FINAL Report

Per- and Poly-fluoroalkyl Substances (PFAS) Whole Sediment Toxicity

Prepared by:

Great Lakes Environmental Center, Inc. (GLEC)
739 Hastings St.
Traverse City, MI 48686
Contact: Dennis McCauley
Phone: 231-941-2230

Email: DMcCauley@glec.com

GLEC.com/

Prepared for:

Michigan Department of Environment, Great Lakes, and Energy (EGLE)
Water Resources Division
Constitution Hall, 3rd Floor
P.O. Box 30458
Lansing, Michigan 48909-7773
Lead Staff Person: Lee Schoen
Phone: 517-342-4500
Email: SchoenL1@Michigan.gov

EGLE Contract Number EP190000000317

GLEC Project Number 4373
Work Assignment #21-20
Goal 1A

Date: May 15, 2023
Final Revision Date: July 26, 2023

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Attachments

ACRONYMS AND ABBREVIATIONS

ASTM	ASTM International, formerly known as American Society for Testing and Materials
BAF	Bioaccumulation factors
BSAF	biota-sediment accumulation factor
CEC	cation exchange capacity
CV	Coefficient of Variation
COC	chain of custody form
DO	Dissolved Oxygen
DOC	dissolved organic carbon
EGLE	Michigan Department of Environment, Great Lakes, and Energy
EPA	U.S. Environmental Protection Agency
GLEC	Great Lakes Environmental Center, Inc.
K_p	sediment water partition coefficients
K_{oc}	sediment water partition coefficients
MDEQ	State of Michigan's Department of Environmental Quality
Pace	Pace Analytical®
PAH	Polycyclic aromatic hydrocarbons
PCB	Polychlorinated biphenyl
PFAS	Per- and Poly-fluoroalkyl substances
PFBS	perfluorobutanesulfonic acid
PFCA	perfluorinated carboxylic acid
PFOA	perfluorooctanoic acid
PFOS	perfluorooctane sulfonate
ppb	parts per billion
QAPP	Quality Assurance Project Plan
RCRA	Resource Conservation and Recovery Act
SED	Sediment
SOP	Standard Operating Procedure
SQG	Sediment Quality Guidelines
TEC	Threshold Effect Concentrations

PROBLEM DEFINITION/BACKGROUND

Problem Definition

Per- and poly-fluoroalkyl substances (PFAS) have been detected in sediments and surface waters of Michigan and are a risk to aquatic life. The Michigan Department of Environment, Great Lakes, and Energy (EGLE) currently has surface water values protective of aquatic life and human health for perfluorooctanoic acid (PFOA) and perfluorooctane sulfonate (PFOS). However, data are not currently available to estimate PFAS sediment screening values protective of aquatic life and human health. The purpose of this work (Goal 1A) was to perform field and laboratory studies that will produce data that will facilitate the determination of PFAS sediment screening values and potentially sediment quality guidelines protective of aquatic life.

Background

Prior to this work, EGLE collected sediment samples for PFAS analysis from six regions of Michigan. These regions represent areas where there are known water quality exceedances for PFAS. Sediment samples were collected from waterbodies within these areas to assist in the identification of potential sources of PFAS (Figure 1). EGLE previously reported that the 24 default analytes analyzed, PFOS was detected in 47 of 63 samples. PFOS was also the PFAS analyte with the highest detected concentration, at 60 parts per billion (ppb) dry weight.

Remucal (2019) provided a review of the spatial variability of PFAS in Great Lakes sediments. In general, PFAS sediment concentrations tended to increase as one moves from west to east (sum of C_8-C_{12} PFAS increasing from Superior < Michigan < Erie < Huron < Ontario). The trend was also apparent for PFOS, which was found at the lowest concentrations in Lake Superior sediments, and highest concentrations in Lake Ontario sediments. The higher concentrations seemed to be correlated with the more urbanized and industrialized land use around Lake Ontario and Lake Erie. PFOA sediment concentrations in the Great Lakes were consistently lower than PFOS concentrations. The long-chain PFAS (≥8 carbons) were more likely to be found at elevated concentrations compared to short-chain (< 8 carbons) PFAS. For example, long-chain PFAS made up >80% of the total perfluorinated carboxylic acid (PFCA) compounds detected in Lake Ontario sediments. While short-chain PFAS were also detected in sediments from the Great Lakes, these PFAS were likely present in the porewater and not sorbed to sediment. Long-chain PFAS tend to partition to sediment more easily than short-chain PFAS, and sediment tends to be enriched in long-chain PFAS compared to the PFAS distribution found in surface water. Consequently, the investigation of PFAS compounds in sediment is a relevant concern.

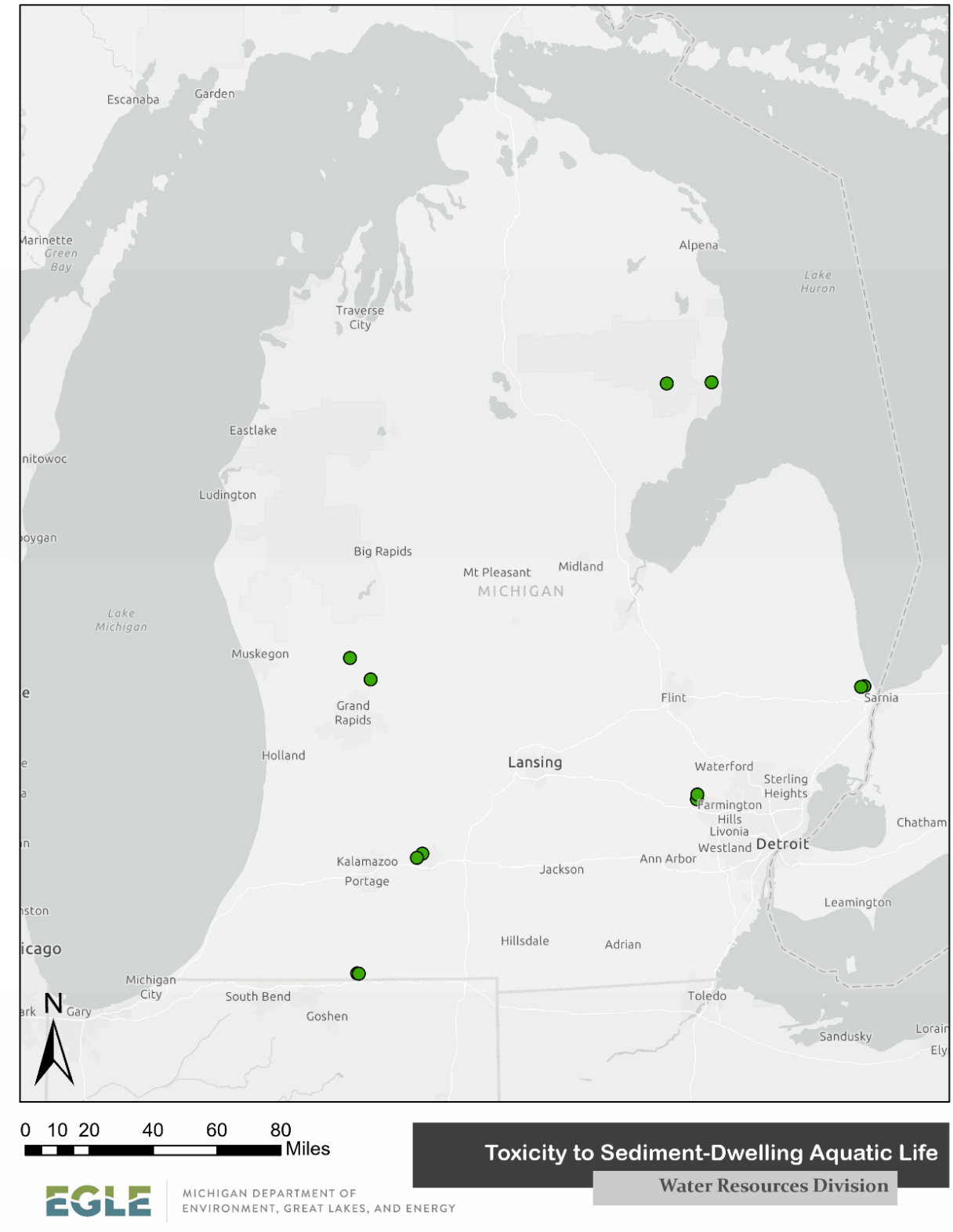

FIGURE 1. Location of PFAS Assessment Sites in Michigan

TABLE 1. EGLE PFAS Sediment Toxicity Assessment Sampling Locations

Goal #1A - Toxicity to Sediment Dwelling Aquatic Life			
Site (contamination source)	**Latitude**	**Longitude**	**Notes and Narrative Description**
Fort Gratiot (biosolids, paper manufacturing)	43.0382	-82.5061	Upstream of Keewahdin Road. Previously UN-0040S 60 PPB PFOS.
Fort Gratiot reference site	43.0359	-82.526	Upstream of North Road. Previously BMT-0030 J-Flag PFOS.
Clark's Marsh (AFFF)	44.44182	-83.391296	Within Clark's Marsh using USFS access.
Clark's Marsh reference site	44.442615	-83.675279	Au Sable R.
Huron/Norton Creek or Kent Lake (plating)	42.552528	-83.562203	Sample on Norton Creek at Buno Road.
Huron/Norton Creek reference site	42.574162	-83.55845	Sample at Proud Lake Recreation Area Canoe Launch, upstream of potential canoe loading area.
Rogue River (tannery)	43.122342	-85.561361	Sample adjacent to former Wolverine Plant. Access from White Pine Trail. Do not sample any farther north or west of coordinates since this area was previously remediated.
Rouge River reference site	43.219901	-85.688519	Upstream of Sparta at 17 Mile/M46.
Pigeon River (paper)	41.788394	-85.652876	Private property. Coordinate access with RRD and WRD Ray Spaulding/Jen Klang.
Pigeon River reference site	41.786977	-85.6429	Sample northern bank, upstream and east of Kalamazoo Street, within depositional area.
Beaver Dam Pond (AFFF)	42.329679	-85.250815	No known boat access from Beaver Dam Road.
Harts Lake reference site	42.311089	-85.28428	Private boat access on Northwest corner.

Based on limited data collected and reviewed by EGLE and Purdue University (cited by EGLE), PFOS appears to be the most-frequently detected analyte at the greatest concentrations in Michigan sediment. In the Great Lakes, PFOS was also the dominant analyte detected in sediment samples (see Remucal 2019 review). Consequently, for Goal 1A, GLEC designed the assessment to measure a suite of 36 PFAS analytes in the sediment and surface water, with an emphasis on PFOS as the primary source of toxicity.

APPROACH TO WORK

To further understand the effect of field collected PFAS contaminated sediment to sediment dwelling organisms, GLEC led the significant effort of collecting the sediment and surface water (Table 1), analyzing the samples for physical and chemical characteristics, and finally conducting whole sediment and bioaccumulation toxicity tests on sediment samples collected from six PFAS impacted areas in Michigan. The ultimate goal of this effort was to provide toxicological data to support the derivation of sediment quality benchmarks or sediment screening values (e.g., PFAS Sediment Quality Guidelines) that can be used to assess whether sediment-dwelling species are adversely impacted by PFAS in sediment. PFOS was the most frequently detected analyte at the greatest concentrations in sediments collected from Michigan, and it has been shown to be more toxic than other PFAS (specifically, PFOA and perfluorobutanesulfonic acid [PFBS]) to aquatic life in water-only and mesocosm exposures (MacDonald et.al., 2004; Marziali et.al., 2019; Simpson et.al.,. 2021; and Stefani et. a., 2014) and in this study GLEC measured a suite of 36 PFAS compounds (including PFOS) in each collected sediment and surface water sample, 35 PFAS compounds in tissue, and 10 PFAS compounds in sediment porewater, to better understand PFAS chemistry in the impacted sediment. For Goal 1A procedures outlined in the approved Quality Assurance Project Plan (QAPP) were followed to complete the assessment of the toxicity to sediment dwelling aquatic life.

Goal #1A: Toxicity to sediment-dwelling aquatic life

Literature Review/Rationale

EGLE reviewed a number of PFAS toxicity references, including Condor et al. (2019), Wang (2017), Remucal (2019), Higgins et al. (2007), and Giesy et al. (2010) that described the chemical distribution, species sensitivity, toxicity in various media types, and apparent data gaps in the published data. GLEC expanded the literature search to include additional data and analysis to further the understanding of PFAS compound chemistry, distribution, and toxicity of PFAS to freshwater benthic organisms via sediment exposure based on the distribution of PFAS compounds in Michigan sediment. The results of the PFAS literature search were reported separately from the Goal 1A toxicity testing. However, this review was utilized in the discussion of the toxicity testing results obtained with the field collected sediment samples (GLEC 2021).

Field Whole Sediment and Surface Water Sample Collection

GLEC collected whole sediment samples and surface water grab samples from six predetermined areas (regions) in Michigan (Figure 1). Each area had two sample locations; one location was the reference sample and the second location was the impacted sample, for a total of 12 sample locations. The sampling events occurred on October 11 through October 14, 2021 and November 23, 2021. These sample locations are summarized in Table 1, the various analyses conducted on the sediment and water samples are summarized in Table 2, and the sample identifications and corresponding collection dates are provided in Table 3.

The sediment and surface water samples were collected following the EGLE's *General PFAS Sampling Guidance* (MDEQ, 2018), *PFAS Sampling Quick Reference Field Guide* (October 2018 revision), and *Surface Water PFAS Sampling Guidance* document (MDEQ, 2018a). Wadeable locations were approached from downstream and collected facing upstream while an inflatable boat was used for non-wadeable locations. Field personnel used gloved hands (i.e., clean hands/dirty hands technique) when collecting samples and site water was used in rinsing all equipment (sampling device, spoons, spatula, mixing bowls, etc.) prior to use and in between sampling stations.
Surface water (grab) samples were collected, at each of the twelve locations (six impacted and six reference) just prior to sediment collection at each location. The water sample was collected by hand in a 250 milliliter (mL) HDPE bottle (laboratory certified as PFAS free) by lowering an inverted sample bottle to a depth of 0.5 meters and then righting it to fill with water. If the water was less than 0.5 meters in depth, a grab sample was collected at the mid-point of the depth using the same technique.

Field measurements (water temperature, pH, dissolved oxygen (D.O.), and conductivity) were obtained at the time of surface water sample collection using a calibrated YSI Pro DSS sonde. The field measurements were recorded on dedicated field data sheets along with project number, sample identification (i.e., site number, location, etc.), date and time of collection, and collector's initials. Those observations and measurements are provided in Appendix A.

The sediment samples were collected at each of the 12 (six impacted and six reference) locations using a Standard Ponar Bottom Grab Sampler following methods outlined in GLEC's SOP: SED 7002, July 29, 2020[1]. At each location, sediment was collected from the approximate coordinates used during previous EGLE investigations at the site. Depositional areas were targeted in riverine areas. Three sediment sample grabs were collected from preestablished locations and composited into one sample to be used for the physical, chemical, and toxicological analysis. Whole sediment was collected using multiple grabs with the Ponar sampler at each location and combined into a clean five-gallon stainless-steel mixing container. Once combined in the stainless-steel mixing container and prior to homogenization, large debris such as rocks, sticks, vegetation mats, etc. were handpicked and discarded from the sediment sample. The sediment was thoroughly homogenized using a battery-powered drill with a stainless-steel mixing paddle to form a composite sample. After homogenization the composite sample was equally distributed, using a clean stainless-steel spoon, to a clean one gallon and to a two-gallon high-density polyethylene (HPDE) sample bucket (U.S. Plastics)(Figure 2).

[1] This SOP is based on *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates*, EPA/600/R-99/064 and ASTM 1706-05, *Standard Test Methods for Measuring the Toxicity of Sediment associated Contaminants with Fresh Water Invertebrates*; and ASTM 1391-03, *Standard guide for Collection, Storage, Characterization, and Manipulation of Sediments for Toxicological Testing*.

