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Evaluation of Toxicity to the Amphipod, *Hyalella azteca*, and to the Midge, *Chironomus dilutus*; and Bioaccumulation by the Oligochaete, *Lumbriculus variegatus*, with Exposure to PCB-Contaminated Sediments from Anniston, Alabama

Scientific Investigations Report 2013–5125

U.S. Department of the Interior
U.S. Geological Survey

Cover. Photographs of test organisms (clockwise): Egg mass of *Chironomus dilutus*, *Hyalella azteca*, *Lumbriculus variegatus*, and *Chironomus dilutus*. Photographs by Doug Hardesty, U.S. Geological Survey.

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Volume comprises chapters 1, 2, 3, 4, and 5

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U.S. Department of the Interior
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U.S. Department of the Interior
SALLY JEWELL, Secretary

U.S. Geological Survey
Suzette M. Kimball, Acting Director

U.S. Geological Survey, Reston, Virginia: 2013

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Preface

The purpose of this report is to provide a compilation of information on the toxicity and bioaccumulation of chemicals of potential concern (COPCs) including polychlorinated biphenyls (PCBs) in sediments collected from the Anniston, Alabama PCB Site (Anniston PCB Site) located in north-central Alabama. Long-term reproduction toxicity tests were done with the amphipod, *Hyalella azteca*, and the midge, *Chironomus dilutus*, and bioaccumulation tests were done with the oligochaete, *Lumbriculus variegatus*, exposed to sediments collected from the Anniston PCB Site. Results of these laboratory toxicity and bioaccumulation tests subsequently will be used by personnel with ARCADIS U.S., Inc. (ARCADIS) as part of a weight-of-evidence assessment to evaluate risks and sediment remediation goals for contaminants to sediment-dwelling organisms inhabiting the Anniston PCB Site. Given that PCBs in sediment are a primary COPC at the Anniston PCB Site, the decision was made to perform longer exposures, focusing on measuring potential reproductive effects of PCBs in sediment on *H. azteca* or *C. dilutus* and bioaccumulation of PCBs by *L. variegatus*. Only a limited number of laboratories had the demonstrated capacity to perform long-term reproduction sediment toxicity tests with *H. azteca* or *C. dilutus* with the large number of samples required for the project. Hence, ARCADIS contracted with the U.S. Geological Survey, Columbia Environmental Research Center in Columbia, Missouri and with the U.S. Army Corps of Engineers, Engineer Research and Development Center, Vicksburg, Mississippi to perform the sediment toxicity tests and the sediment bioaccumulation tests with sediments collected from the Anniston PCB Site.

The goal of this study was to characterize relations between sediment chemistry and sediment toxicity and relations between sediment chemistry and sediment bioaccumulation in samples of sediments collected from the Anniston PCB Site in Alabama. A total of 32 samples were evaluated from six test sites and one reference site to provide a wide range in concentrations of COPCs in samples of whole sediment. The goal of this study was not to determine the extent of sediment contamination or sediment toxicity across the Anniston PCB Site. Hence, the test sites or samples collected from within a test site were not selected to represent the spatial extent of sediment contamination across the Anniston PCB Site. Additional studies may be required to determine the spatial extent of sediment contamination and sediment toxicity or sediment bioaccumulation at the Anniston PCB Site.

Acknowledgments

This research was funded through an agreement between the U.S. Geological Survey Columbia Environmental Research Center, Columbia, Missouri (USGS–Columbia) and ARCADIS U.S., Inc. (ARCADIS) and through an agreement between the U.S. Army Corps of Engineers Engineer Research and Development Center, Vicksburg, Mississippi (USACE–Vicksburg) and ARCADIS. We would like to thank personnel from ARCADIS, U.S. Environmental Protection Agency (USEPA) Region 4, and the U.S. Fish and Wildlife Service Region 4 for providing guidance on the design of the study or for providing assistance in collection or characterization of the sediments.

Many individuals at USGS contributed to the success of this project and report including: John Besser, Eric Brunson, Rebecca Consbrock, Doug Hardesty, Jamie Hughes Chris Ivey Brittney King, Carl Orazio, Ning Wang, Ryan Warbritton, Dave Whites, Jesse Arms, Shannon Earhart, Tom May, Vanessa Melton-Silvey, and Mike Walther at USGS–Columbia.

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Conversion Factors

Inch/Pound to SI

Multiply	By	To obtain
	Length	
centimeter (cm)	0.3937	inch (in.)
millimeter (mm)	0.03937	inch (in.)
meter (m)	3.281	foot (ft)
kilometer (km)	0.6214	mile (mi)
mile, nautical (nmi)	1.852	kilometer (km)
meter (m)	1.094	yard (yd)
	Volume	
liter (L)	33.82	ounce, fluid (fl. oz)
liter (L)	0.2642	gallon (gal)
	Mass	
gram (g)	0.03527	ounce, avoirdupois (oz)
kilogram (kg)	2.205	pound, avoirdupois (lb)

Temperature in degrees Celsius (°C) may be converted to degrees Fahrenheit (°F) as follows:

$$°F=(1.8\times°C)+32$$

Temperature in degrees Fahrenheit (°F) may be converted to degrees Celsius (°C) as follows:

$$°C=(°F-32)/1.8$$

Concentrations of chemical constituents in water are given either in milligrams per liter (mg/L) or micrograms per liter (µg/L).

Concentrations of chemical constituents in sediment are given either in milligrams per liter (mg/L) or micrograms per liter (µg/L). Sediment concentrations are expressed on a dry weight basis, on an organic-carbon normalized basis, or on a molar basis.

Concentrations of chemical constituents in tissue are given in micrograms per kilogram (µg/kg). Tissue concentrations are expressed on a wet weight basis or on a lipid-normalized basis.

