UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

WASHINGTON, D.C. 20460

OFFICE OF CHEMICAL SAFETY AND POLLUTION PREVENTION

January 9, 2024

MEMORANDUM

SUBJECT: Review of Final Study Report for p-Dichlorobenzene (pDCB, CASRN: 106-46-7) 10-day Toxicity with the Midge (*Chironomus dilutus*) using Spiked Whole Sediment in response to January 2021 TSCA Section 4(a) Test Order

FROM: Melody Bernot, PhD
Risk Assessment Branch 5 (RAB5)
Existing Chemicals Risk Assessment Division (ECRAD)
Office of Pollution Prevention and Toxics (OPPT)

Kelley Stanfield
Risk Assessment Branch 5 (RAB5)
Existing Chemicals Risk Assessment Division (ECRAD)
Office of Pollution Prevention and Toxics (OPPT)

Janet Burris, MSPH
Risk Assessment Branch 4 (RAB4)
Existing Chemicals Risk Assessment Division (ECRAD)
Office of Pollution Prevention and Toxics (OPPT)

THROUGH: Karen Eisenreich, PhD
Senior Science Advisor
Existing Chemicals Risk Assessment Division (ECRAD)
Office of Pollution Prevention and Toxics (OPPT)

TO: Virginia Lee
Team Lead
Data Collection Branch (DCB)
Data Gathering Assessment Division (DGAD)
Office of Pollution Prevention and Toxics (OPPT)

David Turk
Branch Chief
Data Collection Branch (DCB)
Data Gathering Assessment Division (DGAD)
Office of Pollution Prevention and Toxics (OPPT)

SUMMARY

In January 2021, EPA issued a Test Order under Section 4 of the Toxic Substances Control Act (TSCA) to require recipients of the Test Order to conduct environmental hazard toxicity tests on aquatic benthic midges (*Chironomus*) following p-Dichlorobenzene (pDCB) acute exposures in sediment (Test Guideline OCSPP 850.1735). Study plans were received and following EPA review and subsequent revision of the study plan, the study plan was approved. The final study report titled "p-Dichlorobenzene: A 10-Day Toxicity Test with the Midge (*Chironomus dilutus*) using Spiked Sediment" was received in November 2023.

We have reviewed the Final Study Report through both our standard Systematic Review process and by cross-referencing with the approved study protocol. The study quality was consistent with a high-quality ranking in our systematic review and there are no concerns about it being an acceptable study for use in risk evaluation. The final study report did deviate from the approved study plan in a few areas and did not fully meet the EPA test guideline (OCSPP 850.1735) requirements. Specifically, the selected test concentrations did not bracket the expected 10-d LC50 for mortality or the 10-d EC50 for growth as specified in the guidelines and resulted in unbounded toxicity endpoints. This limited range of concentrations tested does not provide a full understanding of potential pDCB toxicity to chironomids. Based on current information, concentrations of pDCB in sediment are not expected to be higher than the maximum test concentration (100 mg/kg nominal; 16 mg/kg measured) used in this study. Thus, additional testing to meet the test concentration selection criteria is not essential. Other deviations reflected process variables that did not significantly exceed established guidelines and do not affect the acceptability of the study.

DETAILED REVIEW OF FINAL STUDY REPORT

Review of Chironomid 10-Day Toxicity Test of *p*-Dichlorobenzene

Study title: *p*-Dichlorobenzene: A 10-Day Toxicity Test with the Midge (*Chironomus dilutus*) Using Spiked Whole Sediment
Study number: Easton Study No. 264A-116
Report date: November 20, 2023
Guideline: U.S. EPA OCSPP 850.1735; ASTM Standard E 1706-05

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
Methods				
Range-finding test	A range-finding test may be conducted to establish test concentrations.	EPA recommendation: In the range-finding test, the test organisms are exposed to a series of widely spaced concentrations	Yes	A non-GLP, 10-day range-finding test was conducted under flow-through conditions with chironomids 3-4 days post-hatch at nominal

[1] Final protocol submitted to EPA for review, dated 12/10/2021.
[2] EPA's requirements and recommendations in this column are from EPA's protocol review dated 10/19/2021 (in response to revised protocol dated 9/28/2021), and includes only requirements and recommendations that were not addressed in the latest protocol dated 12/10/2021.
[3] Correspondence between EPA and the Sponsor has been condensed and summarized, where necessary, to conserve space.
[4] Compliance with EPA's requirements (e.g., as indicated by "must" in EPA's comments) is needed to receive a "Yes" in this column; compliance with EPA's recommendations (e.g., as indicated by "should" in EPA's comments) is not needed.

