to the same environmental conditions, other than HHCB concentration.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information suggested differences among groups.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Mortality % is reported for each group. Can perform independent statistical analysis since # of test organisms in each group is known.
	Metric 22:	Reporting of Data	High	Data was presented from each treatment and control (in supplemental material)
	Metric 23:	Explanation of Unexpected Outcomes	High	Unexpected outcome (no concentration dependent relationship in mortality) was adequately explained; HHCB is not acutely toxic to study organisms at the concentrations tested.

Additional Comments: Study notes mortality in HHCB test "was not concentration-dependent and was highest (17%) for0.005 and 0.5 mg/L." Looking at the full data in the supplementary materials, each group had either one, two or zero mortalities, with no dose-dependent pattern detectable (the seawater control group had one mortality). Due to the small sample size, I think it is hard to distinguish between an effect from the chemical, and background mortality.

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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:	Invertebrate; Arthropods; *Palaemon varians*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5400911

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

Study Citation:	Tamura, I., Kimura, K., Kameda, Y., Nakada, N., Yamamoto, H. (2013). Ecological risk assessment of urban creek sediments contaminated by untreated domestic wastewater: potential contribution of antimicrobials and a musk fragrance. Environmental Technology 34(12):1567-1575.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (brackish); Sediment; 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; *Chironomus yoshimatsui*; Larvae
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427931

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCB (Galoxolide 53.5%) was purchased from LGC standards, GmbH (Wesel, Germany), Table 1 provides definitive identifiers for the test material, including chemical structure, CASRN, and chemical name.
	Metric 2:	Test Substance Source	High	"HHCB (Galoxolide 53.5%) was purchased from LGC standards GmbH (Wesel, Germany)."
	Metric 3:	Test Substance Purity	Low	Purity of the test material (Galoxolide) was reported as 53.5%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Control was artificial sediment
	Metric 5:	Negative Control Response	High	No issues with neg. control
	Metric 6:	Randomized Allocation	Low	Study authors did not describe allocation procedures.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Details about the experimental procedure were minimal and the procedures used to maintain the test concentrations were not described. The authors described the procedure to add the test material to the artificial medium and this was consistent with guideline recommended practices. The authors reported that test concentrations in the laboratory experiments were determined using high performance liquid chromatography, with detection limits in the range between 0.1 and 0.5μgL$-$1.
	Metric 8:	Consistency of Exposure Administration	High	Exposure procedure appeared to be the same across all test concentrations and replicates.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations were measured using HPLC and endpoints appeared to be calculated using measured concentrations. Nominal concentrations were not reported.
	Metric 10:	Exposure Duration and Frequency	High	20-d study to access Chironomid emergence (mortality)
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	"10 first-instar larvae added to each vessel." 5 treatment groups + control
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent was used to facilitate the exposure, and this appeared to be appropriate. A solvent control was not included in the test setup, but the acetone solvent was allowed to vaporize prior to the addition of the test organisms. Precipitate was not reported in the test concentrations.
Domain 4: Test Organism				

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Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Aquatic (brackish); Sediment; 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; *Chironomus yoshimatsui*; Larvae
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427931

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	”Chironomid toxicity tests were conducted using Chiromusyoshimatsui purchased from NIES and acclimated in thelaboratory of the University of Tokushima for at least amonth.” Test organisms were at the First instar (larvae) stage.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Chironomids were ”aclimated in the laboratory of the University of Tokushima for at least a month.”
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 first-instar larvae added to each vessel.” 5 treatment groups + control and three replicates.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Organism housing, environmental conditions, food, water, and nutrients were conducive to maintenance of health and biomass loading were not reported. As excess mortality was not reported in the controls, this was not expected to have significant effects on the results.
	Metric 17:	Outcome Assessment Methodology	High	Assessment methodology was sufficient to calculate a NOAEC based on a reduction in larval emergence as significant effects were observed at the highest test concentrations. A separate aspect of the experiment characterized the effects on emergence when organisms were exposed to actual river sediment collected in situ.
	Metric 18:	Consistency of Outcome Assessment	Low	Specific details of the protocol were discussed in a very limited sense.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No reported differences among study groups that would impact the results.
	Metric 20:	Outcomes Unrelated to Exposure	High	No differences among the study groups were identified.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	”20 d-No Effect Concentration (NOEC) was determined using the Ecotox–statics version 2.6d distributed by the Japan Society for Environmental Toxicology”
	Metric 22:	Reporting of Data	High	Results were reported in Figure 4
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes related to the calculation of a NOAEC for HHCB for the effects of interest.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Breitholtz, M., Wollenberger, L., Dinan, L. (2003). Effects of four synthetic musks on the life cycle of the harpacticoid copepod Nitocra spinipes. Aquatic Toxicology 63(2):103-118.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Aquatic (brackish); 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; *Nitocra spinipes*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	1417909

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	The test substance was a technical product with 26% DEP and undefined HHCB % purity.
	Metric 2:	Test Substance Source	High	The manufacturer source was provided. There was no lot number but HHCB was analytically verified by other labs.
	Metric 3:	Test Substance Purity	Medium	HHCB was obtained as a technical product with one 26% impurity identified.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls were not indicated in the study itself, however, the acute toxicity test cited Bengtsson, 1978, which used a solvent control.
	Metric 5:	Negative Control Response	Low	The study did not report negative control outcomes.
	Metric 6:	Randomized Allocation	Low	The random allocation method was not reported.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	This study cited Bengtsson, 1978 for the experimental system and test media preparation. Bengtsson conducted static acute toxicity tests.
	Metric 8:	Consistency of Exposure Administration	High	For the acute test, the authors cited Bengtsson, 1978 test methods.
	Metric 9:	Measurement of Test Substance Concentration	Low	The authors only measured initial test substance concentrations for the acute toxicity test.
	Metric 10:	Exposure Duration and Frequency	High	The authors used a 96-hour test duration for the copepod acute test.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The study authors noted that "The highest nominal concentration in each chronic experiment was set at one-tenth of thecorresponding 96-h-LC50-value and the lowerconcentrations were set on a geometric scale." Therefore the test concentrations appear adequate to obtain an LC50.
	Metric 12:	Testing at or Below Solubility Limit	Medium	There were limited study details, and comments included remarks for other tested chemicals that were near solubility.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The study adequately described the copepod test organism.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The authors cited using Bengtsson, 1978 methods.

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Exposure Route, Media, Path:	Aquatic (brackish); 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; *Nitocra spinipes*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	1417909

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms and replicates were not reported, but the authors cited Bengtsson, 1978 methods, which used 10 test organisms per concentration, without replicates.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Test conditions were reportedly from the Bengtsson, 1978 study.
	Metric 17:	Outcome Assessment Methodology	Medium	The study authors cited Bengtsson, 1978 methods for outcome assessment, but there is uncertainty because negative control outcomes were not reported.
	Metric 18:	Consistency of Outcome Assessment	Medium	Details for the acute toxicity test were not reported, including no outcomes reported for negative controls, but the authors reported following Bengtsson's 1978 study methods.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	The authors did no provide information regarding variables.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest differences among study groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The authors reported using Probit analysis on the acute toxicity test results.
	Metric 22:	Reporting of Data	Low	There were no details reported, only LC50 values and CI for the test concentrations (and not for the control).
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes reported.

Additional Comments: The acute toxicity test was conducted to establish chronic toxicity test concentrations, so there are few details in the study and the authors refer to Bengtsson's 1978 study to describe methodology.

Overall Quality Determination Medium

Study Citation:	Breitholtz, M., Wollenberger, L., Dinan, L. (2003). Effects of four synthetic musks on the life cycle of the harpacticoid copepod Nitocra spinipes. Aquatic Toxicology 63(2):103-118.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (brackish); 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; *Nitocra spinipes*; NA; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	1417909

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	The test substance was technical product with 26% DEP and HHCB with unknown % purity.
	Metric 2:	Test Substance Source	High	The source was indicated and the test substance identitiy was verified by other labs.
	Metric 3:	Test Substance Purity	Medium	HHCB was obtained as a technical product with one impurity identified.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A negative control and solvent control were used.
	Metric 5:	Negative Control Response	High	The study reported no unusual responses to controls.
	Metric 6:	Randomized Allocation	Medium	The study reported randomized allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	There was a significant loss of test substance concentration, especially in the highest concentration.
	Metric 8:	Consistency of Exposure Administration	High	The study author administered exposures consistently across study gropus.
	Metric 9:	Measurement of Test Substance Concentration	High	Test concentrations were measured.
	Metric 10:	Exposure Duration and Frequency	High	The duration and exposure frequency (semi-static renewal) were adequate and appropriate for the study.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and spacing of exposure concentrations were adequate for the purpose of the study.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The measured highest concentration was significantly lower than the nominal value.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test species source and age were well documented and the species characteristics and rationale for use in the study were adequately documented.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were reasonably acclimatized prior to testing.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of test organisms and replicates appear adequate for the purpose of the test.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	There was low variance of test water parameters.

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Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (brackish); 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; *Nitocra spinipes*; NA; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	1417909

Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	The larval mortality rates had reasonable dose response outcomes.
	Metric 18:	Consistency of Outcome Assessment	Medium	Although the highest measured test concentration was significantly lower than the nominal concentration, outcomes were assessed across study groups, including controls.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	DEP was present as a 26% impurity and could possibly effect outcomes.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information regarding negative aspects of organism health, but based on controls, this is not likely to have a substantial impact on results.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The study reported the statistical analysis methods used on the data.
	Metric 22:	Reporting of Data	High	All data for all exposure treatment groups and controls were reported in the study.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected mortality outcomes reported.

Additional Comments: The study included a supplemental mechanistic Ecdysteroid cell line assay.

Overall Quality Determination High

Study Citation:	Breitholtz, M., Wollenberger, L., Dinan, L. (2003). Effects of four synthetic musks on the life cycle of the harpacticoid copepod Nitocra spinipes. Aquatic Toxicology 63(2):103-118.
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Exposure Route, Media, Path:	Aquatic (brackish); 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; *Nitocra spinipes*; NA; Larvae
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	1417909

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	The test substance was a technical product with 26% DEP and HHCB of unknown % purity.
	Metric 2:	Test Substance Source	High	The study reported the source manufacturer and the chemical identity was verified by other labs.
	Metric 3:	Test Substance Purity	Medium	The test substance was obtained as a technical product with one impurity identified.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A negative control and solvent control were used.
	Metric 5:	Negative Control Response	High	There was no unusual response to the controls reported.
	Metric 6:	Randomized Allocation	Medium	The study reported randomized allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	There was a substantial loss of test concentration at the highest concentration tested.
	Metric 8:	Consistency of Exposure Administration	High	The study reported consistent administration of the test substance across study groups.
	Metric 9:	Measurement of Test Substance Concentration	High	Although there was significant loss of test substance at the highest concentration tested, exposure concentrations were measured.
	Metric 10:	Exposure Duration and Frequency	Medium	The duration of the test could have been longer, but the author provided a clear explanation of why that duration was chosen.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The study reported adequate geometric distribution of test concentrations, but had inadequate dose response results.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The highest concentration was significantly lower than the nominal value.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The study reported the source and age of the test organisms.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The test organisms were reasonably acclimatized prior to testing.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The numbers of test organisms and replicates were adequate for evaluating the test outcome.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	There was low variance of test water parameters.
	Metric 17:	Outcome Assessment Methodology	Medium	There was limited dose response reported, but the authored provided reasoning in the study.

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Study Citation:	Breitholtz, M., Wollenberger, L., Dinan, L. (2003). Effects of four synthetic musks on the life cycle of the harpacticoid copepod Nitocra spinipes. Aquatic Toxicology 63(2):103-118.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (brackish); 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; *Nitocra spinipes*; NA; Larvae
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	1417909

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment was consistent across study groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	DEP was present as an impurity and could possibly effect outcomes.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information provided regarding negative aspects of organism health.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The study adequately reported statistical analysis methodology.
	Metric 22:	Reporting of Data	High	The study reported exposure-related outcomes for each treatment group and the controls.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	The short exposure duration for juvenile copepods may have added to outcome uncertainty.

Additional Comments: The study included a supplemental Ecdysteroid cell line assay.

Overall Quality Determination High

Study Citation:	Breitholtz, M., Wollenberger, L., Dinan, L. (2003). Effects of four synthetic musks on the life cycle of the harpacticoid copepod Nitocra spinipes. Aquatic Toxicology 63(2):103-118.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (brackish); 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; *Nitocra spinipes*; NA; Larvae
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	1417909

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	The test substance is a technical product with 26% DEP, and the HHCB % purity was not indicated.
	Metric 2:	Test Substance Source	High	The test substance manufacturer was provided and its identity was verified by other labs.
	Metric 3:	Test Substance Purity	Medium	HHCB was obtained as a technical product with one impurity identified.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A negative control and solvent control were used.
	Metric 5:	Negative Control Response	High	There were no unusual responses to controls reported.
	Metric 6:	Randomized Allocation	Medium	Randomized allocation was reported for the growth and development tests.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	There was a significant loss of the chemical, especially in the highest concentration tested.
	Metric 8:	Consistency of Exposure Administration	High	The researcher consistently exposed the study groups to the test substance.
	Metric 9:	Measurement of Test Substance Concentration	High	The study reported measured concentrations of the test substance.
	Metric 10:	Exposure Duration and Frequency	High	Semi-static renewal exposures to copepod larvae were appropriate for testing growth and development outcomes.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The authors used an adequate number of test concentrations and spacing for the test.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The highest concentration was quite low relative to the nominal value.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The authors documented the copepod source and age and provided detail rationale for use of the test species.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	The study reported reasonable acclimatization conditions for the test species.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The number of nauplii per treatment group (10-15) with 8 replicates appear adequate to characterize toxicological effects.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	There was low variance of test water parameters.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment for development rates was reasonable.

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Study Citation:	Breitholtz, M., Wollenberger, L., Dinan, L. (2003). Effects of four synthetic musks on the life cycle of the harpacticoid copepod Nitocra spinipes. Aquatic Toxicology 63(2):103-118.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Aquatic (brackish); 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; *Nitocra spinipes*; NA; Larvae
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	1417909

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	Medium	Development rates were adjusted for a lower temperature of the HHCB test.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	DEP was present as a 26% impurity and could possibly effect outcomes.
	Metric 20:	Outcomes Unrelated to Exposure	High	There was no information regarding negative aspects of organism health, but this is not expected to affect outcome assessment based on control outcomes.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The study reported the statistical methods used to analyze the data.
	Metric 22:	Reporting of Data	High	The study presented exposure-related outcomes for controls and each treatment group.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected development outcomes reported.

Additional Comments: Note that the study includes supplemental mechanistic information, the Ecdysteroid cell line assay.

Overall Quality Determination High

Study Citation:	Pablos, M.V., Jiménez, M.Á., Segundo, L.S., Martini, F., Beltrán, E., Fernández, C. (2015). Effects of dietary exposure of polycyclic musk HHCB on the metamorphosis of Xenopus laevis. Environmental Toxicology and Chemistry 35(6):1428-1435.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Amphibian; *Xenopus laevis*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	3007206

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCB identified as test substance
	Metric 2:	Test Substance Source	High	Source of HHCB included in paper
	Metric 3:	Test Substance Purity	Medium	Purity of HHCB not stated, but the study was unlikely to be impacted by the lack of this detail
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls used in this study
	Metric 5:	Negative Control Response	High	Responses of negative controls were adequate
	Metric 6:	Randomized Allocation	Medium	Larvae were housed and fed in aquaria, then transferred to test aquaria
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Experimental design and methods detailed
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration was via food, the food was analytically analyzed for concentrations and administered to organisms.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations monitored by GCMS.
	Metric 10:	Exposure Duration and Frequency	High	Exposure durations were reported
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Number of organisms and exposure groups reported
	Metric 12:	Testing at or Below Solubility Limit	High	Concentrations below limit of solubility
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms described in detail
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Organisms acclimated
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of organisms sufficient
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Experimental conditions were conducive to overall health of organisms
	Metric 17:	Outcome Assessment Methodology	High	Outcome methodology addressed

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Study Citation:	Pablos, M.V., Jiménez, M.Á., Segundo, L.S., Martini, F., Beltrán, E., Fernández, C. (2015). Effects of dietary exposure of polycyclic musk HHCB on the metamorphosis of Xenopus laevis. Environmental Toxicology and Chemistry 35(6):1428-1435.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Amphibian; *Xenopus laevis*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	3007206

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes reported
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No reported differences
	Metric 20:	Outcomes Unrelated to Exposure	High	No unexpected outcomes reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistics clearly articulated in paper
	Metric 22:	Reporting of Data	High	Data findings reported
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes

Additional Comments: None

Overall Quality Determination High

Study Citation:	Pablos, M.V., Jiménez, M.Á., Segundo, L.S., Martini, F., Beltrán, E., Fernández, C. (2015). Effects of dietary exposure of polycyclic musk HHCB on the metamorphosis of Xenopus laevis. Environmental Toxicology and Chemistry 35(6):1428-1435.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Amphibian; *Xenopus laevis*; Larvae
Health Outcome:	Endocrine
Chemical:	HHCB
HERO ID:	3007206

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCB identified as test substance
	Metric 2:	Test Substance Source	High	Source of HHCB included in paper
	Metric 3:	Test Substance Purity	Medium	Purity of HHCB not stated, but the study was unlikely to be impacted by the lack of this detail
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls used in this study
	Metric 5:	Negative Control Response	High	Responses of negative controls were adequate
	Metric 6:	Randomized Allocation	Medium	Larvae were housed and fed in aquaria, then transferred to test aquaria
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Experimental design and methods detailed
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration was via food, the food was analytically analyzed for concentrations and administered to organisms.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations monitored by GCMS.
	Metric 10:	Exposure Duration and Frequency	High	Exposure durations were reported
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Number of organisms and exposure groups reported
	Metric 12:	Testing at or Below Solubility Limit	High	Concentrations below limit of solubility
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms described in detail
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Organisms acclimated
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of organisms sufficient
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Experimental conditions were conducive to overall health of organisms
	Metric 17:	Outcome Assessment Methodology	High	Outcome methodology addressed
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes reported

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Study Citation:	Pablos, M.V., Jiménez, M.Á., Segundo, L.S., Martini, F., Beltrán, E., Fernández, C. (2015). Effects of dietary exposure of polycyclic musk HHCB on the metamorphosis of Xenopus laevis. Environmental Toxicology and Chemistry 35(6):1428-1435.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Amphibian; *Xenopus laevis*; Larvae
Health Outcome:	Endocrine
Chemical:	HHCB
HERO ID:	3007206

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No reported differences
	Metric 20:	Outcomes Unrelated to Exposure	High	No unexpected outcomes reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistics clearly articulated in paper
	Metric 22:	Reporting of Data	High	Data findings reported
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes

Additional Comments: None

Overall Quality Determination High

Study Citation:	Pablos, M.V., Jiménez, M.Á., Segundo, L.S., Martini, F., Beltrán, E., Fernández, C. (2015). Effects of dietary exposure of polycyclic musk HHCB on the metamorphosis of Xenopus laevis. Environmental Toxicology and Chemistry 35(6):1428-1435.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Amphibian; *Xenopus laevis*; Larvae
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	3007206

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCB identified as test substance
	Metric 2:	Test Substance Source	High	Source of HHCB included in paper
	Metric 3:	Test Substance Purity	Medium	Purity of HHCB not stated, but the study was unlikely to be impacted by the lack of this detail
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls used in this study
	Metric 5:	Negative Control Response	High	Responses of negative controls were adequate
	Metric 6:	Randomized Allocation	Medium	Larvae were housed and fed in aquaria, then transferred to test aquaria
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Experimental design and methods detailed
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration was via food, the food was analytically analyzed for concentrations and administered to organisms.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations monitored by GCMS.
	Metric 10:	Exposure Duration and Frequency	High	Exposure durations were reported
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Number of organisms and exposure groups reported
	Metric 12:	Testing at or Below Solubility Limit	High	Concentrations below limit of solubility
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms described in detail
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Organisms acclimated
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of organisms sufficient
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Experimental conditions were conducive to overall health of organisms
	Metric 17:	Outcome Assessment Methodology	High	Outcome methodology addressed
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes reported

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Study Citation:	Pablos, M.V., Jiménez, M.Á., Segundo, L.S., Martini, F., Beltrán, E., Fernández, C. (2015). Effects of dietary exposure of polycyclic musk HHCB on the metamorphosis of Xenopus laevis. Environmental Toxicology and Chemistry 35(6):1428-1435.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Amphibian; *Xenopus laevis*; Larvae
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	3007206

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No reported differences
	Metric 20:	Outcomes Unrelated to Exposure	High	No unexpected outcomes reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistics clearly articulated in paper
	Metric 22:	Reporting of Data	High	Data findings reported
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes

Additional Comments: None

Overall Quality Determination High

Study Citation:	Pablos, M.V., Jiménez, M.Á., Segundo, L.S., Martini, F., Beltrán, E., Fernández, C. (2015). Effects of dietary exposure of polycyclic musk HHCB on the metamorphosis of Xenopus laevis. Environmental Toxicology and Chemistry 35(6):1428-1435.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Amphibian; *Xenopus laevis*; Larvae
Health Outcome:	ADME (biotransformation)
Chemical:	HHCB
HERO ID:	3007206

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCB identified as test substance
	Metric 2:	Test Substance Source	High	Source of HHCB included in paper
	Metric 3:	Test Substance Purity	Medium	Purity of HHCB not stated, but the study was unlikely to be impacted by the lack of this detail
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls used in this study
	Metric 5:	Negative Control Response	High	Responses of negative controls were adequate
	Metric 6:	Randomized Allocation	Medium	Larvae were housed and fed in aquaria, then transferred to test aquaria
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Experimental design and methods detailed
	Metric 8:	Consistency of Exposure Administration	High	Exposure administration was via food, the food was analytically analyzed for concentrations and administered to organisms.
	Metric 9:	Measurement of Test Substance Concentration	High	Exposure concentrations monitored by GCMS.
	Metric 10:	Exposure Duration and Frequency	High	Exposure durations were reported
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Number of organisms and exposure groups reported
	Metric 12:	Testing at or Below Solubility Limit	High	Concentrations below limit of solubility
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms described in detail
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Organisms acclimated
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of organisms sufficient
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Experimental conditions were conducive to overall health of organisms
	Metric 17:	Outcome Assessment Methodology	High	Outcome methodology addressed
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes reported

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Study Citation:	Pablos, M.V., Jiménez, M.Á., Segundo, L.S., Martini, F., Beltrán, E., Fernández, C. (2015). Effects of dietary exposure of polycyclic musk HHCB on the metamorphosis of Xenopus laevis. Environmental Toxicology and Chemistry 35(6):1428-1435.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Food/Diet; Dietary
Taxa, Species, Age:	Vertebrate; Amphibian; *Xenopus laevis*; Larvae
Health Outcome:	ADME (biotransformation)
Chemical:	HHCB
HERO ID:	3007206

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No reported differences
	Metric 20:	Outcomes Unrelated to Exposure	High	No unexpected outcomes reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistics clearly articulated in paper
	Metric 22:	Reporting of Data	High	Data findings reported
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes

Additional Comments: This evaluation is for the HHCB measurements in postmetamorphosis frogs shown in Table 3.

Overall Quality Determination High

Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Mollusks; *Achatina fulica*; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by nomenclature and formula.
	Metric 2:	Test Substance Source	High	Purchased from Guoyao Chemical Co. Ltd. (Beijing, China)
	Metric 3:	Test Substance Purity	High	Given as ">98% purity".
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls and solvent controls were used.
	Metric 5:	Negative Control Response	High	No difference between solvent controls and negative controls, no reported mortality in controls.
	Metric 6:	Randomized Allocation	Medium	"Six juvenile snails were randomly selected for each container..."
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	HHCB was mixed with soil and it is unclear whether sealed vessels were used. As HHCB can volatilize from soil in the long term and nominal concentrations were not tested, there may be some discrepancy. The test duration was 28 days, and Schafer 2005 reported a volatilization of up to 16% after 28 days. However, the soil was replaced every 7 days in this test. This discrepancy is unlikely to have a major effect on results since it was replicated across all treatments.
	Metric 8:	Consistency of Exposure Administration	High	Exposure same for all treatments.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were not measured, however HHCB does not rapidly biodegrade and sorbs to soil somewhat strongly. The discrepancy between nominal and actual exposure concentrations is unlikely to substantially effect the results.
	Metric 10:	Exposure Duration and Frequency	High	The study was conducted in accordance with ISO 15952:2006. Duration and frequency were appropriate (replacement of soil every 7 days for a 28-day test).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Range and spacing of exposure levels were given for all treatments & were adequate to develop EC10, EC50, and a NOEC value with reasonable confidence intervals.
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent used (acetone) did not cause measurable responses relative to negative controls.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Snails were obtained from a snail-culturing farm in Beijing, China. Rearing conditions were described and seem adequate for the species chosen.

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Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Mollusks; *Achatina fulica*; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Snails were kept for 2 weeks in the same conditions as the test to acclimate before the experiment began.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	6 snails were used per replicate, with 3 replicates per treatment and controls.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Conditions were appropriate for the snails and were the same for all groups & were consistent before treatment.
	Metric 17:	Outcome Assessment Methodology	High	Endpoints were measured at the end of the test (28d) according to ISO 15952:2006.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed the same across all groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences in environmental conditions across groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported details that suggest differences in attrition or other health outcomes.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was adequately described in the methods section and appropriate for the experiments performed.
	Metric 22:	Reporting of Data	Low	Summary data (ECx, NOEC) was reported for the overall experiment for each endpoint, but individual data for each treatment and control was not reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, and the outcomes that were reported are adequately discussed in the text.

Additional Comments: This study also includes the calculation of an SSD from all 10 tested terrestrial organisms and the derivation of PNECs from said distribution, with discussion of AFs. In my opinion it would provide an important contribution to terrestrial data for HHCB.

Overall Quality Determination High

Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Mollusks; *Achatina fulica*; Juvenile
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by nomenclature and formula.
	Metric 2:	Test Substance Source	High	Purchased from Guoyao Chemical Co. Ltd. (Beijing, China)
	Metric 3:	Test Substance Purity	High	Given as ">98% purity".
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls and solvent controls were used.
	Metric 5:	Negative Control Response	High	No difference between solvent controls and negative controls, no reported mortality in controls.
	Metric 6:	Randomized Allocation	Medium	"Six juvenile snails were randomly selected for each container..."
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	HHCB was mixed with soil and it is unclear whether sealed vessels were used. As HHCB can volatilize from soil in the long term and nominal concentrations were not tested, there may be some discrepancy. The test duration was 28 days, and Schafer 2005 reported a volatilization of up to 16% after 28 days. However, the soil was replaced every 7 days in this test. This discrepancy is unlikely to have a major effect on results since it was replicated across all treatments.
	Metric 8:	Consistency of Exposure Administration	High	Exposure same for all treatments.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were not measured, however HHCB does not rapidly biodegrade and sorbs to soil somewhat strongly. The discrepancy between nominal and actual exposure concentrations is unlikely to substantially effect the results.
	Metric 10:	Exposure Duration and Frequency	High	The study was conducted in accordance with ISO 15952:2006. Duration and frequency were appropriate (replacement of soil every 7 days for a 28-day test).
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Range and spacing of exposure levels were given for all treatments & were adequate to develop EC10, EC50, and a NOEC value with reasonable confidence intervals.
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent used (acetone) did not cause measurable responses relative to negative controls.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Snails were obtained from a snail-culturing farm in Beijing, China. Rearing conditions were described and seem adequate for the species chosen.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Snails were kept for 2 weeks in the same conditions as the test to acclimate before the experiment began.