FIGURE 2. Field Collection Equipment and Sample Containers

During sample collection and for the duration of the field sampling event, the whole sediment samples and surface water samples were preserved on ice in coolers to maintain a sample temperature between 0 and ≤ 6 °Celsius. Chain-of-custody (COC) forms with the appropriate information were completed for each sample. At the completion of the field sampling event, samples were hand delivered to the laboratory, stored between 0 and ≤ 6 °Celsius until toxicity test initiation and to process the samples (from time of collection) to be shipped overnight to Pace laboratories to be analyzed for PFAS and other analytes.

Laboratory Toxicity Tests with Field-Collected Sediment
The twelve field collected sediment samples (six impacted and six reference) were held in the laboratory for a maximum of eight weeks, per EPA and ASTM guidelines. Sediment samples were “aged” a minimum of one week after collection before initiating any toxicity tests, by storing the samples between 0 and ≤ 6 °Celsius in the sample containers used during field collection. Copies of the sample chain of custodies are provided in Appendix A.

The field collected samples were analyzed at time of collection, at time of toxicity test initiation and toxicity test termination and a summary of the samples analyzed by parameter and matrices are provided in Table 2. Surface water, sediment, and sediment porewater samples were analyzed for PFAS as well as other physical and chemical characteristics (listed below). Consistent with MI EGLE sediment dredge procedure WRD-048, the sediment samples were analyzed for other contaminants known to cause toxicity in sediment to aquatic organisms, i.e., trace metals, polychlorinated biphenyls (PCBs), and polycyclic aromatic hydrocarbons (PAHs).

The physical and chemical characteristics that were measured in the sediment/sediment porewater samples include:

- pH (in both sediment and sediment porewater);
- cation exchange capacity (CEC);
- grain size;
- percent solids;
- total organic carbon (TOC);
- 15 elements, including the 8 Resource Conservation and Recovery Act (RCRA) metals (bolded): **arsenic (As)**, **barium (Ba)**, **cadmium (Cd)**, calcium (Ca), **chromium (Cr)**, copper (Cu), iron (Fe), **lead (Pb)**, magnesium (Mg), manganese (Mn), **mercury (Hg),** nickel (Ni), **selenium (Se), silver (Ag)**, and zinc (Zn);
- polychlorinated biphenyls (PCBs) – 209 congeners;
- polycyclic aromatic hydrocarbons (PAHs) – 17 parent PAHs;
- ammonia-nitrogen (NH_3-N, in sediment porewater);
- hardness (in sediment porewater);
- dissolved organic carbon (DOC, in sediment porewater).

As a result, whole sediment toxicity tests were completed with sediment characterized by varying physical properties and at varying PFAS concentrations with each test organism. For each test organism, whole sediment toxicity tests were initiated with; one clean sediment reference, one impacted sediment for each predetermined location, and one clean laboratory control sediment (CS#' 158 and 159). One laboratory control sediment was utilized with all the investigative sediments initiated with one source of the test organisms (i.e., one laboratory control sediment was used for each group of sediment toxicity tests).

Standard test species for whole sediment toxicity and bioaccumulation tests were used. *Hyalella azteca (H. azteca*) *and Chironomus dilutus* (*C. dilutus*) were used to assess acute and chronic toxicity, whereas the *Lumbriculus variegatus* (*L. variegatus*) tests were used to assess PFAS (specifically PFOS) bioconcentration.

The acute and chronic whole sediment toxicity and bioaccumulation tests were conducted using the EPA (2000) and ASTM (2010) methods and the following methodology (see Table 7 in Section 8.5 of the QAPP for GLEC SOP references):

- 10-day Sediment Acute Toxicity Test with the Amphipod *H. azteca;* (endpoints: survival, growth, and biomass);
- 28-day Sediment Chronic Toxicity Test with the Amphipod *H. azteca* (endpoints: survival, growth, and biomass);
- 10-day Sediment Acute Toxicity Test with the Midge *C. dilutus;* (endpoints: survival, growth, and biomass);
- 20-day Sediment Chronic Toxicity Test with the Midge *C. dilutus* (endpoints: survival, growth, and biomass);

- 28-day Bioaccumulation Toxicity Test with *L. variegatus* (4-day screening survival and PFAS bioconcentration).

The above suite of whole sediment toxicity tests addressed acute and chronic toxicity, species sensitivity, and bioconcentration in field collected sediment. The same suite of field collected sediment samples were used in each set of tests (thereby reducing variability in sediment contamination and/or physical characteristics because of multiple field collection efforts).
The concentration of PFAS compounds in the sediment samples used for the toxicity tests was measured at the time of field collection, toxicity test initiation and toxicity test termination, so that the actual sediment exposure concentrations was known. At test termination, 100 mls of sediment from each replicate was composited and subsampled for analysis. Sediment porewater PFAS concentrations were also measured at test initiation and test termination (from “non-biological” replicates for porewater extraction at test termination) at 10-days and 28-days. The design of Goal #1A did not include measurement of PFAS concentrations in the overlying water since it was renewed at least twice daily and would not necessarily represent the PFAS exposure.

36 PFAS compounds were measured in the whole sediment and surface water, and 10 PFAS compounds were measured in sediment porewater. For the *L. variegatus* bioaccumulation tests, 35 PFAS compounds were analyzed in tissue samples. All analyses included the analysis of PFOS. For the list of PFAS compounds analyzed in each matrix, see Appendices B1-B3.

TABLE 2. Summary of Samples Analyzed For The Michigan EGLE Whole Sediment PFAS Toxicity Assessment Project

Analysis Description	Matrix
36 PFAS compounds in sediment	Sediment
10 PFAS compounds in porewater	Porewater
36 PFAS compounds in surface water	Water
35 PFAS compounds in Lumbriculus tissue recovered from bioaccumulation tests	Tissue
Percent lipids: Lumbriculus tissue recovered from bioaccumulation tests	Tissue
pH	Sediment
CEC	Sediment
Grain Size	Sediment
TOC	Sediment
PCB Congeners (209)	Sediment
PAHs (17 parent PAHs)	Sediment
Elements (15): As, Ba, Cd, Ca, Cr, Cu, Fe, Pb, Mg, Mn, Hg, Ni, Se, Ag, and Zn	Sediment
pH	Porewater
Ammonia-Nitrogen	Porewater
DOC	Porewater
Hardness	Porewater
Hardness	Water

Test Methods for the Whole Sediment Toxicity and Bioaccumulation Tests

The acute and chronic whole sediment toxicity and bioaccumulation tests were conducted at GLEC's Traverse City, Michigan laboratory following GLEC written Standard Operating Procedures (SOPs) which are based on the procedures outlined in U.S. EPA Method, EPA/600/R-99/064 *Methods for Measuring the Toxicity and Bioaccumulation of Sediment-Associated Contaminants with Freshwater Invertebrates*, Second Edition; test methods EPA

100.1, 100.2, 100.4, and 100.5, and American Society for Testing and Materials (ASTM) 1706-95B, *Standard Test Methods for Measuring the Toxicity of Sediment Associated Contaminants with Freshwater Invertebrates* (ASTM 2010).

The twelve sediment samples which were collected and hand delivered to GLEC-by-GLEC personnel, were assigned a unique GLEC laboratory identification number and stored between 0 and ≤ 6° C (but not frozen) until test initiation (see table below).

TABLE 3. Summary of Sample Collection Sites, Collection Dates and Test Initiation Dates

Sample I.D. Site Number	Investigative Sample Description	GLEC Lab ID	Date Sampled	Date Received	Temp. Upon Receipt (ºC)	Date Test Initiated
Clarks Marsh	Site Sample	13,204	October 11, 2021	October 11, 2021	10.4	October 22, 26, 29, and November 02, 2021
AuSable Reference	Reference Site Sample	13,205	October 11, 2021	October 11, 2021	8.4	October 22, 26, 29, and November 02, 2021
Fort Gratiot Reference	Reference Site Sample	13,214	October 12, 2021	October 14, 2021	5.0	October 22, 26, 29, and November 02, 2021
Fort Gratiot	Site Sample	13,215	October 12, 2021	October 14, 2021	4.3	October 22, 26, 29, and November 02, 2021
Huron/ Norton Creek Reference	Reference Site Sample	13,216	October 12, 2021	October 14, 2021	4.4	October 22, 26, 29, and November 02, 2021
Huron/Norton Creek	Site Sample	13,217	October 12, 2021	October 14, 2021	2.5	October 22, 26, 29, and November 02, 2021
Beaver Dam Pond	Site Sample	13,218	October 13, 2021	October 14, 2021	3.6	October 22, 26, 29, and November 16, 2021
Harts Lake	Reference	13,219	October	October	4.4	October 22, 26, 29,

Sample I.D. Site Number	Investigative Sample Description	GLEC Lab ID	Date Sampled	Date Received	Temp. Upon Receipt (ºC)	Date Test Initiated
Reference	Site Sample		13, 2021	14, 2021		and November 16, 2021
Rogue River Tannery	Site Sample	13,220	October 13, 2021	October 14, 2021	3.7	October 22, 26, 29, and November 16, 2021
Rogue River Reference	Reference Site Sample	13,221	October 14, 2021	October 14, 2021	3.9	October 22, 26, 29, and November 16, 2021
Pigeon River Reference	Reference Site Sample	13,251	November 23, 2021	November 24, 2021	3.8	January 03, 06, 07 and 011, 2022
Pigeon River Paper	Site Sample	13,250	November 23, 2021	November 24, 2021	3.9	January 03, 06, 07, and 11, 2022

Due to a logistical field complication, the twelve sediment samples were not sampled over the same time period and the toxicity tests were initiated at different times. The ten sediment samples: Clarks Marsh, AuSable Reference, Fort Gratiot Reference, Fort Gratiot, Huron/Norton Creek Reference, Huron/Norton Creek, Beaver Dam Pond, Harts Lake Reference, Rogue River Tannery, and Rogue River Reference were collected October 11 through October 14, 2021 and the toxicity tests were initiated on the following dates:

- 4-day *L. variegatus* screening toxicity tests initiated on October 26, 2021;
- 10-day and 28-day *H. azteca* toxicity tests initiated on October 26, 2021;
- 10-day and 20-day *C. dilutus* toxicity tests initiated on October 29, 2021; and
- 28-day *L. variegatus* Bioaccumulation tests initiated on staggered start dates November 2 and 16, 2021.

The remaining sediment samples: Pigeon River Reference and Pigeon River Paper were collected on November 23, 2021 and the toxicity tests were initiated on the following dates:

- 4-day *L. variegatus* screening toxicity tests initiated on January 3, 2022;
- 10-day and 28-day *H. azteca* toxicity tests initiated on January 3, 2022;
- 10-day and 20-day *C. dilutus* toxicity tests initiated on January 6 and 7, 2022; and
- 28-day *L. variegatus* Bioaccumulation tests initiated on January 11, 2022.

The whole sediment toxicity tests were initiated for each of the twelve sediment samples, one GLEC laboratory control sediment and one water only control, per test organism (*C. dilutus* and *H. azteca)*. Whereas the *L. variegatus* screening toxicity tests and bioaccumulation tests were initiated for each of the twelve sediment samples and one GLEC laboratory control sediment.

Summary of Test Procedures: 10-Day *Chironomus dilutus*, 20-Day *Chironomus dilutus*, 10-Day *Hyalella azteca*, and 28-Day *Hyalella azteca* Whole Sediment Toxicity Tests

Second to third instar *C. dilutus* (approximately nine days old; average head capsule 0.23-0.63 mm measured at test initiation, purchased from Aquatic BioSystems) were used to initiate the 10-day whole sediment toxicity tests and newly-hatched *C. dilutus* larvae (< 24 hours old from egg cases, purchased from Aquatic BioSystems) were used to initiate the 20-day whole sediment toxicity tests. Juvenile *H. azteca* (seven to eight days old at test initiation,) were used to initiate both the 10-day and 28-day whole sediment toxicity tests. Test organisms were received one to two days prior to test initiation. After receipt at the laboratory, the test organisms were held in the culture water for approximately 2 hours to acclimate to test temperature. After acclimation, 50 percent of the shipped culture water was syphoned and replaced with 50 percent laboratoryoverlying test water. This procedure was repeated two more times with overlying test water that same day, resulting in the holding and acclimation of the test organisms in 100% laboratory water prior to test initiation at test temperature.

C. dilutus and *H. azteca* were continuously exposed for the duration of the test (i.e.,10-days, 20-days, or 28-days) to each of the sediment samples, one laboratory control sediment and one water only control. In the water only controls, test organisms were exposed to the overlying water only with clean substrates (e.g., silica sand and nylon screen) and no sediment.

There were eight replicate beakers for each sediment sample, the water only control, and the laboratory control sediment. For the 10-day *C. dilutus* and *H. azteca* and the 28-day *H. azteca*

toxicity tests, each replicate beaker contained 10 test organisms. For the 20-day *C. dilutus* toxicity test, each replicate beakers contained 12 test organisms.

The GLEC laboratory control sediment is a reference sediment that is collected from the Boardman River, a local river that has a primarily forested watershed in the Pere Marquette State Forest. The yearly analytical measurements of the laboratory control sediment are suppled in Appendix E.

The *C. dilutus* and *H. azteca* were exposed in 470 mL glass test chambers, each containing 100 mL of whole sediment and 175 mL of overlying water.

Prior to adding the whole sediment to each test chamber, the GLEC laboratory control sediment was sieved through a one-centimeter mesh stainless steel sieve to remove large debris and again homogenized. The laboratory control sediment and the twelve sediment samples were homogenized using a pre-cleaned stainless steel all-purpose mixer and a stainless-steel spoon until a uniform color and texture was achieved.

The homogenized sediment was then added to each test chamber using a pre-cleaned stainless-steel spoon. After the addition of the sediment to the test chambers, the overlying water was immediately added; this was considered test day -1, the test day prior to day 0 (October 28, 2021 and January 05-06, 2022 for the 10-day and 20-day *C.*

dilutus tests; October 25, 2021 and January 02, 2022 for the 10-day and 28-day *H. azteca* tests). The test organisms for the 10-day and 28-day whole sediment toxicity tests were randomly added using a large bore pipette, to each replicate test chamber the following day; test day 0. The test organisms for the 20-day *C. dilutus* tests were randomly added to each test chamber using a dissecting scope and a Pasteur pipet.

Overlying water was intermittently supplied to each test chamber at least twice daily (once every 12-hours) via a static-renewal water delivery system. The overlying water for each of the investigative sediment samples, the laboratory control sediment, and the water only exposure consisted of de-chlorinated municipal (Traverse City, Michigan) tap (Lake Michigan sourced) water, with an average hardness of 132 mg/L and an average alkalinity of 102 mg/L. Temperature, dissolved oxygen (DO), pH, and specific conductance of the overlying water was measured daily prior to use. Municipal tap water is dechlorinated at GLEC using carbon-bed filtration. Yearly analytical measurements for the laboratory water are suppled in Appendix F.

The *C. dilutus* test chambers were fed 1.5 mL of Tetrafin® goldfish food slurry (4 mg/mL dry solids) once daily. The *H. azteca* test chambers were fed a 1.0 mL mixture of yeast, trout food, and wheatgrass (YTC; ~1800 (1700-1900 +/- 5%) mg/L solids) once daily.

The test chambers were placed in a temperature-controlled water bath under the specified conditions of 23 ± 1°C; photoperiod 16 hours light: eight hours dark; and light intensity of 100-1000 lux.

Temperature (23 ± 1ºC) of the overlying water in the test chambers was measured daily in two alternating replicates for each test sediment. There were no instances of temperature exceedance in the 10-day *C. dilutus,* 20-day *C. dilutus,* 10-day *H. azteca* or the 28-day *H. azteca* whole sediment toxicity tests.

The DO (≥ 2.5 mg/L) concentrations of the overlying water in the 10-day *C. dilutus* and 10-day *H. azteca* test chambers were measured daily in two alternating replicates for each test sediment and the results were recorded on the laboratory bench data sheets and are provided in Appendices C2 and D2.