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
		of the test substance (e.g., 1, 10, 100 mg/kg dry weight). The details of the range-finding test do not have to be the same as for definitive testing in that the number of replicates, the number of test organisms, and duration of exposure may be less than that used in definitive testing, per OCSPP 850.1735.		concentrations of 0, 0.10, 0.80, 4.0, 20, and 100 mg/kg. After 10 days of exposure at test concentrations of 0, 0.10, 0.80, 4.0, 20, and 100 mg/kg, the percent survival per treatment group was 98, 100, 98, 75, 78, and 85%, respectively; the percent reduction in survival relative to the control group was -2.6, 0, 23, 21, and 13%, respectively. The average individual ash-free dry weight (AFDW) was 1.97, 1.91, 1.82, 2.37, 2.27, and 1.93 mg at 0, 0.10, 0.80, 4.0, 20, and 100 mg/kg, respectively; the percent reduction in AFDW relative to the control group was 2.9, 7.8, -20, -15, and 2.1%, respectively. Summary tables of the range-finding test results are provided in Appendix 10.
Test organisms:				
Species	*Chironomus dilutus*		Yes	
Source	Test organisms will be obtained from cultures maintained at the Easton site or from a commercial supplier (e.g., Environmental Consulting and Testing, Superior, Wisconsin). The source of the test organisms and conditions of culture will be provided in the final report. The identity of the species will be verified by the supplier of the original		Yes	Midges used in the test were from cultures maintained by Eurofins-Easton (Maryland). The identity of the species was verified by the supplier of the original culture (Aquatic Bio Systems, Fort Collins, CO).

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
	culture.			
Age and size	Midges to be used in the test will be third instar larvae (~10 days old) at test initiation. The average length of midge larvae at test initiation will be 4-6 mm, and the average dry weight will be 0.08-0.23 mg per individual. A representative sub-sample of the organisms used for testing will be measured at test initiation to verify initial mean length and weight.		Yes (with the exception that average length of midge larvae at test initiation was not reported)	Third instar larvae, ~10 days old at test initiation. The average individual ash-free dry weight (AFDW) of 80 midge larvae collected from the test batch of organisms at the beginning of the test was 0.22 mg. A protocol deviation noted that the lengths of a representative subsample of organisms were not measured at test initiation to verify initial mean length; the study authors considered this to have no impact on the results of the study.
Holding and acclimation	In the laboratory, the organisms will be hatched in water from the same source and at approximately the same temperature as will be used in the test and will be held until they are the appropriate age. Holding water will be well water obtained from a well ~40 meters deep, located on the Easton site (same water as used for test). During holding, midge larvae will be fed an invertebrate slurry diet or equivalent.		Yes	During the 14-day holding period immediately preceding the test, water temperatures in the culture ranged from 21.9-22.4°C, the pH of the water ranged from 8.3-8.5, and the dissolved oxygen concentrations were ≥6.9 mg/L (≥79% of saturation). During the holding period, the midge larvae were fed an invertebrate slurry and chlorella diet.
Health status/ condition	If more than 10% of the organisms in the batch to be used for testing die or appear to be unhealthy, discolored, or		Yes	During the holding period, the midge larvae to be used in the test appeared normal.

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
	otherwise stressed during the post-hatch period (~10 days post-hatch), or if more than 5% die or show signs of stress during the 2 days prior to test initiation, the batch will not be used for testing.			
Care and handling	Organisms will be transferred to test chambers below the water surface, as close to the sediment surface as possible, using a glass wide-bore pipette or a similar device.		Yes	Organisms were transferred to test chambers below the water surface using a glass wide-bore pipette. Organisms were transferred near the sediment; proximity to sediment was not reported
Test substance characterization	The Sponsor is responsible for providing the testing facility with verification that the test substance has been characterized according to GLP prior to its use in the study. A copy of the test substance COA will be included in the study report, along with test substance identification including name, chemical abstract number or code number, strength, purity, composition, and/or other information, such as physicochemical properties, if provided by the Sponsor.	EPA requirement: EPA requires that a statement of test substance purity be submitted prior to initiation of the tests. A copy of the COA must be included in the final report. Sponsor response: The Sponsor will provide EPA with a statement of test substance purity prior to test initiation and Eurofins will include a copy of the COA in the final report if provided by the Sponsor.	Yes	The test substance (*p*-DCB; CASRN 106-46-7) had a purity of 99.9% a.i. (expiration date not stated), according to the Certificate of Analysis provided in Appendix 2 of the study report. It was received from Sigma-Aldrich (St. Louis, MO) on February 14, 2022. [^{14}C]-*p*-DCB had a chemical purity of 99.9%, a radiochemical purity of 99.8%, and a specific radioactivity of 43.21 mCi/mmol (291.2 µCi/mg), according to the Certificate of Analysis provided in Appendix 2. It was received from Eurofins Selcia Ltd.
Exposure duration	10 days		Yes	The exposure duration was 10 days.

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
Number of test concentrations	Minimum of 5		Yes	Six concentrations were tested.
Test concentration selection	Test concentrations will be selected in consultation with the Sponsor based upon information such as exploratory range-finding toxicity data, known toxicity data, physical/chemical properties of the test substance, or other relevant information. Generally, the nominal test substance concentrations used in the definitive test will be in a geometric series with a spacing factor between 1.5 and 3.2 unless information concerning the concentration-effect curve indicates that a different dilution factor would be more appropriate.	EPA requirement: Test concentrations must be selected with the intention of bracketing all endpoint values for mortality and growth (NOEC, LOEC, LC50, and EC50) based on the information available about the toxicity of the test substance (e.g., from a range-finding test).	Yes	Nominal concentrations were selected based on the results of exploratory range-finding data. Nominal concentrations used in the definitive test were 0.32, 1.0, 3.2, 10, 32, and 100 mg/kg sediment dry weight. The highest nominal concentration is equivalent to the limit test concentration suggested in OCSPP 850.1735. No effects were observed on any endpoint up to the highest concentration tested.
Use of controls	Negative control (untreated sediment) and a solvent control, if a solvent is used to prepare test substance concentrations.		Yes	The negative control consisted of formulated sediment and overlying water, without the addition of test substance. The solvent control was prepared using 0.765 mL ethanol in 1200 g dry weight sediment with no test substance added, and was mixed using the same procedures as the treated sediments.
Number of replicates	8 replicates per test concentration and control		Yes	None
Number of test organisms/ replicate	Each test compartment will be initiated with 10		Yes	The number of organisms per replicate was as described in the protocol.