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Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Mollusks; *Achatina fulica*; Juvenile
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	6 snails were used per replicate, with 3 replicates per treatment and controls.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Conditions were appropriate for the snails and were the same for all groups & were consistent before treatment.
	Metric 17:	Outcome Assessment Methodology	High	Endpoints were measured at the end of the test (28d) according to ISO 15952:2006.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed the same across all groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences in environmental conditions across groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported details that suggest differences in attrition or other health outcomes.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was adequately described in the methods section and appropriate for the experiments performed.
	Metric 22:	Reporting of Data	Low	Summary data (ECx, NOEC) was reported for the overall experiment for each endpoint, but individual data for each treatment and control was not reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, and the outcomes that were reported are adequately discussed in the text.

Additional Comments: This study also includes the calculation of an SSD from all 10 tested terrestrial organisms and the derivation of PNECs from said distribution, with discussion of AFs. In my opinion it would provide an important contribution to terrestrial data for HHCB.

Overall Quality Determination High

Study Citation:	Mori, T., Morita, F., Inokuchi, A., Takao, Y., Kohra, S., Tominaga, N., Takemasa, T., Arizono, K. (2006). Ecotoxicological Effect of Polycyclic Musks on Caenorhabditis elegans. Journal of Health Science 52(3):276.
Duration:	Overall Duration: 0 - 4 days (0-96h); 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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5919179

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by IUPAC name and abbreviation, 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-g-2- benzopyran (HHCB)
	Metric 2:	Test Substance Source	High	Obtained from Promochem (Teddington, UK)
	Metric 3:	Test Substance Purity	Low	Purity was not mentioned
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Control group was used
	Metric 5:	Negative Control Response	High	Results were compared to negative controls
	Metric 6:	Randomized Allocation	Medium	Nematodes were randomly selected.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Nematodes were grown on nematode growth medium, then transferred to 24 well plates containing the test concentrations of HHCB.
	Metric 8:	Consistency of Exposure Administration	High	24 hour chemical exposure duration. The number of dead/alive were counted at the end of the treatment period.
	Metric 9:	Measurement of Test Substance Concentration	Low	DMSO solvent was used, but test concentrations were not analytically verified.
	Metric 10:	Exposure Duration and Frequency	High	24 hour exposure
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	10 nematodes/well – HHCB test group concentrations: 3.0, 6.1, 12.2, 24.3, 48.7, 97.3, 194.6 mg/L
	Metric 12:	Testing at or Below Solubility Limit	Low	HHCB was dissolved in DMSO. Study states that DMSO was used as a control for all treatments. However, the authors do not state the concentration of DMSO used to dissolve the HHCB, nor the concentration of DMSO used for the control group.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Nematodes that were mainly in the larval stage were used.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Nematodes were grown on nematode growth medium prior to test initiation.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 nematodes/well were used
Domain 5: Outcome Assessment				

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Study Citation:	Mori, T., Morita, F., Inokuchi, A., Takao, Y., Kohra, S., Tominaga, N., Takemasa, T., Arizono, K. (2006). Ecotoxicological Effect of Polycyclic Musks on Caenorhabditis elegans. Journal of Health Science 52(3):276.
Duration:	Overall Duration: 0 - 4 days (0-96h); 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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Larvae
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5919179

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Nematodes were grown in nematode growth medium prior to test initiation. No indication of inappropriate housing conditions.
	Metric 17:	Outcome Assessment Methodology	High	Lethality was assessed
	Metric 18:	Consistency of Outcome Assessment	High	None noted
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	None identified
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Median lethal concentrations were determined via probit analysis
	Metric 22:	Reporting of Data	High	Statistical differences were determined between controls and treatment groups.
	Metric 23:	Explanation of Unexpected Outcomes	High	The study author referred to other investigations of HHCB exposure to nematodes.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Mori, T., Morita, F., Inokuchi, A., Takao, Y., Kohra, S., Tominaga, N., Takemasa, T., Arizono, K. (2006). Ecotoxicological Effect of Polycyclic Musks on Caenorhabditis elegans. Journal of Health Science 52(3):276.
Duration:	Overall Duration: 0 - 4 days (0-96h); 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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Larvae
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	5919179

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by IUPAC name and abbreviation, 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-g-2- benzopyran (HHCB)
	Metric 2:	Test Substance Source	High	Obtained from Promochem (Teddington, UK)
	Metric 3:	Test Substance Purity	Low	Purity was not mentioned
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Control group was used
	Metric 5:	Negative Control Response	High	Results were compared to negative controls
	Metric 6:	Randomized Allocation	Medium	Nematodes were randomly selected.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Nematodes were grown on nematode growth medium, then transferred to 24 well plates containing the test concentrations of HHCB.
	Metric 8:	Consistency of Exposure Administration	High	24 hour chemical exposure duration. The number of dead/alive were counted at the end of the treatment period.
	Metric 9:	Measurement of Test Substance Concentration	Low	DMSO solvent was used, but test concentrations were not analytically verified.
	Metric 10:	Exposure Duration and Frequency	High	24 hour exposure
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	HHCB test group concentrations: 0.3, 0.6, 1.4, 2.4, 4.9, 9.8, 19.5 mg/L
	Metric 12:	Testing at or Below Solubility Limit	Low	HHCB was dissolved in DMSO. Study states that DMSO was used as a control for all treatments
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Nematodes that were mainly in the larval stage were used.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Nematodes were grown on nematode growth medium prior to test initiation.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 nematodes/well were used, in triplicate
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Nematodes were grown in nematode growth medium prior to test initiation. No indication of inappropriate housing conditions.
	Metric 17:	Outcome Assessment Methodology	High	Brood size was counted everyday and new test solution was added until egg production stopped.

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Study Citation:	Mori, T., Morita, F., Inokuchi, A., Takao, Y., Kohra, S., Tominaga, N., Takemasa, T., Arizono, K. (2006). Ecotoxicological Effect of Polycyclic Musks on Caenorhabditis elegans. Journal of Health Science 52(3):276.
Duration:	Overall Duration: 0 - 4 days (0-96h); 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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Larvae
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	5919179

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	None noted
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	None identified
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Data were analyzed via an ANOVA followed by a Dunnetts multiple comparisons test. If homogeneity was observed, a Kruskal-Wallis test was used followed by a Mann-Whitney U test with Bonferroni adjustment.
	Metric 22:	Reporting of Data	High	Statistical differences were determined between controls and treatment groups.
	Metric 23:	Explanation of Unexpected Outcomes	High	The study author referred to other investigations of HHCB exposure to nematodes.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Mori, T., Morita, F., Inokuchi, A., Takao, Y., Kohra, S., Tominaga, N., Takemasa, T., Arizono, K. (2006). Ecotoxicological Effect of Polycyclic Musks on Caenorhabditis elegans. Journal of Health Science 52(3):276.
Duration:	Overall Duration: 0 - 4 days (0-96h); 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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Larvae
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5919179

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by IUPAC name and abbreviation, 1,3,4,6,7,8-Hexahydro-4,6,6,7,8,8-hexamethylcyclopenta-g-2- benzopyran (HHCB)
	Metric 2:	Test Substance Source	High	Obtained from Promochem (Teddington, UK)
	Metric 3:	Test Substance Purity	Low	Purity was not mentioned
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Control group was used
	Metric 5:	Negative Control Response	High	Results were compared to negative controls
	Metric 6:	Randomized Allocation	Medium	Nematodes were randomly selected.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Nematodes were grown on nematode growth medium, then transferred to 24 well plates containing the test concentrations of HHCB.
	Metric 8:	Consistency of Exposure Administration	High	24 hour chemical exposure duration. The number of dead/alive were counted at the end of the treatment period.
	Metric 9:	Measurement of Test Substance Concentration	Low	DMSO solvent was used, but test concentrations were not analytically verified.
	Metric 10:	Exposure Duration and Frequency	High	24 hour exposure
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	10 nematodes/well – HHCB test group concentrations: 0.3, 0.6, 1.4, 2.4, 4.9, 9.8, 19.5 mg/L
	Metric 12:	Testing at or Below Solubility Limit	Low	HHCB was dissolved in DMSO. Study states that DMSO was used as a control for all treatments. However, the authors do not state the concentration of DMSO used to dissolve the HHCB, nor the concentration of DMSO used for the control group.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Nematodes that were mainly in the larval stage were used.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Nematodes were grown on nematode growth medium prior to test initiation.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 nematodes/well were used, in triplicate
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Nematodes were grown in nematode growth medium prior to test initiation. No indication of inappropriate housing conditions.

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Study Citation:	Mori, T., Morita, F., Inokuchi, A., Takao, Y., Kohra, S., Tominaga, N., Takemasa, T., Arizono, K. (2006). Ecotoxicological Effect of Polycyclic Musks on Caenorhabditis elegans. Journal of Health Science 52(3):276.
Duration:	Overall Duration: 0 - 4 days (0-96h); 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:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Caenorhabditis elegans*; Larvae
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5919179

Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	A determination under a microscope was assessed of number of eggs inside a body as well as nematode length.
	Metric 18:	Consistency of Outcome Assessment	High	None noted
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	None identified
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information to suggest outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Data were analyzed via an ANOVA followed by a Dunnetts multiple comparisons test. If homogeneity was observed, a Kruskal-Wallis test was used followed by a Mann-Whitney U test with Bonferroni adjustment.
	Metric 22:	Reporting of Data	High	Statistical differences were determined between controls and treatment groups.
	Metric 23:	Explanation of Unexpected Outcomes	High	The study author referred to other investigations of HHCB exposure to nematodes.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Ramskov, T., Selck, H., Salvito, D., Forbes, V.E. (2009). INDIVIDUAL- AND POPULATION-LEVEL EFFECTS OF THE SYNTHETIC MUSK, HHCB, ON THE DEPOSIT-FEEDING POLYCHAETE, CAPITELLA SP I. Environmental Toxicology and Chemistry 28(12):2695-2705.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Capitella capitata*; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5428042

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by nomenclature.
	Metric 2:	Test Substance Source	High	The HHCB (98.5% purity) was supplied by InternationalFlavors and Fragrances
	Metric 3:	Test Substance Purity	High	98.5% purity
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative control reported.
	Metric 5:	Negative Control Response	High	No unacceptable outcomes in controls reported.
	Metric 6:	Randomized Allocation	Low	random allocation not reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	reported that conditions were adequate but actual values weren't reported
	Metric 8:	Consistency of Exposure Administration	Medium	overall results reported without details
	Metric 9:	Measurement of Test Substance Concentration	High	Concentrations measured by GC-MS, and measured rather than nominal concentrations were used throughout the paper to refer to groups.
	Metric 10:	Exposure Duration and Frequency	Medium	difficult to judge if renewal was adequate, as details weren't provided
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	adequate for dose response
	Metric 12:	Testing at or Below Solubility Limit	High	no indication of solubility issues
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Capitella capitata from 20-year stock culture.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	well reported pre-exposure routine
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of organisms was sufficient.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	reported as acceptable but details were not provided
	Metric 17:	Outcome Assessment Methodology	High	variables that may influence results were well incorporated into the analysis

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Study Citation:	Ramskov, T., Selck, H., Salvito, D., Forbes, V.E. (2009). INDIVIDUAL- AND POPULATION-LEVEL EFFECTS OF THE SYNTHETIC MUSK, HHCB, ON THE DEPOSIT-FEEDING POLYCHAETE, CAPITELLA SP I. Environmental Toxicology and Chemistry 28(12):2695-2705.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Capitella capitata*; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5428042

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across all groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No reported differences among groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	no unrelated information reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	statistical analysis well reported
	Metric 22:	Reporting of Data	High	Data reported fore each treatment & control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes reported

Additional Comments: None

Overall Quality Determination High

Study Citation:	Ramskov, T., Selck, H., Salvito, D., Forbes, V.E. (2009). INDIVIDUAL- AND POPULATION-LEVEL EFFECTS OF THE SYNTHETIC MUSK, HHCB, ON THE DEPOSIT-FEEDING POLYCHAETE, CAPITELLA SP I. Environmental Toxicology and Chemistry 28(12):2695-2705.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Capitella capitata*; Juvenile
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428042

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by nomenclature.
	Metric 2:	Test Substance Source	High	The HHCB (98.5% purity) was supplied by InternationalFlavors and Fragrances
	Metric 3:	Test Substance Purity	High	98.5%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative control reported.
	Metric 5:	Negative Control Response	High	No unacceptable losses in controls.
	Metric 6:	Randomized Allocation	Low	random allocation not reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	reported that conditions were adequate but actual values weren't reported
	Metric 8:	Consistency of Exposure Administration	Medium	overall results reported without details
	Metric 9:	Measurement of Test Substance Concentration	High	Concentrations measured by GC-MS, and measured rather than nominal concentrations were used throughout the paper to refer to groups.
	Metric 10:	Exposure Duration and Frequency	Medium	difficult to judge if renewal was adequate, as details weren't provided
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	The range of concentrations for juvenile mortality was insufficent to observe an LC50, with survivorship at ~71% even at the highest dose. But this was a chronic exposure.
	Metric 12:	Testing at or Below Solubility Limit	High	no indication of solubility issues
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Capitella capitata from 20-year stock culture.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	well reported pre-exposure routine
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of organisms was sufficient.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	reported as acceptable but details were not provided
	Metric 17:	Outcome Assessment Methodology	High	variables that may influence results were well incorporated into the analysis
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across all groups.

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Study Citation:	Ramskov, T., Selck, H., Salvito, D., Forbes, V.E. (2009). INDIVIDUAL- AND POPULATION-LEVEL EFFECTS OF THE SYNTHETIC MUSK, HHCB, ON THE DEPOSIT-FEEDING POLYCHAETE, CAPITELLA SP I. Environmental Toxicology and Chemistry 28(12):2695-2705.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Capitella capitata*; Juvenile
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428042

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No reported differences among groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	no unrelated information reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	data analysis well reported
	Metric 22:	Reporting of Data	High	Data reported for each treatment & control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes reported

Additional Comments: None

Overall Quality Determination High

Study Citation:	Ramskov, T., Selck, H., Salvito, D., Forbes, V.E. (2009). INDIVIDUAL- AND POPULATION-LEVEL EFFECTS OF THE SYNTHETIC MUSK, HHCB, ON THE DEPOSIT-FEEDING POLYCHAETE, CAPITELLA SP I. Environmental Toxicology and Chemistry 28(12):2695-2705.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Capitella capitata*; Adult
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	5428042

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by nomenclature.
	Metric 2:	Test Substance Source	High	The HHCB (98.5% purity) was supplied by InternationalFlavors and Fragrances
	Metric 3:	Test Substance Purity	High	98.5% purity
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative control reported.
	Metric 5:	Negative Control Response	High	No unacceptable outcomes in controls reported.
	Metric 6:	Randomized Allocation	Low	random allocation not reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	reported that conditions were adequate but actual values weren't reported
	Metric 8:	Consistency of Exposure Administration	Medium	overall results reported without details
	Metric 9:	Measurement of Test Substance Concentration	High	Concentrations measured by GC-MS, and measured rather than nominal concentrations were used throughout the paper to refer to groups.
	Metric 10:	Exposure Duration and Frequency	Medium	difficult to judge if renewal was adequate, as details weren't provided
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	adequate for dose response
	Metric 12:	Testing at or Below Solubility Limit	High	no indication of solubility issues
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Capitella capitata from 20-year stock culture.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	well reported pre-exposure routine
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of organisms was sufficient.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	reported as acceptable but details were not provided
	Metric 17:	Outcome Assessment Methodology	High	variables that may influence results were considered during analysis
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across all groups.

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Study Citation:	Ramskov, T., Selck, H., Salvito, D., Forbes, V.E. (2009). INDIVIDUAL- AND POPULATION-LEVEL EFFECTS OF THE SYNTHETIC MUSK, HHCB, ON THE DEPOSIT-FEEDING POLYCHAETE, CAPITELLA SP I. Environmental Toxicology and Chemistry 28(12):2695-2705.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Capitella capitata*; Adult
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	5428042

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No reported differences among groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	no unrelated information reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	statistical methods well documented
	Metric 22:	Reporting of Data	High	Data reported for each treatment & control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes reported

Additional Comments: None

Overall Quality Determination High

Study Citation:	Ramskov, T., Selck, H., Salvito, D., Forbes, V.E. (2009). INDIVIDUAL- AND POPULATION-LEVEL EFFECTS OF THE SYNTHETIC MUSK, HHCB, ON THE DEPOSIT-FEEDING POLYCHAETE, CAPITELLA SP I. Environmental Toxicology and Chemistry 28(12):2695-2705.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Capitella capitata*; Adult
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5428042

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by nomenclature.
	Metric 2:	Test Substance Source	High	The HHCB (98.5% purity) was supplied by InternationalFlavors and Fragrances
	Metric 3:	Test Substance Purity	High	98.5% purity
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative control reported.
	Metric 5:	Negative Control Response	High	No unacceptable outcomes in controls reported.
	Metric 6:	Randomized Allocation	Low	random allocation not reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	reported that conditions were adequate but actual values weren't reported
	Metric 8:	Consistency of Exposure Administration	Medium	overall results reported without details
	Metric 9:	Measurement of Test Substance Concentration	High	Concentrations measured by GC-MS, and measured rather than nominal concentrations were used throughout the paper to refer to groups.
	Metric 10:	Exposure Duration and Frequency	Medium	difficult to judge if renewal was adequate, as details weren't provided
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	adequate for dose response
	Metric 12:	Testing at or Below Solubility Limit	High	no indication of solubility issues
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Capitella capitata from 20-year stock culture.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	well reported pre-exposure routine
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of organisms was sufficient.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	reported as acceptable but details were not provided
	Metric 17:	Outcome Assessment Methodology	High	variables that may influence results were well incorporated into the analysis
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across all groups.

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Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Capitella capitata*; Adult
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5428042

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No reported differences among groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	no unrelated information reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	statistical analysis well reported
	Metric 22:	Reporting of Data	High	Data reported for each treatment & control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes reported

Additional Comments: None

Overall Quality Determination High

Study Citation:	Ramskov, T., Selck, H., Salvito, D., Forbes, V.E. (2009). INDIVIDUAL- AND POPULATION-LEVEL EFFECTS OF THE SYNTHETIC MUSK, HHCB, ON THE DEPOSIT-FEEDING POLYCHAETE, CAPITELLA SP I. Environmental Toxicology and Chemistry 28(12):2695-2705.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Capitella capitata*; Adult
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	5428042

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by nomenclature.
	Metric 2:	Test Substance Source	High	The HHCB (98.5% purity) was supplied by InternationalFlavors and Fragrances
	Metric 3:	Test Substance Purity	High	98.5% purity reported
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Controls used.
	Metric 5:	Negative Control Response	High	No unacceptable outcomes in controls reported.
	Metric 6:	Randomized Allocation	Low	random allocation not reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	reported that conditions were adequate but actual values weren't reported
	Metric 8:	Consistency of Exposure Administration	Medium	overall results reported without exposure details
	Metric 9:	Measurement of Test Substance Concentration	High	Concentrations measured by GC-MS, and measured rather than nominal concentrations were used throughout the paper to refer to groups.
	Metric 10:	Exposure Duration and Frequency	Medium	difficult to judge if renewal was adequate, as details weren't provided
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	adequate for dose response
	Metric 12:	Testing at or Below Solubility Limit	High	no indication of solubility issues
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Capitella capitata from 20-year stock culture.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	well documented pre-exposure routine
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of organisms was sufficient.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	reported as acceptable but details were not provided
	Metric 17:	Outcome Assessment Methodology	High	variables that may influence results were considered in the analysis
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across all groups.

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Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Capitella capitata*; Adult
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	5428042

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No reported differences among groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	no unrelated information reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	data was thoroughly reported
	Metric 22:	Reporting of Data	High	Data reported for each treatment & control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes reported

Additional Comments: None

Overall Quality Determination High

Study Citation:	Ramskov, T., Selck, H., Salvito, D., Forbes, V.E. (2009). INDIVIDUAL- AND POPULATION-LEVEL EFFECTS OF THE SYNTHETIC MUSK, HHCB, ON THE DEPOSIT-FEEDING POLYCHAETE, CAPITELLA SP I. Environmental Toxicology and Chemistry 28(12):2695-2705.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Capitella capitata*; Juvenile
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	5428042

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by nomenclature.
	Metric 2:	Test Substance Source	High	The HHCB (98.5% purity) was supplied by InternationalFlavors and Fragrances
	Metric 3:	Test Substance Purity	High	98.5% purity
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative control reported.
	Metric 5:	Negative Control Response	High	No unacceptable outcomes in controls reported.
	Metric 6:	Randomized Allocation	Low	random allocation not reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	reported that conditions were adequate but actual values weren't reported
	Metric 8:	Consistency of Exposure Administration	Medium	overall results reported without details
	Metric 9:	Measurement of Test Substance Concentration	High	Concentrations measured by GC-MS, and measured rather than nominal concentrations were used throughout the paper to refer to groups.
	Metric 10:	Exposure Duration and Frequency	Medium	difficult to judge if renewal was adequate, as details weren't provided
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	adequate for dose response
	Metric 12:	Testing at or Below Solubility Limit	High	no indication of solubility issues
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Capitella capitata from 20-year stock culture.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	well reported pre-exposure routine
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of organisms was sufficient.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	reported as acceptable but details were not provided
	Metric 17:	Outcome Assessment Methodology	High	variables that may influence results were considered during analysis
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across all groups.

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Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Capitella capitata*; Juvenile
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	5428042

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No reported differences among groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	no unrelated information reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	statistical methods well documented
	Metric 22:	Reporting of Data	High	Data reported fore each treatment & control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes reported

Additional Comments: None

Overall Quality Determination High

Study Citation:	Ramskov, T., Selck, H., Salvito, D., Forbes, V.E. (2009). INDIVIDUAL- AND POPULATION-LEVEL EFFECTS OF THE SYNTHETIC MUSK, HHCB, ON THE DEPOSIT-FEEDING POLYCHAETE, CAPITELLA SP I. Environmental Toxicology and Chemistry 28(12):2695-2705.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Capitella capitata*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428042

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by nomenclature.
	Metric 2:	Test Substance Source	High	The HHCB (98.5% purity) was supplied by InternationalFlavors and Fragrances
	Metric 3:	Test Substance Purity	High	98.5%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative control reported.
	Metric 5:	Negative Control Response	High	No unacceptable losses in controls.
	Metric 6:	Randomized Allocation	Low	random allocation not reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	reported that conditions were adequate but actual values weren't reported
	Metric 8:	Consistency of Exposure Administration	Medium	overall results reported without details
	Metric 9:	Measurement of Test Substance Concentration	High	Concentrations measured by GC-MS, and measured rather than nominal concentrations were used throughout the paper to refer to groups.
	Metric 10:	Exposure Duration and Frequency	Medium	difficult to judge if renewal was adequate, as details weren't provided
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Survivorship was at 90% at the highest dose. But this was a chronic exposure.
	Metric 12:	Testing at or Below Solubility Limit	High	no indication of solubility issues
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Capitella capitata from 20-year stock culture.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	well reported pre-exposure routine
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Number of organisms was sufficient.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	reported as acceptable but details were not provided
	Metric 17:	Outcome Assessment Methodology	High	variables that may influence results were well incorporated into the analysis
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across all groups.

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Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Sediment; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Capitella capitata*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5428042

Domain	Metric		Rating	Comments
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No reported differences among groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	no unrelated information reported
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	data analysis well reported
	Metric 22:	Reporting of Data	High	Data reported for each treatment & control group.
	Metric 23:	Explanation of Unexpected Outcomes	High	no unexpected outcomes reported

Additional Comments: None

Overall Quality Determination High

Study Citation:	Chen, C., Zhou, Q. (2012). Lipid Peroxidation and Gene Expression of Antioxidant Enzymes in Response to Polycyclic Musks in Earthworm Eisenia Fetida. Advanced Materials Research 365:245-+.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; N/A (e.g., injection); Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Not reported; Adult
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)
Chemical:	HHCB
HERO ID:	4690063

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Authors provide both scientific chemical name and CAS number and source of chemical
	Metric 2:	Test Substance Source	High	Chemical manufacturer is reported.
	Metric 3:	Test Substance Purity	High	Purity is 99%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Both a negative and solvent control were run at the same conditions as chemical exposure.
	Metric 5:	Negative Control Response	Low	The control mortality/survival was not reported.
	Metric 6:	Randomized Allocation	Medium	Organisms were randomly sampled from each group.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The study was conducted according to the filter paper contact test as recommended by OECD guideline 207, 1984.
	Metric 8:	Consistency of Exposure Administration	High	The study was conducted according to the filter paper contact test as recommended by OECD guideline 207, 1984.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Nominal concentrations are reported and actual concentrations are likely to be similar to nominal concentrations.
	Metric 10:	Exposure Duration and Frequency	High	The study was conducted according to the filter paper contact test as recommended by OECD guideline 207, 1984.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	Multiple concentrations (n=3) were tested but were able to show results relevant to the outcome of interest.
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent concentration was similar to the negative controls.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	The source of the animals was not discussed but are unlikely to have a substantial impact on results.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	All organisms were acclimatized for two weeks prior to test initiation.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The number of test organisms and replicates was not reported but the sample numbers were reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Biomass loading and food/nutrient conditions were not reported.

