p-Dichlorobenzene: A 10-DAY TOXICITY TEST WITH THE MIDGE (*Chironomus dilutus*) USING SPIKED WHOLE SEDIMENT

FINAL REPORT

EASTON STUDY NUMBER: 264A-116
eSM PROJECT NUMBER: S21-08512

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STUDY INITIATION DATE: June 2, 2023
STUDY COMPLETION DATE: November 20, 2023

SUBMITTED TO:

American Chemistry Council
p-Dichlorobenzene Test Order Consortium
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Washington, DC 20002
USA

TESTING FACILITY:

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GOOD LABORATORY PRACTICE COMPLIANCE STATEMENT

SPONSOR: American Chemistry Council

TITLE: *p*-Dichlorobenzene: A 10-Day Toxicity Test with the Midge (*Chironomus dilutus*) Using Spiked Whole Sediment

STUDY NUMBER: 264A-116

STUDY COMPLETION: November 20, 2023

This study was conducted and reported in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency (40 CFR Part 792) (1989), which are compatible with the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice (ENV/MC/CHEM (98)17), with the following exceptions:

Annual analyses of the sediment, water and feed for potential contaminants were not performed in compliance with Good Laboratory Practice Standards, but were performed using a certified laboratory and standard US EPA analytical methods.

Preliminary range-finding data are considered exploratory work and were not conducted in accordance with Good Laboratory Practice Standards.

The 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 Good Laboratory Practice Standards.

These exceptions to the stated GLP standards did not adversely affect study integrity or the interpretation of the results generated from this study.

STUDY DIRECTOR:

Suzanne Z. Schneider — 20 November 2023

Suzanne Z. Schneider, Ph.D.
Associate Director of Aquatic Toxicology
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Date

SPONSOR APPROVAL:

C Steven — 21 November 2023

Sponsor's Representative — Date

Applicant/Submitter — Date

QUALITY ASSURANCE STATEMENT

This study was conducted and reported in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency (40 CFR Part 792) (1989), which are compatible with the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice (ENV/MC/CHEM (98)17). The dates of all inspections and audits and the dates that any findings were reported to the Study Director and Laboratory Management were as follows:

		DATE REPORTED TO:	
ACTIVITY:	DATE CONDUCTED:	STUDY DIRECTOR:	MANAGEMENT:
Protocol	June 9, 2023	June 9, 2023	August 14, 2023
Test Substance Preparation	June 5, 2023	June 5, 2023	June 14, 2023
pH Measurements	June 8, 2023	June 8, 2023	June 15, 2023
Analytical Data and Draft Report	September 15 – 18, 2023	October 6, 2023	September 18, 2023
Biological Data and Draft Report	October 13 – 19, 2023	October 20, 2023	November 13, 2023
Final Report	November 17, 2023	November 17, 2023	November 20, 2023

All inspections were study-based unless otherwise noted.

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Darryl Anderson, B.S.
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Nov. 20, 2023

Date

REPORT APPROVAL

SPONSOR: American Chemistry Council *p*-Dichlorobenzene Test Order Consortium

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STUDY DIRECTOR:

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TABLE OF CONTENTS

SUMMARY

SPONSOR:	American Chemistry Council	
STUDY TITLE:	*p*-Dichlorobenzene: A 10-Day Toxicity Test with the Midge (*Chironomus dilutus*) Using Spiked Whole Sediment	
STUDY NUMBER:	264A-116	
GUIDELINE:	U.S. EPA OCSPP 850.1735	
TEST SUBSTANCE:	Name:	1, 4- Dichlorobenzne
	Purity (Content of A.I.):	99.9%
	Batch/Lot Number:	MKBS4401V
	Appearance:	Solid
TEST DATES:	Experimental Start (OECD):	June 5, 2023
	Experimental Start (EPA):	June 6, 2023
	Exposure Termination:	June 16, 2023
	Experimental Termination:	June 16, 2023
LENGTH OF EXPOSURE:	10 Days under flow-through conditions	
TEST ORGANISM:	Name:	Midge (*Chironomus dilutus*)
	Source:	Aquatic Bio Systems
		Fort Collins, Colorado, 80524
	Age:	10 days post-hatch at test initiation
REPLICATION:	8 test compartments per test concentration, 10 midge larvae per compartment (total of 80 midge larvae per concentration)	
ENVIRONMENTAL CONDITIONS IN OVERLYING WATER:	Temperature Range:	22.4 – 23.6°C
	Dissolved Oxygen:	≥78%; no aeration
	pH Range:	6.7 – 8.4
	Hardness Range:	152 – 176 mg/L as $CaCO_3$
	Alkalinity Range:	184 – 200 mg/L as $CaCO_3$
	Specific Conductance Range:	334 – 417 µS/cm
	Ammonia:	<LOQ – 6.19 mg/L as NH_3

SUMMARY (Continued)

TEST CONCENTRATIONS IN SEDIMENT:

Nominal (mg/kg dry sediment)	Mean Measured (mg/kg)
Negative Control	< LOQ
Solvent Control	< LOQ
0.32	0.018
1.0	0.045
3.2	0.25
10	1.0
32	3.8
100	16

MEASURED ENDPOINTS: Survival and growth (ash-free dry weight)

RESULTS: Based on mean measured concentrations in sediment:

10-Day LC_{50} for Survival: >16 mg/kg

Lowest-Observed-Effect Concentration: >16 mg/kg
No-Observed-Effect Concentration: 16 mg/kg

INTRODUCTION

This study was conducted for American Chemistry Council *p*-Dichlorobenzene Test Order Consortium at the Eurofins facility in Easton, Maryland. The in-life phase of the definitive test was conducted from June 6 to June 16, 2023, with dry-weight measurements completed on June 19, 2023. Raw data generated at the testing facility and a copy of the final report are filed under Study Number 264A-116 in archives located on the Easton site.

OBJECTIVE

The objective of this study was to determine the effects of sediment-incorporated *p*-Dichlorobenzene on the midge, *Chironomus dilutus*, during a 10-day exposure period in a flow-through system providing intermittent renewal of overlying water. The measured endpoints of the test were survival and growth as determined by ash-free dry weight (AFDW) measurements.

EXPERIMENTAL DESIGN

Groups of midges were exposed to a geometric series of six test concentrations, a negative control (untreated formulated sediment), and a solvent control (ethanol) for 10 days under flow-through test conditions. Eight replicate test compartments were maintained in each treatment and control group, with 10 midge larvae in each test compartment, for a total of 80 midge larvae per test concentration. Each test compartment contained sediment and overlying water. An additional three replicates were prepared in each treatment and control group for physical/chemical measurements of water and sediment. No midge larvae were placed in the additional replicates sampled on Day 0, but those sampled on Days 5 and 10 had midge larvae 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.

Test concentrations in the sediment were prepared on a mg/kg dry weight basis. Nominal test concentrations were selected in consultation with the Sponsor based on exploratory range-finding toxicity data, and were 0.32, 1.0, 3.2, 10, 32 and 100 mg *p*-Dichlorobenzene/kg of sediment. Test concentrations were measured in samples of overlying water, pore water, and sediment collected at the beginning, approximate middle and end of the test. The results of the study are based on arithmetic mean measured test concentrations in the sediment.

The water/sediment systems in the test compartments were allowed to equilibrate for approximately 24 hours prior to introduction of the organisms. Third instar larvae (10 days of age) were impartially assigned

to exposure chambers at test initiation. Observations of abnormal behavior were made daily during the test, and survival and growth (AFDW) were determined at the end of the 10-day test period. Percent mortality observed in the treatment groups at the end of the test was used to determine the 10-day LC_{50} value. The lowest-observed-effect concentration (LOEC) and the no-observed-effect concentration (NOEC) were determined by the concentration-response pattern and statistical analyses of the survival and dry weight data.

MATERIALS AND METHODS

The study was conducted according to the procedures outlined in the protocol, "*p*-Dichlorobenzene: A 10-Day Toxicity Test with the Midge (*Chironomus dilutus*) Using Spiked Sediment" (Appendix 1). The protocol was based on procedures in the U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines, OCSPP Number 850.1735: *Spiked Whole Sediment 10-Day Toxicity Test, Freshwater Invertebrates* (1) and ASTM Standard E 1706-05: *Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates* (2).

Test Substance

The non-radiolabeled test substance used to prepare the test sediments, the analytical matrix fortification samples and the analytical calibration standards for the study was received from Sigma-Aldrich on February 14, 2022. It was assigned testing facility identification number 17540 upon receipt and was stored under ambient conditions. The test substance, a solid, was identified as: 1, 4-Dichlorobenzene (Batch MKBS4401V; CAS# 106-46-7). The test substance contained 99.9% active ingredient (Appendix 2).

The radiolabeled test substance used to prepare the test sediments, the analytical matrix fortification samples and the analytical calibration standards for the study was received from Selcia on June 16, 2022. It was assigned testing facility identification number 17854 upon receipt and was stored under frozen conditions. The test substance, a liquid, was identified as: [phenyl-U-14C]*p*-Dichlorobenzene; Batch ID 12435JLC006-1. The test substance had a radiochemical purity of 99.8% and a specific activity of 43.21 mCi/mmol (Appendix 2).

Test Water

The water used for holding and testing was freshwater obtained from a well approximately 40 meters deep located on the testing facility site. The well water was passed through a sand filter to remove particles and pumped into a 37,800-L storage tank where the water was aerated with spray nozzles. Prior

to use in the test system, the water was filtered to 0.45 μm to remove fine particles and was passed through an ultraviolet (UV) sterilizer.

The well water is characterized as moderately-hard water. The specific conductance, hardness, alkalinity, pH and total organic carbon (TOC) content of the well water during the approximate four-week period immediately preceding the test are presented in Appendix 3. The results of periodic analyses performed to measure the concentrations of selected organic and inorganic constituents in the well water are presented in Appendix 4.

Test Sediment

Formulated sediment based on the recommendations of OECD Guideline 218 (3) was used as the test sediment. The sediment was composed of approximately 5% air dried peat moss, 20% clay (kaolin clay), 1% of ground limestone and 70% industrial quartz sand (Appendix 5). 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. The initial pH of the sediment was 5.7. A sample of the formulated sediment used in the test was sent to Agvise Laboratories, Northwood, North Dakota, for characterization and analysis of total organic carbon (TOC), and a summary of the sediment characterization is presented in Appendix 6. The percent organic carbon of the sediment was determined to be 2.7%. The results of periodic analyses performed to measure the concentrations of selected organic and inorganic constituents in a representative sample of formulated sediment like that used in the test are presented in Appendix 7.

Test Organism

The midge, *Chironomus dilutus*, was selected as the test species for this study. This species is representative of an important group of aquatic invertebrates and was selected for use in the study based upon past history of use in the laboratory and the recommendations of the study guideline (1). Reference testing with midges from the culture is periodically conducted at Eurofins-Easton laboratories. Midges were exposed for 48 hours under static conditions to five nominal concentrations of potassium chloride ranging from 1200 to 6000 mg/L. Results of the most recent test (Eurofins-Easton study number 100A-160) indicated the 48-hour EC_{50} value was 3629 mg/L, with 95% confidence intervals of 3443 to 4223 mg/L. These reference test results were consistent with previous reference testing at Eurofins-Easton and demonstrate that the commercially supplied *Chironomid dilutus* used for testing by Eurofins-Easton are sensitive to a known toxicant, potassium chloride, and are suitable for use in toxicological testing.

Test organisms used in the test were obtained from cultures maintained by Eurofins-Easton, Maryland. The identity of the species was verified by the supplier of the original culture Aqua Bio Systems, Fort Collins, Colorado. In the laboratory, the organisms were hatched in water from the same source as the water used during the test at approximately the same temperature and held until they were the appropriate age. Midges used in the test were third instar larvae (approximately 10 days old) at test initiation. During the 14-day holding period immediately preceding the test, water temperatures in the culture ranged from 21.9 to 22.4°C, the pH of the water ranged from 8.3 to 8.5, and the dissolved oxygen concentrations were ≥6.9 mg/L (≥79% of saturation). During this holding period, the midge larvae to be used in the test appeared normal.

Feeding

During the holding period, the midge larvae were fed an invertebrate slurry and chlorella diet. 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. The organisms were not fed on the last day of the test. The results of periodic analyses performed to measure the concentrations of selected organic and inorganic constituents in the feed are presented in Appendix 8.

Pore Water Equilibration and Stability Trials

Prior to the definitive study, non-GLP pore water and stability trials (264A-115) were conducted. The results of the pore water trial are presented in Appendix 9. The trial 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*-Dichlorobenzene in the sediment. Equilibration typically is considered to have occurred when two consecutive samples result in relatively similar measurements.

Results of analyses indicated that concentrations of *p*-Dichlorobenzene 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.

A second stability trial was conducted to evaluate the stability of *p*-Dichlorobenzene 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 minutes and 20 hours after mixing, and 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 analysis of sediment, overlying water and pore water are presented in Appendices 9.3, 9.4 and 9.5, respectively. Mass balance calculations are presented in Appendix 9.6 and indicate that the overall concentrations of *p*-Dichlorobenzene 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 and stability trials, mixing of the sediment and preparation of the test compartments were prepared 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*-Dichlorobenzene.

Non-GLP Range-finding Test

A non-GLP range-finding test was conducted to determine the test concentrations used in the definitive test. The nominal concentrations selected for the rangefinder were 0.10, 0.80, 4.0, 20 and 100 mg/kg in addition to a negative control. Four replicates containing 10 organisms in each replicate were maintained for each concentration and control. The procedure for dosing the rangefinder was equivalent to that which was used in the definitive test. The rangefinder was conducted under flow-through conditions for 10 days, under similar environmental conditions as the definitive test using organisms that were approximately 3 – 4 days post hatch. There was no analytical confirmation of test concentrations during the range-finding test. The results of the range-finding test are presented in Appendix 10.

Preparation of Test Concentrations

The test substance was administered to the test organism in sediment. This route of administration was selected because it represents the most likely route of exposure to sediment dwelling organisms.

To prepare a batch of sediment for each treatment level, the appropriate amount of neat non-radiolabeled test material was mixed with 60 grams of sand in a labeled glass beaker and was stirred with a glass stir rod. The appropriate volume of radiolabeled stock solution was added to the sand premix containing neat material and mixed by hand until homogenous. The amounts of radiolabeled and non-radiolabeled material added to the sand premixes can be found in the table below.

	Nominal Test Concentration (mg/kg)					
	0.32	1.0	3.2	10	32	100
Volume of 14C stock added (mL)	0.245	0.765	0.765	0.765	0.765	0.765
Amount of Neat Test material added (mg)	--[1]	--[1]	2.64	10.80	37.2	118.8

[1] The 0.32 and 1.0 mg/kg treatment groups were dosed with radiolabeled material only.

This dosed "sand premix" was placed under a fume hood and the ethanol was allowed to evaporate for approximately 15 minutes. The 60.0-gram sand premix was added to 540.0 grams dry weight (873.8 grams wet weight adjusted for a 38.2% moisture content) of untreated sediment in a 2000-mL plastic Nalgene® bottle and mixed on a roller mixer for approximately 30 minutes. An additional 600.0 grams dry weight (970.9 grams wet weight) of untreated sediment was added to the premix to achieve a final weight of 1200.0 grams dry weight. This 1200.0-g batch of sediment was mixed on a roller mixer for approximately 1 hour prior to transfer of the sediment to the test chambers. Since a solvent (ethanol) was used in the preparation of the test sediments, a solvent control was included in the test design. The solvent control sediment was prepared using 0.765 mL of ethanol, with the same mixing procedures as the treated sediments but with no test substance added. The negative control sediment was prepared without the addition of test substance or solvent.

Preparation of Test Compartments

Fourteen replicate test compartments were prepared for each treatment and control group. Eight replicates per group were used for the evaluation of survival and growth. An additional three replicates per group were used for the purpose of analytical confirmation of concentrations on Days 0, 5 and 10. An additional three replicates per group were used for the physical/chemical measurements of water and sediment on Days 0, 5 and 10. After mixing the batch sediments, approximately 100-mL of the appropriate dosed sediment was added to each of the replicate test compartments (300-mL glass beakers) on a top-loading balance, and the weight of the sediment was recorded. Approximately 175 mL of overlying water slowly filled the test compartments as they were placed into test chambers (diluter tanks) containing untreated test water, while avoiding disturbance of the sediment. 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.

Test Apparatus

The test apparatus consisted of a flow-through diluter system that was designed to maintain test compartments in multiple stainless steel tanks in a temperature controlled water bath. The test compartments in each treatment and control group were indiscriminately positioned in two diluter tanks per group, and were labeled with the project number, test concentration and replicate designation.

The test compartments were 300-mL glass beakers with stainless steel mesh-covered holes on opposite sides of the beaker. Each compartment contained approximately 100 mL of sediment and approximately 175 mL of overlying water. 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 water volume in the test chambers (tanks) was maintained by a standpipe within the test chamber. The water volume in the test compartments was maintained by the water levels in the test chambers and the position of the holes on the opposite sides of the test compartments. Each test compartment received approximately two volume additions of test water per day. The test water was delivered directly into each test compartment, passively forcing water out through the holes in the sides of the compartments to exchange the water overlying the sediment. Test water delivery volume was verified prior to test initiation. The general operation of the test apparatus was checked visually at least once each day during the test.

Analytical Sampling

Samples of stock solutions were collected at preparation to confirm the concentrations used to dose the sediments and were analyzed immediately. The additional replicate test compartments prepared for each treatment and control group were collected for analysis of overlying water, pore water and sediment on Days 0, 5 and at test termination on Day 10. The test compartments were processed and analyzed immediately. The sediment was transferred into centrifuge tubes using a scoopula or similar device and transferred to chemistry for analysis.

Analytical Method

^{14}C-*p*-DCB Radiopurity Check

A primary stock solution of ^{14}C-*p*-Dichlorobenzene (^{14}C-*p*-DCB) radiolabeled test substance was prepared by accurately measuring 5000 µL (0.875 mCi/mL) of test substance using a pipettor and transferring it to an amber glass vial that was filled with 5.00 mL of ethanol, bringing the stock to a total volume of 10.0 mL. The primary stock was mixed by inversion (at least 20 times) until the solution was clear and then stored frozen until use. A radiopurity analysis was conducted on the radiolabeled primary

stock prior to its use in the definitive test to demonstrate that it was appropriate for use. A previous dilution of the primary stock in ethanol was diluted an additional time with HPLC-grade water to achieve a 50 : 50 (v/v) ethanol : HPLC-grade water composition and dilution factor of 800. This dilution of the primary stock was analyzed by HPLC/UV with in line β-Ram detection. The radiopurity was determined to be 96.885% by percent area. The retention time of the UV/VIS signal of the diluted primary stock matched that of a non-radiolabeled standard also prepared in 50 : 50 (v/v) ethanol : HPLC-grade water, and the radioactive signal for the primary stock was limited to a single peak corresponding to the retention time of the parent material. Therefore, it is concluded that this primary stock has remained as listed on the Certificate of Analysis (Appendix 2). Representative chromatograms from the radiopurity analysis are presented in Appendix 11.1. Typical operational parameters for the radiopurity analysis are presented in Appendix 11.2. A method outline for the radiopurity check analysis is presented in Appendix 11.3.

^{14}C-*p*-DCB Primary Stock Concentration Verification

Prior to the experimental start date, the concentration of the ^{14}C-*p*-DCB primary stock solution was verified. To conduct the verification, the primary stock solution of ^{14}C-*p*-DCB was diluted in ethanol by a factor of 400. Three 50.0 µL aliquots of this primary stock dilution and 10 mL of Ultima Gold XR scintillation cocktail were added to scintillation vials and analyzed by liquid scintillation counting (LSC) to verify the primary stock concentration of ^{14}C-*p*-DCB. A method outline for the primary stock concentration verification is presented in Appendix 11.4. The primary stock was used to prepare ^{14}C working stock solutions of 0.32, 1.0, 3.2, 10, 32 and 100 mg/mL nominal concentrations.

Analytical Method

The analytical method used to analyze the test samples was based upon methodology developed by Eurofins-Easton. Concentrations of the test substance in overlying water, pore water, and sediment samples were analyzed by LSC. Sample aliquots of overlying water and dilution water were measured upon collection into scintillation vials to which 10 mL of Ultima Gold XR scintillation cocktail was added. The sediment portion of each sample centrifuged at 1962 relative centrifugal force (RCF) for approximately 10 minutes. The volume of pore water separated from each sediment sample was measured and recorded. Aliquots of pore water samples were transferred to polypropylene tubes and centrifuged for approximately 10 minutes at approximately 4415 RCF. Next, 5.00 mL of each sample were transferred to scintillation vials, and Ultima Gold XR scintillation cocktail (15 mL) was added to each vial and water samples were counted by LSC. Aliquots of the sediment samples (0.200 g) weighed into Combusto-Cones, and 2-3 drops of Combustaid were added to each sample. The samples were oxidized with 8.00 mL Carbosorb E and

10.0 mL Permafluor E+ using a Perkin Elmer A307 Sample Oxidizer, and then analyzed by LSC. Perkin Elmer Model Tri-Carb 2910 TR and Tri-Carb 4910 TR Liquid Scintillation Analyzers were used to determine disintegrations per minute (dpm) in the overlying water, pore water and sediment. A separate aliquot of each sediment sample was weighed for moisture determination. A method outline for separating overlying water, pore water, and sediment is presented in Appendix 11.5. Method outlines for the analysis of pore/overlying water, sediment and dilution water are presented in Appendices 11.6, 11.7 and 11.8, respectively, and instrument parameters for LSC are presented in Appendix 11.9. The calculations for sample quantitation are included in Appendix 11.10.