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
	larvae/replicate. To obtain known age organisms, egg masses will be isolated from the culture and held in test water to stimulate hatch. The newly hatched larvae will be held in water from the same source and at approximately the same temperature as will be used for the test, until the organisms are the appropriate age (third instar, ~10 days old). To initiate the test, one or two ~10-day old midges will be indiscriminately and sequentially added to transfer containers (e.g., glass beakers) containing test water until each contains its complement of 10 individuals. Each group of individuals will then be transferred to an indiscriminately assigned test compartment.			Organisms used to initiate the test were from egg masses isolated from the culture and hatched and held in water from the same source and at approximately the same temperature as used in the test, as described in the protocol.
Additional replicates for analytical sampling	Additional replicate test chambers for each control and test substance concentration will be included, as needed, for analytical sampling and physical/chemical measurements of water and sediment for the Day 0, 5, and 10 sampling time points. No midges will be placed in the		Yes	Three additional replicates were prepared in each treatment and control group for analytical confirmation of concentrations on Days 0, 5, and 10. An additional three replicates per group were used for analysis of physical/chemical measurements of overlying water, pore water, and sediment. No midge larvae were placed

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
	additional replicates to be sampled on Day 0, but those sampled on later days (e.g., Days 5 and 10) will be initiated with 10 midges per replicate. These additional replicates will not be used to evaluate the biological response of the test organisms.			in the additional replicates sampled on Day 0, but those sampled on Days 5 and 10 had midges added at the same time as the biological replicates on Day 0. These additional replicates were not used to evaluate the biological response of the test organisms.
Route of exposure	Direct contact with spiked sediment		Yes	
Renewal conditions	Flow-through system with intermittent renewal of overlying water; each test compartment will receive ~2 volume replacements of overlying water per day. The test water (clean water, not treated with test substance) will be delivered directly into each test compartment, passively forcing water out through the holes in the sides of the compartment to exchange the water overlying the sediment, while minimizing any disturbance to the sediment. Test water delivery flow rates will be verified prior to initiation of exposure and will be recalibrated and/or verified approximately weekly, or as needed during the test. Flow	EPA requirement: The physical-chemical properties of this test substance (*p*-DCB) suggest that it will largely be retained in the sediment, with slow migration to overlying water (log Koc = 2.65 [experimental value from EPI Suite v4.11, U.S. EPA, 2012]); however, the laboratory must be cautioned that the fraction of test substance in overlying water is subject to loss during renewal of overlying water with clean water. The use of flow-through conditions resulting in two daily renewals is acceptable provided that preliminary testing is completed and demonstrates that the test substance loss is not enhanced by the	Yes	A flow-through system providing intermittent renewal of overlying water was used as described in the protocol. Results of preliminary analyses indicated that concentrations of *p*-DCB had attained equilibrium by Day 7 in both the flow-through and static test designs (Appendices 9.1 and 9.2). The results of the analysis showed that there was no difference between the two test designs.

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
	rates through any 2 test compartments should not differ by more than 10%. Proper system operation will be visually checked at least once each day during the test.	renewal methodology. The results of the preliminary testing must be shared with EPA prior to the initiation of definitive testing. Sponsor response: A preliminary (non-GLP) equilibration trial is already scheduled to determine if loss of test substance will be a concern in the test system. The results of the preliminary trial can be shared with EPA/OPPT prior to the initiation of the definitive test.		
Sediment composition	Formulated sediment based on the recommendations of OECD TG 218 will be used as the test sediment. The sediment will be composed of ~5% sphagnum peat moss, 20% silt and kaolin clay, and 75% industrial quartz sand. The dry constituents of the sediment will be mixed in a PK Twinshell mixer or equivalent. The pH of the sediment will be determined, and calcium carbonate will be added as needed to adjust the pH to 7.0 (± 0.5). Organic carbon content of the final mixture of sediment should be 2.0% (±		Yes (with the exception that the pH of prepared sediment was 5.7)	Formulated sediment based on the recommendations of OECD TG 218 was used as the test sediment. The sediment was composed of ~5% air-dried peat moss, 20% kaolin clay, 1% ground limestone, and 70% industrial quartz sand (results presented in Appendix 5 of the study report). The dry constituents of the sediment were mixed using a top-down mixer for 10 minutes, and the batch was stored under ambient conditions until used. A sample of formulated sediment used in the test was sent to Agvise Laboratories for characterization. Organic carbon content was 2.7%; pH was 5.7; percent