Abbreviations

°C	degree Celsius
µm	micrometers
µg/L	microgram per liter
µmol/g	micromole per gram
µS	microsiemens
AFDW	ash-free-dry weight
ANOVA	analysis of variance
ASTM	American Society for Testing and Materials International
AVS	acid volatile sulfide
BSAF	biota sediment accumulation factor
$CaCO_3$	calcium carbonate
CERCLA	Comprehensive Environmental Response, Compensation and Liability Act
C_f	mass of contaminant absorbed by fiber/volume of polydimethylsiloxane
COPC	chemical of potential concern
$\sum ESB\text{-}TU_{FCV}$	chronic sum equilibrium-partitioning sediment benchmark toxic unit
cm	centimeter
C_{pw}	concentration in pore water
CRM	concentration-response model
$Ct_{predicted}$	concentration predicted in lipid-normalized accumulation on individual polychlorinated biphenyl congeners in oligochaetes
DDD	dichlorodiphenyldichloroethane
DDE	dichlorodiphenyldichloroethylene
DDT	dichlorodiphenyltrichloroethane
DOC	dissolved organic carbon
DW	dry weight
f/w	fiber-water
f_{OC}	fraction organic carbon
g	gram
GC-ECD	gas chromatography electron capture detector
GPS	Global Positioning System
HCH	hexachlorocyclohexane
HCl	hydrochloric acid
HPLC	high performance liquid chromatography
HT	highly toxic

ICP-MS	inductively coupled plasma-mass spectrometry
ICP-AES	inductively-coupled plasma-atomic emission spectroscopy
IOT	incidence of toxicity
$K_{f/w}$	polydimethylsiloxane fiber-water partition coefficient
kg	kilogram
K_{lip-w}	lipid-water partition coefficient
K_{oc}	organic carbon-water partition coefficient
K_{ow}	octanol-water partition coefficient
L	liter
LC_{50}	median lethal effect concentration
LR/MS/SIM	low resolution mass spectrometry in selective ion mode
M	molar
MESL	MacDonald Environmental Sciences, Ltd.
mg/kg	milligrams per kilogram
min	minute
mm	millimeter
MS	matrix spike
MSD	matrix spike duplicate
MT	moderately toxic
OC	organic carbon
OCPEST	organochlorine pesticide
OU	operable unit
p	*p* value: probability of observing a test statistic that is as or more extreme as the determined statistic, assuming the null hypothesis is true
PAH	polycyclic aromatic hydrocarbon
PCB	polychlorinated biphenyl
PCDD	polychlorinated dibenzo-*p*-dioxin
PCDF	polychlorinated dibenzofurans
PDMS	polydimethylsiloxane
PEC	probable effect concentration
PEC-Q	probable effect concentration quotient
R^2	coefficient of determination
RI/FS	remedial investigation/feasibility study
r_s	Spearman's rank correlation coefficient
SAOB	sulfide anti-oxidant buffer
SEC	sediment effect concentration

SEM	Simultaneously extracted metal
SEM-AVS	Molar difference between simultaneously extracted metal and acid volatile sulfide
SEM-AVS/f_{OC}	Molar difference between simultaneously extracted metal and acid volatile sulfide normalized to the fraction organic carbon
SPME	solid-phase microextraction
SQG	sediment quality guideline
TAL	target analytical metal
TEC	threshold effect concentration
TFE	tetrafluoroethylene
TOC	total organic carbon
TPAH	total polycyclic aromatic hydrocarbons
TPCB	total polychlorinated biphenyls
TSV	toxicity screening value
TT	toxicity threshold
TT_{HR}	toxicity threshold high range
TT_{LR}	toxicity threshold low range
USACE	U.S. Army Corps of Engineers
USEPA	U.S. Environmental Protection Agency
USGS	U.S. Geological Survey
v/v	volume per volume
WQS	water quality standard
WW	wet weight

Toxicity and Bioaccumulation of PCB-Contaminated Sediments from Anniston, Alabama

By Christopher G. Ingersoll[1], Jeffery A. Steevens[2], Donald D. MacDonald[3], William G. Brumbaugh[1], Matthew R. Coady[3], J. Daniel Farrar[2], Guilherme R. Lotufo[2], Nile E. Kemble[1], James L. Kunz[1], Jacob K. Stanley[2], and Jesse A. Sinclair[3]

Executive Summary

The U.S. Environmental Protection Agency (USEPA) requested that as part of the remedial investigation for the Anniston, Alabama Polychlorinated Biphenyl (PCB) Site (Anniston PCB Site), that Pharmacia Corporation and Solutia Inc. (P/S) perform long-term reproduction toxicity tests with the amphipod, *Hyalella azteca*, and the midge, *Chironomus dilutus*, and bioaccumulation tests with the oligochaete, *Lumbriculus variegatus*, using sediment samples collected from reference locations and from Operable Unit 4 of the Anniston PCB Site. The sediment toxicity testing and sediment bioaccumulation results will be used by ARCADIS U.S., Inc. (ARCADIS) as part of a weight-of-evidence assessment to evaluate risks and establish sediment remediation goals for contaminants to sediment-dwelling organisms inhabiting the Anniston PCB Site.

The goal of this study was to characterize relations between sediment chemistry and sediment toxicity and relations between sediment chemistry and sediment bioaccumulation in samples of sediments collected from the Anniston PCB Site. A total of 32 samples were evaluated from six test sites and one reference site to provide a wide range in concentrations of chemicals of potential concern (COPCs) including PCBs in samples of whole sediment. The goal of this study was not to determine the extent of sediment contamination across the Anniston PCB Site. Hence, the test sites or samples collected from within a test site were not selected to represent the spatial extent of sediment contamination across the Anniston PCB Site.