In addition to the test samples, 10-mL samples of Ultima Gold XR scintillation cocktail were analyzed with overlying water and pore water samples at each interval to determine background radioactivity. A blank Combusto-Cone was oxidized and analyzed with each set of sediment samples to determine background radioactivity. The background contribution for each LSC sequence was automatically subtracted from each measurement by the LSC's software.

Environmental Conditions

The test systems were illuminated using fluorescent tubes that emit wavelengths similar to natural sunlight. The lights were controlled by an automatic timer to provide a photoperiod of 16 hours of light and 8 hours of darkness. A 30-minute transition period of low light intensity was provided when lights went on and off to avoid sudden changes in light intensity. Light intensity at the water surface of one representative test compartment was measured at the beginning of the test using a SPER Scientific Model 840006 light meter.

The test was conducted at a target water temperature of 23 ± 1°C. Temperature was measured in the overlying water of one replicate test compartment of each treatment and control group daily during the test using a digital thermometer. Measurements typically alternated between replicate test compartments in each group at each measurement interval. Water temperature also was monitored continuously in the negative control test chamber using a validated environmental monitoring system (Pointview Central Monitoring System). The system measurements were calibrated prior to exposure initiation with a digital thermometer.

Dissolved oxygen concentrations were measured in samples of overlying water from one replicate test compartment of each treatment and control group daily during the test using a Thermo Scientific Orion Star A213 Benchtop RDO/DO meter. Measurements of pH were made in samples of overlying water from

one replicate test compartment of each treatment and control group at test initiation and daily during the test. Measurements of pH in pore water were also measured in one of the additional replicates maintained for physical/chemical measurements in each experimental group at the beginning and end of the test. Measurements of pH in overlying water were made using a Thermo Scientific Orion Dual Star pH/ISE meter. Measurements of dissolved oxygen and pH typically alternated between replicate test compartments in each group at each measurement interval.

Hardness, alkalinity, specific conductance, and ammonia were measured in composite samples of overlying water from the negative and solvent control group replicates and the highest concentration treatment group replicates at the beginning and end of the test. Ammonia was also measured in samples of the pore water from one of the additional replicates maintained for physical/chemical measurements in each experimental group at the beginning of the test, mid-test (e.g., Day 6), and at the end of the test. Hardness and alkalinity measurements were made by titration based on methods in *Standard Methods for the Examination of Water and Wastewater* (4). Specific conductance was measured using a Thermo Scientific Orion Star A122 Portable Conductivity Meter. Ammonia was measured using a Thermo Scientific Orion Dual Star pH/ISE meter.

The sediment redox potential (Eh) was measured at the beginning of the test, mid-test (e.g., Day 5), and at the end of the test from one of the additional replicates maintained for physical/chemical measurements in each experimental group. Measurements of redox potential were made using a PINPOINT® Redox/ORP Monitor.

Procedures and Biological Observations and Measurements

The test was initiated following the equilibration period for the water/sediment systems. To obtain known age organisms, egg masses were isolated from the culture and held in test water to stimulate hatch. The newly hatched larvae were held in water from the same source and at approximately the same temperature as was used in the test until the organisms were the appropriate age (third instar, approximately 10 days old). To initiate the test, one or two approximately 10-day old midges were indiscriminately and sequentially added to transfer containers (e.g., glass beakers) containing test water until each transfer container contained its complement of 10 individuals. The transfer containers were indiscriminately assigned to each of twelve test compartments per treatment and control group (eight biological replicates plus two analytical sampling replicates and two replicates used for physical/chemical measurements). Organisms were transferred below the water surface, using a glass wide-bore pipette. The test compartments prepared for analytical sampling

and water chemistry measurements on Day 0 did not contain organisms. An additional subset of eighty 10-day old organisms from the test lot of organisms used in the test were impartially selected on Day 0 for measurement of dry weight.

The test compartments were observed daily during the test to make visual assessments of any abnormal behavior (e.g., leaving the sediment, unusual swimming). Any dead organisms observed on the surface of the sediment during the test were counted, recorded, and removed. At test termination, on Day 10, the organisms were segregated from the sediment using a 425-µm sieve and a shallow sorting pan, and the numbers of live or dead organisms were enumerated. Any immobile organisms isolated from the sediment surface or sieved materials were considered dead. If the total number of individuals found in a replicate at test termination was fewer than the number initially placed into the replicate at the beginning of the test, then those missing were considered dead. Surviving organisms were grouped by replicate for determination of ash-free dry weight (AFDW). If pupae were recovered during the sieving procedure, the organisms were included in the survival data but were not included in the growth data.

Statistical Analyses

Statistical analysis was performed to evaluate differences between treatment and control groups for survival and growth (AFDW). The unit of statistical analysis is the test compartment. The percent inhibition (%I) as compared to the pooled control at each test substance treatment level was calculated for survival and growth. The results of the test are based on the mean measured concentrations in the sediment.

Since a solvent control group was used in addition to a negative control group, the data from these two groups was compared using a Fisher's exact test for survival and using a t-test for growth. There were no statistically significant differences ($p > 0.05$), between the controls using the appropriate tests. Therefore, the data from the treatment groups for survival and growth were compared to the pooled control group to evaluate the treatment-related effects.

The statistical analyses used to evaluate the data were based on the procedure provided in the study guideline (3) and OECD 54 (5) to determine the 10-Day NOEC and LOEC values for each parameter. Survival was analyzed using Fisher's Exact test. The growth data were visually evaluated for monotonicity and evaluated for normality and homogeneity of variance ($\alpha = 0.01$) using the Shapiro-Wilk and Bartlett equality of variance test and passed the assumptions of normality and homogeneity. Since the data for growth were determined to be non-monotonic and passed the assumptions of normality and homogeneity, the data in the

treatment groups were compared to the pooled control data using a Dunnett T3 multiple comparison test to identify any significant differences ($\alpha = 0.05$). The statistical tests were conducted using CETIS (6).

The results of the statistical analyses were used to aid in the determination of the NOEC and LOEC. However, scientific judgment was used to determine if any statistical differences were biologically meaningful, and if the data followed a concentration-dependent response. The LOEC was defined as the lowest tested concentration at which the test substance is observed to have had a statistically significant adverse effect ($p \leq 0.05$) on survival or growth when compared to the control. However, all test concentrations above the LOEC should have a harmful effect equal to or greater than those observed at the LOEC. The NOEC was defined as the test concentration immediately below the LOEC, which when compared with the control group, had no statistically significant adverse effect (at $p > 0.05$).

RESULTS AND DISCUSSION

Measurement of Test Concentrations

Nominal concentrations selected for use in this study were 0.32, 1.0, 3.2, 10, 32 and 100 mg/kg dry weight of sediment. During the course of the test, the appearance of the overlying water was observed in the test compartments. At test initiation and termination, the overlying water appeared clear and colorless with no evidence of precipitation.

Results of analyses to measure the concentration of *p*-Dichlorobenzene in the radiolabeled stock solution samples used to dose the sediment are presented in Table 1. Measured concentrations ranged from 104 to 105% of nominal. Results of analysis to measure *p*-Dichlorobenzene in a dilution water sample is presented in Table 2 and was < LOQ. Results of analyses to measure equivalent concentrations of ^{14}C- *p*-Dichlorobenzene in the sediment, pore water and overlying water samples during the test are presented in Tables 3, 4 and 5. respectively. All reported sediment concentrations are expressed on a dry weight basis. The LOQ for sediment, overlying water and pore water analyses was set at the instrument LOQ of 50 dpm. Measured concentrations of the test substance in negative and solvent control sediment and water samples on Days 0, 5 and 10 were below the LOQ.

Measured concentrations in the sediment samples collected from the 0.32, 1.0, 3.2, 10, 32 and 100 mg/kg treatment groups were 0.0205, 0.0484, 0.280, 1.16, 4.92 and 18.3 mg/kg, respectively on Day 0; 0.0174, 0.0425, 0.236, 0.921, 3.24 and 15.8 mg/kg, respectively on Day 5, and 0.0165, 0.0443, 0.240, 0.950, 3.34 and 12.9 mg/kg, respectively on Day 10.

Measured concentrations in the pore water collected from the 0.32, 1.0, 3.2, 10, 32 and 100 mg/kg treatment groups ranged from 0.00125 to 0.708 mg/L on Day 0; ranged from 0.000792 to 0.514 mg/L on Day 5 and ranged from 0.000808 to 0.572 mg/L on Day 10. Measured concentrations in the overlying water collected from the 0.32, 1.0, 3.2, 10, 32 and 100 mg/kg treatment groups ranged from 0.0000242 to 0.0369 mg/L on Day 0; ranged from 0.00000938 to 0.00533 mg/L on Day 5 and ranged from 0.0000322 to 0.0502 mg/L on Day 10.

When measured concentrations of the sediment samples collected during the test were averaged, the mean measured test concentrations for this study were 0.018, 0.045, 0.25, 1.0, 3.8 and 16 mg/kg, representing 5.7, 4.5, 7.9, 10, 12 and 16% of nominal concentrations, respectively. The results of the study were based on the mean measured concentrations in sediment.

Observations and Measurements

Measurements of temperature, dissolved oxygen and pH of the overlying water in the test compartments are summarized in Table 6, and individual measurements are presented in Appendices 12, 13 and 14, respectively. All water quality measurements were within the desired range of the test. Water temperatures were within the 23 ± 1°C range established for the test. Measurements of pH in the overlying water ranged from 7.9 to 8.3 during the test. Dissolved oxygen concentrations remained ≥6.7 mg/L (≥78% of air saturation) throughout the test. Measurements of specific conductance, hardness, alkalinity, and ammonia of the overlying water in the negative and solvent control and the highest concentration treatment group are summarized in Table 6 and individual measurements are presented in Appendix 15. Measurements of specific conductance, hardness, and alkalinity were comparable between the control and treatment group and were typical of the test facility well water. Measurements of ammonia in the overlying water at the beginning of the test ranged from < LOQ – 6.19 mg/L and at the end of the test ranged from <LOQ – 1.03 mg/L (LOQ of 0.17 mg/L, the concentration of the lowest calibration standard). Light intensity at test initiation was 633 lux at the surface of the water of one representative test compartment (negative control replicate A). Measurements of pH and ammonia in the pore water as well as the sediment redox potential are summarized in Table 7 and individual replicate data is presented in Appendices 16 and 17, respectively. The pH in the pore water ranged from 7.3 to 7.8 during the test. Ammonia in the pore water ranged from 1.42 to 12.1 during the test and the sediment redox (Eh) potential ranged from -53 to 326 during the test.

Survival of midges in each treatment and control group at test termination is summarized in Table 8, and individual replicate data is presented in Appendix 18. There were sporadic observations of organisms leaving the sediment or on the surface of the sediment during the test. These observations were noted in the controls and treatment groups and were not considered to be treatment related. There were no apparent treatment-related effects on survival in any of the treatment groups. Mean percent survival at test termination in the negative control and solvent control group was 86% and 93%, respectively. Mean percent survival at test termination in the 0.018, 0.045, 0.25, 1.0, 3.8 and 16 mg/kg treatment groups was 91%, 90%, 94%, 85%, 84% and 93%, respectively. There were no statistically significant differences ($p > 0.05$) in survival in any of the treatment groups in comparison to the pooled control using a Fisher's Exact/Bonferroni-Holm test. Therefore, the LOEC for survival was >16 mg/kg dry sediment and the NOEC was 16 mg/kg dry sediment. The 10-day LC_{50} for survival was determined to be >16°mg/kg dry sediment, the highest concentration tested.

The mean individual ash-free dry weights (AFDW) of surviving midge larvae in each treatment and control group at test termination are summarized in Table 8, and individual replicate data is presented in Appendix 19. Pupae and dead organisms were not included in the determination of ash-free dry weights. There were no apparent treatment-related effects on growth in any of the treatment groups. The mean AFDW per surviving midge larvae at test termination in the negative and solvent control group was 2.30 and 2.13 mg, respectively. The mean AFDW per surviving midge larvae at test termination in the 0.018, 0.045, 0.25, 1.0, 3.8 and 16 mg/kg treatment groups was 2.18, 2.17, 2.20, 2.62, 2.40 and 2.23 mg, respectively. There were no statistically significant differences ($p > 0.05$) in growth in any of the treatment groups in comparison to the pooled control using the Dunnett T3 multicomparison test. Therefore, the LOEC for growth was >16 mg/kg dry sediment and the NOEC was 16 mg/kg dry sediment. The mean individual dry weight of 80 midge larvae collected from the test batch of organisms at the beginning of the test was 0.22 mg (Table 8).

Conditions for the Validity of the Test

The following criteria were used to judge the validity of the test and were met in this study:

1. 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. In this study, mean survival in the negative and solvent control groups was 86% and 93%, respectively.
2. the average larval weight on Day 10 will be ≥0.48 mg per surviving organism as ash-free dry weight (AFDW) in the negative control group and, where relevant, in the solvent

control group. In this study, the average individual AFDW in the negative and solvent control groups was 2.30 and 2.13 mg, respectively.

3. all test vessels will be identical and will contain the same amount of sediment and overlying water. In this study, all test vessels were identical and contained the same volumes of sediment and overlying water.
4. all test organisms were indiscriminately assigned to test vessels.
5. a negative sediment control and a solvent sediment control were included in the test.

CONCLUSIONS

Third instar midge larvae (*Chironomus dilutus*) were exposed for 10 days to six mean measured concentrations of sediment-incorporated *p*-Dichlorobenzene ranging from 0.018 to 16 mg/kg. There were no treatment-related effects observed on survival, or growth at any tested concentration. Based on the mean measured concentrations in sediment, the 10-day LC_{50} value for survival was >16 mg/kg, the highest concentration tested. The NOEC for both survival and growth was 16 mg/kg and the LOEC was >16 mg/kg.

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5 **Organization for Economic Cooperation and Development.** 2006. *Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application.* OECD ENV/JM/MONO(2006)18.

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Table 1

Analysis of Primary Stock Solution of 14C-*p*-DCB

Sample Type	Sample Number	Nominal ^{14}C-*p*-DCB Concentration (mg/mL)	Specific Activity (dpm/mg)	Total [^{14}C] Found (dpm)	^{14}C-*p*-DCB Equivalents[1,2] (mg/mL)	Percent of Nominal[2]
Stock	17854-072822-1G	1.50	646446526	126719.75	1.57	105
Stock	17854-072822-1H	1.50	646446526	126653.78	1.57	104
Stock	17854-072822-1I	1.50	646446526	127283.13	1.58	105
				Mean =	1.57	105

1 ^{14}C-*p*-DCB Equiv. = ({[(Total dpm found/Sample Volume]}/Specific Activity) * Dilution Factor. Dilution factor = 400. Sample volume = 0.0500 mL.

2 Results were calculated using Excel. Manual calculations may differ.

Table 2

Measured Concentrations of ^{14}C-*p*-DCB Pretest Dilution Water Sample Analyzed by LSC

Sample ID (264A-116-)	Nominal Concentration (mg/kg)	Sample Volume (mL)	Specific Activity (dpm/µg)	Total [^{14}C] Found[2] (dpm)	^{14}C-*p*-DCB Equivalents[1,2,3] (mg/kg)
1-DIL	--	10.0	647558	40.06	< LOQ

1 ^{14}C-*p*-DCB Equivalents = (Total [^{14}C] Found/Specific Activity/Sample Volume).
2 Results were calculated using Excel. Manual calculations may differ.
3 The limit of quantitation (LOQ) was set at the instrument LOQ of 50 dpm. Results with a total dpm count below 50 are reported as < LOQ.

Table 3

Measured Concentrations of ^{14}C-*p*-DCB in Sediment Samples Analyzed by LSC

Nominal Sediment Concentration (mg/kg)	Sample Number (264A-116-)	Sampling Interval (Day)	Sample Mass (g)	Specific Activity (dpm/µg)	Total [^{14}C] Found (dpm)	Soil Content[3]	^{14}C-*p*-DCB Equivalents Found [1,2,3,4] (mg a.i./kg)	Percent of Nominal[1,3] (%)	Mean Measured ^{14}C-*p*-DCB Equivalent Concentration[3] (± SD and CV%) (mg/kg)	Mean Percent of Nominal[3]
Negative Control (0.0)	1-SED	0	0.200	647558	0.00	0.684	< LOQ	< LOQ	--	--
	9-SED	5	0.200	647558	0.00	0.698	< LOQ	< LOQ		
	17-SED	10	0.200	647558	0.00	0.702	< LOQ	< LOQ		
Solvent Control (0.0)	2-SED	0	0.200	647558	8.72	0.698	< LOQ	< LOQ	--	--
	10-SED	5	0.200	647558	0.00	0.693	< LOQ	< LOQ		
	18-SED	10	0.200	647558	0.00	0.700	< LOQ	< LOQ		
0.32	3-SED	0	0.200	647558	1830.53	0.691	0.0205	6.40	0.018 ± 0.0021	5.7
	11-SED	5	0.200	647558	1575.58	0.699	0.0174	5.44	CV = 12%	
	19-SED	10	0.200	647558	1461.05	0.685	0.0165	5.15		
1.0	4-SED	0	0.200	647030	4325.01	0.691	0.0484	4.84	0.045 ± 0.0030	4.5
	12-SED	5	0.200	647030	3834.03	0.698	0.0425	4.25	CV = 6.7%	
	20-SED	10	0.200	647030	3902.21	0.680	0.0443	4.43		
3.2	5-SED	0	0.200	202197	7841.43	0.692	0.280	8.76	0.25 ± 0.024	7.9
	13-SED	5	0.200	202197	6612.47	0.692	0.236	7.39	CV = 9.7%	
	21-SED	10	0.200	202197	7406.79	0.762	0.240	7.51		
10	6-SED	0	0.200	64703	10363.10	0.690	1.16	11.6	1.0 ± 0.13	10
	14-SED	5	0.200	64703	8296.56	0.696	0.921	9.21	CV = 13%	
	22-SED	10	0.200	64703	8473.58	0.689	0.950	9.50		

[1] Analytical results were generated using wet weights. The tabulated values are reported on a dry weight basis.

[2] ^{14}C-*p*-DCB Equivalents = ({[Total dpm found/sample mass (g)]}/specific activity)/soil content.

[3] Results were generated in Excel. Manual calculations may differ slightly.

[4] The limit of quantitation (LOQ) was set at the instrument LOQ of 50 dpm. Results with a total dpm count below 50 are reported as < LOQ.

Table 3 (Continued)

Measured Concentrations of ^{14}C-*p*-DCB in Sediment Samples Analyzed by LSC

Nominal Sediment Concentration (mg/kg)	Sample Number (264A-116-)	Sampling Interval (Day)	Sample Mass (g)	Specific Activity (dpm/µg)	Total [^{14}C] Found (dpm)	Soil Content[3]	^{14}C-*p*-DCB Equivalents Found [1,2,3] (mg/kg)	Percent of Nominal[1,3] (%)	Mean Measured ^{14}C-*p*-DCB Equivalent Concentration[3] (± SD and CV%) (mg/kg)	Mean Percent of Nominal[3]
32	7-SED	0	0.200	20220	13781.06	0.693	4.92	15.4	3.8 ± 0.94	12
	15-SED	5	0.200	20220	8992.10	0.687	3.24	10.1	CV = 25%	
	23-SED	10	0.200	20220	9343.53	0.693	3.34	10.4		
100	8-SED	0	0.200	6470	16479.55	0.695	18.3	18.3	16 ± 2.7	16
	16-SED	5	0.200	6470	14172.61	0.692	15.8	15.8	CV = 17%	
	24-SED	10	0.200	6470	11157.70	0.667	12.9	12.9		

[1] Analytical results were generated using wet weights. The tabulated values are reported on a dry weight basis.
[2] ^{14}C-*p*-DCB Equivalents = ({[Total dpm found/sample mass (g)]}/specific activity)/soil content
[3] Results were generated in Excel. Manual calculations may differ slightly.