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Study Citation:	Chen, C., Zhou, Q. (2012). Lipid Peroxidation and Gene Expression of Antioxidant Enzymes in Response to Polycyclic Musks in Earthworm Eisenia Fetida. Advanced Materials Research 365:245-+.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; N/A (e.g., injection); Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Not reported; Adult
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)
Chemical:	HHCB
HERO ID:	4690063

Domain	Metric		Rating	Comments
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcomes, noting NOEL/LOELS for each effect.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed consistently across time periods.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The authors' did not report differences among the study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No study information was provided on organism attrition or outcomes other than the test chemical.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	One-way analysis of variance (ANOVA) followed by LSD post hoctest for multiple comparisons were used.
	Metric 22:	Reporting of Data	High	No concerns or uncertainties in the outcomes.
	Metric 23:	Explanation of Unexpected Outcomes	High	Data are presented as means and standard deviation. No unexpected outcomes were discussed.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Chen, C., Zhou, Q., Liu, S., Xiu, Z. (2011). Acute toxicity, biochemical and gene expression responses of the earthworm Eisenia fetida exposed to polycyclic musks. Chemosphere 83(8):1147-1154.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; N/A (e.g., injection); Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	4690069

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCD was identified as the test substance
	Metric 2:	Test Substance Source	High	HHCB was obtained from Zhongkejian Chemical Co. Ltd (Tianjin, China)
	Metric 3:	Test Substance Purity	High	Purity was reported to be 99%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls were used (both acetone and deionized water)
	Metric 5:	Negative Control Response	High	No mortality was observed in the control group
	Metric 6:	Randomized Allocation	Medium	To control for variation in size, adult worms within a certain size range were selected. After the experiment worms were randomly selected from each treatment to measure mechanistic endpoints
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Experimental tubes were capped to prevent volatilization of HHCB
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent and used acetone as a solvent
	Metric 9:	Measurement of Test Substance Concentration	Low	Concentrations were not verified analytically
	Metric 10:	Exposure Duration and Frequency	High	Exposure was appropriate - 72 hours
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The concentrations of HHCB were prepared as 2.0, 3.2, 5.1, 8.2, 13.1, 21.0,2,33.6, 53.7, and 85.9 ug/cm, equivalent to 125–1.5376 ug/mL
	Metric 12:	Testing at or Below Solubility Limit	Medium	Exposure was via filter paper moistened with water. Some concentrations exceed the water solubility of HHCB, but a solvent was used.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Adult earthworms with a weight of 400 ± 50 mg and a ”well-developed clitellum” were used
	Metric 14:	Acclimatization and Pretreatment Conditions	High	earthworms were acclimatized for 24 hours to allow for egestion of gut contents
	Metric 15:	Number of Organisms and Replicates per Group	Medium	20 replicate individuals per exposure concentration
Domain 5: Outcome Assessment				

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Study Citation:	Chen, C., Zhou, Q., Liu, S., Xiu, Z. (2011). Acute toxicity, biochemical and gene expression responses of the earthworm Eisenia fetida exposed to polycyclic musks. Chemosphere 83(8):1147-1154.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; N/A (e.g., injection); Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	4690069

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Organisms were not fed during the toxicity study, and were housed in test tubes with filter paper.
	Metric 17:	Outcome Assessment Methodology	High	Mortality and sickness were recorded every 24 hours. ”Worms were considered dead when they did not respond togentle mechanical stimulus.” and ”Sick worms were recorded if themorphological symptoms were observed, that is, coelomic fluidexcretion, inflammation, and bleeding.”
	Metric 18:	Consistency of Outcome Assessment	High	Mortality was assessed consistently across groups
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Unclear if there were any confounding variables.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Sick worms were observed, but associated with exposure
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Probit analysis was conducted to estimate LC50 values with 95% CIs
	Metric 22:	Reporting of Data	High	Data were reported for all test concentrations and controls, as well as LC50 endpoints
	Metric 23:	Explanation of Unexpected Outcomes	High	Authors discuss the higher toxicity observed in their study (filter paper) compared with Balk and Ford, 1999 (exposure to artificial soil with 10% peat) due to possible sorption with organic matter reducing the bioavailability.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Chen, C., Zhou, Q., Liu, S., Xiu, Z. (2011). Acute toxicity, biochemical and gene expression responses of the earthworm Eisenia fetida exposed to polycyclic musks. Chemosphere 83(8):1147-1154.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; N/A (e.g., injection); Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)
Chemical:	HHCB
HERO ID:	4690069

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	HHCD was identified as the test substance
	Metric 2:	Test Substance Source	High	HHCB was obtained from Zhongkejian Chemical Co. Ltd (Tianjin, China)
	Metric 3:	Test Substance Purity	High	Purity was reported to be 99%
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls were used (both acetone and deionized water)
	Metric 5:	Negative Control Response	High	No mortality was observed in the control group
	Metric 6:	Randomized Allocation	Medium	To control for variation in size, adult worms within a certain size range were selected. After the experiment worms were randomly selected from each treatment to measure mechanistic endpoints
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Experimental tubes were capped to prevent volatilization of HHCB
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent and used acetone as a solvent
	Metric 9:	Measurement of Test Substance Concentration	Low	Concentrations were not verified analytically
	Metric 10:	Exposure Duration and Frequency	High	Exposure was appropriate - 72 hours
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	For the time dependence of lipid peroxidation levels and gene expression levels qPCR - concentrations of 0.3 and 3.0 ug/cm were used. Goal was not dose-response.
	Metric 12:	Testing at or Below Solubility Limit	Medium	Exposure was via filter paper moistened with water. Some concentrations exceed the water solubility of HHCB, but a solvent was used.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Adult earthworms with a weight of 400 $\pm$ 50 mg and a "well-developed clitellum" were used
	Metric 14:	Acclimatization and Pretreatment Conditions	High	earthworms were acclimatized for 24 hours to allow for egestion of gut contents
	Metric 15:	Number of Organisms and Replicates per Group	Low	Unclear how many organisms were used for the mechanistic endpoints. Also unclear if technical replicates were used to determine variation within qPCR plates and the protein assay.
Domain 5: Outcome Assessment				

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Study Citation:	Chen, C., Zhou, Q., Liu, S., Xiu, Z. (2011). Acute toxicity, biochemical and gene expression responses of the earthworm Eisenia fetida exposed to polycyclic musks. Chemosphere 83(8):1147-1154.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; N/A (e.g., injection); Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)
Chemical:	HHCB
HERO ID:	4690069

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Organisms were not fed during the toxicity study, and were housed in test tubes with filter paper.
	Metric 17:	Outcome Assessment Methodology	Low	Protein concentration and lipid peroxidation (MDA and - methods cited to other papers. RT- qPCR (Transcriptional level of target genes SOD, CAT, and HSP70) methods are reported, however RNA quality (RIN number) was not reported and it is unclear how many replicates there were. Efficiency of the qPCR primers for each target gene was also not reported.
	Metric 18:	Consistency of Outcome Assessment	High	Lipid peroxidation and gene expression were examined at the same time points
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Unclear if there were any confounding variables.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information suggested outcomes unrelated to exposure
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Probit analysis was conducted to estimate LC50 values with 95% CIs
	Metric 22:	Reporting of Data	High	Data were reported for all test concentrations and controls, as well as LC50 endpoints
	Metric 23:	Explanation of Unexpected Outcomes	High	Authors discuss the higher toxicity observed in their study (filter paper) compared with Balk and Ford, 1999 (exposure to artificial soil with 10% peat) due to possible sorption with organic matter reducing the bioavailability.

Additional Comments: Assessment for for mechanistic endpoints:1) Protein concentration (MDA) and lipid peroxidation (LPO), Methods cited to other papers2) RT- qPCR - transcriptional level of target genes SOD, CAT, and HSP70.

Overall Quality Determination High

Study Citation:	Liu, S., Zhou, Q., Chen, C. (2012). Antioxidant enzyme activities and lipid peroxidation in earthworm Eisenia fetida exposed to 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-gamma-2-benzopyran. Environmental Toxicology 27(8):472-479.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; N/A (e.g., injection); Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	3406502

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	The HHCB was identified by name as well as by molecular formula. No CASRN was provided, thus the downgrade to a medium rating.
	Metric 2:	Test Substance Source	Low	The source of the HHCB was reported to be the Zhong Kejian Chemical Co. in Tianjin, China. However, it was not reported if the HHCB was analytically verified.
	Metric 3:	Test Substance Purity	Medium	The purity of the HHCB was reported to be 77.4%. This is a bit lower than is typical, thus the downgrade to medium.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	The study authors reported the use of an appropriate concurrent negative control. Control vials were reported to have 1mL of the appropriate solvent that was later evaporated.
	Metric 5:	Negative Control Response	Low	The negative control response was not reported. All that was reported was that there was no mortality for E. fetida in all tests.
	Metric 6:	Randomized Allocation	Low	It was not reported how the worms were allocated into the study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The test system consisted of flat-bottomed glass vials 27mm in diameter and 75mm in length. They were covered with perforated plastic film. The exposure was via filter paper lining the bottom of the vial with dimensions of 85 x 75mm. The HHCB was prepared by dissolving it with acetone, but details regarding how the concentrations were prepared were limited and did not appear to be measured. 1mL of the HHCB concentrations were pipetted into each vial and were then evaporated. The dry filter paper was then moistened with distilled water; the worms were then added to the test system.
	Metric 8:	Consistency of Exposure Administration	High	All exposures were conducted in glass vials of the same dimensions. 85 x 75mm filter paper was used in each vial for the exposure. 1mL of each test concentrations was placed on each filter paper and allowed to dry. The paper was then moistened with 1mL of distilled water, and the worms were placed in the test system. There was one worm per vial with 5 replicates for each test concentration. All tests were carried out in the dark for 1-3 days.
	Metric 9:	Measurement of Test Substance Concentration	Low	It was not reported if the test concentrations were measured at any point in the study.
	Metric 10:	Exposure Duration and Frequency	High	The study duration of 1-3 days was adequate to observe the outcomes of interest.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure concentrations, ranging from 0.00157 to 3.14ug/cm^2. The spacing was adequate to observe the outcomes of interest.

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Study Citation:	Liu, S., Zhou, Q., Chen, C. (2012). Antioxidant enzyme activities and lipid peroxidation in earthworm Eisenia fetida exposed to 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-gamma-2-benzopyran. Environmental Toxicology 27(8):472-479.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; N/A (e.g., injection); Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	3406502

Domain	Metric		Rating	Comments
	Metric 12:	Testing at or Below Solubility Limit	High	The solubility was reported to be below the water solubility limit. The HHCB was also dissolved in acetone before it was applied to the filter paper.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were reported to be from the Lutai Earthworm Breeding Farm located in Tianjin, China. The earthworms used in the study were mature adults with well developed clitella weighing 350-450mg.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the worms were acclimated to test conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	It was reported that there were 5 replicates for each test concentration. There was one worm per test chamber, which is standard to OECD Guideline 207 that is cited in the reference.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	The earthworms were kept in the loam they were delivered in at 20C and were fed cow excrement prior to testing. Water was added to maintain moist conditions, but moisture content was not reported. It was not reported if they were kept in complete darkness during holding. For testing, the worms were rinsed in water and dried and allowed to void their guts 24h prior to testing. They were then kept at 20C for the duration of the test and kept in the dark.
	Metric 17:	Outcome Assessment Methodology	Low	The study was not designed to determine HHCB effects on survival/mortality. The authors simply state that no worms died. It was not an endpoint of interest.
	Metric 18:	Consistency of Outcome Assessment	Low	Details regarding the execution of the study protocol for outcome assessment were not reported. No details were provided on the assessment of mortality. The authors were not interested in HHCB effects on survival. they simply stated that no worms died in the experiments where oxidative stress measures were the outcomes.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The experiments were carried out for 1, 2, and 3day(s) at a constant temperature(208C6 18C) and in the dark. This indicates that all experimental units were kept in these conditions.
	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 the exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Uninformative	No statistical methods were reported for mortality. No deaths occurred and mortality was not of interest.

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Study Citation:	Liu, S., Zhou, Q., Chen, C. (2012). Antioxidant enzyme activities and lipid peroxidation in earthworm Eisenia fetida exposed to 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-gamma-2-benzopyran. Environmental Toxicology 27(8):472-479.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; N/A (e.g., injection); Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	3406502

Domain	Metric		Rating	Comments
	Metric 22:	Reporting of Data	Low	The results for mortality were reported in the text only under the ”Tested Earthworm Species” section.
	Metric 23:	Explanation of Unexpected Outcomes	Low	Study authors did not report any unexpected outcomes. Results were reported in the text, and no variability was reported.

Additional Comments: This study looked at the effect of HHCB on earthworms. Mortality was not the main outcome of interest. Study authors did report that ”there were no mortality of E. fetida in all tests.” Thus mortality was evaluated here. This evaluation received an unacceptable rating because no statistical analysis was reported for mortality.

Overall Quality Determination High

Study Citation:	Liu, S., Zhou, Q., Chen, C. (2012). Antioxidant enzyme activities and lipid peroxidation in earthworm Eisenia fetida exposed to 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-gamma-2-benzopyran. Environmental Toxicology 27(8):472-479.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; N/A (e.g., injection); Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)
Chemical:	HHCB
HERO ID:	3406502

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	The HHCB was identified by name as well as by molecular formula. No CASRN was provided, thus the downgrade to a medium rating.
	Metric 2:	Test Substance Source	Low	The source of the HHCB was reported to be the Zhong Kejian Chemical Co. in Tianjin, China. However, it was not reported if the HHCB was analytically verified.
	Metric 3:	Test Substance Purity	Medium	The purity of the HHCB was reported to be 77.4%. This is a bit lower than is typical, thus the downgrade to medium.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	The study authors reported the use of an appropriate concurrent negative control. Control vials were reported to have 1mL of the appropriate solvent that was later evaporated.
	Metric 5:	Negative Control Response	High	The negative control response was reported in Figures 1-4. It was adequate for the outcomes of interest.
	Metric 6:	Randomized Allocation	Low	It was not reported how the worms were allocated into the study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	The test system consisted of flat-bottomed glass vials 27mm in diameter and 75mm in length. They were covered with perforated plastic film. The exposure was via filter paper lining the bottom of the vial with dimensions of 85 x 75mm. The HHCB was prepared by dissolving it with acetone, but details regarding how the concentrations were prepared were limited and did not appear to be measured. 1mL of the HHCB concentrations were pipetted into each vial and were then evaporated. The dry filter paper was then moistened with distilled water; the worms were then added to the test system.
	Metric 8:	Consistency of Exposure Administration	High	All exposures were conducted in glass vials of the same dimensions. 85 x 75mm filter paper was used in each vial for the exposure. 1mL of each test concentrations was placed on each filter paper and allowed to dry. The paper was then moistened with 1mL of distilled water, and the worms were placed in the test system. There was one worm per vial with 5 replicates for each test concentration. All tests were carried out in the dark for 1-3 days.
	Metric 9:	Measurement of Test Substance Concentration	Low	It was not reported if the test concentrations were measured at any point in the study.
	Metric 10:	Exposure Duration and Frequency	High	The study duration of 1-3 days was adequate to observe the outcomes of interest.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure concentrations, ranging from 0.00157 to 3.14ug/cm^2. The spacing was adequate to observe the outcomes of interest.
	Metric 12:	Testing at or Below Solubility Limit	High	The solubility was reported to be below the water solubility limit. The HHCB was also dissolved in acetone before it was applied to the filter paper.

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Study Citation:	Liu, S., Zhou, Q., Chen, C. (2012). Antioxidant enzyme activities and lipid peroxidation in earthworm Eisenia fetida exposed to 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-gamma-2-benzopyran. Environmental Toxicology 27(8):472-479.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; N/A (e.g., injection); Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)
Chemical:	HHCB
HERO ID:	3406502

Domain	Metric		Rating	Comments
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organisms were reported to be from the Lutai Earthworm Breeding Farm located in Tianjin, China. The earthworms used in the study were mature adults with well developed clitella weighing 350-450mg.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if the worms were acclimated to test conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	It was reported that there were 5 replicates for each test concentration. There was one worm per test chamber, which is standard to OECD Guideline 207 that is cited in the reference.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	The earthworms were kept in the loam they were delivered in at 20C and were fed cow excrement prior to testing. Water was added to maintain moist conditions, but moisture content was not reported. It was not reported if they were kept in complete darkness during holding. For testing, the worms were rinsed in water and dried and allowed to void their guts 24h prior to testing. They were then kept at 20C for the duration of the test and kept in the dark.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcomes of interest–mechanistic outcomes including SOD, CAT, POD, and MDA levels.
	Metric 18:	Consistency of Outcome Assessment	High	Earthworms were used in 3 different experiments; one experiment had a 1-day exposure, another experiment had a 2-day exposure and a third experiment had a 3-day exposure period. The authors were ambiguous about this, but since the earthworms were destructively sampled, the number of replicate would have changed otherwise. The worms were destructively sampled for SOD, CAT, POD, and MDA levels.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The experiments were carried out for1,2,and3day(s)at a constant temperature(208C6 18C) and in the dark. The authors description seems to include all of the experimental groups receiving these same condition.
	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 the exposure.
Domain 7: Data Presentation and Analysis				

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Study Citation:	Liu, S., Zhou, Q., Chen, C. (2012). Antioxidant enzyme activities and lipid peroxidation in earthworm Eisenia fetida exposed to 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethyl-cyclopenta-gamma-2-benzopyran. Environmental Toxicology 27(8):472-479.
Duration:	Overall Duration: 0 - 4 days (0-96h); Exposure Duration: 0 - 4 days (0-96h)
Exposure Route, Media, Path:	Terrestrial; N/A (e.g., injection); Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)
Chemical:	HHCB
HERO ID:	3406502

Domain	Metric		Rating	Comments
	Metric 21:	Statistical Methods	High	Results were expressed as mean +- standard deviation, and they were subjected to analysis of variance with factors of HHCB concentrations and three time intervals. When significant differences were considered between treatments, multiple comparisons were made by the least significant difference test. Degrees of freedom and F-values were not reported. thus, it is difficult to determine if they did the analyses correctly. However, it should have been a straightforward set of analyses.
	Metric 22:	Reporting of Data	High	Data was reported in Figures 1-4 and were appropriate. Results were reported for SOD, CAT, POD, and MDA.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability was reported in the figures.

Additional Comments: This study looked at the effect of HHCB on earthworm SOD, CAT, POD, and MDA activity. The exposure was via filter paper with 5 test concentrations. The worms were sampled daily for 3 days. Mechanistic outcomes were the outcomes of interest.

Overall Quality Determination High

Study Citation:	Chen, C., Xue, S., Zhou, Q., Xie, X. (2011). Multilevel ecotoxicity assessment of polycyclic musk in the earthworm Eisenia fetida using traditional and molecular endpoints. Ecotoxicology 20(8):1949-1958.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Not reported.; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5352379

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	Chemical name reported- form and specific identifying details not provided but based on available information about the chemical substance, these are not likely to affect the reliability of the study results.
	Metric 2:	Test Substance Source	High	Purchased from Zhongkejian Chemical Co. Ltd, Tianjin, China.
	Metric 3:	Test Substance Purity	High	The purity was 99%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Solvent control reported- No negative control reported, as no mortality was reported in the solvent control, this is not expected to have a substantial effect on the results.
	Metric 5:	Negative Control Response	High	No mortality in the solvent control.
	Metric 6:	Randomized Allocation	Low	Authors did not report allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	System and test preparation were described, and the authors took measures to reduce the potential evaporation of the test material, but ultimately, they did not report the measured test concentrations. In the case of mortality of the test organisms, the highly linear dose response indicated that this lack of measured test concentrations was unlikely to have an effect on the outcome of the test.
	Metric 8:	Consistency of Exposure Administration	High	Exposure was reported and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Nominal concentrations reported but based on the linear dose-response, the lack of reporting is not expected to have and impact on results.
	Metric 10:	Exposure Duration and Frequency	High	Seven-day lethal study.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The geometric concentration series of HHCBwere prepared were 0 (solvent control), 100.0, 140.0, 196.0, 274.4, 384.2, 537.8, 752.9, 1054.1, and 1475.8 lg g-1 air-dried soil, respectively. The dose-series and response of the test organisms was sufficient to calculate an LC50.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent used and no interactions between control and test chemical.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Obtained from the Earthworm Breeder Company in Tianjin, China. They were acclimatized for 2 months under the laboratory conditions.

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Study Citation:	Chen, C., Xue, S., Zhou, Q., Xie, X. (2011). Multilevel ecotoxicity assessment of polycyclic musk in the earthworm Eisenia fetida using traditional and molecular endpoints. Ecotoxicology 20(8):1949-1958.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Not reported.; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5352379

Domain		Metric	Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimatized for 2 months under the laboratory conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 organisms and 4 replicates were used in the study. This is consistent with the test guideline recommendations.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Biomass loading was not reported, but test conditions were reported and the organisms were fed a consistent diet.
	Metric 17:	Outcome Assessment Methodology	High	The study addressed or reported the intended outcome.
	Metric 18:	Consistency of Outcome Assessment	High	The outcomes were consistent and measured at the same durations.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The study did not report differences in the study conditions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	The authors did not report differences attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	A computer program Probit was used to calculate 7 day- and 14 day-LC50 value as following its 95% confidence interval.
	Metric 22:	Reporting of Data	High	Data reported on Figure 1 and p. 1953 for each treatment with an endpoint determination.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Chen, C., Xue, S., Zhou, Q., Xie, X. (2011). Multilevel ecotoxicity assessment of polycyclic musk in the earthworm Eisenia fetida using traditional and molecular endpoints. Ecotoxicology 20(8):1949-1958.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Not reported.; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5352379

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	Chemical name reported- form and specific identifying details not provided but based on available information about the chemical substance, these are not likely to affect the reliability of the study results.
	Metric 2:	Test Substance Source	High	Purchased from Zhongkejian Chemical Co. Ltd, Tianjin, China.
	Metric 3:	Test Substance Purity	High	The purity was 99%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Solvent control reported- No negative control reported, as no mortality was reported in the solvent control, this is not expected to have a substantial effect on the results.
	Metric 5:	Negative Control Response	High	No mortality in the solvent control.
	Metric 6:	Randomized Allocation	Low	Authors did not report allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	System and test preparation were described, and the authors took measures to reduce the potential evaporation of the test material, but ultimately, they did not report the measured test concentrations. In the case of mortality of the test organisms, the highly linear dose response indicated that this lack of measured test concentrations was unlikely to have an effect on the outcome of the test.
	Metric 8:	Consistency of Exposure Administration	High	Exposure was reported and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Nominal concentrations reported but based on the linear dose-response, the lack of reporting is not expected to have and impact on results.
	Metric 10:	Exposure Duration and Frequency	High	14 day Lethal study.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The geometric concentration series of HHCBwere prepared were 0 (solvent control), 100.0, 140.0, 196.0, 274.4, 384.2, 537.8, 752.9, 1054.1, and 1475.8 lg g-1 air-dried soil, respectively. The dose-series and response of the test organisms was sufficient to calculate an LC50.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent used and no interactions between control and test chemical.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Obtained from the Earthworm Breeder Company in Tianjin, China. They were acclimatized for 2 months under the laboratory conditions.

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Study Citation:	Chen, C., Xue, S., Zhou, Q., Xie, X. (2011). Multilevel ecotoxicity assessment of polycyclic musk in the earthworm Eisenia fetida using traditional and molecular endpoints. Ecotoxicology 20(8):1949-1958.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Not reported.; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5352379

Domain	Metric		Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimatized for 2 months under the laboratory conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 organisms and 4 replicates were used in the study. This is consistent with the test guideline recommendations.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Biomass loading was not reported, but test conditions were reported and the organisms were fed a consistent diet.
	Metric 17:	Outcome Assessment Methodology	High	The study addressed or reported the intended outcome.
	Metric 18:	Consistency of Outcome Assessment	High	The outcomes were consistent and measured at the same durations.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The study did not report differences in the study conditions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	The authors did not report differences attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	A computer program Probit was used to calculate 7 day- and 14 day-LC50 value as following its 95% confidence interval.
	Metric 22:	Reporting of Data	High	Data reported on Figure 1 and p. 1952 for each treatment with an endpoint determination.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes.
Additional Comments:	None			

Overall Quality Determination — High

Study Citation:	Goßmann, A. (1997). Effects of HHCB on reproduction and growth of earthworms Eisenia fetida in artificial soil.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application), Dietary
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	7607848

Domain		Metric	Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test material was definitely identified as HHCB with necessary nomenclature and identifier numbers. The Chemical form was also appropriately described in the study report.
	Metric 2:	Test Substance Source	High	The test substance source was described as the test sponsor. Batch number and analytical verification were provided, including physical-Chemical properties.
	Metric 3:	Test Substance Purity	Low	Test substance Purity/Grade were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Concurrent negative and solvent controls were utilized in the study.
	Metric 5:	Negative Control Response	High	Negative control mortality was <10% and no significant changes in body weight, reproduction, or food consumption were reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not explicitly outline randomization procedure.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Exposure through treated artificial soil is appropriate for the test species. Test vessels were covered to prevent loss of test material through evaporation.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups, including the use of acetone as a solvent carrier.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, based on previous testing, there is a potential for degradation of the test material throughout the course of the test.
	Metric 10:	Exposure Duration and Frequency	High	The 14d exposure was appropriate for a range finding test to establish definitive concentrations.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Geometrically increasing concentration series was appropriate.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent (acetone) was utilized to ensure exposure to test medium to account for low solubility. Exposure procedure was appropriately characterized.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The study did not indicate the source of the test organisms, however the test organisms were bred by the study authors under appropriate husbandry guideline (OECD 207). Life-stage of the adults was appropriate and consistent with test guidelines.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Worms were acclimated in artificial soil for 4 days prior to exposure to the test material. It was unclear whether test organism digestive tracts were evacuated prior to the initial exposures to the test media, which could have delayed effects.

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Study Citation:	Goßmann, A. (1997). Effects of HHCB on reproduction and growth of earthworms Eisenia fetida in artificial soil.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application), Dietary
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	7607848

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Low	The range finding test used only 10 worms per test concentration. This is acceptable for a range finding test.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were consistent with the test guideline except for temperature. This deviation was explained and background references were provided, and the deviation had no effect on the control organisms.
	Metric 17:	Outcome Assessment Methodology	High	Assessment was adequate to characterize the effects from chronic exposure to the test material. Exposure concentrations were sufficiently determined and spaced to calculate the endpoints of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Conditions and exposure methodology were consistently applied among replications and test concentrations.
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	High	Details regarding test organism attrition and outcomes unrelated to exposure were reported for each study group and there were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was not reported for the range finding test, but raw data was reported in Table 1, so an independent analysis is possible.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoints of interest
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: This evaluation is for the 14d range finding test. Body weight was assessed at day 14d. Results were reported in Table 1.

Overall Quality Determination High

Study Citation:	Goßmann, A. (1997). Effects of HHCB on reproduction and growth of earthworms Eisenia fetida in artificial soil.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application), Dietary
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	7607848

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test material was definitely identified as HHCB with necessary nomenclature and identifier numbers. The Chemical form was also appropriately described in the study report.
	Metric 2:	Test Substance Source	High	The test substance source was described as the test sponsor. Batch number and analytical verification were provided, including physical-Chemical properties.
	Metric 3:	Test Substance Purity	Low	Test substance Purity/Grade were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Concurrent negative and solvent controls were utilized in the study.
	Metric 5:	Negative Control Response	High	Negative control mortality was <10% and no significant changes in body weight, reproduction, or food consumption were reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not explicitly outline randomization procedure.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Exposure through treated artificial soil is appropriate for the test species. Test vessels were covered to prevent loss of test material through evaporation.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups, including the use of acetone as a solvent carrier.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, based on previous testing, there is a potential for degradation of the test material throughout the course of the test.
	Metric 10:	Exposure Duration and Frequency	High	The 14d exposure was appropriate for a range finding test to establish definitive concentrations.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Geometrically increasing concentration series was appropriate.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent (acetone) was utilized to ensure exposure to test medium to account for low solubility. Exposure procedure was appropriately characterized.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The study did not indicate the source of the test organisms, however the test organisms were bred by the study authors under appropriate husbandry guideline (OECD 207). Life-stage of the adults was appropriate and consistent with test guidelines.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Worms were acclimated in artificial soil for 4 days prior to exposure to the test material. It was unclear whether test organism digestive tracts were evacuated prior to the initial exposures to the test media, which could have delayed effects.
	Metric 15:	Number of Organisms and Replicates per Group	Low	The range finding test used only 10 worms per test concentration. This is acceptable for a range finding test.

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Study Citation:	Goßmann, A. (1997). Effects of HHCB on reproduction and growth of earthworms Eisenia fetida in artificial soil.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application), Dietary
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	7607848

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were consistent with the test guideline except for temperature. This deviation was explained and background references were provided, and the deviation had no effect on the control organisms.
	Metric 17:	Outcome Assessment Methodology	High	Assessment was adequate to characterize the effects from chronic exposure to the test material. Exposure concentrations were sufficiently determined and spaced to calculate the endpoints of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Conditions and exposure methodology were consistently applied among replications and test concentrations.
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	High	Details regarding test organism attrition and outcomes unrelated to exposure were reported for each study group and there were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was not reported for the range finding test, but raw data was reported in Table 1, so an independent analysis is possible.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoints of interest
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: This evaluation is for the 14d range finding test. Mortality was assessed at day 7 and at day 14. Results are presented in Table 1.

Overall Quality Determination High

Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by nomenclature and formula.
	Metric 2:	Test Substance Source	High	Purchased from Guoyao Chemical Co. Ltd. (Beijing, China)
	Metric 3:	Test Substance Purity	High	Given as ">98% purity".
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls and solvent controls were used.
	Metric 5:	Negative Control Response	High	No difference between solvent controls and negative controls, no reported mortality in controls.
	Metric 6:	Randomized Allocation	Medium	Distribution was made by mass ("10 well developed adults (300-400 mg)")
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	HHCB was mixed with soil and it is unclear whether sealed vessels were used. As HHCB can volatilize from soil in the long term and nominal concentrations were not tested, there may be some discrepancy. The test duration was 14 days, and Schafer 2005 reported a volatilization of up to 16% after 28 days. This discrepancy is unlikely to have a major effect on results since it was replicated across all treatments.
	Metric 8:	Consistency of Exposure Administration	High	Exposure same for all treatments.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were not measured, however HHCB does not rapidly biodegrade and sorbs to soil somewhat strongly. The discrepancy between nominal and actual exposure concentrations is unlikely to substantially effect the results.
	Metric 10:	Exposure Duration and Frequency	High	The study was conducted in accordance with OECD 207. Duration and frequency were appropriate.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Range and spacing of exposure levels were given for all treatments & were adequate to develop an LC50 with reasonable confidence intervals.
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent used (acetone) did not cause measurable responses relative to negative controls.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Worms were obtained from in-house culture at the Chinese Research Academy of Environmental Sciences. Rearing conditions were described and seem adequate for the species chosen.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Worms were reared in the same conditions as the toxicity test.