Sediment chemistry, pore-water chemistry, and sediment toxicity data were generated for 26 sediment samples from the Anniston PCB Site. All of the samples were evaluated to determine if they qualified as reference sediment samples. Those samples that met the chemical selection criteria and biological selection criteria were identified as reference samples and used to develop the reference envelope for each toxicity test endpoint. Because interbatch variability in control performance was observed for some of the toxicity test endpoints, all of the response data were control normalized before performing subsequent data analyses.

Because of the large number of samples and capacities of laboratories, sediment toxicity testing and sediment bioaccumulation testing was done in two cycles (cycle 1a and cycle 1b). Results of 10-d range-finding toxicity tests with *H. azteca* and *L. variegatus* and initial characterization of total organic carbon (TOC) and total PCBs were used to select the samples for sediment toxicity and sediment bioaccumulation testing in cycle 1a. Summaries of cycle 1a toxicity data also were used to select samples for toxicity and bioaccumulation testing in cycle 1b. Physical characterization of samples of whole sediment included analyses of grain size, TOC, and nutrients. Organic chemical characterization of samples of whole sediment included PCB homologs and select (13) PCB congeners, parent and alkylated polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides, and polychlorinated dibenzo-*p*-dioxins; and dibenzofurans (PCDD/PCDFs). The PCB aroclors analyzed included 1016, 1221, 1232, 1242, 1248, 1254, 1260, 1262 and 1268. Analyses of whole sediment also included total metals, simultaneously extracted metals, and acid volatile sulfide. Chemical characterization of samples of pore water isolated from samples of whole sediment at the start of the sediment toxicity exposures or at the start of the sediment bioaccumulation exposures included metals, major cations, major anions, dissolved organic carbon (DOC), and additional water-quality characteristics. Concentrations of metals or PCBs in pore water during the sediment toxicity exposures or during sediment bioaccumulation exposures also were determined using peeper samples (for metals) or solid-phase microextraction (SPME) samplers (for PCBs).

[1]U.S. Geological Survey, Columbia, Missouri.

[2]U.S. Army Corps of Engineers, Vicksburg, Mississippi.

[3]MacDonald Environmental Sciences Ltd., Nanaimo, British Columbia.

The bioavailability and bioaccumulation of PCBs in 14 sediment samples were investigated using SPME passive samplers and the 28-d *L. variegatus* whole-sediment bioaccumulation exposures in basic accordance with USEPA and American Society for Testing and Materials International (ASTM) standard methods. Tissue residues predicted using SPME289 derived pore-water data accurately predicted body residues in sediment-exposed oligochaetes and provided information regarding the bioavailability of PCBs in these sediments. In general the accumulation of PCBs consistently was predicted through the use of organic carbon normalization and equilibrium partitioning. In these sediments, PCB homologs were accumulated differently based on bioavailability and potential to accumulate in oligochaetes. As part of this assessment homolog specific biota sediment accumulation factor (BSAF) values were developed that could be applied across the larger site to predict tissue levels of PCBs.

Long-term reproduction sediment toxicity testing with *H. azteca* and *C. dilutus* was done in basic accordance with USEPA and ASTM standard methods. Results of the interlaboratory testing of Anniston PCB Site sediments demonstrated relatively consistent responses between samples tested by U.S. Geological Survey Columbia Environmental Research Center, Columbia, Missouri (USGS–Columbia) and U.S. Army Corps of Engineers Engineer Research and Development Center, Vicksburg, Mississippi (USACE–Vicksburg). Hence, there was not a substantial bias in the results generated by the USACE–Vicksburg or USGS–Columbia toxicity testing laboratories associated with the toxicity testing of Anniston PCB Site sediments. The whole-sediment toxicity tests done with *H. azteca* and *C. dilutus* met the established ASTM and USEPA test acceptability criteria, and intralaboratory control responses between the two cycles were similar. Samples were designated toxic or not toxic based on a reference envelope approach. Relative endpoint sensitivity was assessed by graphing control normalized data points against each other in pairs. This information was used to establish the number of data points that exceeded a 20-percent difference from the line of unity as well as through the assessment of the number of test sediments classified as toxic by falling below the response of the lower distribution of the reference envelope for each toxicity endpoint. This analysis of endpoint responsiveness demonstrated that the most responsive *H. azteca* endpoints were day 42 survival normalized young per female and day 28 biomass and that the most responsive *C. dilutus* endpoints were adult biomass and percent adult emergence. Overall, between the two species, the most responsive endpoint assessed for these two species was *H. azteca* survival-normalized young per female (67 percent of the samples classified as toxic).

A study also was performed with a subset of sediment samples to determine if the age of midge at the start of the toxicity tests exposures affected the endpoint responses in exposures to five Anniston PCB Site sediments. Survival, weight, and biomass of *C. dilutus* were relatively consistent in exposures started with about 7-day (d)-old larvae compared to the responses in exposures started with less than 24-hour (h)-old larvae. These results indicate that long-term exposures in the definitive cycle 1a and cycle 1b tests started with about 7-d-old larvae would not likely underestimate the toxicity of the Anniston PCB Site sediments to *C. dilutus* compared to starting the exposures with less than 24-h-old larvae.