Table 4

Measured Concentrations of ^{14}C-*p*-DCB in Pore Water Sample

Nominal Sediment Treatment Group (mg/kg)	Sample Number (264A-116-)	Sampling Interval (Day)	Sample Volume (mL)	Specific Activity (dpm/µg)	Total [^{14}C] Found (dpm)	^{14}C-*p*-DCB Equivalents Found[1,2,3] (mg/L)
Negative Control (0.0)	1-PW	0	5.00	647558	5.30	< LOQ
	9-PW	5	5.00	647558	7.57	< LOQ
	17-PW	10	5.00	647558	17.55	< LOQ
Solvent Control (0.0)	2-PW	0	5.00	647558	0.30	< LOQ
	10-PW	5	5.00	647558	9.79	< LOQ
	18-PW	10	5.00	647558	6.53	< LOQ
0.32	3-PW	0	5.00	647558	4044.02	0.00125
	11-PW	5	5.00	647558	2563.33	0.000792
	19-PW	10	5.00	647558	2617.03	0.000808
1.0	4-PW	0	5.00	647030	12719.45	0.00393
	12-PW	5	5.00	647030	8483.27	0.00262
	20-PW	10	5.00	647030	7153.73	0.00221
3.2	5-PW	0	5.00	202197	16599.25	0.0164
	13-PW	5	5.00	202197	10041.60	0.00993
	21-PW	10	5.00	202197	8068.41	0.00798
10	6-PW	0	5.00	64703	16900.93	0.0522
	14-PW	5	5.00	64703	9998.21	0.0309
	22-PW	10	5.00	64703	10419.34	0.0322
32	7-PW	0	5.00	20220	20968.38	0.207
	15-PW	5	5.00	20220	12339.32	0.122
	23-PW	10	5.00	20220	12716.52	0.126
100	8-PW	0	5.00	6470	22897.76	0.708
	16-PW	5	5.00	6470	16629.33	0.514
	24-PW	10	5.00	6470	18516.73	0.572

1 ^{14}C-*p*-DCB Equivalents = [{(Total dpm found)/sample volume (mL)}/specific activity]

2 Results were generated using Excel. Manual calculations may differ slightly.

3 The limit of quantitation (LOQ) was set at the instrument LOQ of 50 dpm. Results with a total dpm count below 50 are reported as < LOQ.

Table 5

Measured Concentrations of ^{14}C-*p*-DCB in Overlying Water Samples

Nominal Sediment Treatment Group (mg/kg)	Sample Number (264A-116-)	Sampling Interval (Day)	Sample Volume (mL)	Specific Activity (dpm/µg)	Total [^{14}C] Found (dpm)	^{14}C-*p*-DCB Equivalents Found[1,2,3] (mg/L)
Negative Control (0.0)	1-OW	0	10.0	647558	0.00	< LOQ
	9-OW	5	10.0	647558	27.79	< LOQ
	17-OW	10	10.0	647558	10.89	< LOQ
Solvent Control (0.0)	2-OW	0	10.0	647558	1.88	< LOQ
	10-OW	5	10.0	647558	11.91	< LOQ
	18-OW	10	10.0	647558	4.07	< LOQ
0.32	3-OW	0	10.0	647558	156.80	0.0000242
	11-OW	5	10.0	647558	60.75	0.00000938
	19-OW	10	10.0	647558	208.39	0.0000322
1.0	4-OW	0	10.0	647030	430.75	0.0000666
	12-OW	5	10.0	647030	81.53	0.0000126
	20-OW	10	10.0	647030	686.09	0.000106
3.2	5-OW	0	10.0	202197	842.95	0.000417
	13-OW	5	10.0	202197	150.04	0.0000742
	21-OW	10	10.0	202197	838.77	0.000415
10	6-OW	0	10.0	64703	1136.76	0.00176
	14-OW	5	10.0	64703	168.71	0.000261
	22-OW	10	10.0	64703	1309.69	0.00202
32	7-OW	0	10.0	20220	1516.34	0.00750
	15-OW	5	10.0	20220	375.82	0.00186
	23-OW	10	10.0	20220	1654.80	0.00818
100	8-OW	0	10.0	6470	2387.50	0.0369
	16-OW	5	10.0	6470	344.67	0.00533
	24-OW	10	10.0	6470	3249.01	0.0502

1 ^{14}C-*p*-DCB Equivalents = [{(Total dpm found)/sample volume (mL)}/specific activity].

2 Results were generated using Excel in full precision mode. Manual calculations may differ slightly.

3 The limit of quantitation (LOQ) was set at the instrument LOQ of 50 dpm. Results with a total dpm count below 50 are reported as < LOQ.

Table 6

Means and Ranges of Overlying Water Quality Measurements Taken During the 10-Day Exposure to Sediment-Incorporated *p*-Dichlorobenzene

Mean Measured Sediment Concentration (mg/kg)	Mean ± SD and Range of Measured Parameters						
	Temperature[1] (°C)	DO[2] (mg/L)	pH	Hardness (mg/L as $CaCO_3$)[3,5]	Alkalinity (mg/L as $CaCO_3$)[3,5]	Conductivity[3,5] (µS/cm)	Ammonia (mg/L as NH_3)[4,5]
Negative Control	22.7 ± 0.16 (22.3 – 22.8)	7.8 ± 0.33 (7.3 – 8.4)	8.1 ± 0.09 (7.9 – 8.2)	166 ± 14.14 (156 – 176)	190 ± 8.49 (184 – 196)	374 ± 56.57 (334 – 414)	<LOQ (<LOQ – <LOQ)
Solvent Control	22.7 ± 0.16 (22.4 – 22.9)	7.8 ± 0.38 (7.0 – 8.4)	8.1 ± 0.09 (7.9 – 8.2)	158 ± 8.49 (152 – 164)	194 ± 8.49 (188 – 200)	375 ± 50.91 (339 – 411)	0.48 (<LOQ – 0.88)
0.018	22.9 ± 0.19 (22.4 – 23.0)	7.8 ± 0.35 (7.1 – 8.3)	8.2 ± 0.08 (8.0 – 8.3)	-- --	-- --	-- --	<LOQ (<LOQ – 0.171)
0.045	22.7 ± 0.15 (22.4 – 22.9)	7.7 ± 0.43 (7.0 – 8.4)	8.1 ± 0.10 (7.9 – 8.2)	-- --	-- --	-- --	2.45 (<LOQ – 4.82)
0.25	22.8 ± 0.16 (22.5 – 23.0)	7.9 ± 0.31 (7.2 – 8.4)	8.2 ± 0.11 (7.9 – 8.3)	-- --	-- --	-- --	0.36 (<LOQ – 0.63)
1.0	22.9 ± 0.14 (22.6 – 23.0)	7.8 ± 0.43 (6.7 – 8.4)	8.2 ± 0.11 (7.9 – 8.3)	-- --	-- --	-- --	0.23 (<LOQ – 0.37)
3.8	22.9 ± 0.18 (22.6 – 23.2)	7.8 ± 0.4 (6.8 – 8.1)	8.2 ± 0.08 (8.0 – 8.3)	-- --	-- --	-- --	3.14 (<LOQ – 6.19)
16	22.9 ± 0.1 (22.7 – 23.0)	7.8 ± 0.31 (7.2 – 8.4)	8.2 ± 0.08 (8.0 – 8.3)	164 ± 11.31 (156 – 172)	189 ± 7.07 (184 – 194)	376.5 ± 57.28 (336 – 417)	2.84 (1.03 – 4.65)

[1] Manual temperature measurements. Temperature monitored continuously during the test ranged from 22.42 to 23.59°C, measured to the nearest 0.01°C.
[2] A dissolved oxygen concentration of 5.1 mg/L represents 60% saturation at 23ºC in freshwater.
[3] -- = no measurements scheduled.
[4] The LOQ for ammonia analyses was set at 0.17 mg/L, the concentration of the lowest calibration standard. When a measured concentration of ammonia was <LOQ, a value of ½ LOQ was used to calculate the mean concentration.
[5] No standard deviation is reported when only two measurements are recorded (Day 0 and Day 10).

Table 7

Means and Ranges of Pore Water and Sediment Measurements Taken During the 10-Day Exposure to Sediment-Incorporated *p*-Dichlorobenzene

Mean Measured Sediment Concentration (mg/kg)	Mean ± SD and Range of Measured Parameters		
	Pore Water		Sediment
	pH[1]	Ammonia (mg/L as NH_3) [2]	Eh
Negative Control	7.6 7.3 – 7.8	6.8 ± 4.8 (2.7 – 12.1)	38 ± 222 (-90 – 294)
Solvent Control	7.4 7.3 – 7.5	5.7 ± 4.54 (2.1 – 10.8)	20.3 ± 227 (-137 – 280)
0.018	7.5 7.5 – 7.5	6.3 ± 4.4 (2.6 – 11.2)	-33.3 ± 262 (-225 – 265)
0.045	7.6 7.6 – 7.6	6.5 ± 4.85 (2.7 – 12)	71.7 ± 141 (-76 – 204)
0.25	7.6 7.5 – 7.7	7.7 ± 4.67 (2.4 – 11.4)	83.3 ± 211 (-57 – 326)
1.0	7.6 7.5 – 7.7	5.9 ± 4.62 (1.6 – 10.8)	42.7 ± 153 (-53 – 219)
3.8	7.6 7.5 – 7.7	5.7 ± 4.33 (2.1 – 10.5)	3 ± 251 (-167 – 291)
16	7.7 7.5 – 7.8	5.2 ± 4.5 (1.4 – 10.2)	35.7 ± 226 (-160 – 283)

[1] No standard deviation is reported when only two measurements are recorded (Day 0 and Day 10).

[2] The LOQ for ammonia analyses was set at 0.17 mg/L, the concentration of the lowest calibration standard. When a measured concentration of ammonia was <LOQ, a value of ½ LOQ was used to calculate the mean concentration.

Table 8

Summary of Survival and Growth of the Midge (*Chironomus dilutus*) Exposed to Sediment-Incorporated *p*-Dichlorobenzene for 10 Days

Mean Measured Sediment Concentration (mg/kg)	Mean Number of Surviving Organisms (± SD)[1]	Percent Survival (%)	Percent Reduction in Survival From Pooled Control[2] (%)	Mean Individual Ash-Free Dry Weight (mg) (± SD)	Percent Reduction in Ash-Free Dry Weight From Pooled Control [2] (%)
Negative Control	8.6 (± 1.41)	86	--	2.30 ± 0.34	--
Solvent Control	9.3 (± 0.71)	93	--	2.13 ± 0.08	--
0.018	9.1 (± 1.13)	91	--	2.18 ± 0.22	1.30
0.045	9.0 (± 0.76)	90	--	2.17 ± 0.16	1.83
0.25	9.4 (± 0.74)	94	--	2.20 ± 0.16	0.69
1.0	8.5 (± 0.93)	85	4.90	2.62 ± 0.36	--
3.8	8.4 (± 0.92)	84	6.29	2.40 ± 0.13	--
16	9.3 (± 1.04)	93	--	2.23 ± 0.13	--

[1] Each replicate contained 10 organisms at test initiation. The average individual ash-free dry weight of 80 midge larvae collected from the test batch of organisms at the beginning of the test was 0.22 mg.

[2] Percent reduction was calculated using Excel 2010 in full precision mode; manual calculations may differ slightly.

There were no statistically significant differences ($p > 0.05$) in the mean number of surviving organisms in any treatment group using a Fisher's Exact/Bonferroni-Holm test.

There were no statistically significant differences ($p>0.05$) in the mean individual ash-free dry weight in comparison to the pooled control group using the Dunnett T3 multiple comparison test.

Appendix 1

Study Protocol

PROTOCOL

p-Dichlorobenzene: A 10-DAY TOXICITY TEST WITH THE MIDGE (*Chironomus dilutus*) USING SPIKED WHOLE SEDIMENT

U.S. EPA OCSPP 850.1735

EPA ORDER NO.: TO-2018-0428-XXXXXX-01-A
EPA DOCKET ID No.: EPA-HQ-OPPT-2018-04461

Submitted to

American Chemistry Council
100 2nd Street, N.E.
Washington, DC 20002
USA

Testing Facility

Eurofins EAG Agroscience, LLC
8598 Commerce Drive
Easton, Maryland 21601 USA
1-410-822-8600

May 29, 2023

- 2 -

_p_-Dichlorobenzene: A 10-DAY TOXICITY TEST WITH THE MIDGE (*Chironomus dilutus*) USING SPIKED WHOLE SEDIMENT

SPONSOR:	American Chemistry Council 100 2nd Street, N.E. Washington, DC 20002 USA
SPONSOR'S REPRESENTATIVE:	Colleen Stevens Colleen_Stevens@americanchemistry.com
TESTING FACILITY:	Eurofins EAG Agroscience, LLC 8598 Commerce Drive Easton, Maryland 21601
STUDY DIRECTOR:	Nanditha Billa, M.S., Staff Scientist II Eurofins EAG Agroscience, LLC
LABORATORY MANAGEMENT:	Suzanne Z. Schneider, Ph.D. Associate Director of Aquatic Toxicology

FOR LABORATORY USE ONLY

Proposed Dates:

Experimental Start Date: 5 June 2023 — Experimental Termination Date: 16 June 2023

Easton Study No.: 264A-116 — (eSM Project No.: S21-08512)

Test Concentrations: 0.32, 1.0, 3.2, 10, 32 and 100 mg/kg

Test Substance No.: 17540, 17854 — Reference Substance No.: (if applicable): –

PROTOCOL APPROVAL

Nanditha Billa
STUDY DIRECTOR — DATE: 2 June 2023

Sean P. Gallagher
LABORATORY MANAGEMENT — DATE: 2 June 2023

Cstevens Colleen Stevens
SPONSOR'S REPRESENTATIVE — DATE: 6/2/23

PROTOCOL NO.: 264/052923/CHI-10D/OCSPP/100P-647

INTRODUCTION

Eurofins will conduct a 10-day whole sediment toxicity test with the midge, *Chironomus dilutus* (formerly known as *C. tentans*), for the Sponsor at the Eurofins aquatic toxicology facility in Easton, Maryland. The study will be performed based on procedures in the U.S. Environmental Protection Agency Series 850 - Ecological Effects Test Guidelines, OCSPP 850.1735: *Spiked Whole Sediment 10-Day Toxicity Test, Freshwater Invertebrates* (1) and ASTM Standard E 1706-05: *Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates* (2).

OBJECTIVE

The objective of this study is to determine the effects of a sediment-incorporated test substance on the midge, *Chironomus dilutus*, during a 10-day exposure period in a flow-through system providing intermittent renewal of overlying water. The measured endpoints of the test are survival and growth (ash-free dry weight (AFDW)).

EXPERIMENTAL DESIGN

Groups of midges will be exposed to at least five test concentrations of treated sediment and a negative (untreated sediment) control for 10 days in a flow-through system providing intermittent renewal of overlying water. A solvent control also will be included if a solvent is used to facilitate preparation of the test sediments. Eight replicate test compartments will be maintained in each treatment and control group, with 10 midges in each compartment for a total of 80 individuals per test concentration. Each test compartment will contain a quantity of sediment and overlying water. Additional replicate test compartments for each experimental group will be included, as needed, for analytical sampling and physical/chemical measurements of water and sediment. No midges will be placed in the additional replicate(s) to be sampled at the beginning of the test, but those sampled on later days (e.g., Day 5 and 10) will be initiated with 10 midges per replicate at the same time as the test replicates. These additional replicates will not be used to evaluate the biological response of the test organisms.

Test concentrations in the sediment will be prepared on a mg/kg dry weight basis. Nominal 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 concentrations used in the test will be based on a geometric series with a spacing factor between 1.5 and 3.2 unless information concerning the concentration-effect curve indicates that a different spacing factor would be more appropriate. Overlying water, pore water, and

sediment samples will be collected at specified intervals for analysis of the test substance. Results of the study typically will be based on the measured test concentrations in sediment and/or pore water, depending on the properties of the test substance.

The test will be initiated with third instar larvae (approximately 10 days old). Observations of abnormal behavior will be made throughout the study, and survival and growth (AFDW) will be determined at the end of the 10-day test period. 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). A 10-day LC_{50} value for mortality and a 10-day EC_{50} value for growth will be determined, when possible. Test concentrations will be selected in an attempt to bracket the NOEC and LOEC for survival and growth, the expected LC_{50} value for mortality and the expected 10-day EC_{50} value for growth, based upon preliminary range finding and other data.

To control bias, the assignment of test organisms to test compartments and the positioning of control and treatment groups in the test area (e.g., water bath) will be indiscriminate. No other potential sources of bias are expected to affect the results of the study.

MATERIALS AND METHODS

Test Substance

Information on the characterization of test, control or reference substances is required by Good Laboratory Practice (GLP) Standards and Principles. The Sponsor is responsible for providing the testing facility with verification that the test substance has been characterized according to GLPs prior to its use in the study. A copy of the test substance certificate(s) of analysis provided by the Sponsor will be included in the study report. If verification of the GLP test substance characterization is not provided to the testing facility, it will be noted in the compliance statement of the final report.

The Sponsor is responsible for all information related to the test substance and agrees to accept, or give the testing facility authorization to dispose of, any unused test substance and/or test substance containers remaining at the end of the study.

Test Water

Water used for organism holding and testing will be obtained from a well approximately 40 meters deep 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. Prior to use in the test system, the water will be filtered to 0.45 µm in order to remove fine particles and will be UV-sterilized. The water is characterized as moderately hard. Typical values for hardness, alkalinity, pH, specific conductance and total organic carbon (TOC) are approximately:

Hardness, mg/L as $CaCO_3$	140
Alkalinity, mg/L as $CaCO_3$	180
pH	8.2
Specific Conductance, µS/cm	365
Total Organic Carbon, mg/L	<2

Prior to the test, hardness, alkalinity, pH and specific conductance are measured weekly to monitor the consistency of the well water. TOC is measured monthly. Means and ranges of the measured parameters for the approximate four-week period preceding the test will be provided in the final report. Analyses will be performed at least once annually to determine the concentrations of selected organic and inorganic constituents of the well water and results of the analyses including the analytical methods followed, LODs and LOQs for these methods and other relevant parameters will be summarized in the final report.

Test Sediment

Formulated sediment based on the recommendations of OECD Guideline 218 (3) will be used as the test sediment. The sediment will be composed of approximately 5% sphagnum peat moss, 20% silt and clay (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). 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 at one third bar. Organic carbon content of the final mixture of sediment should be 2.0% (± 0.5%). A summary of the characterization report for the batch of sediment used in the test will be included in the final report. Analyses will be performed at least once annually to determine the concentrations of selected organic and inorganic constituents in a representative batch of formulated sediment and results of the analyses will be summarized in the final report.

Test Organism

The midge, *Chironomus dilutus*, has been selected as the test species for this study. Midges are representative of an important group of aquatic invertebrates, and have been selected for use in the test based

upon past history of use in the laboratory and the recommendations of the study guideline (1). 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 culture. Results of the most recent reference toxicity test for the test organism source will also be included in the final report.

In the laboratory, the organisms will be hatched in water from the same source and at approximately the same temperature as will be used during the test, and will be held until they are the appropriate age. If more than 10% of the organisms in the batch to be used for testing die or appear to be unhealthy, discolored, or otherwise stressed during the approximately 10-day post-hatch period, or if more than 5% die or show signs of stress during the two days prior to test initiation, the batch will not be used for testing. No organisms used in the test will have been used in any previous testing. Midges to be used in the test will be third instar larvae (approximately 10 days old) at test initiation. The average length of midge larvae at test initiation should be 4-6 mm, and average dry weight should be 0.08-0.23 mg per individual. A representative subsample of the lot of organisms used for testing will be measured at test initiation to verify initial mean length and weight.

Feeding

During holding, midge larvae will be fed an invertebrate slurry diet or equivalent. During the test, the larvae in each replicate test compartment 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 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. Analyses will be performed at least once annually to determine the concentrations of selected organic and inorganic constituents of the food and results of the analyses will be summarized in the final report. Specifications for acceptable levels of contaminants in midge diets have not been established. However, there are no known levels of contaminants reasonably expected to be present in the diet that are considered to interfere with the purpose or conduct of the study.

Preparation of Test Concentrations

The test substance will be administered to the test organism in sediment. This route of administration was selected because it represents the most likely route of exposure to sediment-dwelling organisms.

The test sediments are prepared by mixing the test substance into the sediment prior to adding overlying water. A primary stock solution of [^{14}C]-test substance will be prepared in an appropriate volatile solvent. Acceptable solvents will be reagent grade or better, and include, but are not limited to, methanol, ethanol or acetone. To verify the activity of the stock solution, aliquots of the primary stock or aliquots of a dilution of the primary stock 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 would subsequently be mixed into sand and allowed to evaporate before mixing the test substance into the sediment. A solvent control group will be included in the experimental design along with the negative (untreated sediment) control group, and the sediment will be prepared in the same manner as the treatment group sediments. The concentration of organic solvent will be minimized and will be the same in all treatment levels and the solvent control.

To prepare each treatment level, an aliquot of ^{14}C dosing stock solution, and appropriate amount of neat non-radiolabeled test substance will be mixed into a portion of quartz sand. This "sand premix" will be placed under a fume hood to allow evaporation of the solvent. Each sand premix then will be added to a portion of sediment and mixed thoroughly in a rotary mixer, or equivalent. This premix then will be mixed with additional sediment to prepare the final batch of sediment at each concentration. The batches of sediment will be mixed on a motorized rotating mixer, typically overnight, to ensure thorough mixing prior to transfer of the sediment to the test compartments. Homogeneity of the sediment after mixing will be confirmed by visual inspection. The specific methodology used to prepare the test sediments will be documented in the raw data and summarized in the final report.

Preparation of Test Compartments

After mixing, approximately 100 mL (approximately 2.5 to 3 cm) of dosed sediment will be added to tared test compartments (300 mL glass beakers) on a top-loading balance, and the weight of the sediment will be recorded. Approximately 175 mL of overlying water will be slowly added to each test compartment as it is placed in a tank in a flow-through test system so that the water delivery does not disturb the sediment. 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) 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.