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
	0.5%). Samples of the formulated sediment will be sent to Agvise Laboratories (Northwood, ND), or a similar facility, for characterization, including pH, particle size distribution, analysis of total organic carbon (TOC), and percent moisture. Results of the characterization and analyses will be summarized in the final report.			moisture was 14%; and particle size distribution was 83% sand, 4% silt, and 13% clay. Results of the sediment characterization analyses are presented in Appendix 6. A protocol deviation noted that the protocol was inadvertently not updated to reflect that the pH of the sediment should be 6.0 ± 0.5; the study authors considered this to have no impact on the results of the study.
Overlying water composition	Overlying water will be well water obtained from a well ~40 meters deep and located on the Easton site. The water will be passed through a sand filter and pumped into a 37,800-L storage tank where the water will be aerated with spray nozzles. The water will be filtered with a 0.45 µm filter to remove fine particles and will be UV-sterilized. Prior to test initiation, hardness, alkalinity, pH, and specific conductance will be measured weekly and TOC will be measured monthly to monitor consistency of the well water. Means and ranges of the measured parameters for the approximate 4-week period preceding the test will be		Yes	The source of the overlying water, storage, and filtering methods were as described in the protocol. Well water used during the test had a total hardness of 136-156 mg/L as $CaCO_3$ (mean: 147 mg/L as $CaCO_3$), total alkalinity of 166-178 mg/L as $CaCO_3$ (mean: 175 mg/L as $CaCO_3$), pH of 8.1-8.2 (mean: 8.1), specific conductance of 360-384 µS/cm (mean: 369 µS/cm), and TOC of <2 mg C/L (based on weekly measurements for the 4-week period preceding the test for all parameters except TOC, which was measured monthly; results presented in Appendix 3 of the study report).

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
	provided in the final report.			
Dosing of sediment	A primary stock solution of [^{14}C]-test substance will be prepared in an appropriate volatile solvent. To verify the activity of the stock solution, aliquots would be analyzed by liquid scintillation counting (LSC). An appropriate amount of the [^{14}C]-test substance primary stock solution and an appropriate amount of neat test substance will subsequently be mixed into sand to prepare a "sand premix". The sand premix will be placed under a fume hood to allow evaporation of solvent before mixing the spiked sand into the sediment. A portion of dry sediment will be added to the premix to prepare the final batch of sediment for each test concentration. Sediment batches will be mixed in a motorized rotating mixer (typically overnight) to ensure thorough mixing prior to transfer of test sediment to test compartments. Homogeneity of the sediment after mixing will be confirmed by	EPA recommendation: For this test substance, EPA recommends that spiked sediments be prepared by neat addition of the test substance to the sediment. Use of a stock solution or solvent to prepare the spiked sediment is not expected to be necessary. This test substance, *p*-DCB, is a solid and can be ground up and added directly to the sediment in a dry form (see OCSPP 850.1000). EPA requirement: If the laboratory considers a solvent necessary for spiked sediment preparation, preliminary testing must be conducted to ensure that significant volatilization of the test substance from the sediment does not occur during preparation. If a stock solution in a solvent is used, the ability of this method to successfully achieve approximate target sediment concentrations must be confirmed analytically prior to test initiation. Sponsor response: Using a small volume	No (The laboratory used a solvent for preparation of spiked sediments. In a preliminary stability trial, the measured sediment concentrations at 30 minutes after mixing were, on average, 57.3% of nominal, suggesting that volatilization occurred during handling and mixing. Measured sediment concentrations on Day 0 of the definitive test ranged from 4.84-18.3% of nominal.)	Neat non-radiolabeled test substance was added to 60 g of sand in a labeled glass beaker and stirred with a glass rod until homogenous to create a sand premix. An aliquot of stock solution of radiolabeled *p*-DCB ([^{14}C]-DCB) in ethanol was spiked into the sand premix and stirred with a glass rod until homogeneous. The dosed sand premix was then placed under a fume hood for ~15 min to allow the solvent (ethanol) to evaporate. The 60-g sand premix was combined with untreated sediment (540 g dry wt; 873.8 g wet wt, adjusted for a 38.2% moisture content) in a 2000 mL Nalgene bottle and mixed for ~30 min on a roller mixer. Additional untreated sediment (600 g dry wt; 970.9 g wet wt) was added to each sand premix/sediment mixture to achieve a final weight of 1200 g dry wt. The batch sediments were mixed on a roller mixer for ~1 hr prior to transfer to test compartments/chambers. The solvent control sediment was prepared similarly with 0.765 mL ethanol, with no test substance added. The negative control sediment was prepared without the addition of test substance or solvent.