Concentration-response models (CRMs) and site-specific sediment toxicity thresholds (TTs) were generated with matching sediment chemistry and sediment toxicity data. Sediment chemistry, pore-water chemistry, and sediment toxicity data were evaluated for as many as 26 sediment samples from the Anniston PCB Site. The reference-envelope approach was used to identify the sediment samples that were toxic to benthic invertebrates. This procedure involved identification of reference sediment samples, normalizing the toxicity data to reflect control responses, developing a reference envelope for each toxicity test endpoint, and designating each sediment sample as toxic or not toxic for each toxicity test endpoint, for each species, and for all species combined. These results demonstrated percent emergence of adult *C. dilutus*, biomass of adult *C. dilutus*, and reproduction of *H. azteca* normalized to percent survival were among the most responsive endpoints that were evaluated. Therefore, these endpoints were selected for CRM development.

A step-wise process was used to evaluate relations between sediment chemistry and sediment toxicity for the Anniston PCB Site, to develop site-specific sediment TTs, and to evaluate the reliability of the resultant TTs. This process consisted of six main steps:

1. Screening-level evaluation performed to identify the COPCs and COPC mixtures that were unlikely to cause or substantially contribute to sediment toxicity;

2. Identification of the COPCs or COPC mixtures that were significantly correlated with the toxicity test endpoints [based on results of Spearman's rank correlation analyses, Spearman's rank correlation coefficient (r_s) greater than 0.4; p less than 0.005];

3. Development of CRMs for selected COPC/COPC mixtures and toxicity test endpoint pairs;

4. Derivation of site-specific sediment TTs for selected COPC/COPC mixtures and toxicity test endpoint pairs;

5. Evaluation of the reliability of the site-specific sediment TTs for selected COPC/COPC mixtures and toxicity test endpoint pairs; and,

6. Evaluation of the comparability of the site-specific sediment TTs for selected COPCs or COPC mixtures.

First, a series of analyses was performed to identify the COPCs and COPC mixtures that were most likely to be correlated with the responses to toxicity test organisms (for example, an evaluation of sediment chemistry based on the frequency of detection and comparisons to conservative toxicity screening values).

Second, potential relations between the concentrations of COPCs and the responses of toxicity test organisms were identified by performing Spearman's rank correlation analysis on the underlying data. The results of these analyses indicated that the relations between chemical concentration and response tended to be strongest for PCBs, certain metals (that is, lead and mercury), PAHs, organochlorine pesticides, and mean probable effect concentration quotients (PEC-Qs).

Third, concentration-response models were developed for each of the COPCs and COPC mixtures in sediment that were retained following these initial analyses. The CRMs were then examined to identify the COPC/COPC mixtures and toxicity test endpoint pairs that would be most relevant for development of site-specific sediment TTs (that is, R^2 greater than 0.4; p less than 0.05). Overall, 69 COPC/COPC mixtures and toxicity test endpoint pairs were selected for deriving TTs for whole sediment. In addition, 32 COPC or COPC mixture pairs for pore water were selected for deriving TTs for pore water.

Fourth, two types of TTs, including TTs low risk (TT_{LR}s) and TTs high risk (TTT_{HR}s), were developed using the CRMs for 69 COPC/COPC mixtures and toxicity test endpoint pairs for whole sediment and 32 COPC/COPC mixtures and toxicity test endpoint pairs for pore water. The TT_{LR}s were established as concentrations of COPCs or COPC mixtures that corresponded to the lower limit of the reference envelope for the selected toxicity test endpoint. The TT_{HR}s were established as the concentrations of COPCs or COPC mixtures that corresponded to a 10-percent reduction in survival, weight, biomass, emergence, or reproduction, compared to the lower limit of the reference envelope. The reliability of the resultant TTs were then evaluated using sediment chemistry and sediment toxicity data from the Anniston PCB Site. Toxicity thresholds were considered to be reliable and predictive of sediment toxicity if the incidence of toxicity (IOT) was less than 20 percent below the TT, the IOT was greater than 50 percent above the TT, and the rate of correct classification of sediment samples as toxic and not toxic was greater than or equal to 80 percent.

Fifth, the results of this evaluation indicated that most of the site-specific TTs for whole sediment provide a reliable basis for identifying toxic and not toxic sediment samples in the Anniston PCB Site (that is, for correctly classifying the sediment samples used to derive the TTs as toxic or not toxic, for the endpoint used to derive the TTs). Among the 69 TTs for sediment, the TT_{LR}s for total PCB homologs [499 to 1,870 micrograms per kilogram dry weight (µg/kg DW)] and for lead [(9.48 to 10.3 milligrams per kilogram (mg/kg) DW] based on reproduction of *H. azteca* or based on emergence or biomass of adult *C. dilutus*, were the most reliable. Such TTs had low rates of false negative errors (that is, only 0 to 11 percent of the samples below the TT were toxic to benthic invertebrates), low rates of false positive errors (only 0 to 6 percent of the samples greater than the TT were not toxic to benthic invertebrates), and high rates of correct classification (that is, 92 to 96 percent).

Finally, the site-specific TTs for PCBs and other COPCs derived in this study also were compared to empirically based sediment quality guidelines (SQGs), to equilibrium-partitioning based SQGs, and to the results of spiked-sediment toxicity tests. The results of this evaluation indicated that the site-specific sediment TTs for PCBs were comparable to the consensus-based SQGs that were derived for PCBs. In addition, the site-specific sediment TTs for PCBs are well within the range of SQGs derived using the equilibrium partitioning approach. The site-specific sediment TTs for PCBs also are consistent with the results of chronic TTs that have been estimated for benthic invertebrates using the results of spiked-sediment toxicity tests. As the site-specific sediment TTs for PCBs are consistent with empirically based SQGs, equilibrium-partitioning based SQGs, and results of sediment-spiking studies, these site-specific sediment TTs likely represent the concentrations of PCBs that are sufficient to cause toxicity to benthic invertebrates (as opposed to simply being correlated with adverse effects on the survival, weight, or reproduction of benthic invertebrates). Importantly, such site-specific sediment TTs have been demonstrated to accurately classify sediment samples as toxic or not toxic to benthic invertebrates at the Anniston PCB Site. In contrast, the TTs for metals, PAHs, and organochlorine pesticides were generally lower than consensus-based SQGs (that is, PECs), and LC_{50}s (median lethal effect concentrations) generated in spiked-sediment toxicity tests, indicating that these COPCs are likely not the main contributors to the observed toxicity of the site sediments evaluated in this study. The reproduction endpoint for *H. azteca* provided lower TTs compared to the day 28 biomass endpoint for *H. azteca* and the emergence or biomass endpoints for adult *C. dilutus* provided lower TTs compared to the day 13 biomass endpoint for *C. dilutus*.