During the 20-day *C. dilutus* and 28-day *H. azteca* whole sediment toxicity tests, DO concentrations of the overlying water were measured three times per week, unless the DO concentrations decreased by more than 1.0 mg/L from the previous measurements, then the DO measurement frequency would be increased to daily until the DO decreases were no longer greater than 1.0 mg/L.

There were no instances of decreased DO (≥ 2.5 mg/L) in 10-day *C. dilutus,* 20-day *C. dilutus,* 10-day *H. azteca* or the 28-day *H. azteca* whole sediment toxicity tests.

Alkalinity, hardness, pH, conductance, and total ammonia (as N) were measured in the overlying water on test days 0 and 10 for the 10-day *C. dilutus* (Tables 5a, 5b of Appendix D2 and the 10-day *H. azteca* tests (Tables 11a, 11 of Appendix C2). These same measurements: alkalinity, hardness, pH, conductance, and total ammonia (as N)

were also measured on days 0 and 20 for the 20-day *C. dilutus* (Tables 6a, 6b of Appendix D2) and on days 0 and 28 of the 28-day *H. azteca* tests (Tables 12a, 12b of Appendix C2).

For the 20-day *C. dilutus* and 28-day *H. azteca* whole sediment toxicity tests, conductivity was measured weekly and pH was measured at least three times per week from two randomly selected test chambers. The alkalinity, hardness, and total ammonia (as N) samples were a composite sample collected from all replicates of a given treatment. All test exposure water quality measurements were recorded on the laboratory bench data sheets and are provided in Appendices C2 and D2.

Observations of organism behavior and anomalies within the sediment were made daily for each test chamber and recorded on the laboratory bench data sheets.

The number of *C. dilutus* surviving in each replicate test chamber was recorded at test termination (10 days and 20 days) and summarized in Tables 1 and 3 of Appendix D1. The average ash free dry weight [AFDW in milligrams (mg)] of the surviving organisms for each *C. dilutus* replicate, and the biomass [AFDW (mg) of the surviving organisms divided by the initial number of organisms] was also determined at test termination and summarized in Tables 2 and 4 of Appendix D1.

The number of surviving *H. azteca* in each replicate chamber was recorded at test termination (10 days and 28 days) and summarized Tables 7 and 9 of Appendix C1. The average dry weight [in milligrams (mg)] of the surviving organisms for each *H. azteca* replicate, and the biomass [dry weight (mg) of the surviving organisms divided by the initial number of organisms] was also determined at test termination, and summarized Tables 8 and 10 of Appendix D1.

Statistical Analysis Methods

A statistical procedure, using the program TOXCALC (version 5.0.32) and following statistical guidelines provided in U.S. EPA Method 600/R-99/064 and ASTM Method 1706-95B (2010), was used to compare the 10-day, 20-day, and 28-day survival and growth data from the site-specific reference sediment samples to survival and growth data from the corresponding site investigative sediment samples. Prior to analysis, all percent survival data were transformed using an arc sine-square root transformation.

All transformed data were then tested for normality and homogeneity of variances. Next, the data were analyzed using the homoscedastic or heteroscedastic t-tests, which are used for comparing a single treatment to a single control.

The homoscedastic t-test assumes the data are normally distributed (Shapiro-Wilk Test or Kolmogorov D Test) and the variances are equal (F-test). If the variances are not equal, the data are analyzed using the heteroscedastic t-test. If the data are not normally distributed, then the data are analyzed using a nonparametric t-test (e.g., Steel's Many-One Rank Test or Wilcoxon Rank Sum Test with Bonferroni's Adjustment).

Growth data were initially evaluated for normal distribution and homogeneity of variances. In those cases where the data were not normally distributed or homogenous, the data were analyzed using either the nonparametric test or the heteroscedastic t-test. In addition to growth being evaluated as average dry weight of the surviving organisms, growth was also analyzed as biomass (average dry weight of surviving organisms divided by the number of initial organisms).

The survival and growth data for *C. dilutus* and *H. azteca* for each investigative sample were compared statistically (p= 0.05) to the corresponding site-specific reference sediment sample.

Whole Sediment Toxicity Test Quality Criteria

The *C. dilutus* exposed to the laboratory control sediment and to the water only control exceeded the minimum survival (70 percent) and growth (0.48 mg AFDW at test termination) criteria for acceptable controls for the 10-day and 20-day *C. dilutus* tests (Appendix D1, Tables 1 through 4). The acceptability requirements for survival and growth for the *C. dilutus* test can be found in U.S. EPA Method 600/R-99/064, Tables 12.1 and 15.3. After 10-days, *C. dilutus* had 98.8 and 100 percent survival and an average growth (AFDW) of 1.24117 mg and 1.24275 mg in the laboratory control sediments and 100 and 98.8 percent survival and an average growth (AFDW) of 0.85438 mg and 1.01208 mg in the water only controls.

C. dilutus exposed to the laboratory control sediment for 20-days had 93.8 and 96.9 percent survival and an average growth (AFDW) of 1.96654 mg and 1.61938. Whereas after 20-day the *C. dilutus* exposed to the water only controls had 93.8 and 91.7 percent survival and an average growth (AFDW) of 1.30943 mg and 0.99202 mg.

The *H. azteca* test organisms exposed to the GLEC laboratory control sediment and to the water only control exceeded the minimum survival criteria (80%), and displayed acceptable measurable growth (Appendix C1, Tables 7 through 10). The requirements for acceptable survival and growth for the 10-day and 28-day *H. azteca* toxicity tests can be found in U.S. EPA /600/R-99/064, Tables 11.2 and 14.3. After 10-days, the *H. azteca* had 93.8 and 100 percent survival in the laboratory control sediments and there was 97.5 and 96.3 percent survival in the water only controls. Additionally, after 28-days, the *H. azteca* had 96.3 and 97.5 percent survival in the laboratory control sediments and there was 97.5 and 93.8 percent survival in the water only controls.

The overlying water quality measurements (*C. dilutus*: Appendix D1, Tables 5 and 6; *H. azteca:* Appendix C1, Tables 11 and 12) were also within the acceptable limits following the U.S. EPA testing protocol. Overlying water chemistry measurements were completed each day prior to the AM overlying water renewal. Daily mean temperatures were 23°C ± 1 °C, dissolved oxygen (DO) was maintained above 2.5 mg/L in the overlying water; and there were no variations greater than 50% in overlying water hardness or alkalinity measurements within each test type.

In the 10-day *C. dilutus* whole sediment toxicity tests, dissolved oxygen ranged between

2.8 and 7.3 mg/L in all tests. Hardness varied between 120 mg/L$CaCO_3$ and 168 mg/L $CaCO_3$. Alkalinity varied between 92 mg/L$CaCO_3$ and 144 mg/L $CaCO_3$.

In the 20-day *C. dilutus* whole sediment toxicity tests, dissolved oxygen ranged between 2.6 and 7.1 mg/L in all tests. Hardness varied between 116 mg/L$CaCO_3$ and 172 mg/L $CaCO_3$. Alkalinity varied between 94 mg/L$CaCO_3$ and 146 mg/L $CaCO_3$.

In the 10-day *H. azteca* whole sediment toxicity tests, dissolved oxygen ranged between 4.2 and 7.8 mg/L in all tests. Hardness varied between 128 mg/L$CaCO_3$ and 160 mg/L $CaCO_3$. Alkalinity varied between 88 mg/L$CaCO_3$ and 132 mg/L $CaCO_3$.

In the 28-day *H. azteca* whole sediment toxicity tests, dissolved oxygen ranged between 5.0 and 7.5 mg/L in all tests. Hardness varied between 120 mg/L$CaCO_3$ and 172 mg/L $CaCO_3$. Alkalinity varied between 102 mg/L$CaCO_3$ and 146 mg/L $CaCO_3$.

Total ammonia over 10-day and 28-day for *H. azteca* varied between 0.08 mg/L and 3.23 mg/L and 0.04 mg/L and 3.23 mg/L, respectively in the overlying water among all sediment types. During the 10-day and 20-day for *C. dilutus*, total ammonia varied between 0.04 mg/L and 3.25 mg/L in the overlying water among all sediment types. These ammonia concentrations are far below the toxicity threshold for freshwater aquatic organisms.

Reference toxicant tests were conducted on *C. dilutus* and *H. azteca* using sodium chloride

as the toxicant with each batch of test organisms. These tests demonstrate the health and quality of the organisms, food, and test water. The recent 96-hour LC_{50} values and the standard reference toxicant charts associated with these whole sediment toxicity tests for both the *C. dilutus* and *H. azteca*, are included in Appendix F.

Summary of Test Procedure: 4-day *Lumbriculus variegatus* Toxicity Screening Test

Prior to conducting the 28-day bioaccumulation tests, 4-day *L. variegatus* toxicity screening tests were conducted. The 4-day *L. variegatus* toxicity screening tests were initiated with each of the twelve sediment samples (as listed in the previous table) and one GLEC laboratory control sediment to determine if the sediment samples were acutely toxic to *L. variegatus*.

Adult *L. variegatus* (purchased from Eastern Aquatics) were used to initiate the 4-day toxicity screening tests. *L. variegatus* were continuously exposed for 4-days to each of the twelve investigative sediment samples and to the laboratory control sediment.

There were four replicate samples for each investigative sediment sample and the laboratory control sediment sample; each *L. variegatus* replicate was initiated with 10 animals. The *L. variegatus* were exposed in 470 mL glass test chambers, each containing 100 mL of whole sediment and 175 mL of overlying water.

Prior to adding the whole sediment to each test chamber, the GLEC laboratory control sediment as well as each investigative sediment sample were thoroughly homogenized using a pre-cleaned stainless steel all-purpose mixer and a spoon until a uniform color and texture was achieved.

The homogenized sediment was then added to each test chamber using a pre-cleaned stainless-steel spoon. After the addition of the sediment to the test chambers, the overlying water was immediately added; this was considered to be test day -1 (October 21, 2021 and January 2, 2022). Test organisms were randomly added to each replicate test chamber the following day (test day 0) October 22, 2021 and January 3, 2022.

Overlying water was intermittently supplied to each test chamber at least twice daily (once every 12-hours) via a static-renewal water delivery system. The overlying water for each investigative sediment sample and the laboratory control sediment consisted of de-chlorinated municipal (Traverse City, Michigan) tap (Lake Michigan sourced) water, with an average hardness of 132 mg/L and an average alkalinity of 102 mg/L. Temperature, dissolved oxygen (DO), pH, and specific conductance of the overlying water was measured daily prior to use.

The test chambers were placed in a temperature-controlled water bath under the specified conditions of 23 ± 1°C; photoperiod 16 hours light: eight hours dark; and light intensity of 100-1000 lux.

Temperature (23 ± 1ºC) and the DO (≥ 2.5 mg/L) concentrations of the overlying water in the test chambers were measured daily in two alternating replicates for each test sediment, and the results were recorded on the laboratory bench data sheets.

Alkalinity, hardness, pH, conductivity, and total ammonia (as N) were measured on test days 0 and 4, in the overlying water for the *L. variegatus* tests (Table 15 of Appendix E).

Observations of organism behavior and anomalies observed within the sediment were made daily for each test chamber and recorded on the laboratory bench data sheets. The number of *L. variegatus* surviving in each replicate test chamber was recorded at test termination (4 days), and a summary of the percent survival at test termination is provided in Appendix E, Table 13. The *L. variegatus* 4-day toxicity screening test laboratory data sheets are also provided in Appendix E.

4-day Screening Toxicity Test Quality Criteria

L. variegatus survival after 4-days of exposure in the investigative samples and laboratory control sediment were all greater than 90 percent, which the met the performance-based criteria as specified in the EPA method (Table 13.4) The number surviving and the average percent survival are summarized in Table 13 of Appendix E.

The overlying water quality measurements (Tables 15a and 15b of Appendix E) were also within the acceptable limits following the U.S. EPA testing protocol (i.e., daily mean temperatures were 23 ± 1 °C; DO was maintained above 2.5 mg/L in the overlying water and there were no variations greater than 50% in overlying water hardness or alkalinity

measurements within each test type. Total ammonia over the duration of 4 days varied between 0.05 mg/L and 1.85 mg/L in the overlying water among all sediment types). Consequently, the *L. variegatus* 4-day toxicity screening tests were conducted following the standard protocols and are valid assessments of sediment toxicity.

Reference toxicant testing was conducted on *L. variegatus* using sodium chloride as the toxicant with each batch of test organisms. These tests demonstrate the health and quality of the organisms, food, and test water. The recent 96-hour LC_{50} values and the standard reference toxicant charts associated with these whole sediment toxicity tests for *L. variegatus*, are included in Appendix F.

Consequently, the 28-day *L. variegatus* bioaccumulation tests were initiated with all investigative sediments and were divided into a group of six, four, and two investigative sediment samples. These groups of sediment samples had staggered initiation days of November 2, 2021, November 16, 2021, and January 11, 2022.

Summary of Test Procedures: 28-day *L. variegatus* Bioaccumulation Tests

Following the 4-day survival whole sediment toxicity screening tests, adult *L. variegatus* were continuously exposed for 28-days to the investigative sediment samples. The sediment samples were divided into three groups of six, four, and two sediment samples each, each group of sediment samples had one laboratory control sediment, and each group was initiated on a different day. On November 2, 2021, the following six investigative sediment samples and one laboratory control were initiated: Clarks Marsh, AuSable Ref, Fort Gratiot Ref, Fort Gratiot Ref, Huron/Norton Creek Ref, and Huron/Norton Creek. The following four investigative sediment samples; Beaver Dam Pond, Harts Lake Reference, Tannery, and Rogue River Reference, and one GLEC laboratory control sediment was initiated on November 16, 2021. The remaining two investigative sediment samples: Pigeon River Paper and Pigeon River Reference were initiated on January 11, 2022.

Adult *L. variegatus* were exposed in 3-liter (L) glass tanks, each containing 1.0 L of whole sediment and 2.0 L of overlying water. Temperature-controlled overlying water was supplied to each test chamber via a continuous-renewal water delivery system at a rate of 5 mL/min ($\pm$ 2 mL/min). All test chambers were aerated at approximately 100 bubbles per minute for the full duration of the test. The overlying water consisted of de-chlorinated municipal (Lake Michigan) water of moderate hardness (~140 mg/L).

Consistent with the test procedure and the study plan, there were seven replicate tanks for each sediment sample. Of the seven replicate test tanks, two test chambers were used as “non-biological” replicates and five test tanks were used for the 28-day whole sediment bioaccumulation tests. Of the five test tanks, for the controls and investigative sediment samples, each was initiated with 25 grams wet weight of *L. variegatus* in order to meet the minimum tissue weight required by the chemistry laboratory. As specified, the *L. variegatus* tissue sample submitted for laboratory testing represents a composite sample of the tissue recovered from all 5 replicates per sample, to achieve at least 55

grams of wet weight tissue. A subset sample (approximately 100 grams) of *L. variegatus* was frozen and saved for background chemistry analysis on Day 0. The analysis of the two "non-biological" replicates were used as a surrogate to measure total PFAS and PFAS from porewater at test termination on days 10 and 28. Test termination whole sediment and porewater cannot be collected due to the destruction of the sample to recover the organisms

The test chambers were placed in a temperature-controlled water bath under the specified conditions of 23 ± 1°C; photoperiod of 16 hours light and 8 hours dark; and ambient lighting. The rate of the temperature-controlled overlying water supplied to each test chamber was measured daily. Water temperature and dissolved oxygen were monitored daily in two random replicates for each test sample. Alkalinity, hardness, pH, DO, conductivity, temperature, and total ammonia were measured at Day 0 (test initiation) and on days 7, 14, 21, and 27 (Appendix E, Tables 16a, 16b, and 16c). All test chambers were checked daily to assess organism behavior and no unusual observations were noted with the test organisms.