Test Apparatus

The test apparatus will consist of a Eurofins flow-through test system that is designed to maintain test compartments in multiple stainless steel tanks (test chambers) in a temperature-controlled water bath, with an intermittent overlying water renewal system. The test compartments in each treatment group will be indiscriminately positioned in one or more tanks in the test system, with only one concentration per tank, and will be labeled with the study number, test concentration and replicate designation.

Test compartments will consist of 300-mL glass beakers with two stainless steel mesh-covered holes on opposite sides of the beaker. Each compartment will contain approximately 100 mL of sediment and approximately 175 mL of overlying water. The water level in the compartments will be maintained between approximately 150 and 175 mL by the water level in the tanks (test chambers) and the position of the holes on the sides of the test compartments. Each test compartment will receive approximately two 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 recalibrated and/or verified approximately weekly or as needed during the test. Flow rates through any two test compartments should not differ by more than 10%. Proper system operation will be visually checked at least once each day during the test.

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.

Environmental Conditions

The test systems will be illuminated using fluorescent tubes that emit wavelengths similar to natural sunlight. The lights will be controlled by an automatic timer to provide a photoperiod of 16 hours of light and 8 hours of darkness. A 30-minute transition period of low light intensity will be provided when lights go on and off to avoid sudden changes in light intensity. Light intensity at the water surface of at least one representative test compartment will be measured at the beginning of the test with a SPER Scientific Ltd. light meter or equivalent, and should fall within the range of approximately 540 to 1080 lux.

The test will be conducted at a target water temperature of 23 ± 1°C. Temperature will be 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. The system measurements will be verified with a digital thermometer, or equivalent, prior to exposure initiation and as needed during the test.

Dissolved oxygen will be measured in the 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 test compartments in each experimental group at each measurement interval. The test water is aerated prior to use so that dissolved oxygen concentrations typically are at or near 90 to 100% air saturation prior to use in the exposure system. Typically, the intermittent replacement of overlying water in the test compartments will serve to maintain the dissolved oxygen concentration. However, 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.

Measurements of pH will be made in the overlying water from one replicate test compartment of each experimental group daily during the test. Measurements typically will rotate among the replicate test compartments in each experimental group at each measurement interval. The pH should not vary more than 1.0 pH unit in the overlying water within a test vessel or between test groups during the test. Measurements

of pH in pore water also will be measured 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. Measurements of pH will be made using a Thermo Orion Dual Star pH/ISE meter, or equivalent.

Ammonia will be measured in samples of overlying water collected from one or more of the additional replicates maintained for physical/chemical measurements in each experimental group at the beginning and end of the test. The beginning and end measurements for ammonia should not vary by >50%, when possible. Ammonia also will be measured in samples of pore water collected from one or more of the additional replicates maintained for physical/chemical measurements in each experimental group at the beginning of the test, mid-test (e.g., Day 5), and at the end of test. Ammonia will be measured using a Thermo Orion Model 720Aplus pH/ISE meter, or equivalent. Water samples are acidified and stored appropriately for subsequent analysis if measurements on the day of collection are not feasible. All measurements should be completed within 30 days of collection.

The sediment redox potential (Eh) will be measured at the beginning of the test, mid-test (e.g., Day 5), and at the end of the test from one or more of the additional replicates maintained for physical/chemical measurements in each experimental group. Measurements of redox potential will be made using a PINPOINT® Redox/ORP Monitor, or equivalent.

Hardness, alkalinity and specific conductance will be measured in composite samples of overlying water from the control group replicates and from the highest concentration treatment group replicates at the beginning and end of the test. Hardness and alkalinity measurements will be made by titration based on methods in *Standard Methods for the Examination of Water and Wastewater* (4). Specific conductance will be measured using a Thermo Orion Star A122 conductivity meter, or equivalent.

Additional measurements of environmental conditions may be taken as deemed necessary by study personnel in consultation with the Study Director. The reason for the additional measurements will be documented in the raw data and summarized in the final report.

Test Procedures and Biological Measurements

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, approximately 10 days old).

The test will be initiated following the equilibration period for the water/sediment systems. To initiate the test, one to two approximately 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 then will be transferred to an indiscriminately assigned test compartment. Organisms will be transferred below the water surface as close to the sediment surface as possible using a glass wide-bore pipette or similar device. An additional subset of 80 organisms from the known-age culture used for the test will be indiscriminately selected on Day 0 for dry weight measurements for use in growth analyses.

Test compartments will be observed daily and visual assessments of any abnormal behavior (e.g., leaving sediment, unusual swimming) will be recorded. 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. All replicates will be counted in the same standardized manner. Any immobile organisms isolated from the overlying water, sediment surface or from sieved material will be considered dead. The surviving larvae will be grouped by replicate for determination of ash-free dry weight (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.

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, precipitates, mold or fungus on the sediment, or material adhering to the sides of the test compartments will be recorded.

Sampling for Analytical Measurements

Samples of stock solutions, when used to spike the sediment, will be collected for analysis as soon as practical after preparation. Samples of overlying water, pore water, and sediment from the additional test compartments prepared for analytical measurements will be collected from each treatment and control group at the beginning of the test, on Day 5 and at the end of the test. One or more additional sampling intervals between Days 0 and 10 may be included if requested by the Sponsor. Overlying water samples typically will be collected at mid-depth in the water column, and the remainder of the overlying water will be removed from

the test vessel. Alternately, the entire volume of overlying water may be sampled. The remaining sediment will be collected, centrifuged, and split into separate samples of pore water and sediment. Samples will be processed immediately for analysis when possible, or will be stored under the appropriate conditions (e.g., refrigeration or ambient) until analyzed. The sampling scheme is summarized below:

PROPOSED NUMBERS OF VERIFICATION SAMPLES

Experimental Group	Stock Solutions[a]	Day 0[b,d]			Day 5[b,c,d]			Day 10[b,d]		
		Water	Sediment	Pore	Water	Sediment	Pore	Water	Sediment	Pore
Control	-	1	1	1	1	1	1	1	1	1
Solvent Control (if applicable)	-	1	1	1	1	1	1	1	1	1
Level 1-Low Concentration	1	1	1	1	1	1	1	1	1	1
Level 2	1	1	1	1	1	1	1	1	1	1
Level 3	1	1	1	1	1	1	1	1	1	1
Level 4	1	1	1	1	1	1	1	1	1	1
Level 5	1	1	1	1	1	1	1	1	1	1
Totals	5	7	7	7	7	7	7	7	7	7

[a] If applicable, stocks typically will be collected for analysis prior to Day 0 (as soon as practical after preparation). Additional stocks may be analyzed if necessary.
[b] Water = overlying water; Pore = pore water.
[c] Day 5 samples to be collected following renewal of overlying water.
[d] Additional samples may be collected and held as backup samples for possible analysis, if needed.

Proposed Number of Verification Samples = 68

The above numbers of samples represent those collected from the test and do not include quality control (QC) samples such as matrix blanks and fortifications that may be prepared and analyzed during the analytical chemistry phase of the study. If deemed necessary by the Study Director, additional samples from one or more appropriate test vessels and/or stock solutions may be collected for possible analysis (e.g., to allow for confirmation of results). The reason for collecting the additional samples will be documented in the raw data, and any additional analyses will be summarized in the final report.

Analytical Chemistry

Chemical analysis of sediment, overlying water and pore water samples will be performed by Eurofins. Analyses of water samples will be performed using liquid scintillation counting (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 degradates may be conducted using a technique such as HPLC-Beta-RAM or fraction collection followed by LSC of the fractions. The

methodology used to analyze the test samples will be documented in the raw data and summarized in the final report.

Data Analysis

Statistical analyses will be performed to evaluate differences between treatment and control groups for survival and growth (AFDW). The unit of statistical analysis is the test compartment. The percent inhibition (%I) as compared to the control at each test substance treatment level will be calculated for survival and growth.

If a solvent control group is used in addition to a negative control group, the data from these two groups will be compared using an appropriate test. If no statistical differences are found, then the data of the two control groups may be pooled. If statistical differences are found, then the negative control and/or solvent control data, as appropriate, will be used to evaluate treatment-related effects.

Statistical analyses used to evaluate the data will be performed as described in the test guideline (1) and in the OECD 2006 guidance (5) to determine NOEC/LOEC values for each parameter. Growth data will be examined to determine whether concentration-response is fundamentally monotonic (trending in one direction, e.g., response not trending up and then down as concentration increases) or non-monotonic. Monotonicity of the dose response for the endpoints will be determined by a visual interpretation of the data.

Statistical tests will be performed at $\alpha = 0.05$, with the exception of tests for normality and homogeneity which will be performed at $\alpha = 0.01$. Discrete-variable data (survival) will be analyzed using Fisher's Exact test. All statistical tests will be performed using a personal computer with commercially available computer software programs such as SAS (6), CETIS (7) or other statistical software or application. Analyses of data, including additional analyses not otherwise addressed, may be conducted as deemed appropriate by the Study Director. The results of the appropriate supporting analyses will be documented in the raw data and summarized in the final report.

If LC/ECx values can be estimated (e.g., LC50 for survival; EC_50 for reproduction and development), they will be determined from a regression model, and calculations will be based on the following conditions: the test concentrations must bracket the LC/ECx so that the LC/ECx comes from interpolation rather than extrapolation; and the LC/ECx will be estimated so that (i) the 95% confidence interval reported for LC/ECx does not contain zero and is not overly wide, (ii) the 95% confidence interval for the predicted mean at

LC/ECx does not contain the control mean, and (iii) there is no significant lack-of-fit of regression model to the data. When the above conditions for determining the LC/ECx are not satisfied, only the NOEC approach will be used.

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 follows a concentration-dependent response. Additional analysis of the data may be conducted if deemed appropriate by the Study Director. The results of the analysis will be documented in the raw data and summarized in the final report.

Conditions for the Validity of the Test

The following criteria will be used to judge the validity of the test:

1) 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;
2) the average larval weight on Day 10 will be ≥0.48 mg per surviving organism as ash-free dry weight (AFDW) in the negative control group and, where relevant, in the solvent control group;
3) all test vessels will be identical and will contain the same amount of sediment and overlying water;
4) test organisms will be indiscriminately assigned to test vessels; and
5) a negative sediment control and, where relevant, a solvent sediment control, will be included in the test.

RECORDS TO BE MAINTAINED

Records to be maintained for data generated by the testing facility will include, but not be limited to:

1. The signed protocol.
2. Identification and characterization of the test substance, if provided by the Sponsor.
3. Dates of initiation and termination of the test.
4. Test organism history and holding records.
5. Results of exploratory equilibration or range-finding tests, if applicable.
6. Stock solution calculation and preparation, if applicable.
7. Calculation and preparation of test concentrations.
8. Biological observations and measurements (e.g., ash-free dry weight).

9. Test conditions and physical/chemical measurements.
10. The methods used to analyze test substance concentrations, sample collection information and the results of analytical measurements, if applicable.
11. Statistical calculations, if applicable.
12. The final report.

FINAL REPORT

A final report of the results of the study will be prepared by the testing facility. The report will include, but not be limited to the following, when applicable:

1. Name and address of the facility performing the study.
2. Dates upon which the study was initiated and completed, and the definitive experimental start and termination dates.
3. A statement of compliance signed by the Study Director addressing any exceptions to Good Laboratory Practice Standards.
4. Objectives and procedures as stated in the approved protocol, including any changes to the original protocol.
5. The test, control and reference substances identified by name, chemical abstracts number or code number, strength, purity, and composition or other appropriate characteristics such as physicochemical properties (e.g. solubility, vapor pressure, UV absorption, pKa and Kow), if provided by the Sponsor.
6. Stability and solubility of the test, control and reference substances under the conditions of administration, including under storage condition prior to use, if provided by the Sponsor.
7. Procedures and results of a preliminary range-finding test, if conducted.
8. A description of the methods used to conduct the test including a description of the test system (e.g. intermittent flow-through) and turnover rate of overlying water, a description of the test containers including depth and volume of overlying water, a description of the source, type and amounts of ingredients and methods used to prepare artificial sediment.
9. A description of the test organisms, including the source, scientific name, common name, age or life stage, and method for verifying the species.
10. A description of culture practices including holding and acclimation conditions, environmental conditions, acclimation period, water used, substrate, feeding history, feed types and health status of cultures used to obtain larvae or instars.

11. A description of the preparation of the test sediments. If a solvent is used in the preparation of the test sediment, the name and source of the solvent along with nominal test substance concentrations in stock solutions, along with the concentration of the solvent in the test sediments.
12. The percent total organic carbon from the test sediment.
13. A description of the water source used in the test including water pretreatment, source/type, temperature, pH, hardness and alkalinity, total organic carbon, conductivity , and results of metals and pesticides screens, along with frequency of water quality measurements, and use of aeration, if any, and method.
14. A description of preliminary equilibration trials, if conducted, including data to demonstrate if equilibration of the test substance in the system had occurred by initiation of organism exposure.
15. The methods used to allocate organisms to test chambers and begin the test, the number of organisms and chambers per treatment, the duration of the test, and the environmental conditions during the test including photoperiod and light source.
16. Methods and frequency for measuring the test substance (and major degradates where appropriate) in sediment, pore water and overlying water to verify exposure concentrations.
17. A description of circumstances that may have affected the quality or integrity of the data.
18. The name of the Study Director and the names of other scientists, professionals, and supervisory personnel involved in the study.
19. A description of the transformations, calculations, and operations performed on the data, a summary and analysis of the biological data and analytical chemistry data, and a statement of the conclusions drawn from the analyses.
20. Statistical methods used to evaluate the data.
21. The signed and dated reports of each of the individual scientists or other professionals involved in the study.
22. The location where specimens, raw data and the final report are to be stored.
23. A statement prepared by the Quality Assurance Unit listing the dates that study inspections and audits were made, and the dates of any findings reported to the Study Director and Management.

CHANGES TO FINAL REPORT

If it is necessary to make corrections or additions to a final report after it has been accepted, such changes will be made in the form of an amendment by the Study Director. The amendment shall clearly identify the part of the final report that is being added to or corrected and the reasons for the addition or correction. Amendments shall be signed and dated by the Study Director.

CHANGES TO PROTOCOL

Planned changes to the protocol will be in the form of written amendments signed by the Study Director and approved by the Sponsor's Representative. Amendments will be considered as part of the protocol and will be attached to the final protocol. Any other changes will be in the form of written deviations signed by the Study Director and filed with the raw data. All changes to the protocol will be indicated in the final report.

GOOD LABORATORY PRACTICES

This study will be conducted and reported in compliance with Good Laboratory Practice Standards as published by the U.S. Environmental Protection Agency (40 CFR Part 792) (1989), which are compatible with the Organisation for Economic Cooperation and Development (OECD) Principles of Good Laboratory Practice (ENV/MC/CHEM (98) 17). A statement of compliance, signed by the Study Director, will be included in the final report. The Sponsor will be responsible for compliance with Good Laboratory Practices for procedures performed by other laboratories (e.g., residue analyses or pathology). Each study conducted by the testing facility is routinely examined by the testing facility Quality Assurance Unit for compliance with Good Laboratory Practices, Standard Operating Procedures and the specified protocol. Raw data for all work performed at the testing facility and the final report will be filed by study number in archives located on the Easton site or at an alternative location to be specified in the final report.

REFERENCES

1 **U.S. Environmental Protection Agency.** 2016. Series 850 – Ecological Effects Test Guidelines, OCSPP Number 850.1735: *Spiked Whole Sediment 10-Day Toxicity Test, Freshwater Invertebrates.*

2 **American Society for Testing and Materials.** 2010. ASTM Standard E 1706-05: *Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates.*

3 **Organization for Economic Cooperation and Development.** 2004. OECD Guidelines for the Testing of Chemicals, Guideline 218: *Sediment-Water Chironomid Toxicity Test Using Spiked Sediment.* Adopted 13 April 2004.

4 **APHA, AWWA, WEF.** 2012. *Standard Methods for the Examination of Water and Wastewater.* 22nd Edition, American Public Health Association. American Water Works Association. Water Environment Federation. Washington, D.C.

5 **Organization for Economic Cooperation and Development.** 2006. *Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application.* OECD ENV/JM/MONO(2006)18.

6 **The SAS System for Windows.** 2002-2012. Version 9.4. SAS Institute Inc., Cary, North Carolina.

7 **Tidepool Scientific Software.** 2000 – 2022. Comprehensive Environmental Toxicity Information System (CETIS). McKinleyville, CA.

AMENDMENT TO STUDY PROTOCOL

STUDY NUMBER: 264A-116

AMENDMENT NUMBER: 1

EFFECTIVE DATE: Date of Study Director Signature

AMENDMENT: Page 2

CHANGE:	Study Director:	Nanditha Billa, M.S. Staff Scientist II
TO:	Study Director:	Suzanne Z. Schneider, Ph.D. Associate Director of Aquatic Toxicology
CHANGE:	Management:	Suzanne Z. Schneider, Ph.D. Associate Director of Aquatic Toxicology
TO:	Management:	Sean P. Gallagher, B.S. Director of Aquatic Toxicology

REASON: Study Director and management responsibilities were re-assigned by management

Suzanne Z Schneider
STUDY DIRECTOR

28 July 2023
DATE

Sean P. Gallagher
LABORATORY MANAGEMENT

31 July 2023
DATE

Steven
SPONSOR'S REPRESENTATIVE

October 4, 2023
DATE

QAU Review
KHN / 9/13/23
Initials/Date

STUDY NO.:264A-116
Page 1 of 1

DEVIATION TO STUDY PROTOCOL

STUDY NUMBER: 264A-116

DEVIATION NUMBER: 1

DATE OF DE FACTO DEVIATION: June 2, 2023

DEVIATION: The protocol was inadvertently not update to reflect that the pH of the sediment should be 6.0 ± 0.5.

REASON: Study director oversight.

IMPACT: This deviation had no adverse impact on the results of the study.

DATE OF DE FACTO DEVIATION: June 6, 2023

DEVIATION: The lengths of a representative subsample of organisms were not measured at test initiation to verify initial mean length as stated by the protocol.

REASON: Scientist oversight.

IMPACT: This deviation had no adverse impact on the results of the study.

DATE OF DE FACTO DEVIATION: June 6 and 7, 2023

DEVIATION: Pore water samples collected from water chemistry replicate L were inadvertently stored with HCl before pH measurements were taken altering the pH of the sample. pH measurement was taken from the remaining aliquot of the pore water sample from replicate I on Day 1 instead of Day 0.

REASON: Scientist oversight.

IMPACT: This deviation had no adverse impact on the results of the study.

Suzanne Z Schneider
STUDY DIRECTOR

16 NOV 2023
DATE

LABORATORY MANAGEMENT

November 16, 2023
DATE

QAU Review
SL 11/10/23
Initials/Date

Appendix 2

Certificates of Analysis

SIGMA-ALDRICH®

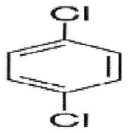
sigma-aldrich.com

3050 Spruce Street, Saint Louis, MO 63103, USA
Website: www.sigmaaldrich.com
Email USA: techserv@sial.com
Outside USA: eurtechserv@sial.com

Certificate of Analysis

Product Name:
1,4-Dichlorobenzene - ≥99%

Product Number:	**D56829**
Batch Number:	**MKBS4401V**
Brand:	ALDRICH
CAS Number:	106-46-7
MDL Number:	MFCD00000604
Formula:	C6H4Cl2
Formula Weight:	147.00 g/mol
Quality Release Date:	06 AUG 2014



Test	Specification	Result
Appearance (Color) Colorless to White	Conforms to Requirements	White
Appearance (Form)	Crystalline Solid	Crystalline Solid
Infrared Spectrum	Conforms to Structure	Conforms
Titration by AgNO3 After Oxygen Combustion	99.0 - 101.0 %	100.8 %
Purity (GC)	≥ 99.0 %	99.9 %

ali ataei

Ali Ataei, Manager
Quality Control
Milwaukee, WI US

eurofins | Selcia

CONFIDENTIAL

CERTIFICATE OF ANALYSIS

Analysis Reference	Document Revision	Page
SEL/12435/3	1	1 of 1

* denotes [phenyl-U-^{14}C] label

Common name	[phenyl-U-^{14}C]*p*-Dichlorobenzene
Chemical name	1,4-Dichloro[phenyl-U-^{14}C]benzene
Batch ID	12435JLC006-1
Molecular formula	$C_6H_4Cl_2$
Storage conditions	Glass bottle, below -15 °C
Date of Manufacture	16 February 2022
Date of Analysis	17 February 2022

Analyses were performed in accordance with internal Quality Program procedures by the Analytical Support Group and the Radiochemistry Department of Eurofins Selcia Ltd. Comparison was with a reference sample (batch BCBW5228) where appropriate.