Overview of the Study to Evaluate the Toxicity and Bioaccumulation of Aquatic Organisms Exposed to PCB-Contaminated Sediments from Anniston, Alabama

By Christopher G. Ingersoll, Jeffery A. Steevens, and Donald D. MacDonald

Chapter 1 of

Evaluation of Toxicity to the Amphipod, *Hyalella azteca*, and to the Midge, *Chironomus dilutus*; and Bioaccumulation by the Oligochaete, *Lumbriculus variegatus*, with Exposure to PCB-Contaminated Sediments from Anniston, Alabama

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Overview of the Study to Evaluate the Toxicity and Bioaccumulation of Aquatic Organisms Exposed to PCB-Contaminated Sediments from Anniston, Alabama

By Christopher G. Ingersoll[1], Jeffery A. Steevens[2], and Donald D. MacDonald[3]

Introduction

The U.S. Environmental Protection Agency (USEPA) requested that as part of the remedial investigation for the Anniston, Alabama Polychlorinated Biphenyl (PCB) Site (Anniston PCB Site), that Pharmacia Corporation and Solutia Inc. (P/S) perform long-term reproduction toxicity tests with the amphipod, *Hyalella azteca*, and the midge, *Chironomus dilutus*, and bioaccumulation tests with the oligochaete, *Lumbriculus variegatus*, using sediment samples collected from reference locations and from Operable Unit 4 of the Anniston PCB Site (ARCADIS, 2010). The sediment toxicity testing and sediment bioaccumulation results will be used by ARCADIS U.S., Inc. (ARCADIS) as part of a weight-of-evidence assessment to evaluate risks and establish sediment remediation goals for contaminants to sediment-dwelling organisms inhabiting the Anniston PCB Site.

The goal of this study was to characterize the relations between sediment chemistry and potential sediment toxicity and relations between sediment chemistry and sediment bioaccumulation of contaminants by benthic macroinvertebrates exposed to sediment samples collected from the Anniston PCB Site. A total of 32 samples were evaluated from six test sites and one reference site (fig. C1–1) to provide a wide range in concentrations of chemicals of potential concern (COPCs; appendix 1, table A1–1) including PCBs in samples of whole sediment. More detailed maps illustrating the locations of the test sites and the reference site are provided in ARCADIS (2010). The goal of this study was not to determine the extent of sediment contamination across the Anniston PCB Site. Hence, the samples collected from within a test site were not selected to represent the spatial extent of sediment contamination across the Anniston PCB Site.