Bioaccumulation Test Quality Criteria

The overlying water quality measurements (Appendix E, Tables 16a, 16b, 16c) were also within the acceptable limits following the U.S. EPA testing protocol (i.e., daily mean temperatures were 23 ± 1 °C; dissolved oxygen (DO) was maintained above 2.5 mg/L in the overlying water and there were no variations greater than 50% in overlying water hardness or alkalinity measurements within each test type. Total ammonia over the duration of 28 days varied between 0.04 mg/L and 1.44 mg/L in the overlying water among all sediment types.). Consequently, the *L. variegatus* 28-day bioaccumulation tests were conducted following the standard protocols and are valid assessments of sediment toxicity.

At test termination, the test organisms were recovered from each replicate chamber per sediment using reasonable effort until approximately 15 grams or a minimum of 11 grams of *L. variegatus* per replicate was recovered. The *L. variegatus* tissue from each of the five replicate chambers per sediment sample were composited and the target composite *L. variegatus* tissue weight per sediment was 55 grams. The final average and total depurated wet weight (g) of surviving *L. variegatus* was determined at test termination and is located in Appendix E, Table 14.

After 28 days of exposure, the surviving *L. variegatus* were depurated for 24 hours in overlying water to purge all gut contents. After the 24-hour depuration period, the surviving *L. variegatus* were weighed, then frozen in glass jars and shipped to PACE laboratory in Green Bay, Wisconsin for tissue analysis.

Analytical Methods

Samples were analyzed for physical and chemical parameters as specified in Table 2 and following the project QAPP. A summary of the sample analysis parameter, matrix and analysis methods is given in Table 4.

TABLE 4. Analytical Methods

Parameter	Matrix	SOP	Reference Method
36 PFAS compounds	Sediment, Surface water	Pace ME003NI-04 Determination of Per- and Polyfluoroalkyl Substances (PFAS) by LC/MS/MS (Isotope Dilution)	Lab SOP, PFAS by ID-SPE
35 PFAS compounds	Biota	Pace ENV-SOP-MIN4-0178 Determination of Selected 36 Per- and Polyfluoroalkyl Substances (PFAS) by LC/MS/MS (Isotope Dilution)	Lab SOP, PFAS by ID
10 PFAS compounds	Sediment porewater	Pace ME00217-04 Determination of Per- and Polyfluoroalkyl Substances (PFAS) by LC/MS/MS (Direct Aqueous Injection)	Lab SOP, PFAS by ID-DAI
pH	Sediment	Pace ME0014S	EPA Method SW846 9040C/9045D
CEC	Sediment	Pace ENV-SOP-SHRT-0046	EPA Method 200.7/SW846 9081
Grain Size	Sediment	Pace 158 Grain Size Analysis	ASTM D422
TOC	Sediment	Pace GBAY-0051	Lloyd Khan Method
PCB Congeners (209)	Sediment	Pace ENV-SOP-MIN4-0031 Preparation and Analysis of Samples for the Determination of Chlorinated Biphenyl Congeners by EPA 1668A/C	EPA Method 1668A and 1668C
PAHs (17 parent PAHs)	Sediment	Pace ENV-SOP-GBAY-0077-Rev.01 Microwave Extraction for the Determination of PAH, BNA, and TPH-DS in a Solid Matrix; ENV-SOP-GBAY-0081 Determination of Semi-Volatile Organics by GC/MS (Selective Ion Monitoring)	EPA Method SW846 3546C (extraction); 8270C SIM (analysis)
Elements As, Ba, Cd, Ca, Cr, Cu, Fe, Pb, Mg, Mn, Ni, Se, Ag, Zn	Sediment	Pace ENV-SOP-GBAY-0009 Determination of Metals by Inductively Coupled Plasma (ICP) Spectroscopy by 6010D and 200.7	EPA Methods 6010D/200.7

Parameter	Matrix	SOP	Reference Method
Mercury (Hg)	Sediment	Pace ENV-SOP-GBAY-0013 Determination of Mercury by Cold Vapor Atomic Absorption Spectroscopy - CETAC M-7500 (7470A/7471B_245.1)	EPA Method SW846 7470A/7471B/245.1
pH	Sediment porewater	Pace ENV-SOP-GBAY-0047-Rev.02 Measurement of pH in Water, Soil, and Waste_SM4500H+_9040C_ 9045D	SM 4500-H+ or EPA SW846 9040C
Ammonia-Nitrogen	Sediment porewater	Pace ME001GZ-06 Ammonia Nitrogen by Gas Diffusion Segmented Flow	EPA Method 350.1
DOC	Sediment porewater	Pace ME0016Q-05 Total Organic Carbon (TOC)	SM 5310 C-2011 and SW-846 9060A
Hardness	Sediment porewater	Pace ME001H5-05 Total Hardness as mg CaCO3/L by EDTA Titrimetric Method and Calculation Method	SM 2340 C-2011/SM 2340 B-2011
Hardness	Water	GLEC SOP LAB 1002 Determination of Total Hardness (mg/L as $CaCO_3$) of a Water Sample (Titrimetric, EDTA)	SM 2340 C
Tissue processing	Biota	Pace ENV-SOP-GBAY-0129 Sample Homogenization, Compositing and Sub-Sampling	Not applicable
Porewater Generation	Sediment porewater	Appendix D of Pace ME003NI-04 Determination of Per- and Polyfluoroalkyl Substances (PFAS) by LC/MS/MS (Isotope Dilution)	Lab SOP, PFAS by ID-SPE

RESULTS

Analytical Chemistry Results

Sediment and surface water samples were field collected as outlined in the QAPP from each of the 12 sites noted in Table 1. These whole sediment and surface water samples were successfully field collected from each of the six investigative areas and the corresponding reference locations and analyzed for PFAS analytes, percent moisture, metals, PAH's, total organic carbon, CEC, and grain size. Sample collection dates and the corresponding toxicity test dates are summarized in Table 3. Surface water samples were also collected for the analysis of PFAS compounds in the surface water. Analytical reports summarizing all PFAS and non-PFAS compounds are given in Appendix B.

Using the PFAS sediment and water concentrations from the field collection samples, we calculated the sediment water partition coefficients (K_p) for total PFAS and seven individual PFAS compounds for each of the six Michigan sampling locations (Table 5). Likewise, the organic carbon normalized partition coefficient (K_{oc}) was calculated, when possible. Total PFAS K_p values ranged between 1.4 at the Huron Norton Creek location and 3.5 at the Pigeon River location.

Toxic metal concentration of As, Cd, Cu, Cr, Pb, Ni, and Zn, as well as total PAH and total PCB were analyzed for each sample. Sediment quality guidelines (SQG) (MacDonald et al 2000) were compared to the measured concentration from each sediment sample to Threshold Effect Concentrations (TEC) and the SQG, for comparison. TEC's were exceeded for several metals, PAHs, and total PCBs at some locations (Tables 6 and 7) listed below:

- Clark's Marsh sediment, the TEC for lead was exceeded (TEC=35.8 mg/Kg vs 55.2 mg/Kg);
- Huron/Norton Creek sediment, the TEC for all metals were exceeded;
- Rogue River sediment, the TEC for Pb, Cu and total PAHs was exceeded;
- Beaver Dam Pond sediment, the TEC for As, Zn, and total PAH were exceeded; and
- Pigeon River sediment, the TEC for total PCB was exceeded.

Published TEC and probable effect concentration (PEC) for lead is approximately 35.8 mg/Kg and 128 mg/Kg, respectively, so the presence of lead in the Clark's Marsh sediment is not overly concerning at 55.2 mg/Kg. However, the presence of multiple metal and PAHs concentrations that exceeded TECs at the Rogue River and Huron/Norton Creek locations, and the concentration of PAHs at the Beaver Dam Pond location likely contributed to sediment toxicity at those locations. The toxicity of metals is known to be additive, so the co-occurrence of metals likely affect the toxicity of whole sediment. The toxicity of metals, PAH and PCB is also affected by total organic carbon, which may help ameliorate the toxicity of those compounds if the TECs are exceeded. Additionally, arsenic TECs were also exceeded at several reference sites, including Fort Gratiot, AuSable River and the Huron Norton Creek reference sediments. Lastly, due to the general structure of a fluorinated carbon chain and a polar or ionizable "head" group

at one or both ends of a PFAS molecule, the toxicity of PFAS compounds may also be affected by cation equivalents due to the presence of calcium, magnesium, and manganese (Table 6). The suspected influence of the non-PFAS compounds and the sediment parameters that may affect the bioavailability of PFAS as well as other toxics are discussed later in the report.

The concentration of total PFAS in sediment at the time of collection ranged from a high of 570 µg/kg at Clark's Marsh to a low concentration of 17 µg/kg at Beaver Dam Pond. Consequently, the order of PFAS impacted sites from high to low was Clark's Marsh at 570 µg/kg, Fort Gratiot at 73 µg/kg, Rogue River Tannery at 73 µg/kg, Pigeon River Paper at 44 µg/kg, Huron/Norton Creek at 31 µg/kg, and Beaver Dam Pond at 17 µg/kg (Tables 8 and 9).

Subsequent analysis for PFAS compounds in sediment was completed with the same sediment samples at toxicity test initiation and termination and resulted in moderately varying results over time (Tables 8 and 9). Analytical data for the PFAS analysis are given in Appendices B1-B3. Analytical PFAS data at test initiation was the same for the *H. azteca* and *C. dilutus* toxicity tests. Total PFAS concentration at 10-day, 20-day and 28-day test terminations were analyzed separately for the *H. azteca* and *C. dilutus* whole sediment toxicity tests. At test termination, sediment from the 10-day and 28-day *H. azteca* toxicity tests was collected directly from each test chamber replicate to form a composite sample to be analyzed for total PFAS. The total PFAS concentrations recorded at 10-day and 20-day *C. dilutus* test termination were obtained from the two "non-biological" test replicates initiated with the 28-day *L. variegatus* bioaccumulation tests and terminated at test days 10 and 28.

1. For the Clark's Marsh sediment, the total PFAS concentration varied between 570 µg/kg at field collection to 655, 664, and 393 µg/kg at test initiation (both acute and chronic) and test termination at 10-days and 28-days, respectively, in the *H. azteca* toxicity tests. At 10-day and 20-day test termination for the *C. dilutus* tests, the total PFAS concentration was 393 µg/kg and 586 µg/kg, respectively.
2. For the Fort Gratiot sediment, the PFAS concentration varied between 73 µg/kg, at field collection to 87, 75, and 42 µg/kg at test initiation and test termination at 10-days and 28-days, respectively in the *H. azteca tests.* At 10-day and 20-day test termination for the *C. dilutus* tests, the total PFAS concentration was 84 µg/kg and 92 µg/kg, respectively.
3. For the Huron/Norton Creek sediment, the total PFAS concentration in the *H. azteca* tests varied between 31 µg/kg at field collection to 46, 28, and 30 µg/kg at test initiation and test termination at 10-days and 28-days, respectively. At 10-day and 20-day test termination for the *C. dilutus* tests, the total PFAS concentration was 55 µg/kg and 32 µg/kg, respectively.

4. For the Beaver Dam Pond sediment, the total PFAS concentration varied from 17 µg/kg at field collection, to 30 µg/kg at test initiation and varied between, 19 µg/kg, and 18 µg/kg, respectively, in the 10-day and 28-day *H. azteca* toxicity tests at test termination. At 10-day and 20-day test termination for the *C. dilutus* tests, the total PFAS concentration was 16 µg/kg and 299 µg/kg, respectively.
5. For the Rogue River Tannery sediment, the total PFAS concentration varied between 73 µg/kg at field collection, to 196, 158, and 115 µg/kg at test initiation and test termination at 10-days and 28-days, respectively, in the *H. azteca* tests. At 10-day and 20-day test termination for the *C. dilutus* tests, the total PFAS concentration was 175 µg/kg and 156 µg/kg, respectively.
6. For the Pigeon River sediment, the total PFAS concentration varied from 44 µg/kg at field collection to 31 µg/kg at the initiation of the 10-day *H. azteca* toxicity tests. However, the concentration of total PFAS at the termination of the 10-day and 28-day toxicity tests increased to 218 µg/kg and 135 µg/kg. The observed increase in total PFAS concentration in the Pigeon River sediment at test termination corresponded with a considerable decrease in the percent solids measured in those samples. At 10-day and 20-day test termination for the *C. dilutus* tests, the total PFAS concentration was 44 µg/kg and 203 µg/kg, respectively.

Although the total PFAS concentrations measured over time between collection, test initiation and test termination were variable, the measured % PFOS at 10- and 28-day test termination was similar to the % PFOS at test initiation (Tables 10-15), which, with the noted exception of the Pigeon River samples, would indicate that the variability is total PFAS concentration was acceptable.

PFOS was the dominant PFAS compound in all samples. PFOS is made up 73-100% of total PFAS in sediment at field collection, except for the Pigeon River sediment samples. Where PFOS made up approximately 23% of the total PFAS.

The total PFAS sediment coefficient of variation (CV) varied between 22% and 39%, which is probably respectable for repeated sediment sampling and analysis from the same sample. However, the CV for the Pigeon River samples was 82% which is problematic and confounds the interpretation of the Pigeon River results.

Likewise, the CV for the PFOS varied between 20% and 27% with again the exception of the Pigeon River sample which has a PFOS CV of 80%.

The total PFAS and PFOS analyses of the Pigeon River sample are inconclusive and difficult to explain and may appear related to the apparent change in % solids over time, which would not be expected.

Sediment PFAS porewater concentrations at test initiation ranged from 3,749 ng/L at Clark's Marsh to 1,744 ng/L at Fort Gratiot, 725 ng/L at Beaver Dam Pond, 660 ng/L at Rogue River Tannery, 283 ng/L at Huron/Norton Creek and 179 ng/L at Pigeon River (Tables 14 and 15). Note that the 10-day and 28-day porewater PFAS concentration

were measured in “non-biological” replicates and conducted concurrently with the 28-day *L. variegatus* bioaccumulation tests.

With the apparent variability of both PFAS and PFOS concentrations over time the biological results from the toxicity tests were compared to the total PFAS and PFOS concentrations measured at the time of field collection for comparison purposes.