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Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 worms were used per replicate, with 4 replicates per treatment and controls.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Conditions were appropriate for the worms and were the same for all groups & were consistent before treatment.
	Metric 17:	Outcome Assessment Methodology	High	Mortality was measured at the end of the test (14d) according to OECD 207.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed the same across all groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences in environmental conditions across groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported details that suggest differences in attrition or other health outcomes.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was adequately described in the methods section and appropriate for the experiments performed.
	Metric 22:	Reporting of Data	Low	Summary data (LC50) was reported for the overall experiment for each endpoint, but individual data for each treatment and control was not reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, and the outcomes that were reported are adequately discussed in the text.

Additional Comments: This study also includes the calculation of an SSD from all 10 tested terrestrial organisms and the derivation of PNECs from said distribution, with discussion of AFs. In my opinion it would provide an important contribution to terrestrial data for HHCB.

Overall Quality Determination High

Study Citation:	Chen, C., Xue, S., Zhou, Q., Xie, X. (2011). Multilevel ecotoxicity assessment of polycyclic musk in the earthworm Eisenia fetida using traditional and molecular endpoints. Ecotoxicology 20(8):1949-1958.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Not reported.; Adult
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5352379

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	Chemical name reported- form and specific identifying details not provided but based on available information about the chemical substance, these are not likely to affect the reliability of the study results.
	Metric 2:	Test Substance Source	High	Purchased from Zhongkejian Chemical Co. Ltd, Tianjin, China.
	Metric 3:	Test Substance Purity	High	The purity was 99%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Solvent control reported- No negative control reported, as no mortality was reported in the solvent control, this is not expected to have a substantial effect on the results.
	Metric 5:	Negative Control Response	High	No mortality was reported in the solvent controls.
	Metric 6:	Randomized Allocation	Low	Authors' did not report allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	System and Test preparation were described, and the authors took measures to reduce the potential evaporation of the test material, but ultimately, they did not report the measured test concentrations. For growth effects, the somewhat linear dose response indicated that this lack of measured test concentrations was unlikely to have an effect on the outcome of the test.
	Metric 8:	Consistency of Exposure Administration	High	Exposure was reported and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Nominal concentrations reported but based on the linear dose-response, the lack of reporting is not expected to have and impact on results.
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration lasted the entirety or the twenty-eight (28) day chronic study.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The geometric concentration series of HHCBwere prepared were 0 (solvent control), 3, 10, 30, 50and 100 lg g-1 (dry mass of soil), respectively. The dose-series was sufficient to calculate a NOAEC for growth effects based on a 40% reduction in growth effects at the highest test concentrations.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent used and no interactions between solvent control and test organisms.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Obtained from the Earthworm Breeder Company in Tianjin, China. They were acclimatized for 2 months under the laboratory conditions.

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Study Citation:	Chen, C., Xue, S., Zhou, Q., Xie, X. (2011). Multilevel ecotoxicity assessment of polycyclic musk in the earthworm Eisenia fetida using traditional and molecular endpoints. Ecotoxicology 20(8):1949-1958.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Not reported.; Adult
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5352379

Domain	Metric		Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimatized for 2 months under the laboratory conditions.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 organisms and 4 replicates were used in the study. This is consistent with the test guideline recommendations.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Biomass loading was not reported, but test conditions were reported and the organisms were fed a consistent diet.
	Metric 17:	Outcome Assessment Methodology	High	The study addressed or reported the intended outcome.
	Metric 18:	Consistency of Outcome Assessment	High	The outcomes were consistent and measured at the same durations.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The study did not report differences in the study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	The authors' did not report differences attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	A one-way analysis of variance (ANOVA) followed by Dunnett's comparison with a control group were used.
	Metric 22:	Reporting of Data	High	Data reported on T. 3 for each treatment with an endpoint determination.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes.
Additional Comments:	None			

Overall Quality Determination High

Study Citation:	Chen, C., Xue, S., Zhou, Q., Xie, X. (2011). Multilevel ecotoxicity assessment of polycyclic musk in the earthworm Eisenia fetida using traditional and molecular endpoints. Ecotoxicology 20(8):1949-1958.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Not reported.; Adult
Health Outcome:	Mechanistic-Genotox (including DNA repair)
Chemical:	HHCB
HERO ID:	5352379

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	Chemical name reported- form and specific identifying details not provided but based on available information about the chemical substance, these are not likely to affect the reliability of the study results.
	Metric 2:	Test Substance Source	High	Purchased from Zhongkejian Chemical Co. Ltd, Tianjin, China.
	Metric 3:	Test Substance Purity	High	The purity was 99%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Solvent control reported- No negative control reported, as no mortality was reported in the solvent control, this is not expected to have a substantial effect on the results.
	Metric 5:	Negative Control Response	High	No mortality was reported in the solvent controls.
	Metric 6:	Randomized Allocation	Low	Authors did not report allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	System and Test preparation were described, and the authors took measures to reduce the potential evaporation of the test material, but ultimately, they did not report the measured test concentrations. For genetic effects, the irregular and non-linear dose response indicated that this lack of measured test concentrations could have had an effect on the outcome of the test.
	Metric 8:	Consistency of Exposure Administration	High	Exposure was reported and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Nominal concentrations reported and based on observed dose-response, the lack of reporting are unlikely to impact the results.
	Metric 10:	Exposure Duration and Frequency	High	Nominal concentrations reported but based on the linear dose-response, the lack of reporting is not expected to have and impact on results.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The geometric concentration series of HHCBwere prepared were 0 (solvent control), 3, 10, 30, 50and 100 lg g-1 (dry mass of soil), respectively. The dose-series was sufficient to calculate a LOAEC for gene expression.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent used and no interactions between control and test organisms.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Obtained from the Earthworm Breeder Company in Tianjin, China. They were acclimatized for 2 months under the laboratory conditions.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimatized for 2 months under the laboratory conditions.

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Study Citation:	Chen, C., Xue, S., Zhou, Q., Xie, X. (2011). Multilevel ecotoxicity assessment of polycyclic musk in the earthworm Eisenia fetida using traditional and molecular endpoints. Ecotoxicology 20(8):1949-1958.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Not reported.; Adult
Health Outcome:	Mechanistic-Genotox (including DNA repair)
Chemical:	HHCB
HERO ID:	5352379

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 organisms and 4 replicates were used in the study. This is consistent with the test guideline recommendations.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Biomass loading was not reported, but test conditions were reported and the organisms were fed a consistent diet.
	Metric 17:	Outcome Assessment Methodology	High	The study addressed or reported the intended outcome.
	Metric 18:	Consistency of Outcome Assessment	High	The outcomes were consistent and measured at the same durations.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The study did not report differences in the study conditions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	The authors did not report differences attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	A one-way analysis of variance (ANOVA) followed by Dunnett's comparison with a control group were used.
	Metric 22:	Reporting of Data	High	Data reported on Figure 2 for each treatment with an endpoint determination.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Chen, C., Xue, S., Zhou, Q., Xie, X. (2011). Multilevel ecotoxicity assessment of polycyclic musk in the earthworm Eisenia fetida using traditional and molecular endpoints. Ecotoxicology 20(8):1949-1958.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Not reported.; Adult
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	5352379

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Medium	Chemical name reported- form and specific identifying details not provided but based on available information about the chemical substance, these are not likely to affect the reliability of the study results.
	Metric 2:	Test Substance Source	High	Purchased from Zhongkejian Chemical Co. Ltd, Tianjin, China.
	Metric 3:	Test Substance Purity	High	The purity was 99%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Solvent control reported- No negative control reported, as no mortality was reported in the solvent control, this is not expected to have a substantial effect on the results.
	Metric 5:	Negative Control Response	High	No mortality was reported in the solvent controls.vv
	Metric 6:	Randomized Allocation	Low	Authors' did not report allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	System and Test preparation were described, and the authors took measures to reduce the potential evaporation of the test material, but ultimately, they did not report the measured test concentrations. For growth effects, the somewhat linear dose response indicated that this lack of measured test concentrations was unlikely to have an effect on the outcome of the test.
	Metric 8:	Consistency of Exposure Administration	High	Exposure was reported and exposures were administered consistently across study groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Nominal concentrations reported but based on the linear dose-response, the lack of reporting is not expected to have and impact on results.
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration lasted the entirety or the twenty-eight (28) day chronic study.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The geometric concentration series of HHCBwere prepared were 0 (solvent control), 3, 10, 30, 50and 100 lg g-1 (dry mass of soil), respectively. The dose-series was sufficient to calculate a NOAEC for growth effects based on a reproductive inhibition of 31% and 69%, at the 50 and 100 ug/g exposure levels, respectively.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent used and no interactions between control and test organisms.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Obtained from the Earthworm Breeder Company in Tianjin, China. They were acclimatized for 2 months under the laboratory conditions.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimatized for 2 months under the laboratory conditions.

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Study Citation:	Chen, C., Xue, S., Zhou, Q., Xie, X. (2011). Multilevel ecotoxicity assessment of polycyclic musk in the earthworm Eisenia fetida using traditional and molecular endpoints. Ecotoxicology 20(8):1949-1958.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Not reported.; Adult
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	5352379

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 organisms and 4 replicates were used in the study.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Biomass loading was not reported, but test conditions were reported and the organisms were fed.
	Metric 17:	Outcome Assessment Methodology	High	The study addressed or reported the intended outcome.
	Metric 18:	Consistency of Outcome Assessment	High	The outcomes were consistent and measured at the same durations.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	The study did not report differences in the study conditions.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	The authors' did not report differences attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	A one-way analysis of variance (ANOVA) followed by Dunnett's comparison with a control group were used.
	Metric 22:	Reporting of Data	High	Data reported on T. 3 for each treatment with an endpoint determination.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Goßmann, A. (1997). Effects of HHCB on reproduction and growth of earthworms Eisenia fetida in artificial soil.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application), Dietary
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	7607848

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test material was definitely identified as HHCB with necessary nomenclature and identifier numbers. The Chemical form was also appropriately described in the study report.
	Metric 2:	Test Substance Source	High	The test substance source was described as the test sponsor. Batch number and analytical verification were provided, including physical-Chemical properties.
	Metric 3:	Test Substance Purity	Low	Test substance Purity/Grade were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Concurrent negative and solvent controls were utilized in the study.
	Metric 5:	Negative Control Response	High	Negative control mortality was <10% and no significant changes in body weight, reproduction, or food consumption were reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not explicitly outline randomization procedure.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Exposure through treated artificial soil is appropriate for the test species. Test vessels were covered to prevent loss of test material through evaporation.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups, including the use of acetone as a solvent carrier.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, based on previous testing, there is a potential for degradation of the test material throughout the course of the test.
	Metric 10:	Exposure Duration and Frequency	High	The 8 week exposure duration was appropriate to characterize chronic effects such as reproduction.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Geometrically increasing concentration series was appropriate.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent (acetone) was utilized to ensure exposure to test medium to account for low solubility. Exposure procedure was appropriately characterized.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The study did not indicate the source of the test organisms, however the test organisms were bred by the study authors under appropriate husbandry guideline (OECD 207). Life-stage of the adults was appropriate and consistent with test guidelines.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Worms were acclimated in artificial soil for 4 days prior to exposure to the test material. It was unclear whether test organism digestive tracts were evacuated prior to the initial exposures to the test media, which could have delayed effects.

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Study Citation:	Goßmann, A. (1997). Effects of HHCB on reproduction and growth of earthworms Eisenia fetida in artificial soil.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application), Dietary
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	7607848

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The study used four replicates of 10 Earthworms/test concentration.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were consistent with the test guideline except for temperature. This deviation was explained and background references were provided, and the deviation had no effect on the control organisms.
	Metric 17:	Outcome Assessment Methodology	High	Assessment was adequate to characterize the effects from chronic exposure to the test material. Exposure concentrations were sufficiently determined and spaced to calculate the endpoints of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Conditions and exposure methodology were consistently applied among replications and test concentrations.
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	High	Details regarding test organism attrition and outcomes unrelated to exposure were reported for each study group and there were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Dunnett's test was used to determine the statistical significance of the effects observed in the test organisms.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoints of interest
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Goßmann, A. (1997). Effects of HHCB on reproduction and growth of earthworms Eisenia fetida in artificial soil.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application), Dietary
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	7607848

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test material was definitely identified as HHCB with necessary nomenclature and identifier numbers. The Chemical form was also appropriately described in the study report.
	Metric 2:	Test Substance Source	High	The test substance source was described as test sponsor. Batch number and analytical verification were provided including physical-chemical properties.
	Metric 3:	Test Substance Purity	Low	The test substance Purity/Grade were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Concurrent negative and solvent controls were utilized in the study.
	Metric 5:	Negative Control Response	High	Negative control mortality was <10% and no significant changes in body weight, reproduction, or food consumption were reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not explicitly outline randomization procedure.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Exposure through treated artificial soil is appropriate for the test species. Test vessels were covered to prevent loss of test material through evaporation.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups, including the use of acetone as a solvent carrier.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, based on previous testing, there is a potential for degradation of the test material throughout the course of the test.
	Metric 10:	Exposure Duration and Frequency	High	The study had an 8 week exposure duration appropriate to characterize chronic effects such as long-term mortality and reproduction.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups in a geometrically increasing concentration series was appropriate.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent (acetone) was utilized to ensure exposure to test medium to account for low solubility. Exposure procedure was appropriately characterized.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The test organism source was not specified, but the test organisms were bred by the study authors under appropriate husbandry guideline (OECD 207). Life-stage of the adults was appropriate and consistent with test guidelines.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Worms were acclimated in artificial soil for 4 days prior to exposure to the test material. It was unclear whether the test organisms digestive tracts were evacuated prior to the initial exposures to the test media, which could have delayed effects.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were four replicates of 10 Earthworms/test concentration.

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Study Citation:	Goßmann, A. (1997). Effects of HHCB on reproduction and growth of earthworms Eisenia fetida in artificial soil.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application), Dietary
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	7607848

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were consistent with the test guideline except for temperature. This deviation was explained and background references were provided, and the deviation had no effect on the control organisms.
	Metric 17:	Outcome Assessment Methodology	High	Assessment was adequate to characterize the effects from chronic exposure to the test material. Exposure concentrations were sufficiently determined and spaced to calculate the endpoints of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Conditions and exposure methodology were consistently applied among replications and test concentrations.
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	High	Details regarding test organism attrition and outcomes unrelated to exposure were reported for each study group and there were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Dunnett's test was used to determine the statistical significance of the effects observed in the test organisms.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoints of interest.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Goßmann, A. (1997). Effects of HHCB on reproduction and growth of earthworms Eisenia fetida in artificial soil.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application), Dietary
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	7607848

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test material was definitely identified as HHCB with necessary nomenclature and identifier numbers. The Chemical form was also appropriately described in the study report.
	Metric 2:	Test Substance Source	High	Test substance source was described as test sponsor. Batch number and analytical verification were provided including physical-Chemical properties.
	Metric 3:	Test Substance Purity	Low	The test substance Purity/Grade were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Concurrent negative and solvent controls were utilized in the study.
	Metric 5:	Negative Control Response	High	Negative control mortality was <10% and no significant changes in body weight, reproduction, or food consumption were reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not explicitly outline randomization procedure.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Exposure through treated artificial soil is appropriate for the test species. Test vessels were covered to prevent loss of test material through evaporation.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups, including the use of acetone as a solvent carrier.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, based on previous testing, there is a potential for degradation of the test material throughout the course of the test.
	Metric 10:	Exposure Duration and Frequency	High	The 8 week exposure duration was appropriate to characterize chronic effects such as reproduction.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	The number of exposure groups and geometrically increasing concentration series was appropriate for the study.
	Metric 12:	Testing at or Below Solubility Limit	High	A solvent (acetone) was utilized to ensure exposure to test medium to account for low solubility and the exposure procedure was appropriately characterized.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	While the source of the test organisms was not identified, the test organisms were bred by the study authors under appropriate husbandry guidelines (OECD 207). The life-stage of the adults was appropriate and consistent with test guidelines.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Worms were acclimated in artificial soil for 4 days prior to exposure to the test material. It was unclear whether test organism digestive tracts were evacuated prior to the initial exposures to the test media, which could have delayed effects.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were four replicates of 10 earthworms per test concentration.

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Study Citation:	Goßmann, A. (1997). Effects of HHCB on reproduction and growth of earthworms Eisenia fetida in artificial soil.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application), Dietary
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	7607848

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were consistent with the test guideline except for temperature. This deviation was explained and background references were provided. This deviation had no effect on the control organisms.
	Metric 17:	Outcome Assessment Methodology	High	Assessment was adequate to characterize the effects from chronic exposure to the test material. Exposure concentrations were sufficiently determined and spaced to calculate the endpoints of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Conditions and exposure methodology were consistently applied among replications and test concentrations.
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	High	Details regarding test organism attrition and outcomes unrelated to exposure were reported for each study group and there were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Dunnett's test was used to determine the statistical significance of the effects observed in the test organisms.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoints of interest.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: None

Overall Quality Determination High

Study Citation:	Goßmann, A. (1997). Effects of HHCB on reproduction and growth of earthworms Eisenia fetida in artificial soil.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application), Dietary
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	7607848

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Test material was definitely identified as HHCB with necessary nomenclature and identifier numbers. The Chemical form was also appropriately described in the study report.
	Metric 2:	Test Substance Source	High	The test substance source was described as the test sponsor. Batch number and analytical verification were provided, including physical-Chemical properties.
	Metric 3:	Test Substance Purity	Low	Test substance Purity/Grade were not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Concurrent negative and solvent controls were utilized in the study.
	Metric 5:	Negative Control Response	High	Negative control mortality was <10% and no significant changes in body weight, reproduction, or food consumption were reported.
	Metric 6:	Randomized Allocation	Low	Researchers did not explicitly outline randomization procedure.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Exposure through treated artificial soil is appropriate for the test species. Test vessels were covered to prevent loss of test material through evaporation.
	Metric 8:	Consistency of Exposure Administration	High	Details of exposure administration were reported and exposures were administered consistently across study groups, including the use of acetone as a solvent carrier.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, based on previous testing, there is a potential for degradation of the test material throughout the course of the test.
	Metric 10:	Exposure Duration and Frequency	High	The 8 week exposure duration was appropriate to characterize chronic effects such as reproduction.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Geometrically increasing concentration series was appropriate.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent (acetone) was utilized to ensure exposure to test medium to account for low solubility. Exposure procedure was appropriately characterized.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	The study did not indicate the source of the test organisms, however the test organisms were bred by the study authors under appropriate husbandry guideline (OECD 207). Life-stage of the adults was appropriate and consistent with test guidelines.
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Worms were acclimated in artificial soil for 4 days prior to exposure to the test material. It was unclear whether test organism digestive tracts were evacuated prior to the initial exposures to the test media, which could have delayed effects.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	The study used four replicates of 10 Earthworms/test concentration.

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Study Citation:	Goßmann, A. (1997). Effects of HHCB on reproduction and growth of earthworms Eisenia fetida in artificial soil.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Dermal (topical application), Dietary
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Behavioral
Chemical:	HHCB
HERO ID:	7607848

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were consistent with the test guideline except for temperature. This deviation was explained and background references were provided, and the deviation had no effect on the control organisms.
	Metric 17:	Outcome Assessment Methodology	High	Assessment was adequate to characterize the effects from chronic exposure to the test material. Exposure concentrations were sufficiently determined and spaced to calculate the endpoints of interest.
	Metric 18:	Consistency of Outcome Assessment	High	Conditions and exposure methodology were consistently applied among replications and test concentrations.
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	High	Details regarding test organism attrition and outcomes unrelated to exposure were reported for each study group and there were no differences among groups that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Dunnett's test was used to determine the statistical significance of the effects observed in the test organisms.
	Metric 22:	Reporting of Data	High	Data for exposure-related findings were presented for each treatment and control group and were adequate to determine values for the endpoints of interest
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes.

Additional Comments: This evaluation is for the food consumption behavioral outcome reported in Table 6.

Overall Quality Determination High

Study Citation:	Liu, S., Zhou, Q., Wang, Y. (2011). Ecotoxicological responses of the earthworm Eisenia fetida exposed to soil contaminated with HHCB. Chemosphere 83(8):1080-6. [Chemosphere].
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)
Chemical:	HHCB
HERO ID:	5918304

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	"HHCB was obtained from the Zhong Kejian Chemical Co. Ltd., Tianjin, China."
	Metric 2:	Test Substance Source	High	Same as above. "HHCB was obtained from the Zhong Kejian Chemical Co. Ltd., Tianjin, China."
	Metric 3:	Test Substance Purity	Low	No indication of purity was mentioned.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Assuming control was an untreated group. "Comparisons between control and treated groups were made by statistical analysis of variance (ANOVA)."
	Metric 5:	Negative Control Response	High	Treatment groups were compared to negative controls.
	Metric 6:	Randomized Allocation	Medium	"Each treatment has three replicates, consisting of ten earthworms per container. Two earthworms were collected from each replicate container on the 3rd, 7th, 14th and 28th day following application of HHCB for enzyme assays."
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	The experiments were conducted in 1 L glass containers with 500 g dry soil. HHCB was applied to soil for final treatment concentrations of 0, 10, 50 and 100 mg kg1 in equal volumes of acetone. The soil samples were vented for 72 h to remove all the solvent, wetted to 50% of water holding capacity, and then stabilized for 24 h prior to adding earthworms."
	Metric 8:	Consistency of Exposure Administration	High	"The experiments were conducted in 1 L glass containers with500 g dry soil. HHCB was applied to soil for final treatment concentrations of 0, 10, 50 and 100 mg kg1 in equal volumes of acetone. The soil samples were vented for 72 h to remove all the solvent, wetted to 50% of water holding capacity, and then stabilized for 24 h prior to adding earthworms."
	Metric 9:	Measurement of Test Substance Concentration	N/A	Soil test
	Metric 10:	Exposure Duration and Frequency	High	"Two earthworms were collected from each replicate container on the 3rd, 7th, 14th and 28th day following application of HHCB for enzyme assays."
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	"Two earthworms were collected from each replicate container on the 3rd, 7th, 14th and 28th day following application of HHCB for enzyme assays." Standard deviations were relatively similar across treatment groups.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Soil test

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Study Citation:	Liu, S., Zhou, Q., Wang, Y. (2011). Ecotoxicological responses of the earthworm Eisenia fetida exposed to soil contaminated with HHCB. Chemosphere 83(8):1080-6. [Chemosphere].
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)
Chemical:	HHCB
HERO ID:	5918304

Domain	Metric		Rating	Comments
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	”Clitellated adult earthworms (wet weight of 350–450 mg) were selected for toxicity tests.”
	Metric 14:	Acclimatization and Pretreatment Conditions	High	”E. fetida were purchased from the Lutai Earthworm-BreedingFarm (Tianjin, China). They were acclimatized for one month underlaboratory conditions in culture pots containing soil and cowexcrements. Water was added as necessary to maintain moist conditions.”
	Metric 15:	Number of Organisms and Replicates per Group	Low	Only two organisms (earthworms) were collected per time point for comparison between each treatment group. Multiple aliquots from each earthworm were tested in order to perform statistical analysis; this may constitute pseudoreplication.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	No uncertainties were noted.
	Metric 17:	Outcome Assessment Methodology	High	No concerns were identified other than what was indicated previously - two organisms/treatment at each time point.
	Metric 18:	Consistency of Outcome Assessment	High	No documented uncertainties or limitations
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No issues identified
	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	Low	”All the values in the work are expressed as means, SD and range.The letters under the x-axis refer to the statistically significant difference ($p < 0.05$) among different concentrations. Comparisonsbetween control and treated groups were made by statistical analysis of variance (ANOVA).”Details of the ANOVA procedure including F-scores and N per group are not given. This makes it impossible to determine how many aliquots or subsamples were taken from each earthworm homogenate & whether they were treated as independent samples (which would constitute pseudoreplication).
	Metric 22:	Reporting of Data	High	Data were presented on each treatment group and negative controls.
	Metric 23:	Explanation of Unexpected Outcomes	High	No unexpected outcomes were identified.

Additional Comments: None

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Study Citation:	Liu, S., Zhou, Q., Wang, Y. (2011). Ecotoxicological responses of the earthworm Eisenia fetida exposed to soil contaminated with HHCB. Chemosphere 83(8):1080-6. [Chemosphere].
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)
Chemical:	HHCB
HERO ID:	5918304

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

Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by nomenclature and formula.
	Metric 2:	Test Substance Source	High	Purchased from Guoyao Chemical Co. Ltd. (Beijing, China)
	Metric 3:	Test Substance Purity	High	Given as ">98% purity".
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls and solvent controls were used.
	Metric 5:	Negative Control Response	High	No difference between solvent controls and negative controls, no reported mortality in controls.
	Metric 6:	Randomized Allocation	Medium	Distribution was made by mass ("10 well developed adults (300-400 mg)")
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	HHCB was mixed with soil and it is unclear whether sealed vessels were used. As HHCB can volatilize from soil in the long term and nominal concentrations were not tested, there may be some discrepancy. The test duration was 56 days, and Schafer 2005 reported a volatilization of up to 16% after 28 days. This discrepancy may have a substantial effect on results.
	Metric 8:	Consistency of Exposure Administration	High	Exposure same for all treatments.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, however HHCB does not rapidly biodegrade and sorbs to soil somewhat strongly. However, since the duration of the test was 56d, and volatilization from soil has been reported in the literature (Schafer 2005), the discrepancy between nominal and actual exposure concentrations may substantially effect the results.
	Metric 10:	Exposure Duration and Frequency	High	The study was conducted in accordance with OECD 222. Duration and frequency were appropriate.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Range and spacing of exposure levels were given for all treatments & were adequate to develop an EC10, EC50, and NOEC with reasonable confidence intervals.
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent used (acetone) did not cause measurable responses relative to negative controls.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Worms were obtained from in-house culture at the Chinese Research Academy of Environmental Sciences. Rearing conditions were described and seem adequate for the species chosen.

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Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Worms were reared in the same conditions as the toxicity test.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 worms were used per replicate, with 4 replicates per treatment and controls.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Conditions were appropriate for the worms and were the same for all groups & were consistent before treatment.
	Metric 17:	Outcome Assessment Methodology	High	Adults were counted and weighed at 28d. Juveniles and cocoons were counted at the end of the test (56d) as per OECD 222.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed the same across all groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences in environmental conditions across groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported details that suggest differences in attrition or other health outcomes.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was adequately described in the methods section and appropriate for the experiments performed.
	Metric 22:	Reporting of Data	Low	Summary data (EC10, EC50, NOEC) was reported for the overall experiment for each endpoint, but individual data for each treatment and control was not reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, and the outcomes that were reported are adequately discussed in the text.

Additional Comments: This study also includes the calculation of an SSD from all 10 tested terrestrial organisms and the derivation of PNECs from said distribution, with discussion of AFs. In my opinion it would provide an important contribution to terrestrial data for HHCB.