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
	visual inspection.	of radioactive stock solution and non-radiolabeled neat test substance will help minimize the volume of solvent used to spike the sediment and the amount of time needed for solvent evaporation.		A stability trial was conducted to evaluate the stability of *p*-DCB in formulated sediment at a nominal concentration of 1000 mg/kg and evaluate mixing procedures for the range-finding and definitive tests. Samples of sediment were collected 30 min and 2 hours after mixing, and on Days 0, 1, and 3 after settling. Overlying and pore water samples were also collected on Days 0, 1, and 3 after settling. Results from the analyses of sediment, overlying water, and pore water are presented in Appendices 9.3, 9.4, and 9.5, respectively, of the final study report. Mass balance calculations are presented in Appendix 9.6 and indicate that the overall concentrations of *p*-DCB remained consistent in sediment, overlying water, and pore water with minimal migration between the matrices. The loss of test material via volatilization primarily occurred during handling and mixing procedures; however, concentrations remained constant once the test compartments were prepared with overlying water. Based on the results of the pore water equilibration

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
				(described below) and stability trials, mixing of the sediment and preparation of the test compartments occurred on the same day and the equilibration time was set at 24 hours to minimize the loss of test material due to volatilization so that the organisms were exposed to the highest amount of *p*-DCB.
Use of organic solvent	The primary stock solution of [^{14}C]-test substance will be prepared in an appropriate volatile solvent. Acceptable solvents will be at least reagent grade and include, but are not limited to, methanol, ethanol, or acetone. The concentration of organic solvent will be minimized and equal in all treatment levels and the solvent control.		Yes	Ethanol was used as an organic solvent for radiolabeled *p*-DCB. The volume of ^{14}C stock (in ethanol) used to prepare each treatment concentration was 0.765 mL per 1200 g dry wt sediment.
Equilibration of test system, prior to adding organisms	The water/sediment systems in the test compartments will be acclimated to establish near-equilibrium conditions among the sediment, pore water, and overlying water prior to adding the test organisms. The length of the acclimation period typically will be determined in preliminary (non-GLP)		Yes	The water/sediment systems in the test compartments were allowed to equilibrate under flow-through conditions for approximately 24 hours prior to introduction of the organisms. This duration was selected to minimize the loss of test material due to volatilization. A pore water equilibration trial

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
	equilibration trials. Information from the equilibration trial will be used to ensure the length of equilibration in the test system minimizes loss of test substance from the test system (e.g. via volatilization) prior to introduction of the test organisms. Results of the preliminary equilibration trial will be shared with the Sponsor and EPA prior to initiation of the definitive test.			was conducted to determine the appropriate acclimation period for the test substance in the water/sediment systems and to compare the equilibration time between the static and intermittent flow-through test design. For each test design, formulated sediment was dosed at two concentrations (0.16 and 100 mg/kg: the low and high concentrations to be used in the range-finding tests) and the treated sediment was held in test compartments under test conditions for 21 days. Samples of the treated sediment were collected after 2, 7, 10, 14, and 21 days of equilibration for analysis of *p*-DCB in the sediment. Results of analyses showed loss of *p*-DCB from sediment through at least Day 7;concentrations of *p*-DCB attained equilibrium by Day 7 in both the flow-through and static test designs (Appendices 9.1 and 9.2). The results of the analysis showed that there was no difference between the two test designs.
Test vessels	Test compartments will consist of 300-mL glass beakers, placed in stainless steel tanks (test chambers), with one test concentration per tank. Test compartments will		Yes (with the exception that the study report did not indicate whether test chambers were covered)	Test chambers and content of sediment and overlying water were as described in the protocol. Overlying water (~175 mL) was added slowly to test compartments containing ~100 mL of sediment

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
	have two stainless steel mesh-covered holes on opposite sides of the beaker. Each compartment will contain ~100 mL of sediment and ~175 mL of overlying water. The water level in the compartments will be maintained at ~150-175 mL by the water level in the tanks (test chambers) and the position of the holes on the sides of the test compartments. Test compartments must be open to allow the twice a day replacement of overlying water within in each replicate and to allow the outflow of overlying water during each replacement. However, to the extent practical, replicates will be covered to reduce the loss of test material or overlying water due to evaporation and to minimize entry of dust and other particles into the test compartments.			while avoiding disturbance of the sediment. The depth of the sediment in a representative compartment (negative control replicate A) was 2.5 cm, and the depth of the overlying water in the same compartment was 5.5 cm. The test compartments in each treatment and control group were indiscriminately positioned in two diluter tanks per group.
Environmental conditions:				
Temperature (overlying water)	The test will be conducted at a target water temperature of 23 ± 1 °C.		Yes	Manual temperature measurements in overlying water during the test were 22.3-23.2 °C Continuous monitoring of temperature: 22.42-23.59 °C

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
Photoperiod	16 hours light:8 hours dark, with a 30-minute transition period of low light intensity when lights go on and off to avoid sudden changes in light intensity		Yes	16 hours light:8 hours dark, with a 30-minute transition period.
Light intensity	~540 to 1080 lux		Yes	Light intensity at test initiation was 633 lux at the surface of the water of one representative test compartment.
pH (overlying water, pore water, and sediment)	The sediment will be adjusted to pH 7.0 ± 0.5 before addition to test chambers. pH in overlying water should not vary more than 1.0 pH unit in the overlying water within a test vessel or between test groups during the test.		No (the pH of prepared sediment was 5.7)	The pH of prepared sediment was adjusted to 6.0 ± 0.5, rather than 7.0 ± 0.5, and the initial pH of the sediment was reported to be 5.7 (Appendix 5). The pH of overlying water during the test was 7.9-8.3. The pH of pore water during the test was 7.3-7.8.