Test	Method	Result
Appearance & physical form	Visual inspection	Colourless solution in ethanol
Structure, identity & residual solvents	^{1}H NMR	Compatible with proposed structure & comparable with supplied reference Total detected residual solvents 1.8 % w/w
Structure & identity	GC-MS	Compatible with proposed structure & comparable with supplied reference
Radiochemical purity	HPLC with radio detection	99.8 area%
Chemical purity & identity	HPLC with UV detection (228 nm)	99.9 area% Retention time comparable with supplied reference sample
Specific activity & labelled molecular weight	Gravimetric analysis by LSC	43.21 mCi/mmol 1599 MBq/mmol 291.2 µCi/mg 10.77 MBq/mg 148.39 g/mol @ 43.21 mCi/mmol
Specific concentration	Volumetric analysis by LSC	0.875 mCi/ml 32.38 MBq/ml

Issuer: Date: 24 February 2022

S. Yau, B.Sc.
Group Leader, Analytical Support

Reviewer: Date: 24 FEBRUARY 2022

P Morgan, Ph.D., MRQA
Quality Assurance

Caution: Radioactive material for research use only. Not suitable for human use.
Expiry date not determined. In the absence of stability data a purity check is recommended before use

Appendix 3

Test Water Chemistry Parameters Measured During the Approximate 4-Week Period Immediately Preceding the Test

Parameter	Mean[1]	Range[1]
Specific Conductance (µS/cm)	369 (N = 4)	360 – 384
Hardness (mg/L as $CaCO_3$)	147 (N = 4)	136 – 156
Alkalinity (mg/L as $CaCO_3$)	175 (N = 4)	166 - 178
pH	8.1 (N = 4)	8.1 – 8.2
Total Organic Carbon (mg C/L)	<2 (N = 1)	--

[1] Parameters are measured weekly with the exception of TOC, which is measured monthly.

Appendix 4

Analyses of Pesticides, Organics and Metals in Eurofins-Easton Well Water

Client Sample Results

Client: Eurofins EAG Agroscience, LLC
Project/Site: Water Samples

Job ID: 410-130339-1

Client Sample ID: Well Water
Date Collected: 06/12/23 10:00
Date Received: 06/13/23 10:05

Lab Sample ID: 410-130339-1
Matrix: Water

Method: SW846 8081B - Organochlorine Pesticides (GC)

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
Aldrin (1C)	<0.021		0.021		ug/L		06/19/23 15:55	06/21/23 11:54	1
alpha-BHC (1C)	<0.021		0.021		ug/L		06/19/23 15:55	06/21/23 11:54	1
alpha-Chlordane (1C)	<0.021		0.021		ug/L		06/19/23 15:55	06/21/23 11:54	1
beta-BHC (1C)	<0.031		0.031		ug/L		06/19/23 15:55	06/21/23 11:54	1
delta-BHC (1C)	<0.021		0.021		ug/L		06/19/23 15:55	06/21/23 11:54	1
Dieldrin (1C)	<0.031		0.031		ug/L		06/19/23 15:55	06/21/23 11:54	1
Endosulfan I (1C)	<0.021		0.021		ug/L		06/19/23 15:55	06/21/23 11:54	1
Endosulfan II (1C)	<0.042		0.042		ug/L		06/19/23 15:55	06/21/23 11:54	1
Endosulfan sulfate (1C)	<0.031		0.031		ug/L		06/19/23 15:55	06/21/23 11:54	1
Endrin (1C)	<0.031		0.031		ug/L		06/19/23 15:55	06/21/23 11:54	1
Endrin aldehyde (1C)	<0.10		0.10		ug/L		06/19/23 15:55	06/21/23 11:54	1
Endrin ketone (1C)	<0.031		0.031		ug/L		06/19/23 15:55	06/21/23 11:54	1
gamma-BHC (Lindane) (1C)	<0.021		0.021		ug/L		06/19/23 15:55	06/21/23 11:54	1
gamma-Chlordane (1C)	<0.042		0.042		ug/L		06/19/23 15:55	06/21/23 11:54	1
Hexachlorobenzene (1C)	<0.010		0.010		ug/L		06/19/23 15:55	06/21/23 11:54	1
Heptachlor (1C)	<0.021		0.021		ug/L		06/19/23 15:55	06/21/23 11:54	1
Heptachlor epoxide (1C)	<0.021		0.021		ug/L		06/19/23 15:55	06/21/23 11:54	1
Kepone (1C)	<0.21		0.21		ug/L		06/19/23 15:55	06/21/23 11:54	1
Methoxychlor (1C)	<0.12		0.12		ug/L		06/19/23 15:55	06/21/23 11:54	1
Mirex (1C)	<0.052		0.052		ug/L		06/19/23 15:55	06/21/23 11:54	1
Telodrin (1C)	<0.010		0.010		ug/L		06/19/23 15:55	06/21/23 11:54	1
Toxaphene (1C)	<1.0	cn	1.0		ug/L		06/19/23 15:55	06/21/23 11:54	1
o,p'-DDD (1C)	<0.094		0.094		ug/L		06/19/23 15:55	06/21/23 11:54	1
o,p'-DDE (1C)	<0.021		0.021		ug/L		06/19/23 15:55	06/21/23 11:54	1
o,p'-DDT (1C)	<0.021		0.021		ug/L		06/19/23 15:55	06/21/23 11:54	1
p,p'-DDD (1C)	<0.031		0.031		ug/L		06/19/23 15:55	06/21/23 11:54	1
p,p'-DDE (1C)	<0.031		0.031		ug/L		06/19/23 15:55	06/21/23 11:54	1
p,p'-DDT (1C)	<0.031		0.031		ug/L		06/19/23 15:55	06/21/23 11:54	1
Chlordane (n.o.s.) (1C)	<0.52		0.52		ug/L		06/19/23 15:55	06/21/23 11:54	1

Surrogate	*%Recovery*	*Qualifier*	*Limits*	*Prepared*	*Analyzed*	*Dil Fac*
DCB Decachlorobiphenyl (Surr) (1C)	*87*		*20 - 149*	*06/19/23 15:55*	*06/21/23 11:54*	*1*
DCB Decachlorobiphenyl (Surr) (2C)	*93*		*20 - 149*	*06/19/23 15:55*	*06/21/23 11:54*	*1*
Tetrachloro-m-xylene (Surr) (1C)	*76*		*20 - 129*	*06/19/23 15:55*	*06/21/23 11:54*	*1*
Tetrachloro-m-xylene (Surr) (2C)	*78*		*20 - 129*	*06/19/23 15:55*	*06/21/23 11:54*	*1*

Method: SW846 8141A - Organophosphorous Pesticides (GC)
Lab: Eurofins Calscience Tustin

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
Azinphos-methyl (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Bolstar (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Chlorpyrifos (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Coumaphos (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Diazinon (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Dichlorvos (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Dimethoate (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Disulfoton (1C)	<9.6	cn	9.6		ug/L		06/16/23 09:05	06/20/23 08:09	1
EPN (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Famphur (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Fensulfothion (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1

Appendix 4 (Continued)

Analyses of Pesticides, Organics and Metals in Eurofins-Easton Well Water

Client Sample Results

Client: Eurofins EAG Agroscience, LLC
Project/Site: Water Samples

Job ID: 410-130339-1

Client Sample ID: Well Water
Date Collected: 06/12/23 10:00
Date Received: 06/13/23 10:05

Lab Sample ID: 410-130339-1
Matrix: Water

Method: SW846 8141A - Organophosphorous Pesticides (GC) (Continued)
Lab: Eurofins Calscience Tustin

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
Fenthion (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Malathion (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Merphos (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Methyl parathion (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Mevinphos (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Naled (1C)	<39	cn	39		ug/L		06/16/23 09:05	06/20/23 08:09	1
Ethyl Parathion (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Phorate (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Ronnel (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Simazine (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Thionazin (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Tokuthion (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Trichloronate (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Demeton-O (2C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Demeton-S (2C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1
Ethoprop (1C)	<4.8	cn	4.8		ug/L		06/16/23 09:05	06/20/23 08:09	1

Method: EPA 300.0 R2.1 - Anions, Ion Chromatography

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
Fluoride	<1.0		1.0		mg/L			06/13/23 23:58	5
Nitrogen, Nitrate	<0.55		0.55		mg/L			06/13/23 23:58	5
Bromide	<3.8		3.8		mg/L			06/13/23 23:58	5
Nitrogen, Nitrite	<0.55		0.55		mg/L			06/13/23 23:58	5
Sulfate	**10**		7.5		mg/L			06/13/23 23:58	5
Chloride	<7.5		7.5		mg/L			06/13/23 23:58	5

Method: EPA 200.7 Rev 4.4 - Metals (ICP) - Total Recoverable

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
Aluminum	<0.30		0.30		mg/L		06/14/23 08:48	06/16/23 21:33	1
Antimony	<0.050		0.050		mg/L		06/14/23 08:48	06/16/23 21:33	1
Arsenic	<0.050		0.050		mg/L		06/14/23 08:48	06/16/23 21:33	1
Barium	<0.0050		0.0050		mg/L		06/14/23 08:48	06/16/23 21:33	1
Beryllium	<0.0050		0.0050		mg/L		06/14/23 08:48	06/16/23 21:33	1
Cadmium	<0.0050		0.0050		mg/L		06/14/23 08:48	06/16/23 21:33	1
Calcium	**38**		0.50		mg/L		06/14/23 08:48	06/16/23 21:33	1
Chromium	<0.015		0.015		mg/L		06/14/23 08:48	06/16/23 21:33	1
Cobalt	<0.0050		0.0050		mg/L		06/14/23 08:48	06/16/23 21:33	1
Copper	<0.020		0.020		mg/L		06/14/23 08:48	06/16/23 21:33	1
Iron	<0.20		0.20		mg/L		06/14/23 08:48	06/16/23 21:33	1
Lead	<0.015		0.015		mg/L		06/14/23 08:48	06/16/23 21:33	1
Magnesium	**14**		0.10		mg/L		06/14/23 08:48	06/16/23 21:33	1
Manganese	<0.010		0.010		mg/L		06/14/23 08:48	06/16/23 21:33	1
Nickel	<0.010		0.010		mg/L		06/14/23 08:48	06/16/23 21:33	1
Potassium	**7.1**		0.50		mg/L		06/14/23 08:48	06/16/23 21:33	1
Selenium	<0.050		0.050		mg/L		06/14/23 08:48	06/16/23 21:33	1
Silver	<0.010		0.010		mg/L		06/14/23 08:48	06/16/23 21:33	1
Sodium	**17**		1.0		mg/L		06/14/23 08:48	06/16/23 21:33	1
Thallium	<0.030		0.030		mg/L		06/14/23 08:48	06/16/23 21:33	1
Vanadium	<0.010		0.010		mg/L		06/14/23 08:48	06/16/23 21:33	1

Appendix 4 (Continued)

Analyses of Pesticides, Organics and Metals in Eurofins-Easton Well Water

Client Sample Results

Client: Eurofins EAG Agroscience, LLC
Project/Site: Water Samples

Job ID: 410-130339-1

Client Sample ID: Well Water
Date Collected: 06/12/23 10:00
Date Received: 06/13/23 10:05

Lab Sample ID: 410-130339-1
Matrix: Water

Method: EPA 200.7 Rev 4.4 - Metals (ICP) - Total Recoverable (Continued)

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
Zinc	<0.020		0.020		mg/L		06/14/23 08:48	06/16/23 21:33	1

Method: EPA 245.1 - Mercury (CVAA)

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
Mercury	<200		200		ng/L		06/13/23 23:41	06/14/23 09:10	1

Appendix 4 (Continued)

Analyses of Pesticides, Organics and Metals in Eurofins-Easton Well Water

Definitions/Glossary

Client: Eurofins EAG Agroscience, LLC Job ID: 410-130339-1
Project/Site: Water Samples

Qualifiers

GC Semi VOA

Qualifier	Qualifier Description
cn	Refer to Case Narrative for further detail

HPLC/IC

Qualifier	Qualifier Description
cn	Refer to Case Narrative for further detail

Metals

Qualifier	Qualifier Description
4	MS, MSD: The analyte present in the original sample is greater than 4 times the matrix spike concentration; therefore, control limits are not applicable.

Glossary

Abbreviation	These commonly used abbreviations may or may not be present in this report.
¤	Listed under the "D" column to designate that the result is reported on a dry weight basis
%R	Percent Recovery
CFL	Contains Free Liquid
CFU	Colony Forming Unit
CNF	Contains No Free Liquid
DER	Duplicate Error Ratio (normalized absolute difference)
Dil Fac	Dilution Factor
DL	Detection Limit (DoD/DOE)
DL, RA, RE, IN	Indicates a Dilution, Re-analysis, Re-extraction, or additional Initial metals/anion analysis of the sample
DLC	Decision Level Concentration (Radiochemistry)
EDL	Estimated Detection Limit (Dioxin)
LOD	Limit of Detection (DoD/DOE)
LOQ	Limit of Quantitation (DoD/DOE)
MCL	EPA recommended "Maximum Contaminant Level"
MDA	Minimum Detectable Activity (Radiochemistry)
MDC	Minimum Detectable Concentration (Radiochemistry)
MDL	Method Detection Limit
ML	Minimum Level (Dioxin)
MPN	Most Probable Number
MQL	Method Quantitation Limit
NC	Not Calculated
ND	Not Detected at the reporting limit (or MDL or EDL if shown)
NEG	Negative / Absent
POS	Positive / Present
PQL	Practical Quantitation Limit
PRES	Presumptive
QC	Quality Control
RER	Relative Error Ratio (Radiochemistry)
RL	Reporting Limit or Requested Limit (Radiochemistry)
RPD	Relative Percent Difference, a measure of the relative difference between two points
TEF	Toxicity Equivalent Factor (Dioxin)
TEQ	Toxicity Equivalent Quotient (Dioxin)
TNTC	Too Numerous To Count

Appendix 5

Formulated Sediment Composition

Constituents[1]	Weight (g)	Percent (%)
Quartz Sand	7,700	70[2]
Kaolin Clay	2,200	20
Air Dried Peat Moss	550	5
Ground Limestone	110	1

[1] The constituents were mixed in a top-down mixer for approximately 10 minutes and the dry sediment was stored under ambient conditions until used. The pH of the sediment was 5.7.

[2] The test substance was spiked into an amount of sand equivalent to 5% of the final weight of the batch sediment. The amount of sand used in preparing this bulk formulated sediment was accordingly adjusted downward by 5%.

Appendix 6

Agvise Laboratories Report of Sediment Characterization

AGVISE LABORATORIES

804 Highway 15 West
P.O. Box 510
Northwood, ND 58267
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AGVISE GLP Soil Characterization Report

Submitting firm = EUROFINS EAG AGROSCIENCE
Protocol or Study No = 2023 AGVISE INTERNAL
Sample ID. = LOT# 053123PH
Sample Depth = MID-DEPTH
Trial ID. = FORMULATED SEDIMENT
Date Received: 7/24/23 Date Reported: 08-01-2023

AGVISE Lab No: 23-982 Acct No: WI2720

Percent Sand	83
Percent Silt	4
Percent Clay	13
USDA Textural Class (hydrometer method)	Sandy Loam
Bulk Density (disturbed) gm/cc	0.98
Cation Exchange Capacity (meq/100 g)	5.7
% Moisture at 1/3 Bar	14.0
% Organic Carbon --Walkley Black	2.7
% Organic Matter--Walkley Black	4.7
pH in 1:1 soil:water ratio	7.7

Base Saturation Data

Cation	Percent	ppm
Calcium	78.8	902
Magnesium	4.4	31
Sodium	0.9	12
Potassium	0.5	11
Hydrogen	15.4	9

These tests were completed in compliance of 40 CFR Part 160.

Laura Simenson
Analytical Investigator

8/3/23
Date

Appendix 7

Analyses of Pesticides, Organics and Metals in Peat Moss

Client Sample Results

Client: Eurofins EAG Agroscience, LLC — Job ID: 410-106628-1
Project/Site: Aquatic Sediment Contaminant Analyses

Client Sample ID: Formulated Sediment with Peat Moss (no PCBs) — **Lab Sample ID: 410-106628-1**
Date Collected: 11/18/22 13:24 — **Matrix: Solid**
Date Received: 11/21/22 09:32 — **Percent Solids: 95.0**

Method: SW846 8081B - Organochlorine Pesticides (GC)

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
Aldrin (1C)	<0.0044		0.0044		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
alpha-BHC (1C)	<0.0044	cn	0.0044		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
alpha-Chlordane (1C)	<0.0044		0.0044		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
beta-BHC (1C)	<0.0053		0.0053		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
delta-BHC (1C)	<0.0053	cn	0.0053		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
Dieldrin (1C)	<0.0089		0.0089		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
Endosulfan I (1C)	<0.0044		0.0044		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
Endosulfan II (1C)	<0.012		0.012		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
Endosulfan sulfate (1C)	<0.0089		0.0089		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
Endrin (1C)	<0.0089		0.0089		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
Endrin aldehyde (1C)	<0.0089	*- cn	0.0089		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
Endrin ketone (1C)	<0.011		0.011		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
gamma-BHC (Lindane) (1C)	<0.0044		0.0044		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
gamma-Chlordane (1C)	<0.0044		0.0044		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
Hexachlorobenzene (1C)	<0.0044		0.0044		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
Heptachlor (1C)	<0.0044		0.0044		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
Heptachlor epoxide (1C)	<0.0044		0.0044		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
Kepone (2C)	<0.037		0.037		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
Methoxychlor (1C)	<0.035		0.035		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
Mirex (1C)	<0.0089		0.0089		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
Telodrin (1C)	<0.0063		0.0063		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
Toxaphene (1C)	<0.17		0.17		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
o,p'-DDD (1C)	<0.0089		0.0089		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
o,p'-DDE (1C)	<0.0089		0.0089		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
o,p'-DDT (1C)	<0.0089		0.0089		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
p,p'-DDD (1C)	<0.0089	cn	0.0089		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
p,p'-DDE (1C)	<0.0089		0.0089		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
p,p'-DDT (1C)	<0.0089		0.0089		mg/Kg	☼	12/02/22 09:09	12/08/22 19:51	5
Chlordane (n.o.s.) (1C)	<89		89		ug/Kg	☼	12/02/22 09:09	12/08/22 19:51	5

Surrogate	*%Recovery*	*Qualifier*	*Limits*	*Prepared*	*Analyzed*	*Dil Fac*
DCB Decachlorobiphenyl (Surr) (1C)	*97*		*54 - 143*	*12/02/22 09:09*	*12/08/22 19:51*	*5*
DCB Decachlorobiphenyl (Surr) (2C)	*106*		*54 - 143*	*12/02/22 09:09*	*12/08/22 19:51*	*5*
Tetrachloro-m-xylene (Surr) (1C)	*77*		*20 - 131*	*12/02/22 09:09*	*12/08/22 19:51*	*5*
Tetrachloro-m-xylene (Surr) (2C)	*80*		*20 - 131*	*12/02/22 09:09*	*12/08/22 19:51*	*5*

Method: SW846 8141A - Organophosphorous Pesticides (GC)
Lab: Eurofins Calscience Tustin

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
Azinphos-methyl (1C)	<0.53	*+ cn	0.53		mg/Kg	☼	11/30/22 06:40	12/14/22 00:10	1
Bolstar (1C)	<0.53	*+ cn	0.53		mg/Kg	☼	11/30/22 06:40	12/14/22 00:10	1
Chlorpyrifos (1C)	<0.53	*+ cn	0.53		mg/Kg	☼	11/30/22 06:40	12/14/22 00:10	1
Coumaphos (1C)	<0.53	*+ cn	0.53		mg/Kg	☼	11/30/22 06:40	12/14/22 00:10	1
Diazinon (1C)	<0.53	cn	0.53		mg/Kg	☼	11/30/22 06:40	12/14/22 00:10	1
Dichlorvos (1C)	<0.53	*+ *1 cn	0.53		mg/Kg	☼	11/30/22 06:40	12/14/22 00:10	1
Disulfoton (1C)	<0.53	*+ cn	0.53		mg/Kg	☼	11/30/22 06:40	12/14/22 00:10	1
EPN (1C)	<0.53	cn	0.53		mg/Kg	☼	11/30/22 06:40	12/14/22 00:10	1
Famphur (1C)	<0.53	cn	0.53		mg/Kg	☼	11/30/22 06:40	12/14/22 00:10	1
Fensulfothion (1C)	<0.53	*+ cn	0.53		mg/Kg	☼	11/30/22 06:40	12/14/22 00:10	1

Appendix 7 (continued)

Analyses of Pesticides, Organics and Metals in Peat Moss

Client Sample Results

Client: Eurofins EAG Agroscience, LLC
Project/Site: Aquatic Sediment Contaminant Analyses

Job ID: 410-106628-1

Client Sample ID: Formulated Sediment with Peat Moss (no PCBs)
Lab Sample ID: 410-106628-1

Date Collected: 11/18/22 13:24
Date Received: 11/21/22 09:32

Matrix: Solid
Percent Solids: 95.0

Method: SW846 8141A - Organophosphorous Pesticides (GC) (Continued)
Lab: Eurofins Calscience Tustin