Overall Quality Determination High

Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by nomenclature and formula.
	Metric 2:	Test Substance Source	High	Purchased from Guoyao Chemical Co. Ltd. (Beijing, China)
	Metric 3:	Test Substance Purity	High	Given as ">98% purity".
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls and solvent controls were used.
	Metric 5:	Negative Control Response	High	No difference between solvent controls and negative controls, no reported mortality in controls.
	Metric 6:	Randomized Allocation	Medium	Distribution was made by mass ("10 well developed adults (300-400 mg)")
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	HHCB was mixed with soil and it is unclear whether sealed vessels were used. As HHCB can volatilize from soil in the long term and nominal concentrations were not tested, there may be some discrepancy. The test duration was 56 days, and Schafer 2005 reported a volatilization of up to 16% after 28 days. This discrepancy may have a substantial effect on results.
	Metric 8:	Consistency of Exposure Administration	High	Exposure same for all treatments.
	Metric 9:	Measurement of Test Substance Concentration	Low	Exposure concentrations were not measured, however HHCB does not rapidly biodegrade and sorbs to soil somewhat strongly. However, since the duration of the test was 56d, and volatilization from soil has been reported in the literature (Schafer 2005), the discrepancy between nominal and actual exposure concentrations may substantially effect the results.
	Metric 10:	Exposure Duration and Frequency	High	The study was conducted in accordance with OECD 222. Duration and frequency were appropriate.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Range and spacing of exposure levels were given for all treatments & were adequate to develop an EC10, EC50, and NOEC with reasonable confidence intervals.
	Metric 12:	Testing at or Below Solubility Limit	High	The solvent used (acetone) did not cause measurable responses relative to negative controls.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Worms were obtained from in-house culture at the Chinese Research Academy of Environmental Sciences. Rearing conditions were described and seem adequate for the species chosen.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Worms were reared in the same conditions as the toxicity test.

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Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Not determined by study authors (i.e., chemical of interest in exposure water, but unable to determine exact uptake route)
Taxa, Species, Age:	Invertebrate; Worms (e.g., Annelids, Nematodes); *Eisenia fetida*; Adult
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 worms were used per replicate, with 4 replicates per treatment and controls.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Conditions were appropriate for the worms and were the same for all groups & were consistent before treatment.
	Metric 17:	Outcome Assessment Methodology	High	Adults were counted and weighed at 28d. Surviving adults, juveniles and cocoons were counted at the end of the test (56d) as per OECD 222.
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed the same across all groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences in environmental conditions across groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There were no reported details that suggest differences in attrition or other health outcomes.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was adequately described in the methods section and appropriate for the experiments performed.
	Metric 22:	Reporting of Data	Low	Summary data (NOEC) was reported for the overall experiment for each endpoint, but individual data for each treatment and control was not reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, and the outcomes that were reported are adequately discussed in the text.

Additional Comments: This study also includes the calculation of an SSD from all 10 tested terrestrial organisms and the derivation of PNECs from said distribution, with discussion of AFs. In my opinion it would provide an important contribution to terrestrial data for HHCB.

Overall Quality Determination High

Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Allium cepa*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784978

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the HHCB was not reported, nor was it reported to be analytically verified, but a batch and lot number were reported.
	Metric 3:	Test Substance Purity	Low	The purity/grade of the HHCB was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	It was reported that a an appropriate negative control and a solvent control were both used in the study.
	Metric 5:	Negative Control Response	High	The biological response of the negative control was reported in Table 8, 29, 30, and 32 as well as in Fig. 6. The response was adequate for the outcome of interest.
	Metric 6:	Randomized Allocation	Low	It was not reported how the seeds were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	It was reported that the test concentrations were prepared by mixing the test substance with acetone and mixing in fine quartz sand before being mixed into the soil. The test system was a 12 cm pot, but the amount of soil was not reported.
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for similar time period in a temperature controlled room. All seeds were from the same source and the same lot number for each study. Test conditions were the same for all treatment groups.
	Metric 9:	Measurement of Test Substance Concentration	High	It was reported the test substance was measured using GC-FI.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration for all the plants tested was reported to be for 14-21d post 50% emergence of the control plants. This is in accordance with OECD Guideline 208, which was cited for the study protocol. 21d for A. cepa and 14d for all other plants
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure levels and spacing was adequate to observe the outcomes of interest.
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via soil.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The seeds were all reported to be from the same source and the same lot number, but the source was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if any acclimation occurred.

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Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Allium cepa*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784978

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 6 pots per test concentration with 5 seeds per pot for each study.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Soil was steam sterilized and was analyzed by the LUFA and reported to be a sandy loam. The test was performed in a growth chamber under controlled conditions. The photoperiod was 16L:8D, and the temperature ranged between 16.4 and 27.5C with a mean humidity of 71%.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–plant fresh weight, plant emergence, and plant phytotoxicity.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Fresh weight was determined 14d (or 21d depending on the plant) after 50% of the seedlings emerged in the control. The above ground portion of the plant was used for this determination. Emergence was checked daily until 50% of the control plants emerged. Phytotoxicity was determined visually by observing chlorosis, necrosis, and abnormal growth and was recorded weekly after 50% control emergence.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No differences were reported among groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The study reported appropriate statistical methods in the ”statistical analysis” section of the report on page 20 of the PDF.
	Metric 22:	Reporting of Data	High	Data for the control response as well as the exposure response were presented in Table 8, 29, 30, and 32 as well as in Fig 6.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. EC values provided 95% confidence intervals.

Additional Comments: This portion of the evaluation was on the effect of HHCB on the development/growth of A. cepa. Fresh weights were taken 14d after 50% emergence of the controls. EC values were determined based off of the fresh weight values. Plant emergences was also included in the development/growth outcome. Phytotoxicity was also considered to be an outcome for this test. This was included in the development/growth outcome because abnormal growth was determined to be a factor in determining this outcome. Chlorosis and necrosis were also included in the phytotoxicity outcome.

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Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Allium cepa*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784978

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

Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Allium cepa*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8784978

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the HHCB was not reported, nor was it reported to be analytically verified, but a batch and lot number were reported.
	Metric 3:	Test Substance Purity	Low	The purity/grade of the HHCB was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	It was reported that a an appropriate negative control and a solvent control were both used in the study.
	Metric 5:	Negative Control Response	High	The biological response of the negative control was reported in Table 8 and Table 31 and was adequate for the outcome of interest.
	Metric 6:	Randomized Allocation	Low	It was not reported how the seeds were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	It was reported that the test concentrations were prepared by mixing the test substance with acetone and mixing in fine quartz sand before being mixed into the soil. The test system was a 12 cm pot, but the amount of soil was not reported.
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for similar time period in a temperature controlled room. All seeds were from the same source and the same lot number for each study. Test conditions were the same for all treatment groups.
	Metric 9:	Measurement of Test Substance Concentration	High	It was reported the test substance was measured using GC-FI.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration for all the plants tested was reported to be for 14-21d post 50% emergence of the control plants. This is in accordance with OECD Guideline 208, which was cited for the study protocol.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure groups and spacing was adequate to observe the outcomes of interest.
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via soil.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The seeds were all reported to be from the same source and the same lot number, but the source was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if any acclimation occurred.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 6 pots per test concentration with 5 seeds per pot for each study.

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Study Citation: IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration: Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path: Terrestrial; Soil; 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; *Allium cepa*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome: Mortality
Chemical: HHCB
HERO ID: 8784978

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Soil was steam sterilized and was analyzed by the LUFA and reported to be a sandy loam. The test was performed in a growth chamber under controlled conditions. The photoperiod was 16L:8D, and the temperature ranged between 16.4 and 27.5C with a mean humidity of 71%.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–plant mortality.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Mortality was assessed on days 7 and 14 (as well as on day 21 if the test extended that long) post 50% emergence of the control. Plants were considered dead when no living tissue could be found on the leaves or the shoots.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No differences were reported among groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The study reported appropriate statistical methods in the ”statistical analysis” section of the report on page 20 of the PDF.
	Metric 22:	Reporting of Data	High	Data for the control response as well as the exposure response were presented in Table 8 and Table 31 and were adequate for the outcome of interest.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Mortality from each pot was reported in Table 31.

Additional Comments: This portion of the evaluation was on the effect of HHCB on the %mortality of A. cepa. Plants were considered to be dead when no living tissue could be found on the leaves or shoots.

Overall Quality Determination High

Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Allium tuberosum*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by nomenclature and formula.
	Metric 2:	Test Substance Source	High	Purchased from Guoyao Chemical Co. Ltd. (Beijing, China)
	Metric 3:	Test Substance Purity	High	Given as ">98% purity".
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls and solvent controls were used.
	Metric 5:	Negative Control Response	Medium	There were differences between untreated and solvent controls (greater growth, and statistically significant differences in all measured parameters for all plants). Therefore comparisons were made to the solvent control.
	Metric 6:	Randomized Allocation	Medium	Tests conducted in accordance with OECD 208 which specifies randomization.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	HHCB was mixed with soil and it is unclear whether sealed vessels were used (doubtful for plant studies). As HHCB can volatilize from soil in the long term and nominal concentrations were not tested, there may be some discrepancy. The test duration was 21 days, and Schafer 2005 reported a volatilization of up to 16% after 28 days. This discrepancy is unlikely to have a major effect on results since it was replicated across all treatments and plants.
	Metric 8:	Consistency of Exposure Administration	High	Exposure same for all treatments.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were not measured, however HHCB does not rapidly biodegrade and sorbs to soil somewhat strongly. The discrepancy between nominal and actual exposure concentrations is unlikely to substantially effect the results.
	Metric 10:	Exposure Duration and Frequency	High	The study was conducted in accordance with OECD 208.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The range and spacing of exposure levels is not given for any plant studies, however the OECD 208 guideline specifies that appropriate rangefinding and exposure level choices must be made, and the text implies (ECx values calculated, NOECs mentioned) that a range of exposure levels was used. Additionally, some exposure levels are given in the Results section.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent used (acetone) caused measurable responses in all plant experiments. Comparisons of HHCB effect were made against solvent controls instead of blank controls.
Domain 4: Test Organism				

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Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Allium tuberosum*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	Seeds were obtained from the Chinese Academy of Agricultural Sciences (Beijing, China)
	Metric 14:	Acclimatization and Pretreatment Conditions	High	OECD 208 guidelines were followed, conditions were appropriate for the plants and were the same for all groups & were consistent before treatment.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 plants were used per replicate, with 4 replicates per treatment including controls.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	OECD 208 guidelines were followed, conditions were appropriate for the plants and were the same for all groups & were consistent before treatment.
	Metric 17:	Outcome Assessment Methodology	High	Growth and biomass were measured at the end of the test according to OECD 208
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed the same across all groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences in environmental conditions across groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	"... there was no significant effect on the survival of the 8 terrestrial plants at the testing concentrations..."
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was adequately described in the methods section and appropriate for the experiments performed.
	Metric 22:	Reporting of Data	Low	Summary data (ECx, NOEC) was reported for the overall experiment for each organism, but individual data for each treatment and control was not reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, and the outcomes that were reported are adequately discussed in the text.

Additional Comments: This study also includes the calculation of an SSD from all 10 tested terrestrial organisms and the derivation of PNECs from said distribution, with discussion of AFs. In my opinion it would provide an important contribution to terrestrial data for HHCB, unfortunately the range and spacing of test concentrations for the 8 plants is not given.

Overall Quality Determination High

Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Avena sativa*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784978

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the HHCB was not reported, nor was it reported to be analytically verified, but a batch and lot number were reported.
	Metric 3:	Test Substance Purity	Low	The purity/grade of the HHCB was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	It was reported that a an appropriate negative control and a solvent control were both used in the study.
	Metric 5:	Negative Control Response	High	The biological response of the negative control was reported in Table 8, 25, 26, and 28 as well as in Fig. 5. The response was adequate for the outcome of interest.
	Metric 6:	Randomized Allocation	Low	It was not reported how the seeds were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	It was reported that the test concentrations were prepared by mixing the test substance with acetone and mixing in fine quartz sand before being mixed into the soil. The test system was a 12 cm pot, but the amount of soil was not reported.
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for similar time period in a temperature controlled room. All seeds were from the same source and the same lot number for each study. Test conditions were the same for all treatment groups.
	Metric 9:	Measurement of Test Substance Concentration	High	It was reported the test substance was measured using GC-FI.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration for all the plants tested was reported to be for 14-21d post 50% emergence of the control plants. This is in accordance with OECD Guideline 208, which was cited for the study protocol.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were only 2 exposure groups for this particular plant.
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via soil.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The seeds were all reported to be from the same source and the same lot number, but the source was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if any acclimation occurred.

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Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Avena sativa*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784978

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 6 pots per test concentration with 5 seeds per pot for each study.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Soil was steam sterilized and was analyzed by the LUFA and reported to be a sandy loam. The test was performed in a growth chamber under controlled conditions. The photoperiod was 16L:8D, and the temperature ranged between 16.4 and 27.5C with a mean humidity of 71%.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–plant fresh weight, plant emergence, and plant phytotoxicity.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Fresh weight was determined 14d (or 21d depending on the plant) after 50% of the seedlings emerged in the control. The above ground portion of the plant was used for this determination. Emergence was checked daily until 50% of the control plants emerged. Phytotoxicity was determined visually by observing chlorosis, necrosis, and abnormal growth and was recorded weekly after 50% control emergence.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No differences were reported among groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	The study reported appropriate statistical methods in the ”statistical analysis” section of the report on page 20 of the PDF.
	Metric 22:	Reporting of Data	High	Data for the control response as well as the exposure response were presented in Table 8, 25, 26, and 28 as well as in Fig 5.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. EC values provided 95% confidence intervals.

Additional Comments: This portion of the evaluation was on the effect of HHCB on the development/growth of A. sativa. Fresh weights were taken 14d after 50% emergence of the controls. EC values were determined based off of the fresh weight values. Plant emergences was also included in the development/growth outcome. Phytotoxicity was also considered to be an outcome for this test. This was included in the development/growth outcome because abnormal growth was determined to be a factor in determining this outcome. Chlorosis and necrosis were also included in the phytotoxicity outcome.

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Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Avena sativa*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784978

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

Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Avena sativa*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8784978

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the HHCB was not reported, nor was it reported to be analytically verified, but a batch and lot number were reported.
	Metric 3:	Test Substance Purity	Low	The purity/grade of the HHCB was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	It was reported that a an appropriate negative control and a solvent control were both used in the study.
	Metric 5:	Negative Control Response	High	The biological response of the negative control was reported in Table 8 and Table 27 and was adequate for the outcome of interest.
	Metric 6:	Randomized Allocation	Low	It was not reported how the seeds were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	It was reported that the test concentrations were prepared by mixing the test substance with acetone and mixing in fine quartz sand before being mixed into the soil. The test system was a 12 cm pot, but the amount of soil was not reported.
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for similar time period in a temperature controlled room. All seeds were from the same source and the same lot number for each study. Test conditions were the same for all treatment groups.
	Metric 9:	Measurement of Test Substance Concentration	High	It was reported the test substance was measured using GC-FI.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration for all the plants tested was reported to be for 14-21d post 50% emergence of the control plants. This is in accordance with OECD Guideline 208, which was cited for the study protocol.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	There were only 2 exposure groups for this particular plant.
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via soil.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The seeds were all reported to be from the same source and the same lot number, but the source was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if any acclimation occurred.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 6 pots per test concentration with 5 seeds per pot for each study.

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Study Citation: IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration: Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path: Terrestrial; Soil; 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; *Avena sativa*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome: Mortality
Chemical: HHCB
HERO ID: 8784978

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Soil was steam sterilized and was analyzed by the LUFA and reported to be a sandy loam. The test was performed in a growth chamber under controlled conditions. The photoperiod was 16L:8D, and the temperature ranged between 16.4 and 27.5C with a mean humidity of 71%.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–plant mortality.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Mortality was assessed on days 7 and 14 (as well as on day 21 if the test extended that long) post 50% emergence of the control. Plants were considered dead when no living tissue could be found on the leaves or the shoots.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No differences among groups were reported.
	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	The study reported appropriate statistical methods in the ”statistical analysis” section of the report on page 20 of the PDF.
	Metric 22:	Reporting of Data	High	Data for the control response as well as the exposure response were presented in Table 8 and Table 27 and were adequate for the outcome of interest.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Mortality from each pot was reported in Table 27.

Additional Comments: This portion of the evaluation was on the effect of HHCB on the %mortality of A. sativa. Plants were considered to be dead when no living tissue could be found on the leaves or shoots.

Overall Quality Determination High

Study Citation:	Zhang, M., Liu, J., Wang, W., Bao, Y. (2019). Responses of Bougainvillea spectabilis to elevated atmospheric CO2 under galaxolide (HHCB) pollution and the mechanisms of its rhizosphere metabolism. Journal of Soils and Sediments 19(1):159-170.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil, Air; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Bougainvillea spectabilis*; Juvenile
Health Outcome:	ADME (biotransformation)
Chemical:	HHCB
HERO ID:	5120365

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	HHCB was identified as the test chemical - however the other components of the test substance were not identified
	Metric 2:	Test Substance Source	High	The HHCB standard (50.0% purity) was purchased from Fluffer Chemical Technology Co., Ltd., of Shanghai, China
	Metric 3:	Test Substance Purity	Low	The HHCB was noted as being of 50.0% purity and the other components of the mixture were not identified. It is thus impossible to tell if observed effects are due to HHCB or the unidentified 50%
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Control plots were used (ambient CO2 and no HHCB) and root samples were taken for analysis of root metabolites. Unclear how many were taken from controls.
	Metric 5:	Negative Control Response	Medium	Metabolic profiles of control roots were analyzed
	Metric 6:	Randomized Allocation	Low	Unclear if plants were allocated at random
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Test chambers used were called calorstats (CO2 was manipulated in some, others were ventilated with ambient air), exposure concentrations in soil were not validated. However, concentrations of HHCB that accumulated in plant tissue were measured.
	Metric 8:	Consistency of Exposure Administration	High	HHCB exposures were to 0, 50, and 100 mg kg−1
	Metric 9:	Measurement of Test Substance Concentration	High	Concentrations of HHCB that accumulated in plant tissue were measured. However, original exposure concentrations were not validated
	Metric 10:	Exposure Duration and Frequency	High	At day 40 metabolites in root samples were analyzed with GC-MS
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Goal was not dose response (two concentrations)
	Metric 12:	Testing at or Below Solubility Limit	N/A	Chemical was mixed with acetone and added to soil
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	Bougainvillea spectabilis - a vining shrub was purchased from a flower market. There is uncertainty surrounding past exposures (for instance of pesticides)
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Uncertainty regarding pre-treatment conditions of plants as they were purchased at a flower market

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Study Citation:	Zhang, M., Liu, J., Wang, W., Bao, Y. (2019). Responses of Bougainvillea spectabilis to elevated atmospheric CO2 under galaxolide (HHCB) pollution and the mechanisms of its rhizosphere metabolism. Journal of Soils and Sediments 19(1):159-170.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil, Air; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Bougainvillea spectabilis*; Juvenile
Health Outcome:	ADME (biotransformation)
Chemical:	HHCB
HERO ID:	5120365

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Low	Each treatment had three replicate plants. For the metabolite analysis, it is unclear how many replicate roots per plant and per treatment were analyzed.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were well described
	Metric 17:	Outcome Assessment Methodology	Medium	After 40 days roots were used for metabolite analysis. Unclear how many roots from each plant/treatment were analyzed.
	Metric 18:	Consistency of Outcome Assessment	High	Metabolites of plant roots were examined consistently across exposure groups
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	No information was provided about the 50% of the chemical that was not pure HHCB. Similarly, prior exposure to pesticides is likely from plants purchased at a market.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information to suggest differences due to outcomes other than exposure
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Sparse details were provided as to how metabolomic data was normalized and analyzed using PLS-DA, variable importance in projection scores, and heatmaps.
	Metric 22:	Reporting of Data	Medium	Raw metabolomic data for each treatment does not appear to be provided in the supplemental. All results from metabolomic analyses were reported (see Figure 5-8).
	Metric 23:	Explanation of Unexpected Outcomes	Low	Unclear how much variation there was for metabolic products identified in roots samples for each treatment and how many replicates were used in each treatment or if roots were pooled across treatments.

Additional Comments: Major concerns are that EDB of 50% purity was used in the experiment, and there is an unknown exposure history of the plants purchased from the garden market. Concentrations in tissue were confirmed.There was sparse reporting of methods and analysis used for identifying metabolic contents in roots.

Overall Quality Determination Low

Study Citation:	Zhang, M., Liu, J., Wang, W., Bao, Y. (2019). Responses of Bougainvillea spectabilis to elevated atmospheric CO2 under galaxolide (HHCB) pollution and the mechanisms of its rhizosphere metabolism. Journal of Soils and Sediments 19(1):159-170.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil, Air; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Bougainvillea spectabilis*; Juvenile
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Photosynthesis
Chemical:	HHCB
HERO ID:	5120365

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	HHCB was identified as the test chemical - however the other components of the test substance were not identified
	Metric 2:	Test Substance Source	High	The HHCB standard (50.0% purity) was purchased from Fluffer Chemical Technology Co., Ltd., of Shanghai, China
	Metric 3:	Test Substance Purity	Low	The HHCB was noted as being of 50.0% purity and the other components of the mixture were not identified. It is thus impossible to tell if observed effects are due to HHCB or the unidentified 50%
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Control plots were used (ambient CO2 and no HHCB) and leaf samples were taken from controls for chlorophyll analysis at day 40. Limitation is that no control was used to account for the unknown components in the 50% HHCB mixture.
	Metric 5:	Negative Control Response	Medium	The chlorophyll a and b concentrations of control plants were reported.
	Metric 6:	Randomized Allocation	Low	Unclear if plants were allocated at random
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Test chambers used were called calorstats (CO2 was manipulated in some, others were ventilated with ambient air), exposure concentrations in soil were not validated. However, concentrations of HHCB that accumulated in plant tissue were measured.
	Metric 8:	Consistency of Exposure Administration	High	HHCB exposures were to 0, 50, and 100 mg kg−1
	Metric 9:	Measurement of Test Substance Concentration	High	Concentrations of HHCB that accumulated in plant tissue were measured. However, original exposure concentrations were not validated
	Metric 10:	Exposure Duration and Frequency	High	Leaf samples for photosynthetic pigments were taken at day 40.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Goal was not dose response (two concentrations)
	Metric 12:	Testing at or Below Solubility Limit	N/A	Chemical was mixed with acetone and added to soil
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	Bougainvillea spectabilis - a vining shrub was purchased from a flower market. There is uncertainty surrounding past exposures (for instance of pesticides)
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Uncertainty regarding pre-treatment conditions of plants as they were purchased at a flower market

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Study Citation:	Zhang, M., Liu, J., Wang, W., Bao, Y. (2019). Responses of Bougainvillea spectabilis to elevated atmospheric CO2 under galaxolide (HHCB) pollution and the mechanisms of its rhizosphere metabolism. Journal of Soils and Sediments 19(1):159-170.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil, Air; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Bougainvillea spectabilis*; Juvenile
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Photosynthesis
Chemical:	HHCB
HERO ID:	5120365

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Low	Each treatment had three replicate plants. For the chlorophyll analysis .5 g of leaves were weighed and pigments were extracted. It is unclear how many leaves per plant and per treatment were analyzed.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were well described
	Metric 17:	Outcome Assessment Methodology	Medium	After 40 days leaves were picked and chlorophyll a, b, and carotenoid concentrations were calculated for each treatment using a spectrometer. Additional details needed about number of replicates and why were leaves with spots not considered - leaves should have been chosen at random.
	Metric 18:	Consistency of Outcome Assessment	High	Pigments of plant material were examined consistently across exposure groups
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	No information was provided about the 50% of the chemical that was not pure HHCB. Similarly, prior exposure to pesticides is likely from plants purchased at a market.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No information to suggest differences due to outcomes other than exposure
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	All treatment data were presented as means and standard deviation and statistical analyses to were conducted to identify differences from controls.
	Metric 22:	Reporting of Data	High	Mean weight and std dev. was reported for all treatment and control groups.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Study determined HHCB affected concentrations of photosynthetic pigments in leaves. Authors could provide discussion on why both increases and decreases in concentration of pigments were observed for the HHCB treatments compared to controls.

Additional Comments: Major concerns are that EDB of 50% purity was used in the experiment, and there is an unknown exposure history of the plants purchased from the garden market. Concentrations in tissue were confirmed.

Overall Quality Determination Low

Study Citation:	Zhang, M., Liu, J., Wang, W., Bao, Y. (2019). Responses of Bougainvillea spectabilis to elevated atmospheric CO2 under galaxolide (HHCB) pollution and the mechanisms of its rhizosphere metabolism. Journal of Soils and Sediments 19(1):159-170.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil, Air; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Bougainvillea spectabilis*; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5120365

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	HHCB was identified as the test chemical - however the other components of the test substance were not identified
	Metric 2:	Test Substance Source	High	The HHCB standard (50.0% purity) was purchased from Fluffer Chemical Technology Co., Ltd., of Shanghai, China
	Metric 3:	Test Substance Purity	Low	The HHCB was noted as being of 50.0% purity and the other components of the mixture were not identified. It is thus impossible to tell if observed effects are due to HHCB or the unidentified 50%
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	Control plots were used (ambient CO2 and no HHCB)
	Metric 5:	Negative Control Response	Medium	The growth of control plants appears to be normal
	Metric 6:	Randomized Allocation	Low	Unclear if plants were allocated at random
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Test chambers used were called calorstats (CO2 was manipulated in some, others were ventilated with ambient air), exposure concentrations in soil were not validated. However, concentrations of HHCB that accumulated in plant tissue were measured.
	Metric 8:	Consistency of Exposure Administration	High	HHCB exposures were to 0, 50, and 100 mg kg−1
	Metric 9:	Measurement of Test Substance Concentration	High	Concentrations of HHCB that accumulated in plant tissue were measured. However, original exposure concentrations were not validated
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration was for 6 weeks
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	N/A	Goal was not dose response (two concentrations)
	Metric 12:	Testing at or Below Solubility Limit	N/A	Chemical was mixed with acetone and added to soil
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	Bougainvillea spectabilis - a vining shrub was purchased from a flower market. Some uncertainty surrounding past exposures (for instance of pesticides)
	Metric 14:	Acclimatization and Pretreatment Conditions	Medium	Some uncertainty regarding pre-treatment conditions of plants as they were purchased at a flower market
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Each treatment had three replicate plants
Domain 5: Outcome Assessment				

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Study Citation:	Zhang, M., Liu, J., Wang, W., Bao, Y. (2019). Responses of Bougainvillea spectabilis to elevated atmospheric CO2 under galaxolide (HHCB) pollution and the mechanisms of its rhizosphere metabolism. Journal of Soils and Sediments 19(1):159-170.
Duration:	Overall Duration: > 21 days; Exposure Duration: > 21 days
Exposure Route, Media, Path:	Terrestrial; Soil, Air; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Bougainvillea spectabilis*; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5120365

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Test conditions were well described
	Metric 17:	Outcome Assessment Methodology	High	After 6 weeks samples were freeze dried and weighed according to root, stem, and leaf portions.
	Metric 18:	Consistency of Outcome Assessment	High	Weight of plant material was done consistently across exposure groups
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	Not information was provided about the 50% of the chemical that was not pure HHCB. Similarly, prior exposure to pesticides is likely from plants purchased at a market.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	Unrelated outcomes were not reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	All treatment data were presented as means and standard deviation and statistical analyses to were conducted to identify differences from controls.
	Metric 22:	Reporting of Data	High	Mean weight and std dev. was reported for all treatment and control groups.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Study determined B. spectabilis growth was not affected by HHCB, but in Figure 1d it appears that the leaf mass was negatively affected by HHCB. Connection between the identified metabolites and photosynthesis and growth could be discussed further.

Additional Comments: Major concerns are that EDB of 50% purity was used in the experiment, and there is an unknown exposure history of the plants purchased from the garden market. Concentrations in tissue were confirmed.