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
Dissolved oxygen (overlying water)	≥2.5 mg/L; in the event that dissolved oxygen levels approach or fall below 2.5 mg/L, appropriate corrective actions will be taken in the following order: (#1) the addition of gentle aeration, (#2) reducing the food ration, or (#3) increasing the volume replacements of overlying water up to a maximum of four times per day), if necessary, in all test compartments. Aeration will be gentle enough as to not disturb or suspend the sediment in the overlying water. The Sponsor will be notified if corrective actions other than reducing the food ration are taken.		Yes	Dissolved oxygen concentrations in overlying water remained ≥6.7 mg/L (≥78% of the air saturation value, ASV), ranging from 6.7-8.4 mg/L.
Hardness (overlying water)	The typical hardness of the well water used for the test is 140 mg/L as $CaCO_3$.		N/A (requirements for hardness during the test were not specified in the protocol or by EPA)	Hardness of overlying water ranged from 152-176 mg/L as $CaCO_3$.
Aeration	Aeration may be used to increase dissolved oxygen levels if they fall below 2.5 mg/L.		N/A (aeration was not used)	None
Feeding frequency/diet	Larvae will be fed 1.5 mL of a 4 g solids/L suspension of flake food in water daily but will not be fed on the last day of the test. If fungal growth is seen		Yes	During the test, the larvae in each replicate test compartment were fed 1.5 mL of a 4 g solids/L suspension of flake food in water daily through Day 9 of the test, as described in

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
	in any control or treatment group test compartments, or if dissolved oxygen concentrations in the overlying water approach or fall below 2.5 mg/L, the food ration may be reduced or suspended in all experimental groups until conditions have readjusted. Feeding will not be suspended for more than 24 hours (i.e., feed withheld for a feeding interval) in response to low dissolved oxygen concentration.			the protocol. The organisms were not fed on the last day of the test.
Method of analytical measurement of test substance concentrations	Chemical analysis of the overlying water, pore water, and stock solution samples will be performed by Eurofins using LSC, and sediment samples will be combusted and analyzed by LSC. If requested by the Sponsor, selected samples may be analyzed for parent material using chromatographic methods (e.g., high performance liquid chromatography [HPLC]), or radio-profiling of sample extracts for degradants may be conducted using a technique such as HPLC-Beta-RAM or fraction collection followed by LSC of the fractions. The		Yes	LSC was used to measure concentrations of test substance in overlying water, pore water, and sediment samples based upon methodology developed by Eurofins-Easton (additional details provided in Appendix 11). The limit of quantitation (LOQ) of the analytical method was 50 dpm.

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
	methodology used to analyze the test samples will be summarized in the final report.			
Separation of pore water from sediment for analytical measurements	Pore water will be separated from sediment by centrifugation.		Yes	The sediment sample collected from each treatment and control group was centrifuged at 1962 relative centrifugal force (RCF) for ~10 minutes. The volume of pore water separated from each sediment sample was measured and recorded. Pore water samples were centrifuged at ~4415 RCF for ~10 minutes prior to analysis.
Storage of samples for analytical measurement of test substance concentrations	Samples will be processed immediately for analysis when possible or will be stored under the appropriate conditions (e.g., refrigeration or ambient) until analyzed.		Yes	Samples collected from replicate test compartments for measurement of test substance concentrations in overlying water, pore water, and sediment on Days 0, 5, and 10, and stock solution samples collected at preparation, were processed immediately for analysis and were not placed in storage.
Results				
Abnormal behavior	Observations of any abnormal behavior (e.g., leaving the sediment, unusual swimming) will be made daily.		Yes	No unusual observations of organisms avoiding the sediment occurred during the test. Although there were a few observations of larvae on the surface of the sediment or swimming in the water column, these observations were noted in the controls and treatment groups and did not appear to be concentration-responsive

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
				or treatment-related.
Survival	The number of dead organisms will be counted during the test and at test termination. Any dead organisms observed on the surface of the sediment during the test will be counted, recorded, and removed. At test termination on Day 10, the organisms will be segregated from the sediment using 425-µm (#40 U.S. standard size) mesh sieves and shallow sorting pans, and the numbers of surviving organisms will be recorded for all replicates. Any immobile organisms isolated from the overlying water, sediment surface, or from sieved material will be considered dead.		Yes	There were no apparent treatment-related effects on survival in any of the treatment groups. Mean percent survival (at test termination on Day 10): 86%, 93%, 91%, 90%, 94%, 85%, 84%, and 93% at mean measured concentrations of 0 (negative control), 0 (vehicle control), 0.018, 0.045, 0.25, 1.0, 3.8, and 16 mg/kg, respectively; treatment groups were not significantly different from the control. The NOEC, LOEC, and 10-d LC_{50} values for survival were reported as 16, >16, and >16 mg/kg dry sediment, respectively, based on no treatment-related effects on survival.
Growth (ash-free dry weight, AFDW)	Surviving larvae will be grouped by replicate for determination of AFDW. If pupae are recovered during the sieving procedure, these organisms will be included in survival data but will not be included in the growth data.		Yes	Mean AFDW of surviving midge larvae at test termination: 2.30, 2.13, 2.18, 2.17, 2.20, 2.62, 2.40, and 2.23 mg at mean measured concentrations of 0 (negative control), 0 (vehicle control), 0.018, 0.045, 0.25, 1.0, 3.8, and 16 mg/kg, respectively; treatment groups were not significantly different from the control. The NOEC and LOEC values for growth (based on mean AFDW of