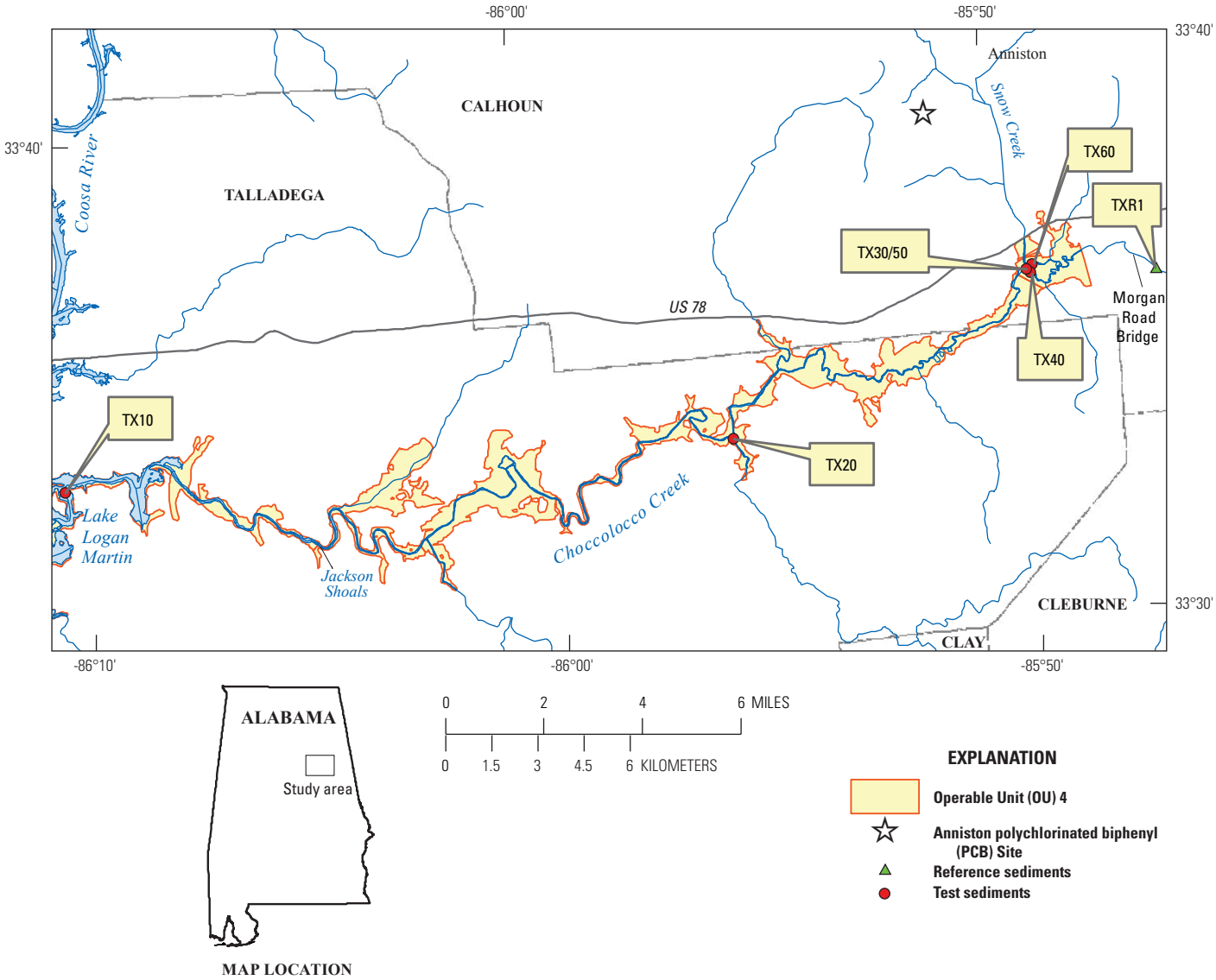
Study Design

American Society for Testing and Materials International (2012a) and U.S. Environmental Protection Agency (2000) describe methods for performing long-term reproduction whole-sediment toxicity tests with *H. azteca* and *C. dilutus*. Toxicity endpoints in these methods include 42-day (d) survival, weight, biomass, and reproduction of *H. azteca* and 48- to 54-d survival, weight, biomass, emergence, and reproduction of *C. dilutus*. American Society for Testing and Materials International (2012b) and U.S. Environmental Protection Agency (2000) also describe methods for performing 28-d whole-sediment bioaccumulation tests with *L. variegatus*. However, routine toxicity testing of field-collected or laboratory-spiked sediments typically report only 10- to 28-d survival or weight of *H. azteca* or 10-d survival or weight of *C. dilutus* (U.S. Environmental Protection Agency, 2000; American Society for Testing and Materials International, 2012a). Given that PCBs in sediment are a primary COPC at the Anniston PCB Site (ARCADIS, 2010), the decision was to perform longer exposures, focusing on measuring potential reproductive effects of PCBs in sediment on *H. azteca* or *C. dilutus* and bioaccumulation of PCBs by *L. variegatus*. Only a limited number of laboratories had the demonstrated capacity to perform these long-term reproduction sediment toxicity tests for the selected sediment samples with *H. azteca* or *C. dilutus* with the number of samples that needed to be tested. Hence, ARCADIS contracted with U.S. Geological Survey Columbia Environmental Research Center, Columbia, Missouri (USGS–Columbia) and U.S. Army Corps of Engineers Engineer Research and Development Center, Vicksburg, Mississippi (USACE–Vicksburg) to perform the laboratory sediment toxicity tests and the sediment bioaccumulation

[1]U.S. Geological Survey, Columbia, Missouri.

[2]U.S. Army Corps of Engineers, Vicksburg, Mississippi.

[3]MacDonald Environmental Sciences Ltd., Nanaimo, British Columbia.



TXR1: Choccolocco Creek, about 500 meters upstream from Morgan Road Bridge; collected along left bank within about a 10-meter radius of the designated sampling coordinates

TX10: Choccolocco Creek near Jackson Shoals; collected along the lacustrine portion of the site (Lake Logan Martin) near an exposed island within about 50 meters of the designated sampling coordinates

TX20: Choccolocco Creek near Jackson Shoals; collected on the inside portion of a river bend across the river from the designated sampling coordinates

TX30: Choccolocco Creek about 125 meters upstream from the confluence with Snow Creek; collected left, middle, right bank within about a 20-meter radius of the designated sampling coordinates

TX40: Choccolocco Creek about 300 meters upstream from the confluence with Snow Creek; collected left, middle, right bank within about a 15-meter radius of the designated sampling coordinates

TX50: Choccolocco Creek about 125 meters upstream from the confluence with Snow Creek; collected left, middle, right bank within about a 20-meter radius of the designated sampling coordinates

Figure C1–1. Anniston PCB site locations where sediment samples were collected from test sites and from one reference site.

tests. USGS–Columbia and USACE–Vicksburg collaborated in the study to increase the number of sediments that could be concurrently tested. USGS–Columbia was the lead laboratory for performing toxicity tests with *C. dilutus* and USACE–Vicksburg was the lead laboratory for performing toxicity tests with *H. azteca* and bioaccumulation tests with *L variegatus*.

A total of 32 samples of sediment were collected from the Anniston PCB Site in August 2010 (table C2–1). Because of the large number of samples and limited capacity of the laboratories, sediment toxicity testing and sediment bioaccumulation testing were done in two cycles (cycle 1a and cycle 1b). Results of 10-d range-finding toxicity tests with *H. azteca* and *L. variegatus* and initial characterization of total organic carbon (TOC) and total PCBs (chapter 2) were used to select the samples for sediment toxicity and sediment bioaccumulation testing in cycle 1a. Summaries of cycle 1a toxicity data also were used to select samples for toxicity and bioaccumulation testing in cycle 1b.