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
Fenthion (1C)	<0.53	*+ cn	0.53		mg/Kg	☼	11/30/22 08:40	12/14/22 00:10	1
Malathion (1C)	<0.53	cn	0.53		mg/Kg	☼	11/30/22 08:40	12/14/22 00:10	1
Merphos (1C)	<0.53	*+ cn	0.53		mg/Kg	☼	11/30/22 08:40	12/14/22 00:10	1
Methyl parathion (1C)	<0.53	*+ cn	0.53		mg/Kg	☼	11/30/22 08:40	12/14/22 00:10	1
Mevinphos (1C)	<0.53	*+ cn	0.53		mg/Kg	☼	11/30/22 08:40	12/14/22 00:10	1
Naled (1C)	<4.2	*1 cn	4.2		mg/Kg	☼	11/30/22 08:40	12/14/22 00:10	1
Ethyl Parathion (1C)	<0.53	cn	0.53		mg/Kg	☼	11/30/22 08:40	12/14/22 00:10	1
Phorate (1C)	<0.53	*+ cn	0.53		mg/Kg	☼	11/30/22 08:40	12/14/22 00:10	1
Ronnel (1C)	<0.53	*+ cn	0.53		mg/Kg	☼	11/30/22 08:40	12/14/22 00:10	1
Tokuthion (1C)	<0.53	*+ cn	0.53		mg/Kg	☼	11/30/22 08:40	12/14/22 00:10	1
Trichloronate (1C)	<0.53	cn	0.53		mg/Kg	☼	11/30/22 08:40	12/14/22 00:10	1
Demeton-O (1C)	<0.53	cn	0.53		mg/Kg	☼	11/30/22 08:40	12/14/22 00:10	1
Demeton-S (1C)	<0.53	cn	0.53		mg/Kg	☼	11/30/22 08:40	12/14/22 00:10	1
Ethoprop (1C)	<0.53	*+ cn	0.53		mg/Kg	☼	11/30/22 08:40	12/14/22 00:10	1

Method: EPA 300.0 R2.1 - Anions, Ion Chromatography - Soluble

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
Fluoride	**5.0**		1.1		mg/Kg	☼		12/18/22 18:56	1
Nitrogen, Nitrate	<1.6		1.6		mg/Kg	☼		12/08/22 18:35	1
Bromide	<5.3		5.3		mg/Kg	☼		12/08/22 18:35	1
Nitrogen, Nitrite	<1.1		1.1		mg/Kg	☼		12/08/22 18:35	1
Sulfate	**120**		16		mg/Kg	☼		12/08/22 18:35	1
Chloride	**14**		11		mg/Kg	☼		12/18/22 18:56	1

Method: SW846 6010C - Metals (ICP)

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
Aluminum	**7500**		17		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1
Antimony	<4.2		4.2		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1
Arsenic	<2.5		2.5		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1
Barium	**120**		0.42		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1
Beryllium	<0.42		0.42		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1
Cadmium	<0.42		0.42		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1
Calcium	**6900**		42		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1
Chromium	**6.4**		1.3		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1
Cobalt	<0.42		0.42		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1
Copper	<1.7		1.7		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1
Iron	**870**		17		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1
Lead	**12**		1.3		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1
Magnesium	**290**		8.5		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1
Manganese	**6.2**		0.85		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1
Nickel	**1.8**		0.85		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1
Potassium	**100**		42		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1
Selenium	<4.2		4.2		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1
Silver	<0.85		0.85		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1
Sodium	<85		85		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1
Thallium	<2.5		2.5		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1
Vanadium	**3.6**		0.85		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1
Zinc	**3.6**		1.7		mg/Kg	☼	12/04/22 04:14	12/06/22 03:33	1

Appendix 7 (continued)

Analyses of Pesticides, Organics and Metals in Peat Moss

Client Sample Results

Client: Eurofins EAG Agroscience, LLC — Job ID: 410-106628-1
Project/Site: Aquatic Sediment Contaminant Analyses

Client Sample ID: Formulated Sediment with Peat Moss (no PCBs) — **Lab Sample ID: 410-106628-1**
Date Collected: 11/18/22 13:24 — **Matrix: Solid**
Date Received: 11/21/22 09:32 — **Percent Solids: 95.0**

Method: SW846 7471A - Mercury (CVAA)

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
Mercury	<0.063		0.063		mg/Kg	☼	12/02/22 15:13	12/04/22 17:55	1

General Chemistry

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
Percent Moisture (EPA Moisture)	5.0		1.0		%			11/23/22 11:01	1
Percent Solids (EPA Moisture)	95.0		1.0		%			11/23/22 11:01	1

Appendix 7 (continued)

Analyses of Pesticides, Organics and Metals in Peat Moss

Definitions/Glossary

Client: Eurofins EAG Agroscience, LLC Job ID: 410-106628-1
Project/Site: Aquatic Sediment Contaminant Analyses

Qualifiers

GC Semi VOA

Qualifier	Qualifier Description
*-	LCS and/or LCSD is outside acceptance limits, low biased.
*+	LCS and/or LCSD is outside acceptance limits, high biased.
*1	LCS/LCSD RPD exceeds control limits.
cn	Refer to Case Narrative for further detail

Glossary

Abbreviation	These commonly used abbreviations may or may not be present in this report.
¤	Listed under the "D" column to designate that the result is reported on a dry weight basis
%R	Percent Recovery
1C	Result is from the primary column on a dual-column method.
2C	Result is from the confirmation column on a dual-column method.
CFL	Contains Free Liquid
CFU	Colony Forming Unit
CNF	Contains No Free Liquid
DER	Duplicate Error Ratio (normalized absolute difference)
Dil Fac	Dilution Factor
DL	Detection Limit (DoD/DOE)
DL, RA, RE, IN	Indicates a Dilution, Re-analysis, Re-extraction, or additional Initial metals/anion analysis of the sample
DLC	Decision Level Concentration (Radiochemistry)
EDL	Estimated Detection Limit (Dioxin)
LOD	Limit of Detection (DoD/DOE)
LOQ	Limit of Quantitation (DoD/DOE)
MCL	EPA recommended "Maximum Contaminant Level"
MDA	Minimum Detectable Activity (Radiochemistry)
MDC	Minimum Detectable Concentration (Radiochemistry)
MDL	Method Detection Limit
ML	Minimum Level (Dioxin)
MPN	Most Probable Number
MQL	Method Quantitation Limit
NC	Not Calculated
ND	Not Detected at the reporting limit (or MDL or EDL if shown)
NEG	Negative / Absent
POS	Positive / Present
PQL	Practical Quantitation Limit
PRES	Presumptive
QC	Quality Control
RER	Relative Error Ratio (Radiochemistry)
RL	Reporting Limit or Requested Limit (Radiochemistry)
RPD	Relative Percent Difference, a measure of the relative difference between two points
TEF	Toxicity Equivalent Factor (Dioxin)
TEQ	Toxicity Equivalent Quotient (Dioxin)
TNTC	Too Numerous To Count

Appendix 8

Analyses of Pesticides, Organics and Metals in TetraMin® Flake Food

Client Sample Results

Client: Eurofins EAG Agroscience, LLC
Project/Site: Water Samples

Job ID: 410-88402-1

Client Sample ID: Aquamax Starter 300
Date Collected: 06/21/22 08:30
Date Received: 06/22/22 10:59

Lab Sample ID: 410-88402-5
Matrix: Solid
Percent Solids: 93.8

Method: 8081B - Organochlorine Pesticides (GC)

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
Aldrin (1C)	<0.16	cn	0.16		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
alpha-BHC (1C)	<0.16	cn	0.16		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
alpha-Chlordane (1C)	<0.16	cn	0.16		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
beta-BHC (1C)	<0.19	cn	0.19		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
delta-BHC (1C)	<0.19	cn	0.19		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
Dieldrin (1C)	<0.33	cn	0.33		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
Endosulfan I (1C)	<0.16	cn	0.16		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
Endosulfan II (1C)	<0.45	cn	0.45		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
Endosulfan sulfate (1C)	<0.33	cn	0.33		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
Endrin (1C)	<0.33	cn	0.33		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
Endrin aldehyde (1C)	<0.33	cn	0.33		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
Endrin ketone (1C)	<0.39	cn	0.39		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
gamma-BHC (Lindane) (1C)	<0.16	cn	0.16		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
gamma-Chlordane (1C)	<0.16	cn	0.16		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
Hexachlorobenzene (1C)	<0.16	cn	0.16		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
Heptachlor (1C)	<0.16	cn	0.16		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
Heptachlor epoxide (1C)	<0.16	cn	0.16		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
Kepone (2C)	<1.4	cn	1.4		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
Methoxychlor (1C)	<1.3	cn	1.3		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
Mirex (1C)	<0.33	cn	0.33		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
Telodrin (1C)	<0.23	cn	0.23		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
Toxaphene (1C)	<6.4	cn	6.4		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
o,p'-DDD (1C)	<0.33	cn	0.33		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
o,p'-DDE (1C)	<0.33	cn	0.33		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
o,p'-DDT (1C)	<0.33	cn	0.33		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
p,p'-DDD (1C)	<0.33	cn	0.33		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
p,p'-DDE (1C)	<0.33	cn	0.33		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
p,p'-DDT (1C)	<0.33	cn	0.33		mg/Kg	☼	07/02/22 09:51	07/05/22 13:36	20
Chlordane (n.o.s.) (1C)	<3300	cn	3300		ug/Kg	☼	07/02/22 09:51	07/05/22 13:36	20

Surrogate	*%Recovery*	*Qualifier*	*Limits*	*Prepared*	*Analyzed*	*Dil Fac*
DCB Decachlorobiphenyl (Surr) (1C)	*136*	*cn*	*54 - 143*	*07/02/22 09:51*	*07/05/22 13:36*	*20*
DCB Decachlorobiphenyl (Surr) (2C)	*135*	*cn*	*54 - 143*	*07/02/22 09:51*	*07/05/22 13:36*	*20*
Tetrachloro-m-xylene (1C)	*120*	*cn*	*20 - 131*	*07/02/22 09:51*	*07/05/22 13:36*	*20*
Tetrachloro-m-xylene (2C)	*118*	*cn*	*20 - 131*	*07/02/22 09:51*	*07/05/22 13:36*	*20*

Method: 8082A - Polychlorinated Biphenyls (PCBs) by Gas Chromatography

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
PCB-1016 (2C)	<0.17	cn	0.17		mg/Kg	☼	07/02/22 09:51	07/05/22 17:34	1
PCB-1221 (2C)	<0.17	cn	0.17		mg/Kg	☼	07/02/22 09:51	07/05/22 17:34	1
PCB-1232 (2C)	<0.17	cn	0.17		mg/Kg	☼	07/02/22 09:51	07/05/22 17:34	1
PCB-1242 (2C)	<0.17	cn	0.17		mg/Kg	☼	07/02/22 09:51	07/05/22 17:34	1
PCB-1248 (2C)	<0.17	cn	0.17		mg/Kg	☼	07/02/22 09:51	07/05/22 17:34	1
PCB-1254 (2C)	<0.17	cn	0.17		mg/Kg	☼	07/02/22 09:51	07/05/22 17:34	1
PCB-1260 (2C)	<0.17	cn	0.17		mg/Kg	☼	07/02/22 09:51	07/05/22 17:34	1

Surrogate	*%Recovery*	*Qualifier*	*Limits*	*Prepared*	*Analyzed*	*Dil Fac*
DCB Decachlorobiphenyl (Surr) (1C)	*57*	*cn*	*45 - 143*	*07/02/22 09:51*	*07/05/22 17:34*	*1*
DCB Decachlorobiphenyl (Surr) (2C)	*73*	*cn*	*45 - 143*	*07/02/22 09:51*	*07/05/22 17:34*	*1*
Tetrachloro-m-xylene (1C)	*78*	*cn*	*53 - 140*	*07/02/22 09:51*	*07/05/22 17:34*	*1*

Appendix 8 (Continued)

Analyses of Pesticides, Organics and Metals in TetraMin® Flake Food

Client Sample Results

Client: Eurofins EAG Agroscience, LLC — Job ID: 410-88402-1
Project/Site: Water Samples

Client Sample ID: Aquamax Starter 300 — **Lab Sample ID: 410-88402-5**
Date Collected: 06/21/22 08:30 — **Matrix: Solid**
Date Received: 06/22/22 10:59 — **Percent Solids: 93.8**

Method: 8082A - Polychlorinated Biphenyls (PCBs) by Gas Chromatography (Continued)

Surrogate	%Recovery	Qualifier	Limits	Prepared	Analyzed	Dil Fac
Tetrachloro-m-xylene (2C)	78	cn	53 - 140	07/02/22 09:51	07/05/22 17:34	1

Method: 8141A - Organophosphorous Pesticides (GC)
Lab: Eurofins Calscience Tustin

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
Azinphos-methyl (2C)	<0.53	cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Bolstar (2C)	<0.53	cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Chlorpyrifos (2C)	<0.53	cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Coumaphos (2C)	<0.53	cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Diazinon (2C)	<0.53	cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Dichlorvos (2C)	<0.53	cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Disulfoton (2C)	<0.53	cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
EPN (1C)	<0.53	cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Famphur (1C)	<0.53	cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Fensulfothion (2C)	<0.53	cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Fenthion (2C)	<0.53	cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Malathion (2C)	<0.53	cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Merphos (2C)	<0.53	cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Methyl parathion (2C)	<0.53	*- cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Mevinphos (2C)	<0.53	cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Naled (2C)	<4.3	cn	4.3		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Ethyl Parathion (1C)	<0.53	cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Phorate (2C)	<0.53	cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Ronnel (2C)	<0.53	cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Tokuthion (2C)	<0.53	cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Trichloronate (2C)	<0.53	cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Demeton-O (2C)	<0.53	cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Demeton-S (2C)	<0.53	*+ cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1
Ethoprop (2C)	<0.53	cn	0.53		mg/Kg	☼	06/25/22 13:01	07/01/22 07:00	1

Method: EPA 300.0 R2.1 - Anions, Ion Chromatography - Soluble

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
Fluoride	**670**		100		mg/Kg	☼		07/19/22 16:49	100
Nitrogen, Nitrate	**10**	**F1**	1.5		mg/Kg	☼		07/19/22 16:32	1
Bromide	<25	F1	25		mg/Kg	☼		07/21/22 16:14	5
Nitrogen, Nitrite	<1.0	F1	1.0		mg/Kg	☼		07/19/22 16:32	1
Sulfate	**1100**		760		mg/Kg	☼		07/21/22 16:25	50
Chloride	**3900**		2000		mg/Kg	☼		07/21/22 16:36	200

Method: 6010C - Metals (ICP)

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
Aluminum	**92**		20		mg/Kg	☼	06/24/22 03:22	06/30/22 16:01	1
Antimony	<5.1		5.1		mg/Kg	☼	06/24/22 03:22	06/28/22 23:13	1
Arsenic	<3.0		3.0		mg/Kg	☼	06/24/22 03:22	06/28/22 23:13	1
Barium	**6.8**		0.51		mg/Kg	☼	06/24/22 03:22	06/28/22 23:13	1
Beryllium	<0.51		0.51		mg/Kg	☼	06/24/22 03:22	06/28/22 23:13	1
Cadmium	<0.51		0.51		mg/Kg	☼	06/24/22 03:22	06/28/22 23:13	1
Calcium	**32000**		51		mg/Kg	☼	06/24/22 03:22	06/28/22 23:13	1
Chromium	**1.7**		1.5		mg/Kg	☼	06/24/22 03:22	06/28/22 23:13	1
Cobalt	**0.69**		0.51		mg/Kg	☼	06/24/22 03:22	06/28/22 23:13	1

Appendix 8 (Continued)

Analyses of Pesticides, Organics and Metals in TetraMin® Flake Food

Client Sample Results

Client: Eurofins EAG Agroscience, LLC
Project/Site: Water Samples

Job ID: 410-88402-1

Client Sample ID: Aquamax Starter 300
Date Collected: 06/21/22 08:30
Date Received: 06/22/22 10:59

Lab Sample ID: 410-88402-5
Matrix: Solid
Percent Solids: 93.8

Method: 6010C - Metals (ICP) (Continued)

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
Copper	22		2.0		mg/Kg	☼	06/24/22 03:22	06/28/22 23:13	1
Iron	510		20		mg/Kg	☼	06/24/22 03:22	06/30/22 16:01	1
Lead	<1.5		1.5		mg/Kg	☼	06/24/22 03:22	06/28/22 23:13	1
Magnesium	1800		10		mg/Kg	☼	06/24/22 03:22	06/28/22 23:13	1
Manganese	210	^5-	1.0		mg/Kg	☼	06/24/22 03:22	06/28/22 23:13	1
Nickel	1.3		1.0		mg/Kg	☼	06/24/22 03:22	06/30/22 16:01	1
Potassium	9100		51		mg/Kg	☼	06/24/22 03:22	06/30/22 16:01	1
Selenium	<5.1		5.1		mg/Kg	☼	06/24/22 03:22	06/28/22 23:13	1
Silver	<1.0	^5-	1.0		mg/Kg	☼	06/24/22 03:22	06/28/22 23:13	1
Sodium	4400		100		mg/Kg	☼	06/24/22 03:22	06/28/22 23:13	1
Thallium	<3.0		3.0		mg/Kg	☼	06/24/22 03:22	06/28/22 23:13	1
Vanadium	<1.0		1.0		mg/Kg	☼	06/24/22 03:22	06/28/22 23:13	1
Zinc	270		2.0		mg/Kg	☼	06/24/22 03:22	06/28/22 23:13	1

Method: 7471A - Mercury (CVAA)

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
Mercury	<6.0	cn	6.0		mg/Kg	☼	06/25/22 03:58	06/26/22 14:26	100

General Chemistry

Analyte	Result	Qualifier	RL	MDL	Unit	D	Prepared	Analyzed	Dil Fac
Percent Moisture	6.2		1.0		%			06/22/22 16:05	1
Percent Solids	93.8		1.0		%			06/22/22 16:05	1

Appendix 8 (Continued)

Analyses of Pesticides, Organics and Metals in TetraMin® Flake Food

Definitions/Glossary

Client: Eurofins EAG Agroscience, LLC Job ID: 410-88402-1
Project/Site: Water Samples

Qualifiers

GC Semi VOA

Qualifier	Qualifier Description
*-	LCS and/or LCSD is outside acceptance limits, low biased.
*+	LCS and/or LCSD is outside acceptance limits, high biased.
*1	LCS/LCSD RPD exceeds control limits.
cn	Refer to Case Narrative for further detail
J	Result is less than the RL but greater than or equal to the MDL and the concentration is an approximate value.
p	The %RPD between the primary and confirmation column/detector is >40%. The lower value has been reported.

HPLC/IC

Qualifier	Qualifier Description
4	MS, MSD: The analyte present in the original sample is greater than 4 times the matrix spike concentration; therefore, control limits are not applicable.
cn	Refer to Case Narrative for further detail
F1	MS and/or MSD recovery exceeds control limits.
F3	Duplicate RPD exceeds the control limit
F5	Duplicate RPD exceeds limit, and one or both sample results are less than 5 times RL, and the absolute difference between results is < the upper reporting limits for both.