TABLE 5. PFAS Sediment Water Partition Coefficients (K_p) for Six Michigan Sampling Locations for The Michigan PFAS Sediment Assessment

Site	**Ft. Gratiot**		**Clark's Marsh**		**Huron Norton Cr.**		**Rogue River**		**Beaver Dam Pond**		**Pigeon River**	
Partition coefficient	log Kp	log Koc	log Kp	log Koc	log Kp	log Koc	log Kp	log Koc	log Kp	log Koc	log Kp	log Koc
PFAS compound	L/kg	L/kg-OC	L/kg	L/kg-OC	L/kg	L/kg-OC	L/kg	L/kg-OC	L/kg	L/kg-OC	L/kg	L/kg-OC
PFOA	1.0	2.3	1.8	2.0			1.9	3.0			2.4	3.4
PFNA	1.7	3.0										
PFHxS			1.7	2.0			2.3	3.3	1.5	2.4		
PFOS	1.6	2.9	2.5	2.8	3.3	4.0	2.6	3.6	2.2	3.1	3.2	4.2
PFOSA			3.3	3.6			3.0	4.1			3.5	4.5
EtFOSAA							3.1	4.1				
MeFOSAA							3.3	4.4				
Total PFAS (Kp)	1.5	2.9	2.4	2.7	1.4	2.0	2.6	3.6	1.8	2.7	3.5	4.4
Water PFAS Conc. (ng/L)	2115.0		2176.0		1434.0		234.4		364.3		15.2	
Sediment PFAS Conc. (mg/Kg)	73		570		31		73		17		44	
Total Organic Carbon (mg/Kg)	47,000		580,000		232,000		92,800		123,000		104,000	
partition coefficients based on sediment and surface water concentrations measured at collection												
PFAS included in table if partition coefficients could be calculted for any sites												

TABLE 6. Percent Moisture And Metal Parameters Analyzed At Time Of Sample Collection From Each Investigative Contaminant And Corresponding Reference Location For The Michigan PFAS Sediment Assessment

		Location	Fort Gratiot	Fort Gratiot Reference	Clark's Marsh	Au Sable River (Clark's Marsh Reference)	Huron/ Norton Creek	Huron/ Norton Creek Reference	Rogue River	Rogue River Reference	Beaver Dam Pond	Hart's Lake (Beaver Dam ond Reference	Pigeon River	Pigeon River Reference	Laboratory Control	Consensus Based TEC
Parameter	**CAS #**	**Units**	**Result**	**Result**	**Result**	**Result**	**Result**	**Result**	**Result**	**Result**	**Result**	**Result**	**Result**	**Result**	**Result**	
Percent Moisture		%	48.9	50.9	95.6	79.1	86.8	75.1	82.5	56.3	83.9	93.0	76.3	62.3	93.9	
Arsenic	7440-38-2	mg/kg	6.6	**9.9**	<32.7	**12.6**	**37.9**	**14.2**	9.3J	3.2J	**17.0**	<19.6	**22.7**	**16.1**	**29.9J**	**9.79**
Barium	7440-39-3	mg/kg	102	101	47.9	166	328	118	163	43.5	181	249	137	80.6	206	
Cadmium	7440-43-9	mg/kg	0.58J	0.60J	<3.0	<0.61	**1.0J**	<0.50	0.80J	<0.28	<0.75	<1.8	0.46 J	0.24 J	<2.2	**0.99**
Calcium	7440-70-2	mg/kg	6360	19100	30400	139000	117000	58600	163000	6320	199000	425000	41200	23900	32500	
Chromium	7440-47-3	mg/kg	20.0	24.4	<6.2	23.2	**73.7**	7.0	**78.4**	4.3	13.4	11.7J	9.2	5.9	37.9	**43.4**
Copper	7440-50-8	mg/kg	12.7	15.4	7.7J	13.2	**40.6**	23.5	29.3	4.6	17.5	13.2J	16.2	7.3	27.9	**31.6**
Iron	7439-89-6	mg/kg	19000	26300	10100	29200	53400	15300	19100	6950	17600	22500	28200	21100	38100	
Lead	7439-92-1	mg/kg	16.2	19.4	**55.2**	26.3	**50.5**	11.2	**47.2**	4.6	32.4	26.6J	23.1	7.2	20.3J	**35.8**
Magnesium	7439-95-4	mg/kg	4680	6810	1840J	15900	9830	4740	8300	1420	6630	12800	4660	2670	10700	
Manganese	7439-96-5	mg/kg	902	610	517	2090	1070	327	421	315	760	5380	1760	1130	3910	
Nickel	7440-02-0	mg/kg	17.3	23.3	<5.9	17.0	**34.7**	6.1	11.2	2.7	10.5	12.3J	9.8	6.0	16.0J	**22.7**
Selenium	7782-49-2	mg/kg	<2.4	<2.6	<29.2	<6.0	<9.7	<4.9	<7.3	<2.8	<7.4	<17.5	<2.7	<1.7	<21.3	
Silver	7440-22-4	mg/kg	<0.56	<0.61	<6.8	<1.4	<2.3	<1.2	<1.7	<0.65	<1.7	<4.1	<0.63	<0.40	<5.0	
Mercury	7439-97-6	mg/kg	0.024J	<0.020	<0.067	<0.043	0.15J	<0.037	0.12J	<0.021	<0.059	<0.13	0.14	0.033J	<0.16	**0.18**
Zinc	7440-66-6	mg/kg	74.4	61.9	78.8J	72.5	**271**	47.4	161	18.5	**132**	82.0	61.0	32.8	80.0	**121**

Note: Bolded and underline values exceeded the TEC

TABLE 7. Total PAH, PH, Total Organic Carbon, Cation Equivalent and Grain Size in Sediment from the Michigan PFAS Sediment Assessment

	Location	Fort Gratiot	Fort Gratiot Reference	Clark's Marsh	Au Sable River (Clark's Marsh Reference)	Huron/ Norton Creek	Huron/ Norton Creek Reference	Rogue River	Rogue River Reference	Beaver Dam Pond	Hart's Lake (Beaver Dam ond Reference	Pigeon River	Pigeon River Reference	Laboratory Control	Consensus Based TEC
Parameter	**Units**	**Result**	**Result**	**Result**	**Result**	**Result**	**Result**	**Result**	**Result**	**Result**	**Result**	**Result**	**Result**	**Result**	
2-Methylnaphthalene	ug/kg	<4.8	<5.0	<55.4	<11.7	<18.6	<9.8	<14.0	<5.4	<15.2	<34.8	<30.9	<19.5	<39.8	
Acenaphthene	ug/kg	<4.2	<4.4	<49.1	<10.3	<16.5	<8.7	<12.4	<4.8	<13.4	<30.8	<27.4	<17.3	<35.3	
Acenaphthylene	ug/kg	<4.1	<4.3	<47.7	<10.1	<16.0	<8.5	14.5J	<4.7	14.7J	<30.0	<26.7	<16.8	<34.3	
Anthracene	ug/kg	<4.1	<4.2	<47.0	<9.9	<15.8	<8.3	22.7J	<4.6	27.3J	<29.5	<26.2	<16.6	<33.8	
Benzo(a)anthracene	ug/kg	7.6J	<4.4	<48.9	<10.3	66.1J	<8.7	122	7.6J	146	<30.7	<27.3	<17.3	<35.2	
Benzo(a)pyrene	ug/kg	4.9J	<3.9	<43.0	<9.1	80.3J	<7.6	158	4.7J	250	<27.0	<24.0	<15.2	<30.9	
Benzo(b)fluoranthene	ug/kg	6.1J	<4.7	<52.6	12.4J	125J	<9.3	226	7.1J	443	<33.0	<29.4	<18.5	<37.8	
Benzo(g,h,i)perylene	ug/kg	<5.7	<6.0	<66.4	<14.0	71.4J	<11.8	109	<6.5	294	<41.7	<37.1	<23.4	<47.8	
Benzo(k)fluoranthene	ug/kg	<4.2	<4.3	<48.4	<10.2	52.5J	<8.6	97.6	<4.8	182	<30.4	<27.0	<17.1	<34.8	
Chrysene	ug/kg	<6.2	<6.4	<71.4	<15.0	96.3J	<12.6	173	<7.0	314	<44.8	<39.9	<25.2	<51.4	
Dibenz(a,h)anthracene	ug/kg	<4.5	<4.7	<52.4	<11.0	<17.6	<9.3	24.3J	<5.2	61.8J	<32.9	<29.3	<18.5	<37.7	
Fluoranthene	ug/kg	10.8J	<4.0	<44.8	18.3J	176	10.2J	302	11.3J	514	37.8J	26.1 J	<15.8	<32.2	
Fluorene	ug/kg	<3.9	<4.1	<45.4	<9.6	<15.2	<8.0	<11.4	<4.5	<12.4	<28.5	<25.4	<16.0	<32.7	
Indeno(1,2,3-cd)pyrene	ug/kg	<6.8	<7.1	<78.9	<16.6	54.5J	<14.0	87.2J	<7.8	237	<49.5	<44.1	<27.8	<56.7	
Naphthalene	ug/kg	<3.2	<3.3	<36.9	<7.8	<12.4	<6.5	<9.3	<3.6	<10.1	<23.2	<20.6	<13.0	<26.5	
Phenanthrene	ug/kg	4.7J	<3.9	<43.4	<9.1	57.8J	<7.7	108	5.9J	129	<27.2	<24.2	<15.3	<31.2	
Pyrene	ug/kg	7.3J	<5.0	<55.6	12.6J	127J	<9.9	232	8.0J	389	<34.9	<31.1	<19.6	<40.0	
TOTAL PAH	ug/kg	41.4	0	0	43.3	907	10.2	**1676**	44.6	**3001**	378	26.1	0	0	**1,610**
TOTAL PCB	ug/kg	1.865	1.3	16.5	29.2	48.5	4.5	**26**	0.7	**7.13**	8.6	74.3	2.6		**60**
pH at 25 Degrees C	Std.	7.4	7.4	7.3	7.0	7.1	7.4	7.3	7.3	7.2	7.3	7.4	7.5	7.6	
Total Organic Carbon	mg/kg	47000	20800	580000	89400	232000	77700	92800	27400	123000	144000	104000	48500	73400	
CEC	meq/100	31.2	20.4	37.7	46.8	90.3	36.5	50.3	20.2	52.4	38.5	52.3	32.1	4.47	
Grain Size Fractional Compon	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	%	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	
	%	0.2	4.3	0.0	0.0	0.9	2.6	0.0	0.2	0.0	0.0	0.1	1.0	0.0	
	%	1.0	4.2	0.0	0.0	0.7	4.2	0.0	0.1	0.0	0.0	0.2	1.5	0.0	
	%	7.7	15.8	22.0	20.2	17.6	13.6	8.5	2.8	5.6	5.4	5.9	10.9	0.8	
	%	33.5	30.2	32.8	14.1	24.6	56.8	19.7	80.3	27.3	36.4	53.7	62.0	95.5	
	%	41.6	31.9	40.3	41.5	45.9	20.9	57.1	15.3	55.6	50.3	34.6	21.9	3.6	
	%	16.0	13.6	4.9	24.2	10.3	1.9	14.7	1.3	11.5	7.9	5.5	2.7	0.1	

Note: Bolded and underline values exceeded the TEC

TABLE 8. Total PFAS Concentrations at Six Investigative Sites and Corresponding Reference Sites at Times of Field Collection, Test Initiation, 10-Day Test Termination and 28-Day Test Termination In *Hyalella Azteca* Survival and Growth Whole Sediment Toxicity Tests from The Michigan PFAS Sediment Assessment

Sample ID	Total PFAS at Sample Field Collection (µg/Kg)	Total PFAS of Sediment at Test Initiation (µg/Kg)	Total PFAS of Sediment at 10-Day Test Termination (µg/Kg)	Total PFAS of Sediment at 28-Day Test Termination (µg/Kg)
AuSable Ref	ND	ND	ND	ND
Clarks Marsh	570	655	664	393
FT Gratiot Ref	ND	ND	ND	ND
FT Gratiot	73	87	75	42
Huron/Norton Cr. Ref	ND	ND	ND	ND
Huron/Norton Cr.	31	46	28	30
Harts Lake Ref	ND	5	ND	ND
Beaver Dam Pond	17	30	19	18
Rogue River Ref	ND	ND	ND	ND
Rogue River Tannery	73	196	158	115
Pigeon River Ref	ND	ND	ND	ND
Pigeon River Paper	44	31	218	135

Table 9. Total PFAS Concentrations At Six Investigative Sites and **Corresponding Reference Sites At Times of Field Collection, Test Initiation, 10-Day Test Termination and 20-Day Test Termination In *Chironomus Dilutus* Survival and Growth Whole Sediment Toxicity Tests from the Michigan PFAS Sediment Assessment**

Sample ID	Total PFAS at Sample Field Collection (µg/Kg)	Total PFAS of Sediment at Test Initiation (µg/Kg)	Total PFAS of Sediment at 10-Day Test Termination (µg/Kg)	Total PFAS of Sediment at 28-Day Test Termination (µg/Kg)
AuSable Ref	ND	ND	ND	ND
Clarks Marsh	570	655	393	586
FT Gratiot Ref	ND	ND	ND	ND
FT Gratiot	73	87	84	92
Huron/Norton Cr. Ref	ND	ND	ND	ND
Huron/Norton Cr.	31	46	55	32
Harts Lake Ref	ND	5	ND	ND
Beaver Dam Pond	17	30	16	299
Rogue River Ref	ND	ND	ND	ND
Rogue River Tannery	73	196	175	156
Pigeon River Ref	ND	ND	ND	ND
Pigeon River Paper	44	31	44	203

Whole Sediment Toxicity Test Results (*Chironomus dilutus and Hyalella azteca)*

GLEC completed the analysis of the 10-day and 20-day *Chironomus dilutus* survival and growth whole sediment toxicity tests and the 10-day and 28-day *Hyalella azteca* survival and growth whole sediment toxicity tests with each of the twelve sediment samples collected from the six predetermined regions (sites) of Michigan with varying physical properties and PFAS concentrations.

The sample identification numbers, survival, and growth test results for the twelve investigative sediment samples and a laboratory control are summarized and provided in the following tables in the specified Appendices:

Appendix D1 Table 1: 10-Day *Chironomus dilutus* (*C. dilutus*) Average Percent Survival
Appendix D1 Table 2: 10-Day *C. dilutus* Average Growth and Biomass Estimates (expressed as average ash-free-dry-weight (AFDW))
Appendix D1 Table 3: 20-Day *C. dilutus* Average Percent Survival
Appendix D1 Table 4: 20-Day *C. dilutus* Average Growth and Biomass Estimates (expressed as average ash-free-dry-weight (AFDW))
Appendix C1 Table 7: 10-Day *Hyalella azteca* (*H. azteca*) Average Percent Survival
Appendix C1 Table 8: 10-Day *H. azteca* Average Growth and Biomass Estimates
Appendix C1 Table 9: 28-Day *H. azteca* Average Percent Survival
Appendix C1 Table 10 :28-Day *H. azteca* Average Growth and Biomass Estimates

Tables 10, 11 and 12 compare the total PFAS, porewater PFAS and PFOS concentrations at sample collection, test initiation and 10- and 28-day test termination for the *H. azteca* whole sediment toxicity tests. Tables 13, 14 and 15 compare the total PFAS, porewater PFAS and PFOS concentrations at sample collection, test initiation and 10- and 28-day test termination for the *C. dilutus* whole sediment toxicity tests.

TABLE 10. Total PFAS Concentrations and Resultant *Hyalella Azteca* Survival and Growth Results from Whole Sediment Toxicity Tests for the Michigan PFAS Sediment Assessment

					10-Day *Hyalella azteca*			28-Day *Hyalella azteca*		
Sample ID	**Total PFAS at Sample Field Collection (µg/Kg)**	**Total PFAS in Sediment at Test Initiation (µg/Kg)**	**Total PFAS in Sediment at 10-Day Test Termination (µg/Kg)**	**Total PFAS in Sediment at 28-Day Test Termination (µg/Kg)**	**10-Day Mean Percent Survival (%)**	**10-Day Average Growth[1] (mg)**	**10-Day Biomass[2] (mg)**	**28-Day Mean Percent Survival(%)**	**28-Day Average Growth[1] (mg)**	**28-Day Biomass[2] (mg)**
AuSable Ref	ND	ND	ND	ND	96.3	0.12344	0.11850	93.8	0.45199	0.41983
Clarks Marsh	570	655	664	393	97.5	0.12797	0.12513	96.3	0.51603	0.49700
FT Gratiot Ref	ND	ND	ND	ND	95.0	0.10678	0.10075	91.3	0.28708	0.25412
FT Gratiot	73	87	75	42	93.8	0.09073	0.08463	97.5	0.28353	0.27713
Huron/Norton Cr. Ref	ND	ND	ND	ND	100.0	0.11437	0.11437	95.0	0.33773	0.32050
Huron/Norton Cr.	31	46	28	30	100.0	0.10200	0.10200	97.5	0.29792	0.29075
Harts Lake Ref	ND	5	ND	ND	95.0	0.13107	0.12391	91.3	0.40110	0.36388
Beaver Dam Pond	17	30	19	18	95.0	0.11349	0.10738	95.0	0.30447	0.28900
Rogue River Ref	ND	ND	ND	ND	97.5	0.12846	0.12538	92.5	0.53058	0.49137
Rogue River Tannery	73	196	158	115	95.0	0.09753	0.09275	95.0	0.36712	0.34737
Pigeon River Ref	ND	ND	ND	ND	96.3	0.10437	0.10037	96.3	0.25663	0.24613
Pigeon River Paper	44	31	218	135	96.3	0.11424	0.10988	95.0	0.27053	0.25575

[1] Growth is average dry weight of surviving organisms.