Samples of sediments selected for cycle 1a testing represented medium to high concentrations of total PCBs [based on dry weight (DW) concentrations or on concentrations normalized to TOC in sediment] with the goal of observing moderate to severe toxicity in the cycle 1a samples. One control sediment, 1 reference sediment, and 10 test sediments were selected for cycle 1a toxicity testing and 1 control sediment, 1 reference sediment, and 6 test sediments were selected for cycle 1a bioaccumulation testing (table C2–1). Results of the cycle 1a toxicity tests demonstrated moderate to severe toxicity in the samples with elevated concentrations of PCBs (chapter 4). Therefore, samples of sediment for cycle 1b toxicity and bioaccumulation testing were selected to represent more of the remaining samples with low to moderate concentrations of PCBs. One control sediment, 5 reference sediments, and 10 test sediments were selected for cycle 1b toxicity testing and 1 control sediment, 1 reference sediment, and 6 test sediments were selected for cycle 1b bioaccumulation testing (table C2–1). In cycle 1a, one control sediment and five test sediments also were evaluated in interlaboratory toxicity testing of *H. azteca* by USGS–Columbia and *C. dilutus* by USACE–Vicksburg (appendix 5). At the end of cycle 1b testing, USGS–Columbia performed a study evaluating the sensitivity *C. dilutus* in toxicity tests started with 7-d old larvae (13-d exposures) compared to toxicity tests started with less than 24-hour (h)-old larvae (20-d exposures; appendix 6).

The sampling of sediments was designed to target six concentration ranges of organic carbon (OC) normalized total PCBs in the samples collected from the Anniston PCB Site (ARCADIS, 2010; table C2–1):

- Less than 100 milligrams PCB per kilogram organic carbon (mg PCB/kg OC);
- 100 to 500 mg PCB/kg OC;
- 500 to 1,000 mg PCB/kg OC;
- 1,000 to 5,000 mg PCB/kg OC;
- 5,000 to 10,000 mg PCB/kg OC; and
- More than 10,000 mg PCB/kg OC.

Data used to select the locations for sediment sampling in the current study were obtained from an Off-Site Resource and Recovery Act Facility Investigation Work Plan and the Phase 1 Field Sampling Plan for Operable Unit (OU-4) (Blasland, Bouck, and Lee, 2006). Based on the results of the Phase 1 toxicity and bioaccumulation testing described in this report, the decision will be made by USEPA and other interested parties if additional data are needed to better refine concentration-response models (chapter 5).

Study Area

Operable Unit 4 (OU-4) of the Anniston PCB Site is the most geographically expansive of the OUs delineated at the site. OU-4 also encompasses most of the potentially suitable habitat for ecological receptors and locations with a variety of human uses of the land (ARCADIS, 2009). OU-4 encompasses the length of Choccolocco Creek and its floodplain from the confluence with Snow Creek downstream to the confluence of the Coosa River (fig. C1–1). The upstream part of OU-4 also includes two additional areas: (1) the lower end of Snow Creek and its floodplain between Highway 78 and the confluence of Snow and Choccolocco Creeks (this area was included in OU-4 because it is more characteristic of the Choccolocco Creek floodplain than the urbanized reaches of Snow Creek); and, (2) the backwater area of Choccolocco Creek at its confluence with Snow Creek was identified in the Resource Conservation and Recovery Act (RCRA) Facility Investigation for the Off-Facility part of the site as an area requiring additional characterization because of the depositional nature of the basin (Blasland, Bouck, and Lee, 1996).

Chemicals of Potential Concern in Anniston PCB Site Sediments

The COPCs in sediments at the Anniston PCB Site were identified in (ARCADIS, 2009). The COPCs include: PCBs, mercury, target analytical metals (TAL), alkylated polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides, and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/PCDFs; chapter 2 and appendix 1, table A1–1). Other constituents including volatile organic compounds, semivolatile organic compounds other than PAHs, and organophosphate pesticides were not observed at frequencies or concentrations of concern (ARCADIS, 2009). Although PAHs and organochlorine pesticides were not likely released from the facility and are likely not of concern relative to the risk posed by

PCBs, these additional compounds were included in the analyses of sediment samples in this study to help to identify other contaminants that might contribute to the observed toxicity of the Anniston PCB Site sediments.

Organization of Report

Chapter 2 provides an overview of methods used to collect, handle, prepare, and characterize sediments used in toxicity testing and in bioaccumulation testing. Chapter 2 also provides an overview of the process that was used to select sediments for cycle 1a and cycle 1b testing. Chapter 3 provides results of bioaccumulation testing with *L. variegatus* and bioavailability analysis using solid-phase microextraction fibers. Chapter 3 also evaluates relations between sediment chemistry and bioaccumulation of PCBs by *L. variegatus*. Chapter 4 provides results of sediment toxicity testing with *H. azteca* and *C. dilutus*. Chapter 5 evaluates relations between sediment toxicity and sediment chemistry. Chapter 5 also describes how toxicity thresholds were established and evaluated to refine the COPCs in sediments at the Anniston PCB Site. The appendixes to the report are available at *http://pubs.usgs.gov/sir/2013/5125*. Appendix 1, 2, 3, and 4 provide summaries of chemistry, bioaccumulation, or toxicity data (in the form of tables or figures). Appendix 5 provides a summary of interlaboratory toxicity testing with the *H. azteca* and *C. dilutus* during cycle 1a testing of sediments. Finally, appendix 6 provides a summary of a study that evaluated the relative sensitivity of two ages of *C. dilutus* with exposure to sediments from the Anniston PCB Site.