Metals

Qualifier	Qualifier Description
*+	LCS and/or LCSD is outside acceptance limits, high biased.
^+	Continuing Calibration Verification (CCV) is outside acceptance limits, high biased.
^1+	Initial Calibration Verification (ICV) is outside acceptance limits, high biased.
^2	Calibration Blank (ICB and/or CCB) is outside acceptance limits.
^3+	Reporting Limit Check Standard is outside acceptance limits, high biased
^5-	Linear Range Check (LRC) is outside acceptance limits, low biased.
^5+	Linear Range Check (LRC) is outside acceptance limits, high biased.
cn	Refer to Case Narrative for further detail

Glossary

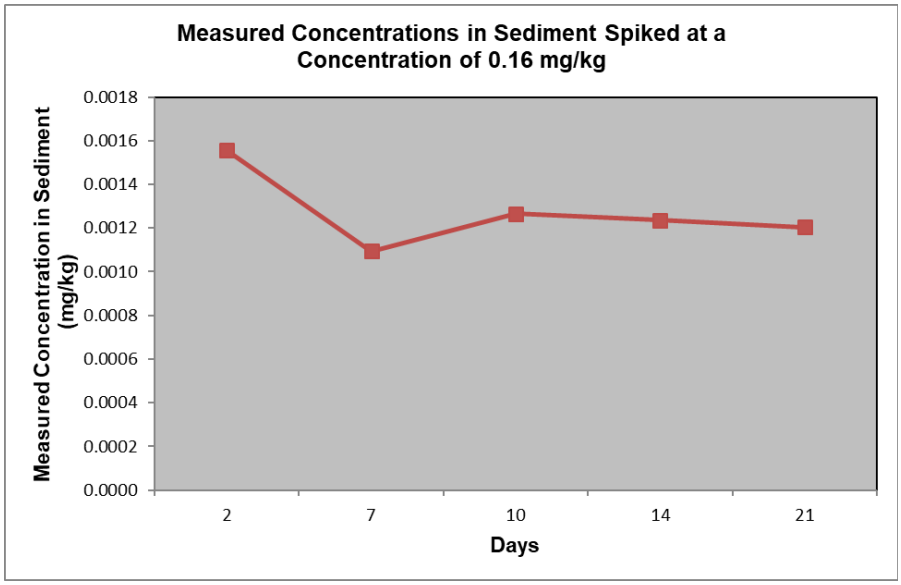
Abbreviation	These commonly used abbreviations may or may not be present in this report.
¤	Listed under the "D" column to designate that the result is reported on a dry weight basis
%R	Percent Recovery
1C	Result is from the primary column on a dual-column method.
2C	Result is from the confirmation column on a dual-column method.
CFL	Contains Free Liquid
CFU	Colony Forming Unit
CNF	Contains No Free Liquid
DER	Duplicate Error Ratio (normalized absolute difference)
Dil Fac	Dilution Factor
DL	Detection Limit (DoD/DOE)
DL, RA, RE, IN	Indicates a Dilution, Re-analysis, Re-extraction, or additional Initial metals/anion analysis of the sample
DLC	Decision Level Concentration (Radiochemistry)
EDL	Estimated Detection Limit (Dioxin)
LOD	Limit of Detection (DoD/DOE)
LOQ	Limit of Quantitation (DoD/DOE)
MCL	EPA recommended "Maximum Contaminant Level"
MDA	Minimum Detectable Activity (Radiochemistry)
MDC	Minimum Detectable Concentration (Radiochemistry)
MDL	Method Detection Limit
ML	Minimum Level (Dioxin)
MPN	Most Probable Number
MQL	Method Quantitation Limit
NC	Not Calculated
ND	Not Detected at the reporting limit (or MDL or EDL if shown)
NEG	Negative / Absent
POS	Positive / Present
PQL	Practical Quantitation Limit
PRES	Presumptive
QC	Quality Control
RER	Relative Error Ratio (Radiochemistry)
RL	Reporting Limit or Requested Limit (Radiochemistry)
RPD	Relative Percent Difference, a measure of the relative difference between two points
TEF	Toxicity Equivalent Factor (Dioxin)
TEQ	Toxicity Equivalent Quotient (Dioxin)
TNTC	Too Numerous To Count

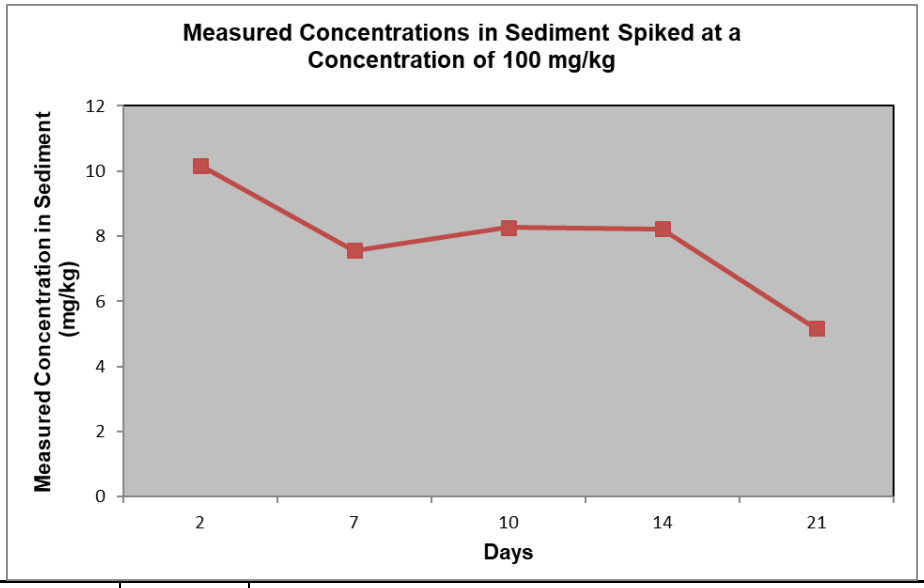
Appendix 9

Summary of Non-GLP Porewater Equilibration Trial and Stability Trial

Appendix 9.1

Concentrations of *p*-Dichlorobenzene in Sediment from a Flow-Through Test Design

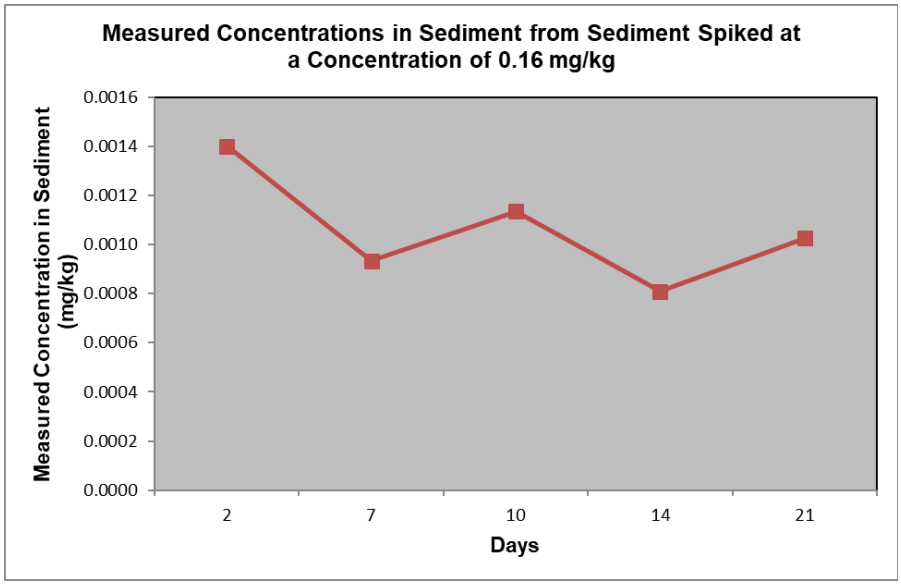


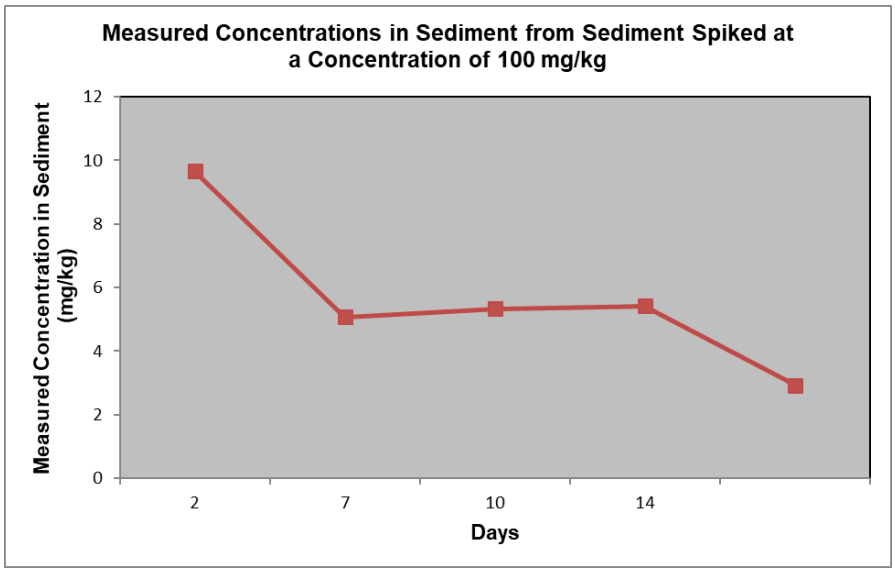


Nominal concentration (mg/kg)		Analytical Result (mg/kg) on Each Day				
		2	7	10	14	21
0.16	Rep. A	0.00162	0.001050	0.001440	0.0013200	0.0011000
	Rep. B	0.001490	0.001140	0.001090	0.001150	0.00131
	Mean	**0.001555**	**0.001095**	**0.001265**	**0.001235**	**0.001205**
100	Rep. A	9.440	6.9500	8.1800	7.790	5.680
	Rep. B	10.900	8.180	8.360	8.670	4.650
	Mean	**10.170**	**7.565**	**8.270**	**8.230**	**5.165**

Appendix 9.2

Concentrations of *p*-Dichlorobenzene in Sediment from a Static Test Design





Nominal concentration (mg/kg)		Analytical Result (mg/kg) on Each Day				
		2	7	10	14	21
0.16	Rep. A	0.00139	0.000943	0.001190	0.0009100	0.0011100
	Rep. B	0.001410	0.000925	0.001080	0.0007080	0.000939
	Mean	**0.001400**	**0.000934**	**0.001135**	**0.000809**	**0.001025**
100	Rep. A	12.700	5.840	6.680	5.2200	3.020
	Rep. B	6.630	4.310	3.980	5.620	2.820
	Mean	**9.665**	**5.075**	**5.330**	**5.420**	**2.920**

Appendix 9.3

Measured Concentrations of *p*-Dichlorobenzene in Sediment from a Stability Trial

Sample ID 264A-115-	Nominal 14C *p*-DCB Concentration (mg/kg)	Test Day	Total [14C] Found (dpm)	14C *p*-DCB Found (wet) (mg/kg)	Soil Content	14C *p*-DCB Found (dry) (mg/kg)	Percent of Nominal (%)	Average % Nominal
--	--		0.00	--	--	--	--	
1-SED	1000	30-min mix	7200.3	720.0	1.000	720.0	72.0	57.3
2-SED	1000	30-min mix	4259.3	425.9	1.000	425.9	42.6	
3-SED	1000	2-hour mix	3122.7	312.3	1.000	312.3	31.2	27.9
4-SED	1000	2-hour mix	2454.5	245.5	1.000	245.5	24.5	
5-SED	1000	Immediately after Settling	3970.6	397.1	0.760	522.2	52.2	48.5
6-SED	1000	Immediately after Settling	3320.8	332.1	0.743	447.2	44.7	
7-SED	1000	Day 1	3422.0	342.2	0.731	468.4	46.8	50.6
8-SED	1000	Day 1	3982.7	398.3	0.734	542.8	54.3	
9-SED	1000	Day 3	3573.9	357.4	0.725	492.8	49.3	47.4
10-SED	1000	Day 3	3330.4	333.0	0.730	456.1	45.6	

14C *p*-DCB Found (Wet) = {[(Total dpm Found - Background Contribution)/Sample Mass]}/Specific Activity

14C *p*-DCB Found (Dry) = 14C *p*-DCB Found (Wet)/soil content

Percent of Nominal = [14C *p*-DCB Found (Dry)/Nominal 14C *p*-DCB Concentration] x 100

Wet Soil Mass=((Wet Soil Weight + Pan Weight)-Pan Weight)*1000

Dry Soil Mass=((Dry Soil Weight + Pan Weight)-Pan Weight)*1000

Soil Content=(Dry Soil Mass/Wet Soil Mass)

Appendix 9.4

Measured Concentrations of *p*-Dichlorobenzene in Overlying Water from a Stability Trial

Sample Type	Sample Number (264A-115 -)	Test Day	Nominal 14C *p*-DCB Concentration (mg/kg)	Sample Volume (mL)	Specific Activity (dpm/µg)	Total [14C] Found (dpm)	14C *p*-DCB Equivalents Found (mg/L)	Mean Measured (mg/L)
Background	--		--	--	--	0.00	--	
Overlying Water	5-OW	Immediately after Settling	1000	10.0	50	1037.3	2.07	2.26
Overlying Water	6-OW	Immediately after Settling	1000	10.0	50	1218.5	2.44	
Overlying Water	7-OW	Day 1	1000	10.0	50	195.0	0.390	0.34
Overlying Water	8-OW	Day 1	1000	10.0	50	148.2	0.296	
Overlying Water	9-OW	Day 3	1000	10.0	50	192.7	0.385	0.51
Overlying Water	10-OW	Day 3	1000	10.0	50	313.4	0.627	

Appendix 9.5

Measured Concentrations of *p*-Dichlorobenzene in Pore Water from a Stability Trial

Sample Type	Sample Number (264A-115 -)	Test Day	Nominal 14C *p*-DCB Concentration (mg/kg)	Sample Volume (mL)	Specific Activity (dpm/µg)	Total [14C] Found (dpm)	14C *p*-DCB Equivalents Found (mg/L)	Mean Measured (mg/L)
Pore Water	5-PW	Immediately after Settling	1000	5.00	50	10759.0	43.0	41.04
Pore Water	6-PW	Immediately after Settling	1000	5.00	50	9759.4	39.0	
Pore Water	7-PW	Day 1	1000	5.00	50	11455.2	45.8	44.28
Pore Water	8-PW	Day 1	1000	5.00	50	10686.9	42.7	
Pore Water	9-PW	Day 3	1000	5.00	50	9345.9	37.4	40.13
Pore Water	10-PW	Day 3	1000	5.00	50	10720.9	42.9	

14C *p*-DCB Equivalents Found = Total dpm found / Sample Volume / Specific Activity

Appendix 9.6

Percentage of *p*-Dichlorobenzene in Sediment, Overlying Water and Pore Water based on Measured Concentration and Mass Balance Calculations

Nominal Concentration (mg/kg)	Study Day	% in Sediment	% in Overlying Water	% in Pore Water	Total %
1000	Time-0	52.22	0.0192	5.80	58.04
	Time-0	44.72	0.0226	5.27	50.01
1000	1	46.84	0.0036	6.19	53.03
	1	54.28	0.0027	5.77	60.05
1000	2	49.28	0.0036	5.05	54.34
	2	45.61	0.0058	5.77	51.39

Appendix 10

Non-GLP Rangefinding Summary

eurofins

p-*DCB*

RESULTS OF A NON-GLP RANGEFINDER FOR A 10-DAY SURVIVAL AND GROWTH TOXICITY TEST WITH THE MIDGE (*Chironomus dilutus*) USING SPIKED SEDIMENT

(Preliminary results not audited by Quality Assurance)

STUDY: p-*DCB*: A 10-Day Survival and Growth Toxicity Test with the Midge (*Chironomus dilutus*) Using Spiked Sediment
SPONSOR: American Chemical Council
PROJECT NO.: 264A-116

Nominal Concentration (mg/kg)[1]	Number of Organisms Surviving to Day 10[2]				Percent Survival by Treatment[3]	Percent Reduction from the Control (%)
	Rep A	Rep B	Rep C	Rep D		
Negative Control	9	10	10	9	98	--
0.10	10	10	10	10	100	-2.6
0.80	10	10	10	9	98	0.0
4.0	10	4	10	6	75	23
20	8	9	8	6	78	21
100	7	10	8	9	85	13

[1] Nominal test concentrations were prepared by dosing the batch sediments with calculated amounts of neat non-radiolabeled p-DCB. Negative control group was clean formulated sediment with no test material.
[2] Four replicates were initiated in each treatment group with 10 midge larvae (3rd instar at initiation) each for a total of 40 midges per treatment group.
[3] Calculated using Excel 2016; manual calculations may slightly differ

Nominal Concentration (mg/kg)[1]	Average Individual Ash-Free Dry Weight (AFDW) (mg)[2]				Average Individual AFDW by Treatment (mg)[3]	Percent Reduction from the Control (%)
	Rep A	Rep B	Rep C	Rep D		
Negative Control	2.09	1.80	--	1.93	1.97 ± 0.13	--
0.10	1.93	1.90	-2.6	1.93	1.91 ± 0.02	2.9
0.80	1.98	1.67	0.0	1.79	1.82 ± 0.13	7.8
4.0	2.06	2.85	23	2.59	2.37 ± 0.43	-20
20	2.15	2.08	21	2.48	2.27 ± 0.19	-15
100	2.28	1.63	13	1.77	1.93 ± 0.29	2.1

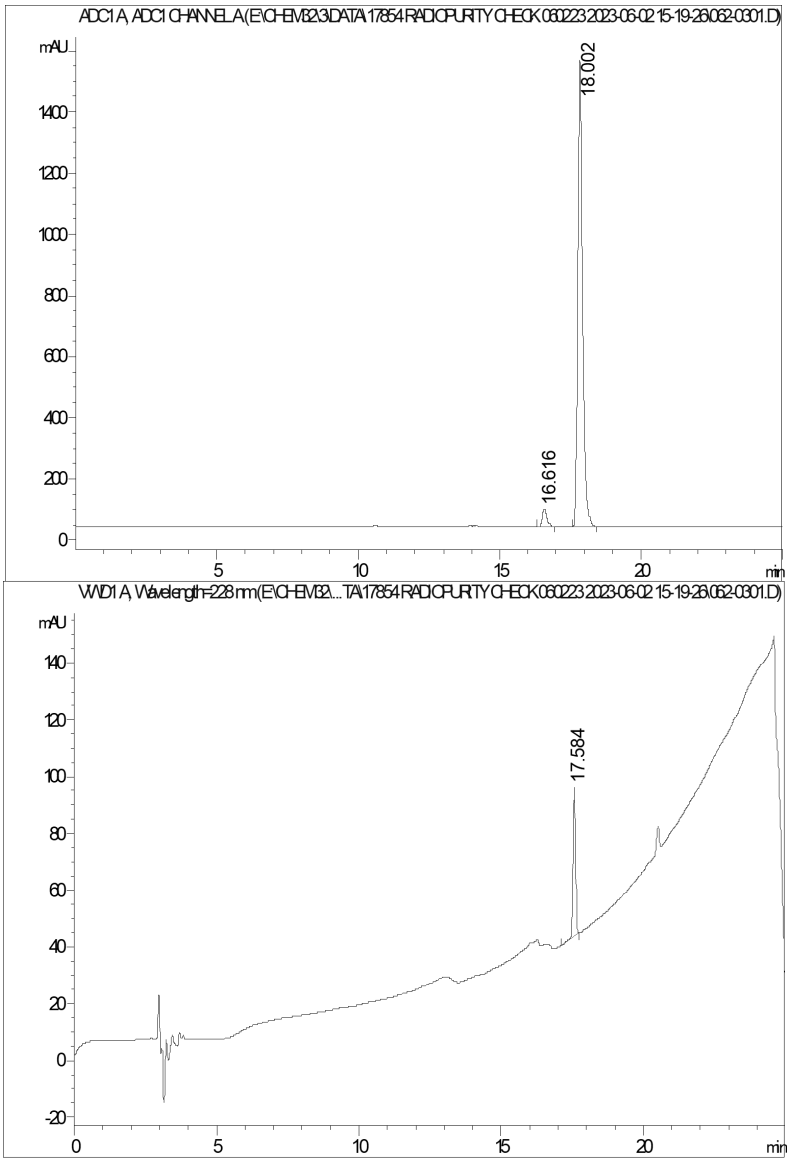
[1] Nominal test concentrations were prepared by dosing the batch sediments with calculated amounts of neat non-radiolabeled p-DCB. Negative control group was clean formulated sediment with no test material.
[2] Only midge larvae were counted towards the average individual dry weights. Any pupae that were recovered at the time of termination was counted towards the total survival but removed from the weight measurements.
[3] Calculated using Excel 2016; manual calculations may slightly differ.
[4] Average dry weight of 80 larvae collected from the culture was calculated to be 0.24 mg.

Appendix 11

The Analysis of *p*-Dichlorobenzene in Sediment and Water

Appendix 11.1

Chromatograms from Radiopurity Analysis



The top chromatogram is the β-Ram output, the bottom chromatogram is the UV output. The approximate retention time of ^{14}C-*p*-DCB by HPLC/UV is 17.6 minutes, which corresponds to the approximate peak retention time of the β-Ram output.

Appendix 11.2

Typical Operational Parameters for Radiopurity Analysis

INSTRUMENT:	Agilent Series 1260 Infinity High Performance Liquid Chromatograph with an Agilent Series 1260 Infinity Variable Wavelength Detector and LabLogic Systems β-Ram Model 3B Detector
ANALYTICAL COLUMN:	Eclipse XDB-Phenyl (250 x 4.6 mm, 5 µm)
OVEN TEMPERATURE:	40.0°C
FLOW RATE:	1.000 mL/min
MOBILE PHASE:	A: 0.1% Trifluoroacetic acid in HPLC-grade water B: 0.1% Trifluoroacetic acid in acetonitrile
GRADIENT TIMETABLE:	Time (min) / % A / % B 0.00 / 95.0 / 5.0 20.00 / 5.0 / 95.0 20.10 / 95.0 / 5.0 25.00 / 95.0 / 5.0
INJECTION VOLUME:	50.00 µL
APPROXIMATE RETENTION TIME OF ^{14}C-*p*-DCB:	Approximately 17.6 minutes
UV DETECTOR WAVELENGTH:	228 nm
β-RAM DETECTOR:	Cell Volume: 200 µL Scintillator: FlowLogic U Flow Rate: 1 mL/min

Appendix 11.3

Method Outline for β-Ram analysis of ^{14}C-*p*-DCB in Primary Stock Solution

1. First prepare a retention time standard using a stock solution of the (non-radiolabeled) test substance, 50 : 50 (v/v) ethanol : HPLC-grade water as the dilution solvent, and a gas-tight syringe and volumetric flask.

Stock Concentration (mg/mL)	Final Concentration (mg/L)	Spike (µL)	Final Volume (mL)
0.100	2.00	200	10.0

2. Using the stock dilution used for LSC counting, prepare a further dilution in HPLC-grade water, to achieve the desired solvent composition for radiopurity analysis. Use a pipettor to add equal volumes of stock and HPLC-grade water to an autosampler vial, crimp the vial, and vortex to mix prior to submission for analysis.