[2] Biomass weight is the total dry weight of surviving organisms divided by the initial number of organisms

Shaded and underlined values are statistically significantly different ($p < 0.05$) from corresponding reference sediment.

TABLE 11. Total PFAS in Sediment Porewater And *Hyalella Azteca* Survival and Growth Whole Sediment Toxicity Test Results from the Michigan PFAS Sediment Assessment

		"Non-biological" Replicates		10-Day *Hyalella azteca*			28-Day *Hyalella azteca*		
Sample ID	Total PFAS Porewater at Test Initiation (ng/L)	Total PFAS* Porewater at 10-day Test Termination (ng/L)	Total PFAS* Porewater at 28-day Test Termination (ng/L)	10-Day Mean Percent Survival (%)	10-Day Average Growth[1] (mg)	10-Day Biomass[2] (mg)	28-Day Mean Percent Survival (%)	28-Day Average Growth[1] (mg)	28-Day Biomass[2] (mg)
AuSable Ref	ND	ND	ND	96.3	0.12344	0.11850	93.8	0.45199	0.41983
Clarks Marsh	3,749	1,780	1,188	97.5	0.12797	0.12513	96.3	0.51603	0.49700
FT Gratiot Ref	103	59	22	95.0	0.10678	0.10075	91.3	0.28708	0.25412
FT Gratiot	1,744	1,754	1,994	93.8	0.09073	0.08463	97.5	0.28353	0.27713
Huron/Norton Cr. Ref	14	11	5	100.0	0.11437	0.11437	95.0	0.33773	0.32050
Huron/Norton Cr.	283	253	232	100.0	0.10200	0.10200	97.5	0.29792	0.29075
Harts Lake Ref	ND	16	ND	95.0	0.13107	0.12391	91.3	0.40110	0.36388
Beaver Dam Pond	725	370	351	95.0	0.11349	0.10738	95.0	0.30447	0.28900
Rogue River Ref	22	ND	ND	97.5	0.12846	0.12538	92.5	0.53058	0.49137
Rogue River Tannery	660	515	496	95.0	0.09753	0.09275	95.0	0.36712	0.34737
Pigeon River Ref	ND	175	5	96.3	0.10437	0.10037	96.3	0.25663	0.24613
Pigeon River Paper	179	88	65	96.3	0.11424	0.10988	95.0	0.27053	0.25575

[1] Growth is average dry weight of surviving organisms.

[2] Biomass weight is the total dry weight of surviving organisms divided by the initial number of organisms.

Shaded and underlined values are statistically significantly different ($p < 0.05$) from corresponding reference sediment.

* Reported analytical results are from the "Non-biological" replicates conducted concurrently with the 28-Day *Lumbriculus* bioaccumulation tests.

TABLE 12. PFOS in Sediment and *Hyalella Azteca* Survival and Growth Whole Sediment Toxicity Test Results from the Michigan PFAS Sediment Assessment

				10-Day *Hyalella azteca*			28-Day *Hyalella azteca*		
Sample ID	**PFOS of Sediment at Test Initiation (µg/Kg)**	**PFOS of Sediment at 10-Day Test Termination (µg/Kg)**	**PFOS of Sediment at 28-Day Test Termination (µg/Kg)**	**10-Day Mean Percent Survival (%)**	**10-Day Average Growth[1] (mg)**	**10-Day Biomass[2] (mg)**	**28-Day Mean Percent Survival (%)**	**28-Day Average Growth[1] (mg)**	**28-Day Biomass[2] (mg)**
AuSable Ref	ND	ND	ND	96.3	0.12344	0.11850	93.8	0.45199	0.41983
Clarks Marsh	580	590	340	97.5	0.12797	0.12513	96.3	0.51603	0.49700
FT Gratiot Ref	ND	ND	ND	95.0	0.10678	0.10075	91.3	0.28708	0.25412
FT Gratiot	83	73	41	93.8	0.09073	0.08463	97.5	0.28353	0.27713
Huron/Norton Cr. Ref	ND	ND	ND	100.0	0.11437	0.11437	95.0	0.33773	0.32050
Huron/Norton Cr.	40	26	28	100.0	0.10200	0.10200	97.5	0.29792	0.29075
Harts Lake Ref	ND	ND	ND	95.0	0.13107	0.12391	91.3	0.40110	0.36388
Beaver Dam Pond	25	17	16	95.0	0.11349	0.10738	95.0	0.30447	0.28900
Rogue River Ref	ND	ND	ND	97.5	0.12846	0.12538	92.5	0.53058	0.49137
Rogue River Tannery	98	81	70	95.0	0.09753	0.09275	95.0	0.36712	0.34737
Pigeon River Ref	ND	ND	ND	96.3	0.10437	0.10037	96.3	0.25663	0.24613
Pigeon River Paper	7	45	24	96.3	0.11424	0.10988	95.0	0.27053	0.25575

[1] Growth is average dry weight of surviving organisms.
[2] Biomass weight is the total dry weight of surviving organisms divided by the initial number of organisms.
Shaded and underlined values are statistically significantly different ($p < 0.05$) from corresponding reference sediment.

TABLE 13. Total PFAS Concentrations and Resultant *Chironomus Dilutus* Survival and Growth Results from Whole Sediment Toxicity Tests for the Michigan PFAS Sediment Assessment

					10-Day *Chironomus dilutus*			20-Day *Chironomus dilutus*		
Sample ID	**Total PFAS at Sample Field Collection (µg/Kg)**	**Total PFAS of Sediment at Test Initiation (µg/Kg)**	**Total PFAS of Sediment at 10-Day Test Termination (µg/Kg)**	**Total PFAS of Sediment at 20-Day Test Termination (µg/Kg)**	**10-Day Mean Percent Survival (%)**	**10-Day Average Growth[1] AFDW (mg)**	**10-Day Biomass[2] AFDW (mg)**	**20-Day Mean Percent Survival (%)**	**20-Day Average Growth[1] AFDW (mg)**	**20-Day Biomass[2] AFDW (mg)**
AuSable Ref	ND	ND	ND	ND	95.0	1.55836	1.47138	91.7	2.28956	2.07749
Clarks Marsh	570	655	393	586	98.8	1.42832	1.41025	91.7	1.98148	1.81329
FT Gratiot Ref	ND	ND	ND	ND	87.1	1.08171	0.95500	86.5	1.74919	1.41468
FT Gratiot	73	87	84	92	98.8	0.97619	0.96338	92.7	1.83552	1.69560
Huron/Norton Cr. Ref	ND	ND	ND	ND	100.0	1.24275	1.24275	95.8	2.00953	1.89714
Huron/Norton Cr.	31	46	55	32	97.5	1.16486	1.13500	88.5	1.92468	1.66940
Harts Lake Ref	ND	5	ND	ND	93.8	1.30075	1.20988	86.5	2.19374	1.83741
Beaver Dam Pond	17	30	16	299	95.0	1.40166	1.32425	96.9	1.79885	1.74655
Rogue River Ref	ND	ND	ND	ND	96.3	1.39909	1.33213	87.5	2.33005	1.94606
Rogue River Tannery	73	196	175	156	100.0	1.19525	1.19525	88.5	2.13504	1.86604
Pigeon River Ref	ND	ND	ND	ND	100.0	1.38300	1.38300	95.8	1.43021	1.37208
Pigeon River Paper	44	31	44	203	100.0	1.42200	1.42200	93.8	1.53825	1.44167

[1] Growth is average Ash-Free-Dry-Weight (AFDW) of surviving organisms

[2] Biomass weight is the total Ash-Free-Dry-Weight of surviving organisms divided by the initial number of organisms

Shaded and underlined values are statistically significantly different ($p < 0.05$) from corresponding reference sediment.

TABLE 14. Total PFAS Concentrations in Porewater and Resultant Chironomus Dilutus Survival and Growth Results from Whole Sediment Toxicity Tests for the Michigan PFAS Sediment Assessment

		"Non-biological" Replicates		10-Day *Chironomus dilutus*			20-Day *Chironomus dilutus*		
Sample ID	**Total PFAS Porewater at Test Initiation (ng/L)**	**Total PFAS* Porewater at 10-day Test Termination (ng/L)**	**Total PFAS* Porewater at 28-day Test Termination (ng/L)**	**10-Day Mean Percent Survival (%)**	**10-Day Average Growth[1] AFDW (mg)**	**10-Day Biomass[2] AFDW (mg)**	**20-Day Mean Percent Survival (%)**	**20-Day Average Growth[1] AFDW (mg)**	**20-Day Biomass[2] AFDW (mg)**
AuSable Ref	ND	ND	ND	95.0	1.55836	1.47138	91.7	2.28956	2.07749
Clarks Marsh	3,749	1,780	1,188	98.8	1.42832	1.41025	91.7	1.98148	1.81329
FT Gratiot Ref	103	59	22	87.1	1.08171	0.95500	86.5	1.74919	1.41468
FT Gratiot	1,744	1,754	1,994	98.8	0.97619	0.96338	92.7	1.83552	1.69560
Huron/Norton Cr. Ref	14	11	5	100.0	1.24275	1.24275	95.8	2.00953	1.89714
Huron/Norton Cr.	283	253	232	97.5	1.16486	1.13500	88.5	1.92468	1.66940
Harts Lake Ref	ND	16	ND	93.8	1.30075	1.20988	86.5	2.19374	1.83741
Beaver Dam Pond	725	370	351	95.0	1.40166	1.32425	96.9	1.79885	1.74655
Rogue River Ref	22	ND	ND	96.3	1.39909	1.33213	87.5	2.33005	1.94606
Rogue River Tannery	660	515	496	100.0	1.19525	1.19525	88.5	2.13504	1.86604
Pigeon River Ref	ND	175	5	100.0	1.38300	1.38300	95.8	1.43021	1.37208
Pigeon River Paper	179	88	65	100.0	1.42200	1.42200	93.8	1.53825	1.44167

[1] Growth is average Ash-Free-Dry-Weight (AFDW) of surviving organisms

[2] Biomass weight is the total Ash-Free-Dry-Weight of surviving organisms divided by the initial number of organisms

* Reported analytical results are from the "Non-biological" replicates conducted concurrently with the 28-Day *Lumbriculus* bioaccumulation tests.

Shaded and underlined values are statistically significantly different ($p < 0.05$) from corresponding reference sediment.

TABLE 15. Total PFOS Concentrations and Resultant *Chironomus Dilutus* Survival and Growth Results from Whole Sediment Toxicity Tests for the Michigan PFAS Sediment Assessment

				10-Day *Chironomus dilutus*			20-Day *Chironomus dilutus*		
Sample ID	**PFOS of Sediment at Test Initiation µg/Kg**	**PFOS* of Sediment at 10-Day Test Termination µg/Kg**	**PFOS* of Sediment at 28-Day Test Termination µg/Kg**	**10-Day Mean Percent Survival (%)**	**10-Day Average Growth[1] AFDW (mg)**	**10-Day Biomass[2] AFDW (mg)**	**20-Day Mean Percent Survival (%)**	**20-Day Average Growth[1] AFDW (mg)**	**20-Day Biomass[2] AFDW (mg)**
AuSable Ref	ND	ND	ND	95.0	1.55836	1.47138	91.7	2.28956	2.07749
Clarks Marsh	580	350	530	98.8	1.42832	1.41025	91.7	1.98148	1.81329
FT Gratiot Ref	ND	ND	ND	87.1	1.08171	0.95500	86.5	1.74919	1.41468
FT Gratiot	83	81	90	98.8	0.97619	0.96338	92.7	1.83552	1.69560
Huron/Norton Cr. Ref	ND	ND	ND	100.0	1.24275	1.24275	95.8	2.00953	1.89714
Huron/Norton Cr.	40	52	30	97.5	1.16486	1.13500	88.5	1.92468	1.66940
Harts Lake Ref	ND	ND	ND	93.8	1.30075	1.20988	86.5	2.19374	1.83741
Beaver Dam Pond	25	16	270	95.0	1.40166	1.32425	96.9	1.79885	1.74655
Rogue River Ref	ND	ND	ND	96.3	1.39909	1.33213	87.5	2.33005	1.94606
Rogue River Tannery	98	96	92	100.0	1.19525	1.19525	88.5	2.13504	1.86604
Pigeon River Ref	ND	ND	ND	100.0	1.38300	1.38300	95.8	1.43021	1.37208
Pigeon River Paper	7	7	38	100.0	1.42200	1.42200	93.8	1.53825	1.44167

[1] Growth is average Ash-Free-Dry-Weight (AFDW) of surviving organisms

[2] Biomass weight is the total Ash-Free-Dry-Weight of surviving organisms divided by the initial number of organisms

* Reported analytical results are from the "Non-biological" replicates conducted concurrently with the 28-Day *Lumbriculus* bioaccumulation tests.

Shaded and underlined values are statistically significantly different ($p < 0.05$) from corresponding reference sediment.

As summarized in the previous section "Whole Sediment Toxicity Test Quality Criteria" the test organisms exposed to the GLEC laboratory control sediment and the water only exposure achieved acceptable survival and growth. GLEC's laboratory control sediment and water only exposure results confirmed test acceptability and the health of the test organisms. Consequently, the *C. dilutus* and *H. azteca* whole sediment toxicity tests were conducted following the standard protocols, met the toxicity test quality criteria, and are valid assessments of sediment toxicity.

Neither *H. azteca* nor *C. dilutus* survival was significantly affected in either of the acute (10-day) or chronic (20-day *C. dilutus* and 28-day *H. azteca*) whole sediment toxicity tests with any sediment sample. However, *H. azteca* and *C. dilutus* growth was significantly affected in the acute (10-day) and chronic (20-day *C. dilutus* and 28-day *H. azteca*) whole sediment toxicity tests with the sediment samples listed in the Tables 16 and 17 below.

TABLE 16. Significant (P=0.05) Affects on Survival and Growth In *Hyalella Azteca* Whole Sediment Toxicity Tests

Location	*H. azteca* Survival	10-day Growth	10-day Biomass	28-day Growth	28-day Biomass
Clark's Marsh	No	No	No	No	No
Fort Gratiot	No	Yes	Yes	No	No
Huron/Norton	No	Yes	Yes	Yes	No
Beaver Dam Pond	No	Yes	Yes	Yes	Yes
Rogue River	No	Yes	Yes	Yes	Yes
Pigeon River	No	No	No	No	No

TABLE 17. Significant (P=0.05) Affects on Survival and Growth in *Chironomus Dilutus* Whole Sediment Toxicity Tests

Location	*C. dilutus* Survival	10-day Growth	10-day Biomass	20-day Growth	20-day Biomass
Clark's Marsh	No	No	No	Yes	Yes
Fort Gratiot	No	No	No	No	No
Huron/Norton	No	No	Yes	No	Yes
Beaver Dam Pond	No	No	No	Yes	No
Rogue River	No	Yes	Yes	No	No
Pigeon River	No	No	No	No	No

There was no significant effect on *H. azteca* growth after 10-day or 28-day exposures with the Clark's Marsh or Pigeon River sediments (Table 10), yet the Clark's Marsh and Pigeon River sediments contained the highest and lowest concentrations of PFAS, respectively. After 10-days of exposure, *H. azteca* growth and biomass were significantly affected with the Fort Gratiot sediment. *H. azteca* 10-day growth and biomass and 28-day growth endpoints were also significantly affected with the

Huron/Norton Creek sediment. The Huron/Norton Creek sediment sample negatively affected three of the four growth endpoints in the *H. azteca* 10-day and 28-day whole sediment toxicity tests. The Beaver Dam Pond and Rogue River Tannery sediments appear to be the most toxic locations in that both *H. azteca* growth and biomass were significantly affected in 10- and 28-day whole sediment toxicity tests. However, the Beaver Dam sediment had the lowest measured PFAS concentration at 17 µg/Kg. No toxicity was evident with the Pigeon River sediment at 44 µg/Kg PFAS. The Rogue River Tannery sediment total PFAS concentration was 73 µg/Kg.