Overall Quality Determination Low

Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Brassica napus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8784978

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the HHCB was not reported, nor was it reported to be analytically verified, but a batch and lot number were reported.
	Metric 3:	Test Substance Purity	Low	The purity/grade of the HHCB was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	It was reported that a an appropriate negative control and a solvent control were both used in the study.
	Metric 5:	Negative Control Response	High	The biological response of the negative control was reported in Table 8 and Table 11 and was adequate for the outcome of interest.
	Metric 6:	Randomized Allocation	Low	It was not reported how the seeds were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	It was reported that the test concentrations were prepared by mixing the test substance with acetone and mixing in fine quartz sand before being mixed into the soil. The test system was a 12 cm pot, but the amount of soil was not reported.
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for similar time period in a temperature controlled room. All seeds were from the same source and the same lot number for each study. Test conditions were the same for all treatment groups.
	Metric 9:	Measurement of Test Substance Concentration	High	It was reported the test substance was measured using GC-FI.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration for all the plants tested was reported to be for 14-21d post 50% emergence of the control plants. This is in accordance with OECD Guideline 208, which was cited for the study protocol.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure levels reported. Spacing was adequate to observe a response.
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via soil.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The seeds were all reported to be from the same source and the same lot number, but the source was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if any acclimation occurred.

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Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Brassica napus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8784978

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 6 pots per test concentration with 5 seeds per pot for each study.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Soil was steam sterilized and was analyzed by the LUFA and reported to be a sandy loam. The test was performed in a growth chamber under controlled conditions. The photoperiod was 16L:8D, and the temperature ranged between 16.4 and 27.5C with a mean humidity of 71%.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–plant mortality.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Mortality was assessed on days 7 and 14 (as well as on day 21 if the test extended that long) post 50% emergence of the control. Plants were considered dead when no living tissue could be found on the leaves or the shoots.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No differences between groups were reported.
	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	The study reported appropriate statistical methods in the ”statistical analysis” section of the report on page 20 of the PDF.
	Metric 22:	Reporting of Data	High	Data for the control response as well as the exposure response were presented in Table 8 and Table 11 and were adequate for the outcome of interest.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Mortality from each pot was reported in Table 11

Additional Comments: This portion of the evaluation was on the effect of HHCB on the %mortality of B. napus. Plants were considered to be dead when no living tissue could be found on the leaves or shoots.

Overall Quality Determination High

Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Brassica napus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784978

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the HHCB was not reported, nor was it reported to be analytically verified, but a batch and lot number were reported.
	Metric 3:	Test Substance Purity	Low	The purity/grade of the HHCB was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	It was reported that a an appropriate negative control and a solvent control were both used in the study.
	Metric 5:	Negative Control Response	High	The biological response of the negative control was reported in Table 8, 9, 10, and 12, as well as in Fig.1. The response was adequate for the outcome of interest.
	Metric 6:	Randomized Allocation	Low	It was not reported how the seeds were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	It was reported that the test concentrations were prepared by mixing the test substance with acetone and mixing in fine quartz sand before being mixed into the soil. The test system was a 12 cm pot, but the amount of soil was not reported.
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for similar time period in a temperature controlled room. All seeds were from the same source and the same lot number for each study. Test conditions were the same for all treatment groups.
	Metric 9:	Measurement of Test Substance Concentration	High	It was reported the test substance was measured using GC-FI.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration for all the plants tested was reported to be for 14-21d post 50% emergence of the control plants. This is in accordance with OECD Guideline 208, which was cited for the study protocol.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure levels reported. Spacing was adequate to observe a response.
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via soil.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The seeds were all reported to be from the same source and the same lot number, but the source was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if any acclimation occurred.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 6 pots per test concentration with 5 seeds per pot for each study.

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Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Brassica napus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784978

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Soil was steam sterilized and was analyzed by the LUFA and reported to be a sandy loam. The test was performed in a growth chamber under controlled conditions. The photoperiod was 16L:8D, and the temperature ranged between 16.4 and 27.5C with a mean humidity of 71%.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–plant fresh weight, plant emergence, and plant phytotoxicity.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Fresh weight was determined 14d (or 21d depending on the plant) after 50% of the seedlings emerged in the control. The above ground portion of the plant was used for this determination. Emergences was checked daily until 50% of the control plants emerged. Phytotoxicity was determined visually by observing chlorosis, necrosis, and abnormal growth and was recorded weekly after 50% control emergence.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No differences among groups were reported.
	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	The study reported appropriate statistical methods in the ”statistical analysis” section of the report on page 20 of the PDF.
	Metric 22:	Reporting of Data	High	Data for the control response as well as the exposure response were presented in Table 8 and Table 11 and were adequate for the outcome of interest.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. EC values provided 95% confidence intervals.

Additional Comments: This portion of the evaluation was on the effect of HHCB on the development/growth of B. napus. Fresh weights were taken 14d after 50% emergence of the controls. EC values were determined based off of the fresh weight values. Plant emergences was also included in the development/growth outcome. Phytotoxicity was also considered to be an outcome for this test. This was included in the development/growth outcome because abnormal growth was determined to be a factor in determining this outcome. Chlorosis and necrosis were also included in the phytotoxicity outcome.

Overall Quality Determination High

Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Brassica pekinensis*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by nomenclature and formula.
	Metric 2:	Test Substance Source	High	Purchased from Guoyao Chemical Co. Ltd. (Beijing, China)
	Metric 3:	Test Substance Purity	High	Given as ">98% purity".
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls and solvent controls were used.
	Metric 5:	Negative Control Response	Medium	There were differences between untreated and solvent controls (greater growth, and statistically significant differences in all measured parameters for all plants). Therefore comparisons were made to the solvent control.
	Metric 6:	Randomized Allocation	Medium	Tests conducted in accordance with OECD 208 which specifies randomization.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	HHCB was mixed with soil and it is unclear whether sealed vessels were used (doubtful for plant studies). As HHCB can volatilize from soil in the long term and nominal concentrations were not tested, there may be some discrepancy. The test duration was 21 days, and Schafer 2005 reported a volatilization of up to 16% after 28 days. This discrepancy is unlikely to have a major effect on results since it was replicated across all treatments and plants.
	Metric 8:	Consistency of Exposure Administration	High	Exposure same for all treatments.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were not measured, however HHCB does not rapidly biodegrade and sorbs to soil somewhat strongly. The discrepancy between nominal and actual exposure concentrations is unlikely to substantially effect the results.
	Metric 10:	Exposure Duration and Frequency	High	The study was conducted in accordance with OECD 208.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The range and spacing of exposure levels is not given for any plant studies, however the OECD 208 guideline specifies that appropriate rangefinding and exposure level choices must be made, and the text implies (ECx values calculated, NOECs mentioned) that a range of exposure levels was used. Additionally, some exposure levels are given in the Results section.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent used (acetone) caused measurable responses in all plant experiments. Comparisons of HHCB effect were made against solvent controls instead of blank controls.
Domain 4: Test Organism				

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Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Brassica pekinensis*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	Seeds were obtained from the Chinese Academy of Agricultural Sciences (Beijing, China)
	Metric 14:	Acclimatization and Pretreatment Conditions	High	OECD 208 guidelines were followed, conditions were appropriate for the plants and were the same for all groups & were consistent before treatment.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 plants were used per replicate, with 4 replicates per treatment including controls.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	OECD 208 guidelines were followed, conditions were appropriate for the plants and were the same for all groups & were consistent before treatment.
	Metric 17:	Outcome Assessment Methodology	High	Growth and biomass were measured at the end of the test according to OECD 208
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed the same across all groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences in environmental conditions across groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	"... there was no significant effect on the survival of the 8 terrestrial plants at the testing concentrations..."
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was adequately described in the methods section and appropriate for the experiments performed.
	Metric 22:	Reporting of Data	Low	Summary data (ECx, NOEC) was reported for the overall experiment for each organism, but individual data for each treatment and control was not reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, and the outcomes that were reported are adequately discussed in the text.

Additional Comments: This study also includes the calculation of an SSD from all 10 tested terrestrial organisms and the derivation of PNECs from said distribution, with discussion of AFs. In my opinion it would provide an important contribution to terrestrial data for HHCB, unfortunately the range and spacing of test concentrations for the 8 plants is not given.

Overall Quality Determination High

Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Cucumis sativus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8784978

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the HHCB was not reported, nor was it reported to be analytically verified, but a batch and lot number were reported.
	Metric 3:	Test Substance Purity	Low	The purity/grade of the HHCB was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	It was reported that a an appropriate negative control and a solvent control were both used in the study.
	Metric 5:	Negative Control Response	High	The biological response of the negative control was reported in Table 8 and Table 23 and was adequate for the outcome of interest.
	Metric 6:	Randomized Allocation	Low	It was not reported how the seeds were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	It was reported that the test concentrations were prepared by mixing the test substance with acetone and mixing in fine quartz sand before being mixed into the soil. The test system was a 12 cm pot, but the amount of soil was not reported.
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for similar time period in a temperature controlled room. All seeds were from the same source and the same lot number for each study. Test conditions were the same for all treatment groups.
	Metric 9:	Measurement of Test Substance Concentration	High	It was reported the test substance was measured using GC-FI.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration for all the plants tested was reported to be for 14-21d post 50% emergence of the control plants. This is in accordance with OECD Guideline 208, which was cited for the study protocol.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure levels reported. Spacing was adequate to observe a response.
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via soil.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The seeds were all reported to be from the same source and the same lot number, but the source was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if any acclimation occurred.

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Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Cucumis sativus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8784978

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 6 pots per test concentration with 5 seeds per pot for each study.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Soil was steam sterilized and was analyzed by the LUFA and reported to be a sandy loam. The test was performed in a growth chamber under controlled conditions. The photoperiod was 16L:8D, and the temperature ranged between 16.4 and 27.5C with a mean humidity of 71%.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–plant mortality.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Mortality was assessed on days 7 and 14 (as well as on day 21 if the test extended that long) post 50% emergence of the control. Plants were considered dead when no living tissue could be found on the leaves or the shoots.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No differences among groups were reported.
	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	The study reported appropriate statistical methods in the ”statistical analysis” section of the report on page 20 of the PDF.
	Metric 22:	Reporting of Data	High	Data for the control response as well as the exposure response were presented in Table 8 and Table 23 and were adequate for the outcome of interest.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Mortality from each pot was reported in Table 23.

Additional Comments: This portion of the evaluation was on the effect of HHCB on the %mortality of C. sativus. Plants were considered to be dead when no living tissue could be found on the leaves or shoots.

Overall Quality Determination High

Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Cucumis sativus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784978

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the HHCB was not reported, nor was it reported to be analytically verified, but a batch and lot number were reported.
	Metric 3:	Test Substance Purity	Low	The purity/grade of the HHCB was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	It was reported that a an appropriate negative control and a solvent control were both used in the study.
	Metric 5:	Negative Control Response	High	The biological response of the negative control was reported in Table 8, 21,22, and 24 as well as in Fig. 4. The response was adequate for the outcome of interest.
	Metric 6:	Randomized Allocation	Low	It was not reported how the seeds were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	It was reported that the test concentrations were prepared by mixing the test substance with acetone and mixing in fine quartz sand before being mixed into the soil. The test system was a 12 cm pot, but the amount of soil was not reported.
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for similar time period in a temperature controlled room. All seeds were from the same source and the same lot number for each study. Test conditions were the same for all treatment groups.
	Metric 9:	Measurement of Test Substance Concentration	High	It was reported the test substance was measured using GC-FI.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration for all the plants tested was reported to be for 14-21d post 50% emergence of the control plants. This is in accordance with OECD Guideline 208, which was cited for the study protocol.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure levels reported. Spacing was adequate to observe a response.
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via soil.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The seeds were all reported to be from the same source and the same lot number, but the source was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if any acclimation occurred.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 6 pots per test concentration with 5 seeds per pot for each study.

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Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Cucumis sativus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784978

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Soil was steam sterilized and was analyzed by the LUFA and reported to be a sandy loam. The test was performed in a growth chamber under controlled conditions. The photoperiod was 16L:8D, and the temperature ranged between 16.4 and 27.5C with a mean humidity of 71%.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–plant fresh weight, plant emergence, and plant phytotoxicity.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Fresh weight was determined 14d (or 21d depending on the plant) after 50% of the seedlings emerged in the control. The above ground portion of the plant was used for this determination. Emergence was checked daily until 50% of the control plants emerged. Phytotoxicity was determined visually by observing chlorosis, necrosis, and abnormal growth and was recorded weekly after 50% control emergence.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	No differences among groups were reported.
	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	The study reported appropriate statistical methods in the ”statistical analysis” section of the report on page 20 of the PDF.
	Metric 22:	Reporting of Data	High	Data for the control response as well as the exposure response were presented in Table 8, 21, 22, and 24 as well as in Fig 4.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. EC values provided 95% confidence intervals.

Additional Comments: This portion of the evaluation was on the effect of HHCB on the development/growth of C. sativus. Fresh weights were taken 14d after 50% emergence of the controls. EC values were determined based off of the fresh weight values. Plant emergences was also included in the development/growth outcome. Phytotoxicity was also considered to be an outcome for this test. This was included in the development/growth outcome because abnormal growth was determined to be a factor in determining this outcome. Chlorosis and necrosis were also included in the phytotoxicity outcome.

Overall Quality Determination High

Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Cucumis sativus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by nomenclature and formula.
	Metric 2:	Test Substance Source	High	Purchased from Guoyao Chemical Co. Ltd. (Beijing, China)
	Metric 3:	Test Substance Purity	High	Given as ">98% purity".
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls and solvent controls were used.
	Metric 5:	Negative Control Response	Medium	There were differences between untreated and solvent controls (greater growth, and statistically significant differences in all measured parameters for all plants). Therefore comparisons were made to the solvent control.
	Metric 6:	Randomized Allocation	Medium	Tests conducted in accordance with OECD 208 which specifies randomization.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	HHCB was mixed with soil and it is unclear whether sealed vessels were used (doubtful for plant studies). As HHCB can volatilize from soil in the long term and nominal concentrations were not tested, there may be some discrepancy. The test duration was 21 days, and Schafer 2005 reported a volatilization of up to 16% after 28 days. This discrepancy is unlikely to have a major effect on results since it was replicated across all treatments and plants.
	Metric 8:	Consistency of Exposure Administration	High	Exposure same for all treatments.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were not measured, however HHCB does not rapidly biodegrade and sorbs to soil somewhat strongly. The discrepancy between nominal and actual exposure concentrations is unlikely to substantially effect the results.
	Metric 10:	Exposure Duration and Frequency	High	The study was conducted in accordance with OECD 208.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The range and spacing of exposure levels is not given for any plant studies, however the OECD 208 guideline specifies that appropriate rangefinding and exposure level choices must be made, and the text implies (ECx values calculated, NOECs mentioned) that a range of exposure levels was used. Additionally, some exposure levels are given in the Results section.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent used (acetone) caused measurable responses in all plant experiments. Comparisons of HHCB effect were made against solvent controls instead of blank controls.
Domain 4: Test Organism				

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Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Cucumis sativus*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	Seeds were obtained from the Chinese Academy of Agricultural Sciences (Beijing, China)
	Metric 14:	Acclimatization and Pretreatment Conditions	High	OECD 208 guidelines were followed, conditions were appropriate for the plants and were the same for all groups & were consistent before treatment.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 plants were used per replicate, with 4 replicates per treatment including controls.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	OECD 208 guidelines were followed, conditions were appropriate for the plants and were the same for all groups & were consistent before treatment.
	Metric 17:	Outcome Assessment Methodology	High	Growth and biomass were measured at the end of the test according to OECD 208
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed the same across all groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences in environmental conditions across groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	"... there was no significant effect on the survival of the 8 terrestrial plants at the testing concentrations..."
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was adequately described in the methods section and appropriate for the experiments performed.
	Metric 22:	Reporting of Data	Low	Summary data (ECx, NOEC) was reported for the overall experiment for each organism, but individual data for each treatment and control was not reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, and the outcomes that were reported are adequately discussed in the text.

Additional Comments: This study also includes the calculation of an SSD from all 10 tested terrestrial organisms and the derivation of PNECs from said distribution, with discussion of AFs. In my opinion it would provide an important contribution to terrestrial data for HHCB, unfortunately the range and spacing of test concentrations for the 8 plants is not given.

Overall Quality Determination High

Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Glycine max*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784978

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the HHCB was not reported, nor was it reported to be analytically verified, but a batch and lot number were reported.
	Metric 3:	Test Substance Purity	Low	The purity/grade of the HHCB was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	It was reported that a an appropriate negative control and a solvent control were both used in the study.
	Metric 5:	Negative Control Response	High	The biological response of the negative control was reported in Table 8, 13, 14, and 16 as well as in Fig.2. The response was adequate for the outcome of interest.
	Metric 6:	Randomized Allocation	Low	It was not reported how the seeds were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	It was reported that the test concentrations were prepared by mixing the test substance with acetone and mixing in fine quartz sand before being mixed into the soil. The test system was a 12 cm pot, but the amount of soil was not reported.
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for similar time period in a temperature controlled room. All seeds were from the same source and the same lot number for each study. Test conditions were the same for all treatment groups.
	Metric 9:	Measurement of Test Substance Concentration	High	It was reported the test substance was measured using GC-FI.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration for all the plants tested was reported to be for 14-21d post 50% emergence of the control plants. This is in accordance with OECD Guideline 208, which was cited for the study protocol.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 7 exposure levels reported. Spacing was adequate to observe a response.
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via soil.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The seeds were all reported to be from the same source and the same lot number, but the source was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if any acclimation occurred.

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Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Glycine max*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784978

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 6 pots per test concentration with 5 seeds per pot for each study.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Soil was steam sterilized and was analyzed by the LUFA and reported to be a sandy loam. The test was performed in a growth chamber under controlled conditions. The photoperiod was 16L:8D, and the temperature ranged between 16.4 and 27.5C with a mean humidity of 71%.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–plant fresh weight, plant emergence, and plant phytotoxicity.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Fresh weight was determined 14d (or 21d depending on the plant) after 50% of the seedlings emerged in the control. The above ground portion of the plant was used for this determination. Emergence was checked daily until 50% of the control plants emerged. Phytotoxicity was determined visually by observing chlorosis, necrosis, and abnormal growth and was recorded weekly after 50% control emergence.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No differences among groups were reported.
	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	The study reported appropriate statistical methods in the ”statistical analysis” section of the report on page 20 of the PDF.
	Metric 22:	Reporting of Data	High	Data for the control response as well as the exposure response were presented in Table 8, 13, 14, and 16 as well as in Fig 2.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. EC values provided 95% confidence intervals.

Additional Comments: This portion of the evaluation was on the effect of HHCB on the development/growth of G. max. Fresh weights were taken 14d after 50% emergence of the controls. EC values were determined based off of the fresh weight values. Plant emergences was also included in the development/growth outcome. Phytotoxicity was also considered to be an outcome for this test. This was included in the development/growth outcome because abnormal growth was determined to be a factor in determining this outcome. Chlorosis and necrosis were also included in the phytotoxicity outcome.

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Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Glycine max*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784978

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

Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Glycine max*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8784978

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the HHCB was not reported, nor was it reported to be analytically verified, but a batch and lot number were reported.
	Metric 3:	Test Substance Purity	Low	The purity/grade of the HHCB was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	It was reported that a an appropriate negative control and a solvent control were both used in the study.
	Metric 5:	Negative Control Response	High	The biological response of the negative control was reported in Table 8 and Table 15 and was adequate for the outcome of interest.
	Metric 6:	Randomized Allocation	Low	It was not reported how the seeds were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	It was reported that the test concentrations were prepared by mixing the test substance with acetone and mixing in fine quartz sand before being mixed into the soil. The test system was a 12 cm pot, but the amount of soil was not reported.
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for similar time period in a temperature controlled room. All seeds were from the same source and the same lot number for each study. Test conditions were the same for all treatment groups.
	Metric 9:	Measurement of Test Substance Concentration	High	It was reported the test substance was measured using GC-FI.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration for all the plants tested was reported to be for 14-21d post 50% emergence of the control plants. This is in accordance with OECD Guideline 208, which was cited for the study protocol.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 7 exposure levels reported. Spacing was adequate to observe a response.
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via soil.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The seeds were all reported to be from the same source and the same lot number, but the source was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if any acclimation occurred.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 6 pots per test concentration with 5 seeds per pot for each study.

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Study Citation: IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration: Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path: Terrestrial; Soil; 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; *Glycine max*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome: Mortality
Chemical: HHCB
HERO ID: 8784978

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Soil was steam sterilized and was analyzed by the LUFA and reported to be a sandy loam. The test was performed in a growth chamber under controlled conditions. The photoperiod was 16L:8D, and the temperature ranged between 16.4 and 27.5C with a mean humidity of 71%.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–plant mortality.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Mortality was assessed on days 7 and 14 (as well as on day 21 if the test extended that long) post 50% emergence of the control. Plants were considered dead when no living tissue could be found on the leaves or the shoots.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No differences among groups were reported.
	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	The study reported appropriate statistical methods in the ”statistical analysis” section of the report on page 20 of the PDF.
	Metric 22:	Reporting of Data	High	Data for the control response as well as the exposure response were presented in Table 8 and Table 15 and were adequate for the outcome of interest.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Mortality from each pot was reported in Table 15.

Additional Comments: This portion of the evaluation was on the effect of HHCB on the %mortality of G. max. Plants were considered to be dead when no living tissue could be found on the leaves or shoots.

Overall Quality Determination High

Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Glycine max*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by nomenclature and formula.
	Metric 2:	Test Substance Source	High	Purchased from Guoyao Chemical Co. Ltd. (Beijing, China)
	Metric 3:	Test Substance Purity	High	Given as ">98% purity".
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls and solvent controls were used.
	Metric 5:	Negative Control Response	Medium	There were differences between untreated and solvent controls (greater growth, and statistically significant differences in all measured parameters for all plants). Therefore comparisons were made to the solvent control.
	Metric 6:	Randomized Allocation	Medium	Tests conducted in accordance with OECD 208 which specifies randomization.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	HHCB was mixed with soil and it is unclear whether sealed vessels were used (doubtful for plant studies). As HHCB can volatilize from soil in the long term and nominal concentrations were not tested, there may be some discrepancy. The test duration was 21 days, and Schafer 2005 reported a volatilization of up to 16% after 28 days. This discrepancy is unlikely to have a major effect on results since it was replicated across all treatments and plants.
	Metric 8:	Consistency of Exposure Administration	High	Exposure same for all treatments.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were not measured, however HHCB does not rapidly biodegrade and sorbs to soil somewhat strongly. The discrepancy between nominal and actual exposure concentrations is unlikely to substantially effect the results.
	Metric 10:	Exposure Duration and Frequency	High	The study was conducted in accordance with OECD 208.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The range and spacing of exposure levels is not given for any plant studies, however the OECD 208 guideline specifies that appropriate rangefinding and exposure level choices must be made, and the text implies (ECx values calculated, NOECs mentioned) that a range of exposure levels was used.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent used (acetone) caused measurable responses in all plant experiments. Comparisons of HHCB effect were made against solvent controls instead of blank controls.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Seeds were obtained from the Chinese Academy of Agricultural Sciences (Beijing, China)

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Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Glycine max*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	High	OECD 208 guidelines were followed, conditions were appropriate for the plants and were the same for all groups & were consistent before treatment.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 plants were used per replicate, with 4 replicates per treatment including controls.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	OECD 208 guidelines were followed, conditions were appropriate for the plants and were the same for all groups & were consistent before treatment.
	Metric 17:	Outcome Assessment Methodology	High	Growth and biomass were measured at the end of the test according to OECD 208
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed the same across all groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences in environmental conditions across groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	"... there was no significant effect on the survival of the 8 terrestrial plants at the testing concentrations..."
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was adequately described in the methods section and appropriate for the experiments performed.
	Metric 22:	Reporting of Data	Low	Summary data (ECx, NOEC) was reported for the overall experiment for each organism, but individual data for each treatment and control was not reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, and the outcomes that were reported are adequately discussed in the text.

Additional Comments: This study also includes the calculation of an SSD from all 10 tested terrestrial organisms and the derivation of PNECs from said distribution, with discussion of AFs. In my opinion it would provide an important contribution to terrestrial data for HHCB.

Overall Quality Determination High

Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Lactuca sativa*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by nomenclature and formula.
	Metric 2:	Test Substance Source	High	Purchased from Guoyao Chemical Co. Ltd. (Beijing, China)
	Metric 3:	Test Substance Purity	High	Given as ">98% purity".
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls and solvent controls were used.
	Metric 5:	Negative Control Response	Medium	There were differences between untreated and solvent controls (greater growth, and statistically significant differences in all measured parameters for all plants). Therefore comparisons were made to the solvent control.
	Metric 6:	Randomized Allocation	Medium	Tests conducted in accordance with OECD 208 which specifies randomization.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	HHCB was mixed with soil and it is unclear whether sealed vessels were used (doubtful for plant studies). As HHCB can volatilize from soil in the long term and nominal concentrations were not tested, there may be some discrepancy. The test duration was 21 days, and Schafer 2005 reported a volatilization of up to 16% after 28 days. This discrepancy is unlikely to have a major effect on results since it was replicated across all treatments and plants.
	Metric 8:	Consistency of Exposure Administration	High	Exposure same for all treatments.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were not measured, however HHCB does not rapidly biodegrade and sorbs to soil somewhat strongly. The discrepancy between nominal and actual exposure concentrations is unlikely to substantially effect the results.
	Metric 10:	Exposure Duration and Frequency	High	The study was conducted in accordance with OECD 208.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The range and spacing of exposure levels is not given for any plant studies, however the OECD 208 guideline specifies that appropriate rangefinding and exposure level choices must be made, and the text implies (ECx values calculated, NOECs mentioned) that a range of exposure levels was used. Additionally, some exposure levels are given in the Results section.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent used (acetone) caused measurable responses in all plant experiments. Comparisons of HHCB effect were made against solvent controls instead of blank controls.
Domain 4: Test Organism				

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Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Lactuca sativa*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	Seeds were obtained from the Chinese Academy of Agricultural Sciences (Beijing, China)
	Metric 14:	Acclimatization and Pretreatment Conditions	High	OECD 208 guidelines were followed, conditions were appropriate for the plants and were the same for all groups & were consistent before treatment.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 plants were used per replicate, with 4 replicates per treatment including controls.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	OECD 208 guidelines were followed, conditions were appropriate for the plants and were the same for all groups & were consistent before treatment.
	Metric 17:	Outcome Assessment Methodology	High	Growth and biomass were measured at the end of the test according to OECD 208
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed the same across all groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences in environmental conditions across groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	"... there was no significant effect on the survival of the 8 terrestrial plants at the testing concentrations..."
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was adequately described in the methods section and appropriate for the experiments performed.
	Metric 22:	Reporting of Data	Low	Summary data (ECx, NOEC) was reported for the overall experiment for each organism, but individual data for each treatment and control was not reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, and the outcomes that were reported are adequately discussed in the text.

Additional Comments: This study also includes the calculation of an SSD from all 10 tested terrestrial organisms and the derivation of PNECs from said distribution, with discussion of AFs. In my opinion it would provide an important contribution to terrestrial data for HHCB, unfortunately the range and spacing of test concentrations for the 8 plants is not given.