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
				surviving midge larvae at test termination) were reported as 16 and >16, mg/kg dry sediment, respectively, based on no treatment-related effects on growth.
NOEC/LOEC	Observations of the effects of the test substance on survival and growth will be used to determine the no-observed-effect concentration (NOEC) and the lowest-observed-effect concentration (LOEC). The results of the statistical analyses will be used to aid in the determination of the NOEC, LOEC, MATC and EC/ICx values. However, scientific judgment will be used to determine if statistical differences are biologically meaningful, and if the data follow a concentration-dependent response.	EPA recommendation: EPA recommends that endpoint values be based on time-weighted average measured concentrations that are calculated using the initial, interim, and ending measurements.	Yes	Based on the mean measured concentrations in sediment, the overall study NOEC was determined to be 16 mg/kg based on no treatment-related effects on survival or growth at the highest concentration tested. The LOEC was >16 mg/kg.
LC50/EC50	A 10-day LC_{50} value for mortality and a 10-day EC_{50} value for growth, with 95% confidence intervals, will be determined, when possible.		Yes	The LC_{50} was determined to be >16 mg/kg, the highest mean measured concentration tested, since there was no greater than 50% reduction in survival.
Analytical measurements of test substance concentration	Samples of stock solutions, when used to spike the sediment, will be collected for analysis as soon as practical after preparation.		Yes	Samples were collected as specified in the protocol. During the test, the appearance of the overlying water was observed in the test compartments. At test

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
	Samples for measurement of test substance concentrations in overlying water, pore water, and sediment will be collected on Day 0, 5, and Day 10 of the study from replicate test vessels assigned for measurement of test substance concentrations. One sample from overlying water, sediment, and pore water will be collected for each test concentration at each time point. Overlying water samples will be collected from test chambers at mid-depth in the water column of test vessels. Alternately, the entire volume of overlying water may be sampled. Sediment will be collected from test chambers and centrifuged to isolate sediment and pore water samples. Observations of the appearance of the overlying water and test sediments will be conducted at the beginning and end of the test, and daily during the test. The appearance of any surface slicks,			initiation and termination, the overlying water appeared clear and colorless with no evidence of precipitation. Mean measured test substance concentrations in stock solutions used for spiking sediment (nominal = 1.50 mg/mL): 1.57-1.58 mg/mL. Mean measured test substance concentrations in sediment (average of Day 0, 5, and 10 measurements): 0.018, 0.045, 0.25, 1.0, 3.8, and 16 mg/kg dry weight, representing 5.7, 4.5, 7.9, 10, 12, and 16% of nominal concentrations, respectively. Negative and solvent controls: <LOQ. Measured pore water test substance concentrations (ranges on Days 0, 5, and 10 across all test concentrations): 0.00125 - 0.708 mg/L on Day 0, 0.000792-0.514 mg/L on Day 5, and 0.000808-0.572 mg/L on Day 10. Measured overlying water concentrations (ranges for Days 0, 5, and 10 across all test concentrations): 0.0000242-0.0369 mg/L on Day 0, 0.00000938-0.00533 mg/L on Day 5, and 0.0000322-0.0502 mg/L on Day 10.

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
	precipitates, mold, or fungus on the sediment, or material adhering to the sides of the test compartments will be recorded.			
Measurements of environmental conditions (in overlying water, unless otherwise specified):				
Temperature	Measured in the overlying water in one replicate test compartment of each experimental group daily during the test using a digital thermometer, or equivalent. Measurements typically will rotate among the replicate test compartments in each experimental group at each measurement interval. Water temperature also will be monitored continuously during the test in one or more representative tanks in the flow-through test system using an automatic monitoring system.		Yes	Temperature of the overlying water was measured daily in one replicate test chamber per test concentration and control, and otherwise, as described in the protocol. Water temperature also was monitored continuously in the negative control test chamber using a validated environmental monitoring system (Pointview Central Monitoring System). Values are provided above under Environmental Conditions.
Dissolved oxygen	Measured in overlying water from one replicate test compartment of each experimental group daily during the test using a Thermo Orion Model 850Aplus dissolved oxygen meter, or equivalent. Measurements typically will rotate among the replicate		Yes	Dissolved oxygen in the overlying water was measured daily in one replicate test chamber per test concentration and control, and otherwise, as described in the protocol. Values are provided above under Environmental Conditions.

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
	test compartments in each experimental group at each measurement interval.			
pH	Measured in overlying water from one replicate test compartment of each experimental group daily during the test using a Thermo Orion Dual Star pH/ISE meter, or equivalent. Measurements typically will rotate among the replicate test compartments in each experimental group at each measurement interval. Also, measured in pore water in one or more of the additional replicates maintained for physical/chemical measurements in each experimental group at the beginning and end of the test.		Yes	pH was measured in the overlying water at test initiation and daily during the test in one replicate test chamber per test concentration and control, and otherwise, as described in the protocol. pH was measured in pore water as described in the protocol. Overlying and pore water pH values are provided above under Environmental Conditions. A protocol deviation noted that pore water samples collected from one water chemistry replicate were inadvertently stored with HCl before pH measurements were taken, altering the pH of the sample. The pH measurement was taken from the remaining aliquot of the pore water sample on Day 1 instead of 0; the study authors considered this to have no impact on the results of the study.
Hardness	Measured in overlying water by titration on Days 0 and 10 in the control and highest test concentration in composite samples collected from replicate test chambers.		Yes	Values are provided above under Environmental Conditions.