C. dilutus 20-day growth and biomass were significantly affected with the Clark's Marsh sediment sample, which had total PFAS concentrations between 570 and 393 µg/Kg. *C. dilutus* 10-day growth and biomass were also significantly affected with the Rogue River Tannery sediment. The third most toxic sediment sample to *C. dilutus* was the Huron/Norton Creek sediment sample, which had a measured PFAS at 31 µg/Kg. There was no significant effect on *C. dilutus* growth after 10-days or 20-days exposure with either the Fort Gratiot or the Pigeon River sediments. Consequently, it is clear from the data that whole sediment toxicity did not correlate with total PFAS, PFOS or sediment porewater PFAS concentrations in all instances.

As noted earlier, TEC's were exceeded for several metals, PAHs, and total PCBs at some locations (Tables 6 and 7). Total PAH TECs were exceeded with Rogue River and Beaver Dam Pond sediment. Multiple TECs were exceeded with Huron/Norton Creek and Rogue River sediment. And a single metal (i.e., Pb) TEC was exceeded with the Clark's Marsh sediment. The toxicity of metals is known to be additive, so the co-occurrence of metals likely affected the toxicity of whole sediment with the Huron/Norton Creek sediment. A mixture of two metals and PAHs that exceed the TEC with the Beaver Dam Pond and Rogue River Tannery sediments also likely contributed to the toxicity with those samples. Consequently, given the relatively low concentration of PFAS compounds in the whole sediment and sediment porewater at those same locations (Tables 11 and 14), the toxicity noted with those samples was not likely contributed by PFAS compounds but rather due to metals or metal/PAH mixtures. For example, using the published more conservative probable effect concentrations (PEC) from MacDonald for metals and the measured concentration, we calculated Hazard Quotients (HQ) for each metal with the assumption metal toxicity is additive. Summing the HQs resulted in an overall HQ much greater than 1.0, indicating that the metals collectively are at concentrations that may contribute to toxicity at the Huron/Norton Creek and Rogue River locations. PAHs were the dominant contaminant at the Beaver Dam location.

The observed effect on *C. dilutus* growth and biomass with the Clark's Marsh sediment, in the absence of other toxic compounds (with the noted exception of Pb at 55.2 mg/Kg) was likely due to the PFAS in whole sediment and porewater. For the Clark's Marsh sediment, total PFAS was measured at 570 µg/kg at collection, and at 655 µg/kg at test initiation (Table 9). Total PFAS concentration in porewater at test initiation was 3,749 ng/L and diminished to 1,780 ng/L and 1,188 ng/L at 10-day and 28-day test

terminations, respectively. Normalized for organic carbon, the Clark's Marsh sediment PFAS concentration was 993µg/Kg-$_{OC}$. PFOS made up nearly 89% of the PFAS mixture with the Clark's Marsh toxicity tests. Porewater PFAS concentrations were also greatest at Clark's Marsh and Ft. Gratiot at 3,749 and 1,744 ng/L, respectively, (Table 14) which also understandably had the greatest surface water concentrations.

The observed effect on *H. azteca* growth and biomass with the Fort Gratiot sediment, with the absence of other toxic compounds was likely due to the PFAS in whole sediment and porewater. The Fort Gratiot sediment, total PFAS was measured at 73 µg/kg at collection and at 87 µg/kg at test initiation (Table 8). Total PFAS concentration in porewater at test initiation ranged between 1,744 ng/L at test initiation and diminished to 1,754 ng/L and 1,994 ng/L at 10-day and 28-day test terminations, respectively. Normalized for organic carbon, the total PFAS concentration increased to 1,559 µg/Kg-OC in the Fort Gratiot sediment. PFOS made up over 95% of the PFAS mixture in the Fort Gratiot sediment ranging between 41 and 83 µg/kg.

Consequently, with the above data it is reasonable to conclude that although the Huron/Norton Creek, Beaver Dam Pond, Rogue River Tannery, and Pigeon River Paper sediments are impacted with PFAS, whole sediment toxicity with those samples was likely due to mixtures of metals, PAHs, and PFAS compounds. However, it is also reasonable to conclude that the toxicity observed in the Clark's Marsh and Fort Gratiot sediments was due to PFAS compounds at elevated concentrations and the absence of other contaminants in those sediment samples. The data also suggest, based on whole sediment toxicity test results and measured PFAS concentrations, that adverse ecological effects on freshwater invertebrates may be expected at organic carbon normalized PFAS concentration ($PFAS_{oc}$) between 999 µg/Kg-$_{oc}$ and 1,559 µg/Kg-$_{oc}$, which coincides with the $PFAS_{oc}$ concentrations at Clark's Marsh and Fort Gratiot. $PFAS_{oc}$ at the other four locations ranged from 134 at Huron/Norton Creek to 786 at the Rogue River Tannery location.

Lumbriculus variegatus **Bioaccumulation Tests**

Bioaccumulation factors (BAFs) were calculated for PFAS compounds based on concentrations measured at the end of the test ("termination"). The results are shown in Table 18 and Figure 3. Across tests conducted with all six sediments, BAFs were consistently highest for PFOS.

TABLE 18. Log BAFs Calculated for PFAS Compounds Based on *Lumbriculus* Test Termination (and Comparison to Kent Lake Goal 2 Field-Based Log BAFs)

PFAS	Fort Gratiot	Clark's Marsh	Huron Norton Creek	Rogue R.	Beaver Dam Pond	Pigeon R.	Kent Lake (Goal 2)	
							Field-based log BAF	SD
PFBA	1.4		1.1		1.1		1.2	
PFPeA			0.68					
PFHxA	0.9				0.77		1.2	
PFHpA	1.3		1.5	1.4	1.3		1.1	
PFOA	1.5	1.8		1.2	1.3	1.6	2	0.46
PFNA	2.2							
PFHxS		2.2		1.7	1.5			
PFOS	2.6	2.5	2.8	2.5	2.6	2.7	3.2	0.28

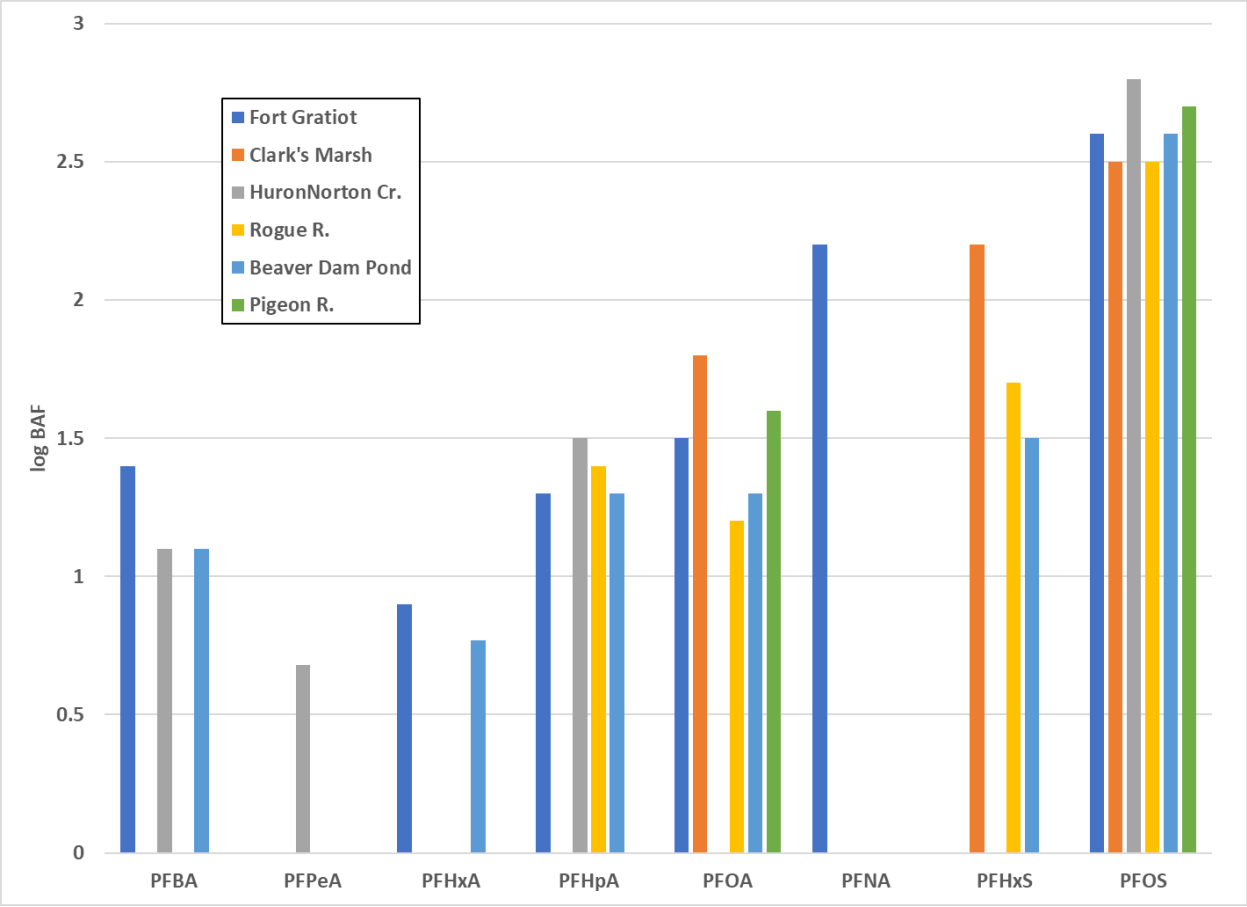

FIGURE 3. Log BAFS Calculated for PFAS Compounds Based on *Lumbriculus* Test Termination Concentrations

We compared log BAFs calculated for PFAS compounds in the *L. variegatus* tests to log BAFs measured for PFAS in invertebrates exposed in Kent Lake (Goal 2). As shown in Table 18, there is a solid agreement between log BAFs for PFAS compounds measured in the laboratory when compared to the field exposure in Kent Lake.

Biota-sediment concentration ratios (BSAFs) were also calculated for PFAS compounds based on concentrations measured at the termination of each test. The results are shown in Table 19 and Figure 4. The three largest BSAFs were measured for 6:2 FTS and PFOS in Huron-Norton Creek sediment (BSAFs of 7.2 and 4.0, respectively) and PFOS in Fort Gratiot sediment (6.3).

TABLE 19. Log BSAFs Calculated for PFAS Compounds Based on *Lumbriculus* Test Termination Concentrations (and Comparison to Kent Lake Goal 2 Field-Based BSAFs)

PFAS	Fort Gratiot	Clark's Marsh	Huron Norton Creek	Rogue R.	Beaver Dam Pond	Pigeon R.	Kent Lake (Goal 2) Field-based BSAF
PFOA	5.5	0.29		0.60			
PFHxDA				0.072			
PFHxS		0.73		0.57	0.16		
PFOS	6.3	0.74	4.0	1.0	0.17	0.58	0.60 – 2.4
PFOSA		0.24		1.3		0.37	
EtFOSE				0.10		0.074	
MeFOSE				0.15			
EtFOSAA				0.35		0.05	
MeFOSAA				0.29			
6:2 FTS			7.2				2.2 – 14

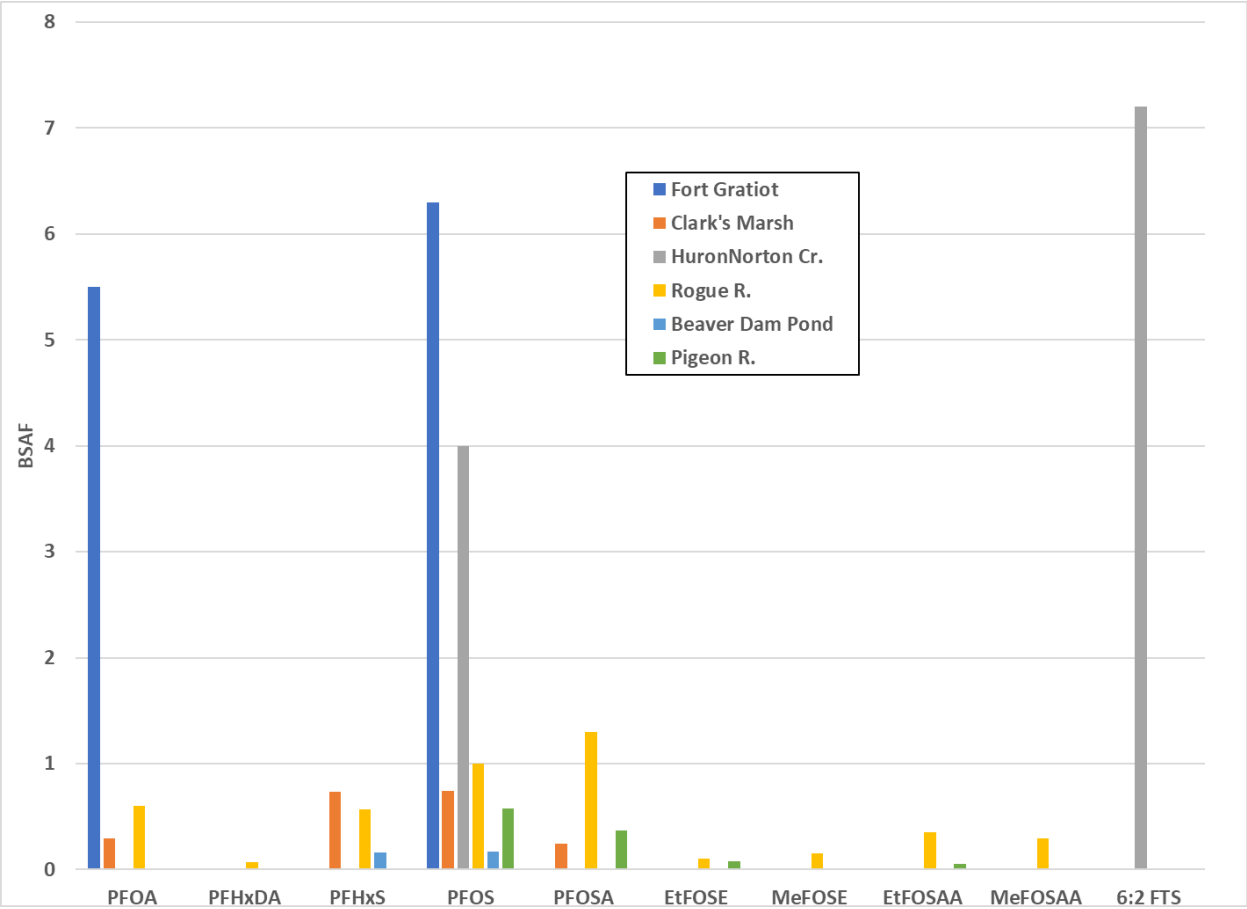

FIGURE 4. BSAF Calculated for PFAS Compounds Based On Lumbriculus Test Termination Concentrations

Seven PFAS compounds were not detected in sediment samples from test termination but were detected in sediment samples at the time of collection or at test initiation. For these 7 PFAS, we calculated BSAFs using the available detected sediment concentrations (Table 20).

TABLE 20. BSAFs Calculated for PFAS Compounds In Lumbriculus Based on Sediment Concentrations Measured at the Time of Collection or at Test Initiation

PFAS	Fort Gratiot	Clark's Marsh	Huron Norton Creek	Rogue R.	Beaver Dam Pond	Pigeon R.
PFHpA	2.8					
PFNA	5.9					
PFUdA				0.057		
PFTrDA				0.046		
PFTeDA				0.49		
PFHpS				0.88		
PFDS			1.8			
6:2 FTS		0.7				

We compared BSAFs calculated for PFAS compounds in the *Lumbriculus* tests to field-based BSAFs measured for PFOS and 6:2 FTS in invertebrates exposed in Kent Lake (Goal 2), as shown in Table 20. BSAFs calculated for PFOS at test termination for three of the sediments (Clark's Marsh, Rouge River, and Pigeon River) fell within (or almost within) the range of BSAFs measured for invertebrates in Kent Lake (0.6-2.4). The BSAF calculated for 6:2 FTS at test termination for Huron-Norton Creek sediment fell within the range of BSAFs measured for invertebrates in Kent Lake. This comparison demonstrates a sound agreement between BSAFs measured in laboratory and field exposures.