Overall Quality Determination High

Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Solaneum lycopersicum*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by nomenclature and formula.
	Metric 2:	Test Substance Source	High	Purchased from Guoyao Chemical Co. Ltd. (Beijing, China)
	Metric 3:	Test Substance Purity	High	Given as ">98% purity".
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls and solvent controls were used.
	Metric 5:	Negative Control Response	Medium	There were differences between untreated and solvent controls (greater growth, and statistically significant differences in all measured parameters for all plants). Therefore comparisons were made to the solvent control.
	Metric 6:	Randomized Allocation	Medium	Tests conducted in accordance with OECD 208 which specifies randomization.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	HHCB was mixed with soil and it is unclear whether sealed vessels were used (doubtful for plant studies). As HHCB can volatilize from soil in the long term and nominal concentrations were not tested, there may be some discrepancy. The test duration was 21 days, and Schafer 2005 reported a volatilization of up to 16% after 28 days. This discrepancy is unlikely to have a major effect on results since it was replicated across all treatments and plants.
	Metric 8:	Consistency of Exposure Administration	High	Exposure same for all treatments.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were not measured, however HHCB does not rapidly biodegrade and sorbs to soil somewhat strongly. The discrepancy between nominal and actual exposure concentrations is unlikely to substantially effect the results.
	Metric 10:	Exposure Duration and Frequency	High	The study was conducted in accordance with OECD 208.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The range and spacing of exposure levels is not given for any plant studies, however the OECD 208 guideline specifies that appropriate rangefinding and exposure level choices must be made, and the text implies (ECx values calculated, NOECs mentioned) that a range of exposure levels was used. Additionally, some exposure levels are given in the Results section.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent used (acetone) caused measurable responses in all plant experiments. Comparisons of HHCB effect were made against solvent controls instead of blank controls.
Domain 4: Test Organism				

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Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Solaneum lycopersicum*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	Seeds were obtained from the Chinese Academy of Agricultural Sciences (Beijing, China)
	Metric 14:	Acclimatization and Pretreatment Conditions	High	OECD 208 guidelines were followed, conditions were appropriate for the plants and were the same for all groups & were consistent before treatment.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 plants were used per replicate, with 4 replicates per treatment including controls.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	OECD 208 guidelines were followed, conditions were appropriate for the plants and were the same for all groups & were consistent before treatment.
	Metric 17:	Outcome Assessment Methodology	High	Growth and biomass were measured at the end of the test according to OECD 208
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed the same across all groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences in environmental conditions across groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	"... there was no significant effect on the survival of the 8 terrestrial plants at the testing concentrations..."
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was adequately described in the methods section and appropriate for the experiments performed.
	Metric 22:	Reporting of Data	Low	Summary data (ECx, NOEC) was reported for the overall experiment for each organism, but individual data for each treatment and control was not reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, and the outcomes that were reported are adequately discussed in the text.

Additional Comments: This study also includes the calculation of an SSD from all 10 tested terrestrial organisms and the derivation of PNECs from said distribution, with discussion of AFs. In my opinion it would provide an important contribution to terrestrial data for HHCB, unfortunately the range and spacing of test concentrations for the 8 plants is not given.

Overall Quality Determination High

Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Solanum lycopersicum*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8784978

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the HHCB was not reported, nor was it reported to be analytically verified, but a batch and lot number were reported.
	Metric 3:	Test Substance Purity	Low	The purity/grade of the HHCB was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	It was reported that a an appropriate negative control and a solvent control were both used in the study.
	Metric 5:	Negative Control Response	High	The biological response of the negative control was reported in Table 8 and Table 19vand was adequate for the outcome of interest.
	Metric 6:	Randomized Allocation	Low	It was not reported how the seeds were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	It was reported that the test concentrations were prepared by mixing the test substance with acetone and mixing in fine quartz sand before being mixed into the soil. The test system was a 12 cm pot, but the amount of soil was not reported.
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for similar time period in a temperature controlled room. All seeds were from the same source and the same lot number for each study. Test conditions were the same for all treatment groups.
	Metric 9:	Measurement of Test Substance Concentration	High	It was reported the test substance was measured using GC-FI.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration for all the plants tested was reported to be for 14-21d post 50% emergence of the control plants. This is in accordance with OECD Guideline 208, which was cited for the study protocol.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure levels reported. Spacing was adequate to observe a response.
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via soil.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The seeds were all reported to be from the same source and the same lot number, but the source was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if any acclimation occurred.

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Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Solanum lycopersicum*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	8784978

Domain	Metric		Rating	Comments
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 6 pots per test concentration with 5 seeds per pot for each study.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Soil was steam sterilized and was analyzed by the LUFA and reported to be a sandy loam. The test was performed in a growth chamber under controlled conditions. The photoperiod was 16L:8D, and the temperature ranged between 16.4 and 27.5C with a mean humidity of 71%.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–plant mortality.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Mortality was assessed on days 7 and 14 (as well as on day 21 if the test extended that long) post 50% emergence of the control. Plants were considered dead when no living tissue could be found on the leaves or the shoots.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No differences among groups were reported.
	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	The study reported appropriate statistical methods in the ”statistical analysis” section of the report on page 20 of the PDF.
	Metric 22:	Reporting of Data	High	Data for the control response as well as the exposure response were presented in Table 8 and Table 19 and were adequate for the outcome of interest.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Mortality from each pot was reported in Table 19.

Additional Comments: This portion of the evaluation was on the effect of HHCB on the %mortality of S. lycopersicum. Plants were considered to be dead when no living tissue could be found on the leaves or shoots.

Overall Quality Determination High

Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Solanum lycopersicum*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784978

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	The HHCB was identified by CASRN.
	Metric 2:	Test Substance Source	Low	The source of the HHCB was not reported, nor was it reported to be analytically verified, but a batch and lot number were reported.
	Metric 3:	Test Substance Purity	Low	The purity/grade of the HHCB was not reported.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	It was reported that a an appropriate negative control and a solvent control were both used in the study.
	Metric 5:	Negative Control Response	High	The biological response of the negative control was reported in Table 8, 17,18, and 20 as well as in Fig. 3. The response was adequate for the outcome of interest.
	Metric 6:	Randomized Allocation	Low	It was not reported how the seeds were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	It was reported that the test concentrations were prepared by mixing the test substance with acetone and mixing in fine quartz sand before being mixed into the soil. The test system was a 12 cm pot, but the amount of soil was not reported.
	Metric 8:	Consistency of Exposure Administration	High	All exposures were for similar time period in a temperature controlled room. All seeds were from the same source and the same lot number for each study. Test conditions were the same for all treatment groups.
	Metric 9:	Measurement of Test Substance Concentration	High	It was reported the test substance was measured using GC-FI.
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration for all the plants tested was reported to be for 14-21d post 50% emergence of the control plants. This is in accordance with OECD Guideline 208, which was cited for the study protocol.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 5 exposure levels reported. Spacing was adequate to observe a response.
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via soil.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Low	The seeds were all reported to be from the same source and the same lot number, but the source was not reported.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if any acclimation occurred.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	There were 6 pots per test concentration with 5 seeds per pot for each study.

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Study Citation:	IFF, (2019). HHCB: Effects on terrestrial (non-target) plants: Seedling emergence and seedling growth test (sanitized).
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; 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; *Solanum lycopersicum*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	8784978

Domain	Metric		Rating	Comments
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Soil was steam sterilized and was analyzed by the LUFA and reported to be a sandy loam. The test was performed in a growth chamber under controlled conditions. The photoperiod was 16L:8D, and the temperature ranged between 16.4 and 27.5C with a mean humidity of 71%.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcome of interest–plant fresh weight, plant emergence, and plant phytotoxicity.
	Metric 18:	Consistency of Outcome Assessment	High	Details of the outcome assessment protocol were reported, and outcomes were assessed consistently across study groups. Fresh weight was determined 14d (or 21d depending on the plant) after 50% of the seedlings emerged in the control. The above ground portion of the plant was used for this determination. Emergence was checked daily until 50% of the control plants emerged. Phytotoxicity was determined visually by observing chlorosis, necrosis, and abnormal growth and was recorded weekly after 50% control emergence.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No differences among groups were reported.
	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	The study reported appropriate statistical methods in the ”statistical analysis” section of the report on page 20 of the PDF.
	Metric 22:	Reporting of Data	High	Data for the control response as well as the exposure response were presented in Table 8, 17, 18, and 20 as well as in Fig 3.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. EC values provided 95% confidence intervals.

Additional Comments: This portion of the evaluation was on the effect of HHCB on the development/growth of S. lycopersicum. Fresh weights were taken 14d after 50% emergence of the controls. EC values were determined based off of the fresh weight values. Plant emergences was also included in the development/growth outcome. Phytotoxicity was also considered to be an outcome for this test. This was included in the development/growth outcome because abnormal growth was determined to be a factor in determining this outcome. Chlorosis and necrosis were also included in the phytotoxicity outcome.

Overall Quality Determination High

Study Citation:	An, J., Zhou, Q., Sun, Y., Xu, Z. (2009). Ecotoxicological effects of typical personal care products on seed germination and seedling development of wheat (Triticum aestivum L.). Chemosphere 76(10):1428-1434.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Terrestrial; Water; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum*; Embryo
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	5427815

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified as HHCB
	Metric 2:	Test Substance Source	High	Source provided as Wuhan Yuancheng Technology Development Co. Ltd., China,
	Metric 3:	Test Substance Purity	High	99% purity reported – analytical grade.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	negative controls used
	Metric 5:	Negative Control Response	High	Response of negative control group was acceptable
	Metric 6:	Randomized Allocation	Low	allocation not reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Static system with test solutions renewed every day to avoid any change in the concentration.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	The test solutions were renewed every day to avoid any change in the concentration, but concentrations were not analytically verified
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration and concentrations reported.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	5 exposure groups with 3 replicates per group were used.
	Metric 12:	Testing at or Below Solubility Limit	Low	Authors did not report using a solvent. I could not find any mention of HHCB's solubility in the study, and did not see an appendix or supplementary materials section which might have more details. In this test "HHCB in the formal solution culturewere ascertained and equal to 0, 50, 100, 150, 200 and 250 mg/ L" which is far higher than the 1.75mg/L water solubility reported in the HHCB scope document
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms adequately described.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimation conditions wee reported and consistent across groups.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	3 replicates per group.
Domain 5: Outcome Assessment				

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Study Citation:	An, J., Zhou, Q., Sun, Y., Xu, Z. (2009). Ecotoxicological effects of typical personal care products on seed germination and seedling development of wheat (Triticum aestivum L.). Chemosphere 76(10):1428-1434.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Terrestrial; Water; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum*; Embryo
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	5427815

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Test conditions wee reported, adequate, and consistent across groups.
	Metric 17:	Outcome Assessment Methodology	High	Outcomes of interest were reported.
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessments were appropriate and consistent across groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No reported non-treatment variables that were inconsistent across groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	No outcomes unrelated to exposure reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	ANOVA was used for analyses.
	Metric 22:	Reporting of Data	High	All exposure related data.
	Metric 23:	Explanation of Unexpected Outcomes	High	All outcomes satisfactorily explained.

Additional Comments: This evaluation is for the effect of HHCB on seed germination.

Overall Quality Determination High

Study Citation:	An, J., Zhou, Q., Sun, Y., Xu, Z. (2009). Ecotoxicological effects of typical personal care products on seed germination and seedling development of wheat (Triticum aestivum L.). Chemosphere 76(10):1428-1434.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Terrestrial; Water; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum*; Embryo
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427815

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified as HHCB
	Metric 2:	Test Substance Source	High	Source provided as Wuhan Yuancheng Technology Development Co. Ltd., China,
	Metric 3:	Test Substance Purity	High	99% purity reported – analytical grade.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	negative controls used
	Metric 5:	Negative Control Response	High	Response of negative control group was acceptable
	Metric 6:	Randomized Allocation	Low	allocation not reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Static system with test solutions renewed every day to avoid any change in the concentration.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	The test solutions were renewed every day to avoid any change in the concentration, but concentrations were not analytically verified
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration and concentrations reported.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	5 exposure groups with 3 replicates per group were used.
	Metric 12:	Testing at or Below Solubility Limit	Low	Authors did not report using a solvent. I could not find any mention of HHCB's solubility in the study, and did not see an appendix or supplementary materials section which might have more details. In this test "HHCB in the formal solution culturewere ascertained and equal to 0, 50, 100, 150, 200 and 250 mg/ L" which is far higher than the 1.75mg/L water solubility reported in the HHCB scope document
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms adequately described.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimation conditions wee reported and consistent across groups.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	3 replicates per group.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Test conditions wee reported, adequate, and consistent across groups.
	Metric 17:	Outcome Assessment Methodology	High	Outcomes of interest were reported.

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Study Citation:	An, J., Zhou, Q., Sun, Y., Xu, Z. (2009). Ecotoxicological effects of typical personal care products on seed germination and seedling development of wheat (Triticum aestivum L.). Chemosphere 76(10):1428-1434.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Terrestrial; Water; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum*; Embryo
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427815

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessments were appropriate and consistent across groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No reported non-treatment variables that were inconsistent across groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	No outcomes unrelated to exposure reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	ANOVA was used for analyses.
	Metric 22:	Reporting of Data	High	All exposure related data.
	Metric 23:	Explanation of Unexpected Outcomes	High	All outcomes satisfactorily explained.

Additional Comments: None

Overall Quality Determination High

Study Citation:	An, J., Zhou, Q., Sun, Y., Xu, Z. (2009). Ecotoxicological effects of typical personal care products on seed germination and seedling development of wheat (Triticum aestivum L.). Chemosphere 76(10):1428-1434.
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; *Triticum aestivum*; Juvenile
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)-Photosynthesis
Chemical:	HHCB
HERO ID:	5427815

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified as HHCB
	Metric 2:	Test Substance Source	High	Source provided as Wuhan Yuancheng Technology Development Co. Ltd., China,
	Metric 3:	Test Substance Purity	High	99% purity reported – analytical grade.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	negative controls used
	Metric 5:	Negative Control Response	High	Response of negative control group was acceptable
	Metric 6:	Randomized Allocation	Low	allocation not reported
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	Static system with test solutions renewed every day to avoid any change in the concentration.
	Metric 8:	Consistency of Exposure Administration	High	Exposures were consistent across groups.
	Metric 9:	Measurement of Test Substance Concentration	Medium	The test solutions were renewed every day to avoid any change in the concentration, but concentrations were not analytically verified
	Metric 10:	Exposure Duration and Frequency	High	Exposure duration and concentrations reported.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	5 exposure groups with 3 replicates per group were used.
	Metric 12:	Testing at or Below Solubility Limit	Low	Authors did not report using a solvent. I could not find any mention of HHCB's solubility in the study, and did not see an appendix or supplementary materials section which might have more details. In this test "HHCB in the formal solution culturewere ascertained and equal to 0, 50, 100, 150, 200 and 250 mg/ L" which is far higher than the 1.75mg/L water solubility reported in the HHCB scope document
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Test organisms adequately described.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	Acclimation conditions wee reported and consistent across groups.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	3 replicates per group.
Domain 5: Outcome Assessment				

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Study Citation:	An, J., Zhou, Q., Sun, Y., Xu, Z. (2009). Ecotoxicological effects of typical personal care products on seed germination and seedling development of wheat (Triticum aestivum L.). Chemosphere 76(10):1428-1434.
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; *Triticum aestivum*; Juvenile
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)-Photosynthesis
Chemical:	HHCB
HERO ID:	5427815

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Test conditions wee reported, adequate, and consistent across groups.
	Metric 17:	Outcome Assessment Methodology	High	Outcomes of interest were reported.
	Metric 18:	Consistency of Outcome Assessment	High	Outcome assessments were appropriate and consistent across groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	No reported non-treatment variables that were inconsistent across groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	No outcomes unrelated to exposure reported.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	ANOVA was used for analyses.
	Metric 22:	Reporting of Data	High	All exposure related data.
	Metric 23:	Explanation of Unexpected Outcomes	High	All outcomes satisfactorily explained.

Additional Comments: This evaluation is for the effect of HHCB on seedling oxidative stress enzymes, photosynthesis, and soluble protein.

Overall Quality Determination High

Study Citation:	Chen, C., Cai, Z. (2015). Physiological and antioxidant responses in wheat (Triticum aestivum) to HHCB in soil. Bulletin of Environmental Contamination and Toxicology 95(2):272-277.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum*; Juvenile
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)
Chemical:	HHCB
HERO ID:	4690052

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The test chemical was only identified by name.
	Metric 2:	Test Substance Source	Low	The HHCB was purchased from Dr. Ehrenstorfer Co., Ltd (Germany). It was not reported if the test substance was analytically verified.
	Metric 3:	Test Substance Purity	High	The purity was reported as 98%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	An appropriate concurrent negative control group was used.
	Metric 5:	Negative Control Response	High	Control responses were reported and adequate.
	Metric 6:	Randomized Allocation	Low	Authors did not report how organisms were allocated to study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Some details were provided on the experimental system. The preparation of the test solution was not described.
	Metric 8:	Consistency of Exposure Administration	Medium	Since some details were lacking, there could have been some issues with consistency. It is unclear.
	Metric 9:	Measurement of Test Substance Concentration	High	HHCB was analyzed by Agilent 7890A GC-5975C MS instrument (Agilent Technologies, Santa Clara, CA, USA).
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	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 was via soil.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	The seeds were obtained from Tianjin Academy of Agricultural Sciences, Tianjin, CN. No details were given on culturing, but authors mentioned they followed methods outlined in Chen et al. (2014).
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	There was no mention of specific culture conditions prior to test start.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Each treatment had three replicates.
Domain 5: Outcome Assessment				

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Study Citation:	Chen, C., Cai, Z. (2015). Physiological and antioxidant responses in wheat (Triticum aestivum) to HHCB in soil. Bulletin of Environmental Contamination and Toxicology 95(2):272-277.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum*; Juvenile
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)
Chemical:	HHCB
HERO ID:	4690052

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions during the test were not reported.
	Metric 17:	Outcome Assessment Methodology	High	Methods to assess lipid peroxidation and the antioxidant system were described.
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment was consistent across treatment groups. Assessment occurred after 7, 14, and 21 days.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	There was no reporting of acclimation prior to test start and details on environmental conditions were not provided.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups that could influence the outcome.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were described and adequate.
	Metric 22:	Reporting of Data	High	Results were described in the text. MDA results were shown in Figure 2. SOD activity results were shown in Figure 3. CAT activity results were shown in Figure 4. POD activity results were shown in Figure 5.
	Metric 23:	Explanation of Unexpected Outcomes	High	Measures of variability were shown in the figures.

Additional Comments: This evaluation is for the assessment of lipid peroxidation and the antioxidant system.

Overall Quality Determination Medium

Study Citation:	Chen, C., Cai, Z. (2015). Physiological and antioxidant responses in wheat (Triticum aestivum) to HHCB in soil. Bulletin of Environmental Contamination and Toxicology 95(2):272-277.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum*; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	4690052

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The test chemical was only identified by name.
	Metric 2:	Test Substance Source	Low	The HHCB was purchased from Dr. Ehrenstorfer Co., Ltd (Germany). It was not reported if the test substance was analytically verified.
	Metric 3:	Test Substance Purity	High	The purity was reported as 98%.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	An appropriate concurrent negative control group was used.
	Metric 5:	Negative Control Response	High	The control responses were reported and seemed appropriate.
	Metric 6:	Randomized Allocation	Low	Authors did not report how organisms were allocated.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Some details were provided on the experimental system. The preparation of the test solution was not described.
	Metric 8:	Consistency of Exposure Administration	Medium	Since some details were lacking, there could have been some issues with consistency. It is unclear.
	Metric 9:	Measurement of Test Substance Concentration	High	HHCB was analyzed by Agilent 7890A GC-5975C MS instrument (Agilent Technologies, Santa Clara, CA, USA).
	Metric 10:	Exposure Duration and Frequency	High	The exposure duration was appropriate for the study type.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	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 was via soil.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	Medium	The seeds were obtained from Tianjin Academy of Agricultural Sciences, Tianjin, CN. No details were given on culturing, but authors mentioned they followed methods outlined in Chen et al. (2014).
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	There was no mention of specific culture conditions prior to test start.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Each treatment had three replicates.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Low	Environmental conditions during the test were not reported.
	Metric 17:	Outcome Assessment Methodology	High	Methods to assess growth (chlorophyll concentration) were described.

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Study Citation:	Chen, C., Cai, Z. (2015). Physiological and antioxidant responses in wheat (Triticum aestivum) to HHCB in soil. Bulletin of Environmental Contamination and Toxicology 95(2):272-277.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum*; Juvenile
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	4690052

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	The outcome assessment was consistent across treatment groups. Assessment occurred after 7, 14, and 21 days.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Low	There was no reporting of acclimation prior to test start and details on environmental conditions were not provided.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups that could influence the outcome.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical methods were described and adequate.
	Metric 22:	Reporting of Data	High	Results were described in the text. Chlorophyll results were shown in Figure 1.
	Metric 23:	Explanation of Unexpected Outcomes	High	Measures of variability were shown in Figure 1.

Additional Comments: This evaluation is for the assessment of growth (measurement of chlorophyll concentration).

Overall Quality Determination Medium

Study Citation:	Chen, C., Zhou, Q., Cai, Z. (2014). Effect of soil HHCB on cadmium accumulation and phytotoxicity in wheat seedlings. Ecotoxicology 23(10):1996-2004.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum*; Embryo
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)-Photosynthesis
Chemical:	HHCB
HERO ID:	3399556

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The HHCB was identified by name only.
	Metric 2:	Test Substance Source	Low	The HHCB was reported to be from Dr. Ehrenstorfer in Germany. It was not reported if it was analytically verified.
	Metric 3:	Test Substance Purity	High	It was reported that all reagents used in this study were of analytical grade.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Study authors reported the use of a concurrent negative control in which no HHCB or cadmium were used.
	Metric 5:	Negative Control Response	High	The negative control responses were adequate for the outcomes of interest. The negative control responses for CHL, MDA, SOD, CAT, and POD were reported in figures 2-6.
	Metric 6:	Randomized Allocation	Low	It was not reported how the seeds were allocated into study groups.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Low	The preparation of the test concentrations in the soil was not reported. There were two concentrations of HHCB in the soil, 1 and 10mg/kg dry weight of soil. Seeds were germinated in a biochemical culturing box. 50g of soil with different concentrations of cadmium and HHCB were placed in 90mm Petri dishes. 10 seeds germinated for 24h were sowed in the soil, and the Petri dishes were put in an illumination chamber. The soil was maintained at a moisture of 24% (v:w). The seedling were allowed to grow for 14d, and then the tissues were harvested for analysis.
	Metric 8:	Consistency of Exposure Administration	Medium	The preparation of the test substance and soil preparation was not reported, creating concern about consistency. All exposures were for 14 days in 90mm Petri dishes with the same number of seedlings. The photoperiod was 12h light with a temperature of 25C and a soil moisture of 24% (v:w). Each treatment had 3 replicates.
	Metric 9:	Measurement of Test Substance Concentration	Low	It was not reported if the HHCB concentrations were analyzed.
	Metric 10:	Exposure Duration and Frequency	Medium	The exposure duration was reported to be 14d, this was adequate to observe the outcomes of interest.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Medium	There were only two exposure levels for HHCB, 1 and 10mg/kg HHCB dry weight of soil. This still allowed for adequate results for the outcomes of interest to be observed. More exposure levels may have yielded more data.
	Metric 12:	Testing at or Below Solubility Limit	N/A	This exposure was via soil.
Domain 4: Test Organism				

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Study Citation: Chen, C., Zhou, Q., Cai, Z. (2014). Effect of soil HHCB on cadmium accumulation and phytotoxicity in wheat seedlings. Ecotoxicology 23(10):1996-2004.
Duration: Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path: Terrestrial; Soil; Root uptake
Taxa, Species, Age: Vegetation; Vascular Plants; *Triticum aestivum*; Embryo
Health Outcome: Mechanistic-Oxidative stress (including redox biology)-Photosynthesis
Chemical: HHCB
HERO ID: 3399556

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	The wheat sees were obtained from Tianjin Academy of Agricultural Sciences in China.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	It was not reported if any acclimation occurred prior to the start of the exposure.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	It was reported that there were 10 seeds germinated per Petri dish. There were three replicates for each treatment.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	It was not reported if the soil was fertilized or the plants fed in any manner during the test. The soil was reported to be collected from an uncontaminated field in an arboretum in Tianjin, China; the soil characteristics are reported in Table 1. The soil moisture was maintained at 24% (v:w). The seeds were kept in an illumination incubator with a photoperiod of 12hr light, and the temperature was kept at 25C.
	Metric 17:	Outcome Assessment Methodology	High	The outcome assessment methodology addressed or reported the intended outcomes of interest–mechanistic outcomes for chlorophyll, MDA, SOD, CAT, and POD.
	Metric 18:	Consistency of Outcome Assessment	High	At the end of the 14d growing period, the wheat tissues were harvested separately and immediately stored in a -80C freezer until analysis. Each assay was performed consistently for each test run.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences among the study groups.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	There was no information in the study to suggest differences among groups in animal attrition or health outcomes unrelated to exposure.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	It was reported that results were presented with mean standard deviations, and statistical analyses were made with SPSS 13.0. However, actual statistical methods were not reported or were reported with very limited details. Variance analysis was mentioned, but little information was provided.
	Metric 22:	Reporting of Data	High	Data for HHCB only is presented for all mechanistic outcomes in figures 2-6. Data is presented in terms of soil cadmium, but the HHCB only data can be found under the 0mg/kg dry weight of soil of cadmium portion of the graphs.
	Metric 23:	Explanation of Unexpected Outcomes	High	Study authors did not report any unexpected outcomes. Variability is reported in each figure.

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Study Citation:	Chen, C., Zhou, Q., Cai, Z. (2014). Effect of soil HHCB on cadmium accumulation and phytotoxicity in wheat seedlings. Ecotoxicology 23(10):1996-2004.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum*; Embryo
Health Outcome:	Mechanistic-Oxidative stress (including redox biology)-Photosynthesis
Chemical:	HHCB
HERO ID:	3399556

Domain	Metric	Rating	Comments
Additional Comments:	This study looked at the effect of HHCB and cadmium on chlorophyll in wheat, as well as levels of MDA, SOD, CAT, and POD levels in the roots and leaves of wheat. Mechanistic outcomes for photosynthesis and oxidation were chosen as the outcomes of interest. Results were reported in terms of cadmium levels, but the HHCB only results can be found at cadmium level 0mg/kg dry weight soil. Results are reported in figures 2-6.		

Overall Quality Determination Medium

Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by nomenclature and formula.
	Metric 2:	Test Substance Source	High	Purchased from Guoyao Chemical Co. Ltd. (Beijing, China)
	Metric 3:	Test Substance Purity	High	Given as ">98% purity".
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls and solvent controls were used.
	Metric 5:	Negative Control Response	Medium	There were differences between untreated and solvent controls (greater growth, and statistically significant differences in all measured parameters for all plants). Therefore comparisons were made to the solvent control.
	Metric 6:	Randomized Allocation	Medium	Tests conducted in accordance with OECD 208 which specifies randomization.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	HHCB was mixed with soil and it is unclear whether sealed vessels were used (doubtful for plant studies). As HHCB can volatilize from soil in the long term and nominal concentrations were not tested, there may be some discrepancy. The test duration was 21 days, and Schafer 2005 reported a volatilization of up to 16% after 28 days. This discrepancy is unlikely to have a major effect on results since it was replicated across all treatments and plants.
	Metric 8:	Consistency of Exposure Administration	High	Exposure same for all treatments.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were not measured, however HHCB does not rapidly biodegrade and sorbs to soil somewhat strongly. The discrepancy between nominal and actual exposure concentrations is unlikely to substantially effect the results.
	Metric 10:	Exposure Duration and Frequency	High	The study was conducted in accordance with OECD 208.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The range and spacing of exposure levels is not given for any plant studies, however the OECD 208 guideline specifies that appropriate rangefinding and exposure level choices must be made, and the text implies (ECx values calculated, NOECs mentioned) that a range of exposure levels was used. Additionally, some exposure levels are given in the Results section.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent used (acetone) caused measurable responses in all plant experiments. Comparisons of HHCB effect were made against solvent controls instead of blank controls.
Domain 4: Test Organism				

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Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	Seeds were obtained from the Chinese Academy of Agricultural Sciences (Beijing, China)
	Metric 14:	Acclimatization and Pretreatment Conditions	High	OECD 208 guidelines were followed, conditions were appropriate for the plants and were the same for all groups & were consistent before treatment.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 plants were used per replicate, with 4 replicates per treatment including controls.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	OECD 208 guidelines were followed, conditions were appropriate for the plants and were the same for all groups & were consistent before treatment.
	Metric 17:	Outcome Assessment Methodology	High	Growth and biomass were measured at the end of the test according to OECD 208
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed the same across all groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences in environmental conditions across groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	"... there was no significant effect on the survival of the 8 terrestrial plants at the testing concentrations..."
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was adequately described in the methods section and appropriate for the experiments performed.
	Metric 22:	Reporting of Data	Low	Summary data (ECx, NOEC) was reported for the overall experiment for each organism, but individual data for each treatment and control was not reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, and the outcomes that were reported are adequately discussed in the text.