Expected Stock Concentration (mg/mL)	Stock Dilution Volume (µL)	HPLC-Grade Water Volume (µL)
1.50	750	750

3. Inject 50.0 µL of the retention time standard via HPLC/UV to determine the retention time of the test substance. Inject 50.0 µL (in triplicate) of the radiopurity analysis standard via HPLC/UV to determine the radiopurity.

4. Store remaining stock dilution in frozen. The retention time and radiopurity analysis standards should be disposed following injection.

Appendix 11.4

Method Outline for the Analysis of ^{14}C-*p*-DCB in Primary Stock Solution

1. Use a volumetric pipette or equivalent to add 10.0 mL of ethanol to an amber glass vial.

2. Use a pipettor to remove 25.0 µL of ethanol from the amber glass vial.

3. Then use a verified pipettor to transfer 25.0 µL of the primary stock to the amber glass vial and mix well.

Expected Stock Concentration (mg/mL)	Stock Aliquot (µL)	Final Volume (mL)
1.50	25.0	10.0

4. Use a verified pipettor to add 50.0 µL aliquots of the diluted stock prepared above to each of three scintillation vials. Use an empty scintillation vial for the Background sample. Add 10 mL of Ultima Gold XR to each scintillation vial. Shake to mix well.

Sample Type	Sample Quantity (µL)	Ultima Gold XR Volume (mL)
Background	--	10
Diluted Stock Sample	50.0	10
Diluted Stock Sample	50.0	10
Diluted Stock Sample	50.0	10

5. Analyze samples by LSC. This will provide confirmation of the total radioactivity in the diluted stock.

6. Remaining stock dilutions will be stored in the freezer when not in use.

Appendix 11.5

Method Outline for Separating Overlying Water, Pore Water and Sediment for the Analysis of ^{14}C-*p*-DCB

Sample Type	Type of Analysis
Pore Water	Hot
Overlying Water	Hot
Sediment	Hot

1. Centrifuge the sediment samples at 1962 RCF for approximately 10 minutes.

2. Decant and measure the volume of each pore water sample using an appropriately sized graduated cylinder and record the volume of each sample. Transfer the pore water from graduated cylinders into appropriately sized polypropylene centrifuge tubes.

3. Weigh an aliquot of each sediment sample for moisture determination. Document data on a Moisture Content Determination outline.

4. Process sediment and water samples for analysis as necessary. Document data on the appropriate sediment and water method outlines.

5. Transfer sediment into sediment cups and then store all samples in refrigerated storage when samples are not being used.

Appendix 11.6

Method Outline of the Analysis of ^{14}C-*p*-DCB in Overlying Water and Pore Water

1. Remove an aliquot of 10.0 mL of overlying water from mid-depth of each sample using a volumetric pipette or equivalent and transfer to a scintillation vial. Add the requisite volume of Ultima Gold XR to each scintillation vial using a bottle top dispenser.

Sample Type	Nominal Concentration (mg/kg)	Sample Quantity (mL)	Ultima Gold XR Volume (mL)
Background	--	--	10
Overlying Water Negative Control	0.0	10.0*	10
Overlying Water Solvent Control	0.0	10.0*	10
Overlying Water Level-1	0.32	10.0*	10
Overlying Water Level-2	1.0	10.0*	10
Overlying Water Level-3	3.2	10.0*	10
Overlying Water Level-4	10	10.0*	10
Overlying Water Level-5	32	10.0*	10
Overlying Water Level-6	100	10.0*	10

*measured by upon collection.

2. Centrifuge the pore water samples for approximately 10 min. at approximately 4415 RCF.

3. Remove an aliquot of 5.00 mL of pore water using a volumetric pipette or equivalent and transfer to a scintillation vial. Add the requisite volume of Ultima Gold XR to each scintillation vial using a bottle top dispenser.

Sample Type	Nominal Concentration (mg/kg)	Sample Quantity (mL)	Ultima Gold XR Volume (mL)
Pore Water Negative Control	0.0	5.00	15
Pore Water Solvent Control	0.0	5.00	15
Pore Water Level-1	0.32	5.00	15
Pore Water Level-2	1.0	5.00	15
Pore Water Level-3	3.2	5.00	15
Pore Water Level-4	10	5.00	15
Pore Water Level-5	32	5.00	15
Pore Water Level-6	100	5.00	15

4. Analyze water samples by LSC.

Appendix 11.7

Method Outline of the Analysis of ^{14}C-*p*-DCB in Sediment

1. Weigh an aliquot of 0.200 g of each sediment sample into a Combusto-Cone. Add 2-3 drops of Combustaid to each sample. Oxidize samples with combustion timer set to 45 seconds.

Sample Type	Nominal Concentration (mg/kg)	Sample Mass (g)
Background (*)	--	--
Sediment Water Negative Control	0.0	0.200
Sediment Water Solvent Control	0.0	0.200
Sediment Water Level-1	0.32	0.200
Sediment Water Level-2	1.0	0.200
Sediment Water Level-3	3.2	0.200
Sediment Water Level-4	10	0.200
Sediment Water Level-5	32	0.200
Sediment Water Level-6	100	0.200

(*) Blank from verification.

2. Analyze combusted sediment samples by LSC.

Appendix 11.8

Method Outline of the Analysis of ^{14}C-*p*-DCB in Dilution Water

1. A dilution water sample will be measured upon collection. Add the requisite volume of Ultima Gold XR to the scintillation vial using a bottle top dispenser.

Sample Type	Nominal Concentration (mg/kg)	Sample Quantity (mL)	Ultima Gold XR Volume (mL)
Background	--	--	10
Dilution Water Sample	--	10.0*	10

*Volume measured upon collection.

2. Analyze water sample by LSC.

Appendix 11.9

Typical Counting Conditions for Liquid Scintillation Counting

INSTRUMENT:	Perkin Elmer Model Tri-Carb 2910 TR or Tri-Carb 4910 TR Liquid Scintillation Analyzer
COUNTING TIME:	3.00 minutes (Sediment, water, radiopurity and primary stock check)
ASSAY TYPE:	DPM (Single)
NUCLIDE:	14C-UG (Water, radiopurity and primary stock check) 14C (Sediment)
QUENCH INDICATOR:	tSIE/AEC
EXT. STD. TERMINATOR (sec):	0.5 2s%
BACKGROUND SUBTRACT:	ON – 1st vial
QUENCH SET:	14C-UG (Water) 14C (Sediment)

Appendix 11.10

Calculations for Sample Quantitation During LSC Analysis

Sample Quantitation

The equivalent concentrations of ^{14}C-*p*-DCB for water were calculated from the following equation:

Equivalent Concentration (mg/L) of ^{14}C-*p*-DCB = Total [^{14}C] found (dpm) / Specific activity (dpm/µg) ÷ Sample volume (mL)

Note: the background contribution was automatically subtracted by the liquid scintillation counter's software.

The equivalent concentrations of ^{14}C-*p*-DCB for sediment were calculated from the following equation:

Equivalent Concentration (mg/kg) of ^{14}C-*p*-DCB = [Total [^{14}C] found (dpm)/ Specific activity (dpm/µg) ÷ Sample mass (g) for sediment] / Soil content

Note: the background contribution was automatically subtracted by the liquid scintillation counter's software.

Limit of Quantitation for Freshwater and Sediment

The method limit of quantitation (LOQ) for the analyses of ^{14}C-*p*-DCB in sediment, overlying water and pore water was set at the liquid scintillation counter (LSC) instrument LOQ of 50 dpm.

Appendix 12

Temperature of Overlying Water in the Test Compartments During Exposure to Sediment-Incorporated *p*-Dichlorobenzene

Mean Measured Sediment Concentration (mg/kg)		Temperature (°C) [1]										
	Day:	0	1	2	3	4	5	6	7	8	9	10
	Rep:	A	B	C	D	E	F	G	H	A	B	C
Negative Control		22.8	22.6	22.5	22.8	22.7	22.8	22.3	22.8	22.8	22.7	22.7
Solvent Control		22.8	22.9	22.5	22.9	22.8	22.8	22.4	22.9	22.7	22.7	22.7
0.018		22.9	23.0	22.6	22.9	22.9	23.0	22.4	22.9	23.0	22.9	22.9
0.045		22.8	22.7	22.5	22.8	22.7	22.8	22.4	22.9	22.7	22.8	22.8
0.25		23.0	22.9	22.7	23.0	22.7	23.0	22.5	22.9	22.9	22.8	22.8
1.0		23.0	23.0	22.7	23.0	22.9	23.0	22.6	23.0	23.0	22.8	22.9
3.8		23.2	22.9	22.6	23.0	22.8	22.9	22.6	23.0	23.0	22.9	23.0
16		22.9	22.9	22.7	23.0	22.8	22.9	22.7	22.9	22.9	22.8	23.0

[1] Manual temperature measurements. Temperature monitored continuously during the test ranged from 22.42 to 23.59°C, measured to the nearest 0.01°C.

Appendix 13

Dissolved Oxygen of Overlying Water in the Test Compartments During Exposure to Sediment-Incorporated *p*-Dichlorobenzene

Mean Measured Sediment Concentration (mg/kg)		Dissolved Oxygen (mg/L) [1]										
	Day:	0	1	2	3	4	5	6	7	8	9	10
	Rep:	A	B	C	D	E	F	G	H	A	B	C
Negative Control		7.5	7.7	8.1	7.3	7.9	8.4	7.7	7.5	7.7	7.8	8.2
Solvent Control		7.0	7.9	8.2	7.6	8.1	8.4	7.9	7.5	7.8	7.6	8.0
0.018		7.1	7.8	8.2	7.5	8.1	8.3	8.0	7.6	7.7	7.9	8.1
0.045		7.1	7.9	8.1	7.3	8.0	8.4	7.9	7.8	7.0	7.7	7.9
0.25		7.2	7.9	8.2	7.7	8.1	8.4	8.0	7.9	7.7	7.9	8.0
1.0		6.7	7.9	8.1	7.6	8.1	8.4	8.0	7.7	7.9	7.8	8.0
3.8		6.8	8.0	8.1	7.4	8.0	8.1	7.8	7.5	7.9	7.9	8.1
16		7.2	7.9	8.0	7.5	7.8	8.4	7.7	7.7	7.6	8.0	7.9

[1] A dissolved oxygen concentration of 5.1 mg/L represents 60% saturation at 23ºC in freshwater.

Appendix 14

pH of Overlying Water in the Test Compartments During Exposure to Sediment-Incorporated *p*-Dichlorobenzene

Mean Measured Sediment Concentration (mg/kg)	pH										
	Day:	0	1	2	3	4	5	6	7	8	9	10
	Rep:	A	B	C	D	E	F	G	H	A	B	C
Negative Control		7.9	8.1	8.2	8.0	8.1	8.2	8.0	8.1	8.1	8.1	8.1
Solvent Control		7.9	8.1	8.2	8.1	8.2	8.2	8.2	8.1	8.1	8.1	8.1
0.018		8.0	8.2	8.2	8.1	8.2	8.3	8.2	8.2	8.2	8.2	8.1
0.045		7.9	8.2	8.2	8.1	8.2	8.2	8.2	8.2	8.0	8.2	8.1
0.25		7.9	8.2	8.3	8.1	8.2	8.3	8.2	8.2	8.2	8.2	8.2
1.0		7.9	8.2	8.3	8.1	8.2	8.3	8.2	8.2	8.2	8.2	8.2
3.8		8.0	8.1	8.3	8.1	8.2	8.2	8.1	8.1	8.2	8.2	8.2
16		8.0	8.1	8.3	8.2	8.2	8.2	8.2	8.2	8.2	8.2	8.2

Appendix 15

Hardness, Alkalinity, Specific Conductance and Ammonia of Overlying Water During Exposure to Sediment-Incorporated *p*-Dichlorobenzene

Mean Measured Sediment Concentration (mg/kg)	Day 0 [1]				Day 10 [1]			
	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Specific Conductance (µS/cm)	Ammonia (mg/L as NH_3) [2]	Hardness (mg/L as $CaCO_3$)	Alkalinity (mg/L as $CaCO_3$)	Specific Conductance (µS/cm)	Ammonia (mg/L as NH_3) [2]
Negative Control	176	196	414	0.0218	156	184	334	0.161
Solvent Control	172	194	417	0.0189	156	184	336	0.882
0.018	--	--	--	0.0229	--	--	--	0.171
0.045	--	--	--	4.82	--	--	--	0.113
0.25	--	--	--	0.0419	--	--	--	0.634
1.0	--	--	--	0.013	--	--	--	0.372
3.8	--	--	--	6.19	--	--	--	0.0976
16	164	200	411	4.65	152	188	339	1.03

[1] All analyses were performed on a composite sample of overlying water from the respective experimental group.
[2] The LOQ for ammonia analyses was set at 0.17 mg/L, the concentration of the lowest calibration standard.

Appendix 16

pH and Ammonia in Pore Water During Exposure to Sediment-Incorporated *p*-Dichlorobenzene

Mean Measured Sediment Concentration (mg/kg)	Day 0		Day 6	Day 10	
	pH	Ammonia (mg/L as NH_3) [1]	Ammonia (mg/L as NH_3) [1]	pH	Ammonia (mg/L as NH_3) [1]
Replicate	I	L	M	N	N
Negative Control	7.8	12.1	5.69	7.3	2.71
Solvent Control	7.5	10.8	4.26	7.3	2.08
0.018	7.5	11.2	5.20	7.5	2.62
0.045	7.6	12.0	4.91	7.6	2.73
0.25	7.7	11.4	9.18	7.5	2.44
1.0	7.7	10.8	5.18	7.5	1.63
3.8	7.7	10.5	4.42	7.5	2.13
16	7.8	10.2	4.11	7.5	1.42

[1] The LOQ for ammonia analyses was set at 0.17 mg/L, the concentration of the lowest calibration standard.

Appendix 17

Redox Potential (Eh) in Sediment During Exposure to Sediment-Incorporated *p*-Dichlorobenzene

Mean Measured Sediment Concentration (mg/kg)	Day 0	Day 5	Day 10
Replicate	N	N	N
Negative Control	294	-90	-90
Solvent Control	280	-82	-137
0.018	265	-140	-225
0.045	204	87	-76
0.25	326	-19	-57
1.0	219	-38	-53
3.8	291	-115	-167
16	283	-16	-160

Appendix 18

Survival and Observations by Replicate of the Midge (*Chironomus dilutus*) at Termination of a 10-Day Exposure to Sediment-Incorporated *p*-Dichlorobenzene

Mean Measured Sediment Concentration (mg/kg)	Replicate	Number Originally Exposed	Day 10 [1]		Number Surviving by Replicate	Mean Number Surviving (± SD)	Percent Survival (%)
			Number Found Dead	Observations			
Negative Control	A	10	0	10 AN	10	8.6 (± 1.41)	86
	B	10	1 MAD	1 pupae; 8 AN	9		
	C	10	2 MAD	8 AN	8		
	D	10	0	10 AN	10		
	E	10	2 MAD	8 AN	8		
	F	10	0	10 AN	10		
	G	10	4 MAD	6 AN	6		
	H	10	2 MAD	8 AN	8		
Solvent Control	A	10	2 MAD	8 AN	8	9.3 (± 0.71)	93
	B	10	1 MAD	9 AN	9		
	C	10	1 MAD	9 AN	9		
	D	10	1 MAD	1 pupae; 8 AN	9		
	E	10	1 MAD	9 AN	9		
	F	10	0	10 AN	10		
	G	10	0	10 AN	10		
	H	10	0	10 AN	10		

[1] MAD = missing and assumed dead ; Observations: AN = appear normal.

Appendix 18 (Continued)

Survival and Observations by Replicate of the Midge (*Chironomus dilutus*) at Termination of a 10-Day Exposure to Sediment-Incorporated *p*-Dichlorobenzene

Mean Measured Sediment Concentration (mg/kg)	Replicate	Number Originally Exposed	Day 10 [1]		Number Surviving by Replicate	Mean Number Surviving (± SD)	Percent Survival (%)
			Number Found Dead	Observations			
0.018	A	10	1 MAD	9 AN	9	9.1 (± 1.13)	91
	B	10	0	10 AN	10		
	C	10	0	10 AN	10		
	D	10	2 MAD	8 AN	8		
	E	10	0	10 AN	10		
	F	10	1 MAD	9 AN	9		
	G	10	0	10 AN	10		
	H	10	3 MAD	7 AN	7		
0.045	A	10	1 MAD	9 AN	9	9.0 (± 0.76)	90
	B	10	0	10 AN	10		
	C	10	1 MAD	9 AN	9		
	D	10	2 MAD	8 AN	8		
	E	10	1 MAD	1♂ ;8 AN	9		
	F	10	0	10 AN	10		
	G	10	1 MAD	9 AN	9		
	H	10	2 MAD	8 AN	8		

[1] Observations: MAD = missing and assumed dead ; AN = appear normal; ♂ = male.

Appendix 18 (Continued)

Survival and Observations by Replicate of the Midge (*Chironomus dilutus*) at Termination of a 10-Day Exposure to Sediment-Incorporated *p*-Dichlorobenzene

Mean Measured Sediment Concentration (mg/kg)	Replicate	Number Originally Exposed	Day 10 [1]		Number Surviving by Replicate	Mean Number Surviving (± SD)	Percent Survival (%)
			Number Found Dead	Observations			
0.25	A	10	2 MAD	8 AN	8	9.4 (± 0.74)	94
	B	10	1 MAD	9 AN	9		
	C	10	0	1 pupae; 9 AN	10		
	D	10	1 MAD	9 AN	9		
	E	10	0	10 AN	10		
	F	10	0	10 AN	10		
	G	10	0	10 AN	10		
	H	10	1 MAD	9 AN	9		
1.0	A	10	1 MAD	9 AN	9	8.5 (± 0.93)	85
	B	10	0	10 AN	10		
	C	10	2 MAD	1 pupae; 7 AN	8		
	D	10	1 MAD	9 AN	9		
	E	10	2 MAD	8 AN	8		
	F	10	3 MAD	7 AN	7		
	G	10	2 MAD	8 AN	8		
	H	10	1 MAD	9 AN	9		

[1] Observations: MAD = missing and assumed dead ; AN = appear normal.

Appendix 18 (Continued)

Survival and Observations by Replicate of the Midge (*Chironomus dilutus*) at Termination of a 10-Day Exposure to Sediment-Incorporated *p*-Dichlorobenzene

Mean Measured Sediment Concentration (mg/kg)	Replicate	Number Originally Exposed	Day 10 [1]		Number Surviving by Replicate	Mean Number Surviving (± SD)	Percent Survival (%)
			Number Found Dead	Observations			
3.8	A	10	2 MAD	8 AN	8	8.4 (± 0.92)	84
	B	10	2 MAD	8 AN	8		
	C	10	1 MAD	9 AN	9		
	D	10	2 MAD	8 AN	8		
	E	10	0	10 AN	10		
	F	10	2 MAD	8 AN	8		
	G	10	1 MAD	9 AN	9		
	H	10	3 MAD	7 AN	7		
16	A	10	3 MAD	7 AN	7	9.3 (± 1.04)	93
	B	10	1 MAD	9 AN	9		
	C	10	0	10 AN	10		
	D	10	1 MAD	9 AN	9		
	E	10	0	10 AN	10		
	F	10	0	10 AN	10		
	G	10	0	10 AN	10		
	H	10	1 MAD	9 AN	9		

[1] Observations: MAD = missing and assumed dead ; AN = appear normal.

Appendix 19

Average Individual Ash-Free Dry Weights (AFDW) by Replicate of the Midge (*Chironomus dilutus*)

At Termination of a 10-Day Exposure to Sediment-Incorporated *p*-Dichlorobenzene

	Average Individual Ash-Free Dry Weights (mg) by Mean Measured Sediment Concentration (mg/kg) [1]							
Replicate	Negative Control	Solvent Control	0.018	0.045	0.25	1.0	3.8	16
A	2.19	2.26	2.23	2.01	2.46	2.31	2.60	2.46
B	2.29	2.08	2.02	2.01	2.23	2.54	2.48	2.23
C	2.20	2.10	2.02	2.04	1.97	3.06	2.42	2.10
D	1.76	2.02	2.59	2.39	2.36	2.44	2.41	2.14
E	2.61	2.19	2.14	2.40	2.22	2.48	2.21	2.20
F	2.17	2.13	2.31	2.10	2.17	3.23	2.34	2.17
G	2.92	2.19	1.89	2.28	2.09	2.70	2.25	2.15
H	2.24	2.04	2.28	2.15	2.09	2.21	2.47	2.40
Mean ± SD:	2.30 ± 0.34	2.13 ± 0.08	2.18 ± 0.22	2.17 ± 0.16	2.20 ± 0.16	2.62 ±0.36	2.40 ±0.13	2.23 ±0.13

[1] The average individual dry weight of 80 organisms collected from the test batch of organisms at the beginning of the test was 0.22 mg.

Appendix 20

Personnel Involved in the Study

The following key personnel were involved in the conduct or management of this study:

1. Suzanne Z. Schneider, Ph.D.
2. Nanditha Bill, M.S.
3. Sean P. Gallagher B.S.
4. Jessica M. Griebel, M.S.
5. Rachel Woodward, B.A.
6. Kathy H. Martin, M.S.
7. Ling Zhang, Ph.D.
8. Elizabeth Ostermann, M.S.
9. Amanda Pevey, B.S.