DISCUSSION

We evaluated the toxicity of whole sediment at six PFAS impacted sites located in the lower Peninsula of Michigan. Although there was measurable sublethal toxicity measured in five of the six whole sediment samples, effects on *H. azteca* and *C. dilutus* growth could not be attributed solely to PFAS compounds with three of the five sites, but rather due to a mixture of metals and PAHs that exceeded published Sediment Quality Guidelines and PFAS compounds (Tables 6 and 7). The concentration of total PFAS in whole sediment and in sediment porewater with those samples was not great enough to attribute the toxicity to PFAS compounds, yet the effect of PFAS, PAH and metal mixtures is largely unknown. Two of the five locations (Clark's Marsh and Fort Gratiot) were relatively free of other contaminants and exhibited the greatest $PFAS_{oc}$ concentrations, the greatest porewater PFAS concentration and surface water concentrations.

Sublethal toxicity, measured as significant reductions in *H. azteca* or *C. dilutus* growth and biomass was observed with two of the six samples that also contained the highest concentrations of PFAS compounds in sediment and sediment porewater (i.e., Clark's Marsh and Fort Gratiot). Concentrations of non-PFAS contaminants in Clark's Marsh and Fort Gratiot samples were not at concentrations exceeding sediment quality guidelines. Correspondingly, these two locations also contained the highest PFOS concentrations in sediment and sediment porewater. To help understand the observed toxicity, total PFAS concentrations were organic carbon normalized with the whole sediment and with sediment porewater at the Clark's Marsh location and again with whole sediment only with the Fort Gratiot sediment. When normalized for organic carbon, the Fort Gratiot sediment appears to have the greatest concentration of PFAS (1,559 µg/Kg-oc), compared to the Clark's Marsh sediment at 993 µg/Kg-oc. The toxicity test results are conflicting when comparing the *H. azteca* and *C. dilutus* growth and biomass results between the two samples. For instance, *C. dilutus* 20-day growth and biomass was significantly affected with the Clark's Marsh sediment, whereas *H. azteca* growth and biomass was not significantly affected at the same PFAS concentration. Likewise, *H. azteca* 10-day growth and biomass was significantly affected with the Fort Gratiot sediment, whereas *C. dilutus* growth was not significantly affected in either the 10 or 20-day exposures at the same test concentrations. *H. azteca* may have different

tolerances than *C. dilutus* and the interaction with sediment water interface are somewhat different. *C. dilutus* appears to be more sensitive species, or both species showed sensitivity in different conditions. For example, *H. azteca* is an epibenthic organisms whereas C. dilutus actively burrows into the sediment. The Clark's Marsh carbon normalized PFAS porewater concentration was 312 ng/mg-oc.

Our preliminary analysis of the PFAS/PFOS data and those factors suspected of attenuating PFAS toxicity (e.g., TOC, CEC, Ca, Mn, Mg, and grain size) do not explain the varying species sensitivity, especially since the Fort Gratiot and Clark's Marsh sediments had similar total PFAS porewater concentrations and CEC equivalents. However, one parameter that largely stood out between the two locations was the calcium concentration in the sediment, where Clark's Marsh was observed at 30,400 mg/Kg Ca and Fort Gratiot was 6,360 mg/Kg Ca.CEC equivalents and the concentration of major cations was greater at the other locations. Higgins and Luthy (2006) demonstrated that the calcium concentration affects the sorption of the PFAS analyte to sediment particles which may help explain the varying species sensitivity we observed. While the effect of TOC on PFAS toxicity is fairly well understood, the effect of CEC and major cations on toxicity is less understood and requires further consideration.

We compared the BSAFs calculated for PFAS compounds measured in the *Lumbriculus* tests to Higgins et al. (2007) who reported steady-state BSAFs for PFAS accumulating in *Lumbriculus* from spiked sediment. Higgins et al. (2007) organic carbon-normalized the sediment concentrations and calculated BSAFs on a carbon-normalized basis ($BSAF_{oc}$), so the same carbon normalization was applied to our BSAFs in order to compare the results. As an added complication, Higgins et al. (2007) also measured the kinetics of PFAS exposure and reported that steady state was not attained in 56 days. For example, Higgins et al.'s $BSAF_s$ for *Lumbriculus* exposed to field-impacted sediments for 56 days were 17 to 82% lower than their steady-state BSAFs for the same PFAS compounds. This means that BSAFs measured in our 28-day tests probably also underestimated steady-state values, and our BSAFs might therefore be expected to be somewhat smaller than Higgins et al.'s.

For the $BSAF_{oc}$'s based on test termination concentrations, our maximum 28-day values were 27% (PFOA and EtFOSAA) to 76% (PFOS) of the steady-state values determined by Higgins et al. (2007). For the $BSAF_{oc}$'s that could only be calculated using sediment concentrations from samples at the time of collection or at test initiation, our maximum 28-day values were 17% (PFNA), 0.85% (PFUdA), and 84% (PFDS) of the steady-state values determined by Higgins et al. (2007). The BSAFs for two of the five PFAS compounds (PFOS and PFDS) measured during the bioaccumulation tests were within a factor of 2 of BSAFs reported by Higgins once carbon-normalization was applied. The BSAFs for the other three PFAS compounds (PFOA, PFNA and PFUdA) were much smaller than BSAFs reported by Higgins, by factors ranging from 4 to 100.

In summary, *H. azteca* growth was significantly affected at 73-84 µg/Kg total PFAS, 1744-1754 ng/L total PFAS in sediment porewater in the Fort Gratiot sediment. PFOS

contributed the majority >95% of the total PFAS in that sample. *C. dilutus* growth was significantly affected at 570 ug/Kg PFAS, 3,749 ng/LPFAS in porewater and with 580 ug/Kg PFOS (test initiation concentrations).

PFAS concentration was also measured in surface water samples at the time of collection and compared to whole sediment test porewater concentrations. Total PFAS in Fort Gratiot surface water was determined to be 4.44 x 10^{-3}µM/L, whereas in sediment porewater from the same locations it was similar at 3.87x 10^{-3}µM/L. Total PFAS in Clark's Marsh surface water was determined to be 4.8 x 10^{-3}µM/L and 8.10 x 10^{-3}µM/L in sediment porewater. These data suggest that PFAS maybe transported between sediment and overlying surface waters due to the similarity in concentrations between the two phases.

Because PFOS is the dominant form of PFAS in these sediment samples, we compared the Fort Gratiot and Clark's Marsh porewater concentrations to published EC_{50} estimates for *C. dilutus*. For comparison purposes, we converted the porewater concentrations to molar concentrations (µM/L). PFOS in Fort Gratiot porewater was measured at 0.002 µM/L; in Clark's Marsh it was 0.006 µM/L. Unpublished preliminary data presented by EPA-Duluth suggested PFOS EC_{50} estimates for *C. dilutus* in water only tests at approximately 0.05 µM/L, or an order of magnitude higher than the porewater concentrations, where we observed sublethal effects with *C. dilutus* and *H. azteca*. However, Krupa et.al., (2022) reported reduced survival of *C. dilutus* at a concentration of 0.002 µM/L, which is equal to the Fort Gratiot porewater concentration in this study.

These data support our conclusions that PFAS/PFOS compounds may have contributed to reductions in growth and biomass within the Clark's Marsh and Fort Gratiot sediment samples. Collectively, these data also suggest, based on whole sediment toxicity test results and measured PFAS concentrations, that adverse ecological effects on freshwater invertebrates may be expected at organic carbon normalized PFAS concentrations between 999 µg/Kg-oc and 1,559 µg/Kg-oc. PFOS was the dominant PFAS compound measured in sediment and sediment porewater, thus the most likely contributor to the observed reductions in growth and biomass whole sediment toxicity.

REFERENCES

Asher BJ, Wang Y, De Silva AO, Backus S, Muir DCG, Wong CS, and Martin JW. 2012. Enantiospecific Perfluorooctane Sulfonate (PFOS) Analysis Reveals Evidence for the Source Contribution of PFOS-Precursors to the Lake Ontario Foodweb . Environmental Science and Technology. 46:7653-7660.

ASTM. 2020. Standard test method for measuring the toxicity of sediment-associated contaminants with freshwater invertebrates. ASTM E1706-20.

Bakke T, Källqvist T, Ruus A, Breedveld G, Hylland K. 2010. Development of sediment quality criteria in Norway. J. Soils Sedim. 10:172-178.

Bertin, D., Ferrari, B. J. D., Labadie, P., Sapin, A., Garric, J., Budzinski, H., … Babut, M. (2014). Bioaccumulation of perfluoroalkyl compounds in midge (*Chironomus riparius*) larvae exposed to sediment. *Environmental Pollution*, *189*, 27–34. https://doi.org/10.1016/j.envpol.2014.02.018

Bertin, D., P. Labadie, B. J. D. Ferrari, A. Sapin, J. Garric, O. Geffard, H. Budzinski and M. Babut. 2016. Potential exposure routes and accumulation kinetics for poly- and perfluorinated alkyl compounds for a freshwater amphipod: Gammarus spp. (Crustacea). Chemosphere. 155: 380-387.

Condor, et. al., (2019). Guidance for Assessing the Ecological Risks of PFASs to Threatened and Endangered Species at Aqueous Film Forming Foam-Impacted Sites. Defense Strategic Environmental Research and Development Program (SERDP). SERDP Project ER18-1614, July 2019.

EPA 2000. Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates. Second Edition. EPA/600/R-99/064.EPA QA/R-5. U.S. Environmental Protection Agency, Quality Assurance Management Staff, Washington, DC, March 2001. EPA/240/B-01/003.

Giesy, John P., Jonathan E. Naile, Jong Seong Khim, Paul D. Jones, and John L. Newste. 2010. Aquatic Toxicology of Perfluorinated Chemicals. Reviews of Environmental Contamination and Toxicology 202.

Great Lakes Environmental Center, Inc. 2022. Per- and Poly-fluoroalkyl Substances (PFAS) in Sediment Goal 2 - Sediment as a Source of PFAS to the Food Web

WA 21-20 PFAS Goal 2: Final Report 2.6.23. GLEC 739 Hastings St., Traverse City, Michigan 49686.

Higgins CP and RG Luthy. 2006. Sorption of Perfluorinated Surfactants on Sediments

Environmental Science & Technology 2006 *40* (23), 7251-7256; DOI: 10.1021/es061000n

Higgins, C. P., Mcleod, Pamela, B., Macmanus-Spencer, L. A., & Luth. (2007). Bioaccumulation of Perfluorochemicals in Sediments by the Aquatic Oligochaete *Lumbriculus variegatus*. *Environ. Sci. Technol*, *41*(13), 4600–4606. https://doi.org/10.1021/es062792o

Krupa PM, Lotufo GR, Mylroie EJ, may LK, Gust KA, Kimble AN, Junug MG, Boyda JA, Garcia-Reyero N and Moore DW. 2022. Chronic aquatic toxicity of perfluorooctane sulfonic acid (PFOS) to *Ceriodaphnia dubia, Chironomus dilutus, Danio rerio and Hyalella azteca*. Ecology and environmental Safety. Volume 241. 2022.

Lasier PJ, Washington JW, Hassan SM, Jenkins TM. 2011. Perfluorinated chemicals in surface waters and sediments from northwest Georgia, USA, and their bioaccumulation in Lumbriculus variegatus. Environmental Toxicology and Chemistry. 30(10):2194–2201.

Lazorchak, J.M., Griffith, M.B., Mills, M., Schubauer-Berigan, J., McCormick, F., Brenner, R. and Zeller, C. (2015), Proof of concept for the use of macroinvertebrates as indicators of polychlorinated biphenyls (PCB) contamination in Lake Hartwell. Environ Toxicol Chem, 34: 1277-1282.

Lazorchak, Jim, K. Fritz, J. Meier, H. Haring, R. Yeardley, M. Mills, P. Wernsing, D. Walters, R. Otter, B. Armstrong, AND J. Kraus. Assessing remediation of contaminated sediments on the Ottawa River using chemical and biological endpoints (Big Rivers Ecology Course). Big Rivers Ecology Course, Thomas More Field Station, KY, July 30, 2018.

MacDonald, M. M., A. L. Warne, N. L. Stock, S. A. Mabury, K. R. Solomon, and P. K. Sibley. 2004. Toxicity of Perfluorooctane Sulfonic Acid and Perfluorooctanoic Acid to *Chironomus tentans*. Environ. Toxicol. Chem., **23:** 2116-2123.

MacDonald, D., Ingersoll, C. & Berger, T. Development and Evaluation of Consensus-Based Sediment Quality Guidelines for Freshwater Ecosystems. Arch. Environ. Contam. Toxicol. 39, 20–31 (2000).

Marziali, L., F. Rosignoli, S. Valsecchi, S. Polesello and F. Stefani. 2019. Effects of Perfluoralkyl Substances on a Multigenerational Scale: A Case Study with *Chironomus riparius* (Diptera, Chironomidae). Environ Toxicol Chem. 38(5): 988-999.

McCauley, D.J., G.M. DeGraeve, and T.K. Linton. 2000. Sediment Quality Guidelines and Assessment: Overview and Research Needs. Env. Sci and Policy. 3(2000) 5122-5144.

Michigan Department of Environmental Quality (MDEQ). 2018a. PFSA Surface Water Sampling Guidance. http://www.michigan.gov/-/media/Project/Websites/PFAS-Response/Sampling-Guidance/Surface-Water.pdf?rev=a4a35607c5ba4a5c83de17928c693aec

NCPA. 2008. Screening of polyfluorinted compounds at four fire training facilities in Norway. Norwegian Pollution Control Authority (NPCA). TA-2444/2008. 89 p. Available from: https://www.miljodirektoratet.no/globalassets/publikasjoner/klif2/publikasjoner/2444/ta2444.pdf

Prosser, R.S., K. Mahon, P.K. Sibley, D. Poirier, and T. Watson-Leung. 2016. Bioaccumulation of perfluorinated carboxylates and sulfonates and polychlorinated biphenyls in laboratory-cultured Hexagenia spp., Lumbriculus variegatus and Pimephales promelas from field-collected sediments. Sci. Total Environ. 543: 715-726.

Remucal CK. 2019. Spatial and temporal variability of perfluoroalkyl substances in the Laurentian Great Lakes. Environmental Science: Processes & Impacts. 21(11):1816–1834.

Simpson, S.L., Y. Liu, D.A. Spadaro, X. Wang, R.S. Kookana, and G.E. Batley. 2021. Chronic effects and thresholds for estuarine and marine benthic organism exposure to perfluorooctane sulfonic acid (PFOS)-contaminated sediments: Influence of organic carbon and exposure. Sci. Tot. Environ. Vol. 776. Science Direct: 146008

Stefani, F., M. Rusconi, S. Valsecchi, and L. Marziali. 2014. Evolutionary ecotoxicology of perfluoralkyl substances (PFASs) inferred from multigenerational exposure: A case study with *Chironomus riparius* (Diptera, Chironomidae). Aquat Toxicol. 156: 41-51.

Wang, Zhanyun et al. “A Never-Ending Story of Per- and Polyfluoroalkyl Substances (PFASs)?” Environmental Science & Technology 51(5) (2017): 2508-2518. ACES Publications. Web. 11, June 2018.

Xia, X., X. Chen, X. Zhao, H. Chen, and M. Shen. 2012. Effects of carbon nanotubes, chars, and ash on bioaccumulation of perfluorochemicals by Chironomus plumosus larvae in sediment. Environ. Sci. Technol. 46: 12467-12475.