Additional Comments: This study also includes the calculation of an SSD from all 10 tested terrestrial organisms and the derivation of PNECs from said distribution, with discussion of AFs. In my opinion it would provide an important contribution to terrestrial data for HHCB, unfortunately the range and spacing of test concentrations for the 8 plants is not given.

Overall Quality Determination High

Study Citation:	Chen, C., Zhou, Q., Bao, Y., Li, Y., Wang, P. (2010). Ecotoxicological effects of polycyclic musks and cadmium on seed germination and seedling growth of wheat (Triticum aestivum). Journal of Environmental Sciences 22(12):1966-1973.
Duration:	Overall Duration: Not-reported; Exposure Duration: Not-reported
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum*; Embryo
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	4650262

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified as 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[g]-2-benzopyran (HHCB)
	Metric 2:	Test Substance Source	High	HHCB was obtained from Tianjin Flavors and Fragrance Corporation
	Metric 3:	Test Substance Purity	Medium	HHCB purity reported 77.4%
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	There are aspects of the reported negative control group that differ between control and treated groups
	Metric 5:	Negative Control Response	High	The control group responses were adequate for the test.
	Metric 6:	Randomized Allocation	Low	Did not report how organisms were allocated
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	test conditions were appropriate for the test.
	Metric 8:	Consistency of Exposure Administration	High	Same exposure protocol
	Metric 9:	Measurement of Test Substance Concentration	Medium	Nominal concentrations used.
	Metric 10:	Exposure Duration and Frequency	Uninformative	When at least 65% of the seeds in the control had germinated, and developed roots grew above 20 mm long, the test period was finished.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Six concentrations with three replicates were used.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent concentrations were apropriate for the test.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Seeds of T. aestivum were obtained the Tianjin Academy of Agricultural Sciences, China.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	No acclimation was reported for seeds, however acclimation for seeds in this test were not necessary.
	Metric 15:	Number of Organisms and Replicates per Group	Low	Number of seeds not reported. Percent germinated were reported.
Domain 5: Outcome Assessment				

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Study Citation:	Chen, C., Zhou, Q., Bao, Y., Li, Y., Wang, P. (2010). Ecotoxicological effects of polycyclic musks and cadmium on seed germination and seedling growth of wheat (Triticum aestivum). Journal of Environmental Sciences 22(12):1966-1973.
Duration:	Overall Duration: Not-reported; Exposure Duration: Not-reported
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum*; Embryo
Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	4650262

Domain	Metric		Rating	Comments
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions were adequate and consistent across groups.
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes were reported.
	Metric 18:	Consistency of Outcome Assessment	High	the outcomes were reported and assessed consistently.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no confounding variable were reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No unrelated outcomes were reported. However because only percents were reported it is difficult to interpret the results.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical methods used were reported, but details of the results were lacking.
	Metric 22:	Reporting of Data	Medium	Results were reported in percent only.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Uncertainties in the reporting make it difficult to asses the presence of unexpected outcomes.

Additional Comments: This evaluation is for the reproductive outcome reported in Fig 1 in terms of seed germination.

Overall Quality Determination Uninformative

Study Citation:	Chen, C., Zhou, Q., Bao, Y., Li, Y., Wang, P. (2010). Ecotoxicological effects of polycyclic musks and cadmium on seed germination and seedling growth of wheat (Triticum aestivum). Journal of Environmental Sciences 22(12):1966-1973.
Duration:	Overall Duration: Not-reported; Exposure Duration: Not-reported
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum*; Embryo
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	4650262

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified as 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[g]-2-benzopyran (HHCB)
	Metric 2:	Test Substance Source	High	HHCB was obtained from Tianjin Flavors and Fragrance Corporation
	Metric 3:	Test Substance Purity	Medium	HHCB purity reported 77.4%
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	There are aspects of the reported negative control group that differ between control and treated groups
	Metric 5:	Negative Control Response	High	The control group responses were adequate for the test.
	Metric 6:	Randomized Allocation	Low	Did not report how organisms were allocated
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	test conditions were appropriate for the test.
	Metric 8:	Consistency of Exposure Administration	High	Same exposure protocol
	Metric 9:	Measurement of Test Substance Concentration	Medium	Nominal concentrations used.
	Metric 10:	Exposure Duration and Frequency	Uninformative	When at least 65% of the seeds in the control had germinated, and developed roots grew above 20 mm long, the test period was finished.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Six concentrations with three replicates were used.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent concentrations were apropriate for the test.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Seeds of T. aestivum were obtained the Tianjin Academy of Agricultural Sciences, China.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	No acclimation was reported for seeds, however acclimation for seeds in this test were not necessary.
	Metric 15:	Number of Organisms and Replicates per Group	Low	Number of seeds not reported. Percent germinated were reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions were adequate and consistent across groups.
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes were reported.

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Study Citation:	Chen, C., Zhou, Q., Bao, Y., Li, Y., Wang, P. (2010). Ecotoxicological effects of polycyclic musks and cadmium on seed germination and seedling growth of wheat (Triticum aestivum). Journal of Environmental Sciences 22(12):1966-1973.
Duration:	Overall Duration: Not-reported; Exposure Duration: Not-reported
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum*; Embryo
Health Outcome:	Mortality
Chemical:	HHCB
HERO ID:	4650262

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	the outcomes were reported and assessed consistently.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no confounding variable were reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No unrelated outcomes were reported. However because only percents were reported it is difficult to interpret the results.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical methods used were reported, but details of the results were lacking.
	Metric 22:	Reporting of Data	Medium	Results were reported in percent only.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Uncertainties in the reporting make it difficult to asses the presence of unexpected outcomes.

Additional Comments: This evaluation is for the mortality outcome reported in Table 1 where LC10 and LC50 values were reported in terms of mg/kg.

Overall Quality Determination Uninformative

Study Citation:	Chen, C., Zhou, Q., Bao, Y., Li, Y., Wang, P. (2010). Ecotoxicological effects of polycyclic musks and cadmium on seed germination and seedling growth of wheat (Triticum aestivum). Journal of Environmental Sciences 22(12):1966-1973.
Duration:	Overall Duration: Not-reported; Exposure Duration: Not-reported
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum*; Embryo
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	4650262

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified as 1,3,4,6,7,8-hexahydro-4,6,6,7,8,8-hexamethylcyclopenta[g]-2-benzopyran (HHCB)
	Metric 2:	Test Substance Source	High	HHCB was obtained from Tianjin Flavors and Fragrance Corporation
	Metric 3:	Test Substance Purity	Medium	HHCB purity reported 77.4%
Domain 2: Test Design				
	Metric 4:	Negative Controls	Low	There are aspects of the reported negative control group that differ between control and treated groups
	Metric 5:	Negative Control Response	High	The control group responses were adequate for the test.
	Metric 6:	Randomized Allocation	Low	Did not report how organisms were allocated
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	High	test conditions were appropriate for the test.
	Metric 8:	Consistency of Exposure Administration	High	Same exposure protocol
	Metric 9:	Measurement of Test Substance Concentration	Medium	Nominal concentrations used.
	Metric 10:	Exposure Duration and Frequency	Uninformative	When at least 65% of the seeds in the control had germinated, and developed roots grew above 20 mm long, the test period was finished.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	Six concentrations with three replicates were used.
	Metric 12:	Testing at or Below Solubility Limit	High	Solvent concentrations were apropriate for the test.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	Seeds of T. aestivum were obtained the Tianjin Academy of Agricultural Sciences, China.
	Metric 14:	Acclimatization and Pretreatment Conditions	High	No acclimation was reported for seeds, however acclimation for seeds in this test were not necessary.
	Metric 15:	Number of Organisms and Replicates per Group	Low	Number of seeds not reported. Percent germinated were reported.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	Environmental conditions were adequate and consistent across groups.
	Metric 17:	Outcome Assessment Methodology	High	Intended outcomes were reported.

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Study Citation:	Chen, C., Zhou, Q., Bao, Y., Li, Y., Wang, P. (2010). Ecotoxicological effects of polycyclic musks and cadmium on seed germination and seedling growth of wheat (Triticum aestivum). Journal of Environmental Sciences 22(12):1966-1973.
Duration:	Overall Duration: Not-reported; Exposure Duration: Not-reported
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum*; Embryo
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	4650262

Domain	Metric		Rating	Comments
	Metric 18:	Consistency of Outcome Assessment	High	the outcomes were reported and assessed consistently.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	no confounding variable were reported.
	Metric 20:	Outcomes Unrelated to Exposure	Medium	No unrelated outcomes were reported. However because only percents were reported it is difficult to interpret the results.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	Statistical methods used were reported, but details of the results were lacking.
	Metric 22:	Reporting of Data	Medium	Results were reported in percent only.
	Metric 23:	Explanation of Unexpected Outcomes	Medium	Uncertainties in the reporting make it difficult to asses the presence of unexpected outcomes.

Additional Comments: None

Overall Quality Determination Uninformative

Study Citation:	Wang, M., Peng, C., Chen, W., Markert, B. (2013). Ecological risks of polycyclic musk in soils irrigated with reclaimed municipal wastewater. Ecotoxicology and Environmental Safety 97:242-247.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum L.*; Embryo
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)
Chemical:	HHCB
HERO ID:	3187166

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The chemical identified in study: "HHCB(1,3,4,6,7,8-hexahydro-4,6,6,7,8-hexamethylcyclopenta-c-2-benzopyrane", as reported in Table 1. However, it was identified by name only.
	Metric 2:	Test Substance Source	Low	HHCB-spiked wastewater irrigated park lands.
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported; Impurities in irrigated soils were not identified.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A 0 mg/kg dose group was included in study.
	Metric 5:	Negative Control Response	Low	The biological response of the negative control groups was not reported.
	Metric 6:	Randomized Allocation	Low	Authors do not state random allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	Preparation of stock substance not described nor how it was incorporated into the soil (details of how soils were spiked with HHCB could be better described).
	Metric 8:	Consistency of Exposure Administration	Medium	Study lacking detail on how seeds were sowed into 100 mm dish (e.g. same depth? using an instrument?). 10 seeds sowed per 100 mm dish.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations stated in the study. Exposure concentrations were not measured but based on professional judgment of experimental design and nature of test substance, actual concentrations are likely to be similar to nominal concentrations. These minor uncertainties or limitations are unlikely to have a substantial impact on results.
	Metric 10:	Exposure Duration and Frequency	High	"Biochemical parameters i.e. SOD and POD activities were determined 7 days after the culturing".
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	There were 7 soil concentration levels tested, 0, 50, 100, 150, 200, 250, and 300 mg/kg.
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposures were via soil.
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	"The variety of the tested wheat (T. aestivum) is LiaoningSpring No. 14 and was obtained as seeds from Shenyang Agricultural University, China." From cited reference Wang and Zhou 2006.

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Study Citation:	Wang, M., Peng, C., Chen, W., Markert, B. (2013). Ecological risks of polycyclic musk in soils irrigated with reclaimed municipal wastewater. Ecotoxicology and Environmental Safety 97:242-247.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum L.*; Embryo
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)
Chemical:	HHCB
HERO ID:	3187166

Domain	Metric		Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pretreatment conditions were the same for control and exposed groups, but observed/not observed biological effects were consistent with other studies in the literature.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Authors state they followed OECD 208 TG. Authors state they exposed 10 seedlings exposed per petri dish with three replicate petri dishes per concentration.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Authors state OECD TG 208 was followed but do not provide details of lighting cycles.
	Metric 17:	Outcome Assessment Methodology	High	"The activity of POD was determined using the guaiacol, C6H4(OH)(OCH3), substrates as outlined in Wu and Von Tiedemann (2002). The activity of SOD was also determined according to Wu and Von Tiedemann (2002)." Reference obtained and reviewed as appropriate.
	Metric 18:	Consistency of Outcome Assessment	High	The authors reported results for SOD and POD activity 7 days after culturing.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Data on attrition and/or outcomes unrelated to controlled variables for each study group were not reported because only substantial differences among groups were noted (as indicated by study authors), and it is unlikely there were any substantial impacts 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 (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	"The dose-effect equations were obtained by the regression analysis between the HHCB...concentration and the inhibitory rate of each parameters compared to the control. EC50 of HHCB...for wheat was estimated accordingly"
	Metric 22:	Reporting of Data	Low	EC50 and NOEC values were provided in Tables 3 and 4. Dose-response data were not represented or discussed in text. "Data were only reported for some outcomes (i.e., less than half of the outcomes that were measured)."
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability (e.g., SE, SD, confidence intervals) and/or insufficient information was provided to determine if excessive variability or unexpected outcomes occurred.

Additional Comments: This evaluation is for the mechanistic outcomes reported in this paper, including SOD and POD inhibition, as well as soluble protein content in the roots and leaves.

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Study Citation:	Wang, M., Peng, C., Chen, W., Markert, B. (2013). Ecological risks of polycyclic musk in soils irrigated with reclaimed municipal wastewater. Ecotoxicology and Environmental Safety 97:242-247.
Duration:	Overall Duration: 4 - 10 days; Exposure Duration: 4 - 10 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum L.*; Embryo
Health Outcome:	Mechanistic-Biomarkers (exposure and effect)-Oxidative stress (including redox biology)
Chemical:	HHCB
HERO ID:	3187166

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

Study Citation:	Wang, M., Peng, C., Chen, W., Markert, B. (2013). Ecological risks of polycyclic musk in soils irrigated with reclaimed municipal wastewater. Ecotoxicology and Environmental Safety 97:242-247.
Duration:	Overall Duration: Not-reported; Exposure Duration: Not-reported
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum L.*; Embryo
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	3187166

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The chemical identified in study: "HHCB(1,3,4,6,7,8-hexahydro-4,6,6,7,8-hexamethylcyclopenta-c-2-benzopyrane", as reported in Table 1. However, it was identified by name only.
	Metric 2:	Test Substance Source	Low	HHCB in soils from park land irrigated with reclaimed waste water; other impurities not analytically quantified
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported. Impurities from extracted soils were not identified.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A 0 mg/kg dose group was included in study.
	Metric 5:	Negative Control Response	Low	Authors state they follow OECD 208 TG which includes parameters regarding control response but the authors do not state control response in the study.
	Metric 6:	Randomized Allocation	Low	Authors cite OECD TG 208 but do not state random allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	It is unclear whether HHCB-spiked soils collected from park land irrigated with reclaimed waste water were used in the testing.
	Metric 8:	Consistency of Exposure Administration	Medium	Study lacking detail on how seeds were sowed into 100 mm dish (e.g. same depth? using an instrument?). 10 seeds sowed per 100 mm dish.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations stated in the study. Exposure concentrations were not measured but based on professional judgment of experimental design and nature of test substance, actual concentrations are likely to be similar to nominal concentrations. These minor uncertainties or limitations are unlikely to have a substantial impact on results.
	Metric 10:	Exposure Duration and Frequency	Uninformative	Authors state "Germination rate was determined at the time when shoot elongation in more than 60% of the germinated seeds in control had exceeded 3cm" and a cited method OECD 208 TG states study is conducted generally within 14-21 days. However, the author do not state the duration of exposure/study in their methods.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	"concentration gradient of HHCB....included seven levels soil concentration levels namely 0, 50, 100, 150, 200, 250, and 300 mg/kg."
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure via soil
Domain 4: Test Organism				

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Study Citation:	Wang, M., Peng, C., Chen, W., Markert, B. (2013). Ecological risks of polycyclic musk in soils irrigated with reclaimed municipal wastewater. Ecotoxicology and Environmental Safety 97:242-247.
Duration:	Overall Duration: Not-reported; Exposure Duration: Not-reported
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum L.*; Embryo
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	3187166

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	"The variety of the tested wheat (T. aestivum) is LiaoningSpring No. 14 and was obtained as seeds from Shenyang Agricultural University, China." From cited reference Wang and Zhou 2006.
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pretreatment conditions were the same for control and exposed groups.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Authors state they followed OECD 208 TG. Authors state they exposed 10 seedlings exposed per petri dish with three replicate petri dishes per concentration.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Authors state OECD TG 208 was followed but do not provide details of lighting cycles.
	Metric 17:	Outcome Assessment Methodology	Medium	"Germination rate was determined at the time when shoot elongation in more than 60% of the germinated seeds in control had exceeded 3cm....The length from seed to root tip and shoot tip was defined as root and shoot elongation respectively and were manually measured using a ruler." Authors did not state if germination rate was determined at the same time as shoot and root elongation.
	Metric 18:	Consistency of Outcome Assessment	Medium	Assumption made that authors took measurements for shoot and root elongation across all treatment groups at the same time but this was not explicitly stated. Germination rate determined when 60% of control seeds had exceeded 3 cm height.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Data on attrition and/or outcomes unrelated to controlled variables for each study group were not reported because only substantial differences among groups were noted (as indicated by study authors), and it is unlikely there were any substantial impacts 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 (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	"The dose-effect equations were obtained by the regression analysis between the HHCB...concentration and the inhibitory rate of each parameters compared to the control. EC50 of HHCB...for wheat was estimated accordingly"
	Metric 22:	Reporting of Data	Low	EC50 and NOEC values provided in Tables 3 and 4. Dose-response data not represented in figure or discussed in text. "Data were only reported for some outcomes (i.e., less than half of the outcomes that were measured)."

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Study Citation:	Wang, M., Peng, C., Chen, W., Markert, B. (2013). Ecological risks of polycyclic musk in soils irrigated with reclaimed municipal wastewater. Ecotoxicology and Environmental Safety 97:242-247.
Duration:	Overall Duration: Not-reported; Exposure Duration: Not-reported
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Triticum aestivum L.*; Embryo
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	3187166

Domain	Metric		Rating	Comments
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability (e.g., SE, SD, confidence intervals) and/or insufficient information was provided to determine if excessive variability or unexpected outcomes occurred.

Additional Comments: Given the authors did not state a timeline for exposure duration/study duration for the apical endpoints (germination rate, shoot length, root length), the rating 'unacceptable' was not adjusted.

Overall Quality Determination Uninformative

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Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	3187166

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	Low	The chemical identified in study: "HHCB(1,3,4,6,7,8-hexahydro-4,6,6,7,8-hexamethylcyclopenta-c-2-benzopyrane", as reported in Table 1. However, it was identified by name only.
	Metric 2:	Test Substance Source	Low	HHCB in soils from park land irrigated with reclaimed waste water; other impurities not analytically quantified
	Metric 3:	Test Substance Purity	Low	Purity and/or grade of test substance were not reported. Impurities from extracted soils were not identified.
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	A 0 mg/kg dose group was included in study.
	Metric 5:	Negative Control Response	Low	Authors state they follow OECD 208 TG which includes parameters regarding control response but the authors do not state control response in the study.
	Metric 6:	Randomized Allocation	Low	Authors cite OECD TG 208 but do not state random allocation.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	It is unclear whether HHCB-spiked soils collected from park land irrigated with reclaimed waste water were used in the testing.
	Metric 8:	Consistency of Exposure Administration	Medium	Study lacking detail on how seeds were sowed into 100 mm dish (e.g. same depth? using an instrument?). 10 seeds sowed per 100 mm dish.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations stated in the study. Exposure concentrations were not measured but based on professional judgment of experimental design and nature of test substance, actual concentrations are likely to be similar to nominal concentrations. These minor uncertainties or limitations are unlikely to have a substantial impact on results.
	Metric 10:	Exposure Duration and Frequency	Uninformative	Authors state "Germination rate was determined at the time when shoot elongation in more than 60% of the germinated seeds in control had exceeded 3cm" and a cited method OECD 208 TG states study is conducted generally within 14-21 days. However, the author do not state the duration of exposure/study in their methods.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	High	"concentration gradient of HHCB....included seven levels soil concentration levels namely 0, 50, 100, 150, 200, 250, and 300 mg/kg."
	Metric 12:	Testing at or Below Solubility Limit	N/A	Exposure via soil
Domain 4: Test Organism				
	Metric 13:	Test Organism Characteristics	High	"The variety of the tested wheat (T. aestivum) is LiaoningSpring No. 14 and was obtained as seeds from Shenyang Agricultural University, China." From cited reference Wang and Zhou 2006.

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Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	3187166

Domain	Metric		Rating	Comments
	Metric 14:	Acclimatization and Pretreatment Conditions	Low	The study did not report whether test organisms were acclimatized and/or whether pre-treatment conditions were the same for control and exposed groups.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	Authors state they followed OECD 208 TG. Authors state they exposed 10 seedlings exposed per petri dish with three replicate petri dishes per concentration.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	Medium	Authors state OECD TG 208 was followed but do not provide details of lighting cycles.
	Metric 17:	Outcome Assessment Methodology	Medium	"Germination rate was determined at the time when shoot elongation in more than 60% of the germinated seeds in control had exceeded 3cm....The length from seed to root tip and shoot tip was defined as root and shoot elongation respectively and were manually measured using a ruler." Authors did not state if germination rate was determined at the same time as shoot and root elongation.
	Metric 18:	Consistency of Outcome Assessment	Medium	Assumption made that authors took measurements for shoot and root elongation across all treatment groups at the same time but this was not explicitly stated. Germination rate determined when 60% of control seeds had exceeded 3 cm height.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	Medium	Data on attrition and/or outcomes unrelated to controlled variables for each study group were not reported because only substantial differences among groups were noted (as indicated by study authors), and it is unlikely there were any substantial impacts 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 (e.g., infection) that could influence the outcome assessment.
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	Low	"The dose-effect equations were obtained by the regression analysis between the HHCB...concentration and the inhibitory rate of each parameters compared to the control. EC50 of HHCB...for wheat was estimated accordingly"
	Metric 22:	Reporting of Data	Low	EC50 and NOEC values provided in Tables 3 and 4. Dose-response data not represented in figure or discussed in text. "Data were only reported for some outcomes (i.e., less than half of the outcomes that were measured)."
	Metric 23:	Explanation of Unexpected Outcomes	Low	The study did not report any measures of variability (e.g., SE, SD, confidence intervals) and/or insufficient information was provided to determine if excessive variability or unexpected outcomes occurred.

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Health Outcome:	Reproductive/Teratogenic
Chemical:	HHCB
HERO ID:	3187166

Domain	Metric	Rating	Comments
Additional Comments:	Given the authors did not state a timeline for exposure duration/study duration for the apical endpoints (germination rate, shoot length, root length), the rating 'unacceptable' was not adjusted. This evaluation is for the germination rate, which falls under reproductive outcomes.		

Overall Quality Determination Uninformative

Study Citation:	Wang, X., Liu, Z., Wang, W., Zhang, C., Chen, L. (2015). Derivation of predicted no effect concentration (PNEC) for HHCB to terrestrial species (plants and invertebrates). Science of the Total Environment 508:122-127.
Duration:	Overall Duration: 11 - 21 days; Exposure Duration: 11 - 21 days
Exposure Route, Media, Path:	Terrestrial; Soil; Root uptake
Taxa, Species, Age:	Vegetation; Vascular Plants; *Zea mays*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
Domain 1: Test Substance				
	Metric 1:	Test Substance Identity	High	Identified by nomenclature and formula.
	Metric 2:	Test Substance Source	High	Purchased from Guoyao Chemical Co. Ltd. (Beijing, China)
	Metric 3:	Test Substance Purity	High	Given as ">98% purity".
Domain 2: Test Design				
	Metric 4:	Negative Controls	High	Negative controls and solvent controls were used.
	Metric 5:	Negative Control Response	Medium	There were differences between untreated and solvent controls (greater growth, and statistically significant differences in all measured parameters for all plants). Therefore comparisons were made to the solvent control.
	Metric 6:	Randomized Allocation	Medium	Tests conducted in accordance with OECD 208 which specifies randomization.
Domain 3: Exposure Characterization				
	Metric 7:	Experimental System/Test Media Preparation	Medium	HHCB was mixed with soil and it is unclear whether sealed vessels were used (doubtful for plant studies). As HHCB can volatilize from soil in the long term and nominal concentrations were not tested, there may be some discrepancy. The test duration was 21 days, and Schafer 2005 reported a volatilization of up to 16% after 28 days. This discrepancy is unlikely to have a major effect on results since it was replicated across all treatments and plants.
	Metric 8:	Consistency of Exposure Administration	High	Exposure same for all treatments.
	Metric 9:	Measurement of Test Substance Concentration	Medium	Exposure concentrations were not measured, however HHCB does not rapidly biodegrade and sorbs to soil somewhat strongly. The discrepancy between nominal and actual exposure concentrations is unlikely to substantially effect the results.
	Metric 10:	Exposure Duration and Frequency	High	The study was conducted in accordance with OECD 208.
	Metric 11:	Number of Exposure Groups/ Spacing of Exposure Levels	Low	The range and spacing of exposure levels is not given for any plant studies, however the OECD 208 guideline specifies that appropriate rangefinding and exposure level choices must be made, and the text implies (ECx values calculated, NOECs mentioned) that a range of exposure levels was used. Additionally, some exposure levels are given in the Results section.
	Metric 12:	Testing at or Below Solubility Limit	Medium	The solvent used (acetone) caused measurable responses in all plant experiments. Comparisons of HHCB effect were made against solvent controls instead of blank controls.
Domain 4: Test Organism				

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Taxa, Species, Age:	Vegetation; Vascular Plants; *Zea mays*; Not Applicable (e.g., fungi or algae studies) or Not Reported
Health Outcome:	Development/Growth
Chemical:	HHCB
HERO ID:	5427885

Domain	Metric		Rating	Comments
	Metric 13:	Test Organism Characteristics	High	Seeds were obtained from the Chinese Academy of Agricultural Sciences (Beijing, China)
	Metric 14:	Acclimatization and Pretreatment Conditions	High	OECD 208 guidelines were followed, conditions were appropriate for the plants and were the same for all groups & were consistent before treatment.
	Metric 15:	Number of Organisms and Replicates per Group	Medium	10 plants were used per replicate, with 4 replicates per treatment including controls.
Domain 5: Outcome Assessment				
	Metric 16:	Adequacy of Test Conditions	High	OECD 208 guidelines were followed, conditions were appropriate for the plants and were the same for all groups & were consistent before treatment.
	Metric 17:	Outcome Assessment Methodology	High	Growth and biomass were measured at the end of the test according to OECD 208
	Metric 18:	Consistency of Outcome Assessment	High	Outcomes were assessed the same across all groups.
Domain 6: Confounding / Variable Control				
	Metric 19:	Confounding Variables in Test Design and Procedures	High	There were no reported differences in environmental conditions across groups.
	Metric 20:	Outcomes Unrelated to Exposure	High	"... there was no significant effect on the survival of the 8 terrestrial plants at the testing concentrations..."
Domain 7: Data Presentation and Analysis				
	Metric 21:	Statistical Methods	High	Statistical analysis was adequately described in the methods section and appropriate for the experiments performed.
	Metric 22:	Reporting of Data	Low	Summary data (ECx, NOEC) was reported for the overall experiment for each organism, but individual data for each treatment and control was not reported.
	Metric 23:	Explanation of Unexpected Outcomes	High	There were no unexpected outcomes, and the outcomes that were reported are adequately discussed in the text.

Additional Comments: This study also includes the calculation of an SSD from all 10 tested terrestrial organisms and the derivation of PNECs from said distribution, with discussion of AFs. In my opinion it would provide an important contribution to terrestrial data for HHCB, unfortunately the range and spacing of test concentrations for the 8 plants is not given.

Overall Quality Determination High