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
Alkalinity	Measured in overlying water by titration on Days 0 and 10 in the control and highest test concentration in composite samples collected from replicate test chambers.		Yes	Range of alkalinity in overlying water: 184-200 mg/L as $CaCO_3$
Conductivity	Measured in overlying water on Days 0 and 10 in the control and highest test concentration in composite samples collected from replicate test chambers. Measurements will be made using a Thermo Orion Star A122 conductivity meter, or equivalent.		Yes	Range of conductivity in overlying water: 334-417 µS/cm
Ammonia	Measured in overlying water on Days 0 and 10 in each experimental group in samples collected from one or more additional replicates maintained for physical/chemical measurements. The beginning and end measurements for ammonia should not vary by >50%, when possible. Also, measured in pore water on Days 0, 5, and 10 in each experimental group in samples collected from one or more additional replicates maintained for physical/chemical measurements.		Yes (with the exception that beginning and ending measurements in overlying water varied by >50%; ammonia concentration generally decreased during the test)	Range of ammonia in overlying water (Day 0): <LOQ-6.19 mg/L as NH_3 Range of ammonia in overlying water (Day 10): <LOQ-1.03 mg/L as NH_3 Range of ammonia in pore water (Days 0-10): 1.42-12.1 mg/L as NH_3

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
	Measurements will be made using a Thermo Orion Model 720Aplus pH/ISE meter, or equivalent.			
Light intensity	Light intensity will be measured at the beginning of the test at the water surface using an SPER Scientific Ltd. light meter or equivalent.		Yes	Values are provided above under Environmental Conditions.
Contaminant analysis	Analyses will be performed at least once annually to determine the concentrations of selected organic and inorganic constituents of the well water and formulated sediment. Results of these analyses, including the analytical methods followed, LODs, and LOQs, will be summarized in the final report.	EPA requirement: The laboratory must ensure that recent samples of the dilution water conform to the specifications provided in OCSPP 850.1000 (p. 13, section c.3.ii, Table 2) and OCSPP 850.1735.	Yes (with the exception that dilution water analysis does not appear to have included particulate matter, ammonia, or chlorine values to confirm that measurements conform to OCSPP guidelines; ammonia was measured in overlying water during the test, and the water was not chlorinated)	The results of periodic analyses to measure selected organic and inorganic constituents (selected pesticides, organics, and metals) of representative samples of well water, formulated sediment, and TetraMin® flake food are presented in Appendix 4, 7, and 8, respectively.
Test validity:				
Control response – survival/ recovery	The average survival/recovery of test organisms on Day 10 will be ≥70% in the negative control group and, where relevant, in the solvent control group.		Yes	In this study, mean survival in the negative and solvent control groups was 86% and 93%, respectively.
Control response – growth (AFDW)	The average larval weight on Day 10 will be ≥0.48 mg per		Yes	In this study, the average individual AFDW in the negative and solvent

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
	surviving organism as AFDW in the negative control group and, where relevant, in the solvent control group.			control groups was 2.30 and 2.13 mg, respectively.
Identical test vessels	All test vessels will be identical and will contain the same amount of sediment and overlying water.		Yes	In this study, all test vessels were identical and contained the same volumes of sediment and overlying water.
Random/ indiscriminate assignment of organisms to test vessels	Test organisms will be indiscriminately assigned to test vessels.		Yes	All test organisms were indiscriminately assigned to test vessels.
Control groups	A negative sediment control and, where relevant, a solvent sediment control, will be included in the test.		Yes	A negative sediment control and a solvent sediment control were included in the test.
Reference toxicant test results	Results of the most recent reference toxicity test for the test organism source will be included in the final report.		Yes	Reference toxicant test results were included in the study report. Summary results were provided on p. 12 of the study report for midges exposed to potassium chloride at concentrations ranging from 1200-6000 mg/L under static conditions (48-hr EC50 = 3629 mg/L, 95% C.I. 3443-4223 mg/L).
GLP/QA compliance	The study will be conducted in compliance with GLP standards. The final report will contain statements of GLP compliance and quality assurance.		Yes	The GLP compliance statement noted the following exceptions: annual analyses of the sediment, water, and feed for potential contaminants were not performed according to GLP standards, but were performed using a certified laboratory and standard EPA analytical methods; preliminary range-finding data are

Parameter	Specifications in Protocol[1]	Additional Information from Correspondence between EPA[2] and Sponsor[3]	Adherence to Protocol and EPA's Requirements[4]	Deviations/ Adjustments/ Clarification/ Comments
				considered exploratory work and were not conducted in accordance with GLP standards; characterization and stability of the non-radiolabeled and radiolabeled test substance under the conditions of storage at the test site were not determined in compliance with GLP standards.

N/A = Not applicable