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Sediment Sample Collection, Handling, Preparation, and Characterization

By Christopher G. Ingersoll, William G. Brumbaugh, Jeffery A. Steevens, Guilherme R. Lotufo, Jacob K. Stanley, Donald D. MacDonald, and Jesse A. Sinclair

Chapter 2 of

Evaluation of Toxicity to the Amphipod, *Hyalella azteca*, and to the Midge, *Chironomus dilutus*; and Bioaccumulation by the Oligochaete, *Lumbriculus variegatus*, with Exposure to PCB-Contaminated Sediments from Anniston, Alabama

Edited by Christopher G. Ingersoll, Jeffery A. Steevens, and Donald D. MacDonald

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Sediment Sample Collection, Handling, Preparation, and Characterization

By Christopher G. Ingersoll[1], William G. Brumbaugh[1], Jeffery A. Steevens[2], Guilherme R. Lotufo[2], Jacob K. Stanley[2], Donald D. MacDonald[3], and Jesse A. Sinclair[3]

Abstract

This chapter provides a summary of methods used to collect and handle sediments as well as methods for characterizing the physical and chemical characteristics of the sediments used to perform the toxicity tests and bioaccumulation tests. Methods also are described for analyses of tissue samples generated from the bioaccumulation tests. This chapter further summarizes data for the physical and chemical characteristics of the sediments and summarizes data for the characteristics of the tissue samples. A total of 32 samples of sediment were collected from the Anniston Polychlorinated Biphenyl (PCB) Site in August 2010. Because of the large number of samples and capacities of laboratories, sediment toxicity testing and sediment bioaccumulation testing were done in two cycles (cycle 1a and cycle 1b). Results of 10-day (d) range-finding toxicity tests with the amphipod *Hyalella azteca* and with the oligochaete *Lumbriculus variegatus* and initial characterization of total organic carbon content and concentration of total PCBs in the sediment samples were used to select the samples for sediment toxicity and sediment bioaccumulation testing in cycle 1a (chapters 3 and 4). Summaries of cycle 1a toxicity data were also used to select samples for toxicity and bioaccumulation testing in cycle 1b and nutrients (chapter 2). Physical characterization of samples of whole sediment included analyses of grain size, total organic carbon, and nutrients. Organic chemical characterization of samples of whole sediment included PCB homologs and select (13) PCB congeners, parent and alkylated polycyclic aromatic hydrocarbons, organochlorine pesticides, and polychlorinated dibenzo-*p*-dioxins and dibenzofurans. The PCB aroclors analyzed included 1016, 1221, 1232, 1242, 1248, 1254, 1260, 1262, and 1268. Analyses of whole sediment also included total metals, simultaneously extracted metals, and acid volatile sulfide. Chemical characterization of samples of pore water isolated from samples of whole sediment at the start of the sediment toxicity exposures or at the start of the sediment bioaccumulation exposures included metals, major cations, major anions, dissolved organic carbon, and additional water-quality characteristics. Concentrations of metals or PCBs in pore water during the sediment toxicity exposures or during sediment bioaccumulation exposures also were determined using peeper samples (for metals) or solid-phase microextraction samplers (for PCBs).

Introduction

The goal of this study was to characterize relations between (1) sediment chemistry and sediment toxicity, and, (2) sediment chemistry and sediment bioaccumulation, in samples of sediments collected from the Anniston Polychlorinated Biphenyl (PCB) Site (chapter 1). A total of 32 samples were evaluated from six test sites and one reference site (fig. C1–1) to provide a wide range in concentrations of chemicals of potential concern (COPCs) including PCBs in samples of whole sediment (table C2–1 and appendix 1, table A1–1). More detailed maps illustrating the locations of the test sites where test and reference samples were collected are provided in ARCADIS (2010). The goal of this study was not to determine the extent of sediment contamination or sediment toxicity across the Anniston PCB Site. Hence, the test sites or the samples collected from within a test site were not selected to represent the spatial extent of sediment contamination across the Anniston PCB Site. Additional studies may be required to determine the spatial extent of sediment contamination and sediment toxicity or sediment bioaccumulation at the Anniston PCB Site.

This chapter provides a summary of analytical methods used to collect, handle, prepare and characterize sediments, as well as methods used to measure the physical and chemical characteristics of the samples of sediment used to perform toxicity or bioaccumulation tests (table C2–2 and appendix 1,

[1] U.S. Geological Survey, Columbia, Missouri.

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[3] MacDonald Environmental Sciences Ltd., Nanaimo, British Columbia.

Table C2–1. Sediment sample identification and results of initial analyses for concentrations of total organic carbon and total polychlorinated biphenyls and results of a 10-day range-finding toxicity test with *Hyalella azteca* and *Lumbriculus variegatus*.

[WB, West Bearskin Lake; UTM, Universal Transverse Mercator; --, not applicable; cm, centimeter; mm, millimeter; mL, milliliter; %, percent; A, cycle 1a; B, cycle 1b; I, interlaboratory toxicity testing; L, life-stage toxicity testing of *Chironomus dilutus*; C, USGS–Columbia interlaboratory testing; V, USACE–Vicksburg interlaboratory toxicity testing; NT, not tested; TOC, total organic carbon; PCB, polychlorinated biphenyls; mg/kg, milligram per kilogram; OC, organic carbon; S, small; A, avoidance of sediment; In the 10-day range-finding toxicity test, 1 replicate/sediment was tested by USGS–Columbia (10 organisms added to 100 mL sediment, 175 mL overlying water, 2 volume additions/day, no feeding of *Lumbriculus variegatus*). Red text designates samples with *Lumbriculus variegatus* that were small or were avoiding sediment or <80% recovery of *Hyalella azteca* or *Lumbriculus variegatus*]

Field identification number	Field sample number	Sampling coordinates (UTM Zone 16: Easting)	Sampling coordinates (UTM Zone 16: Northing)	Field sampling date	Type sampler	Sample depth (range in cm from surface)	Weight of initial sample <2mm (%)	Laboratory sample number	Cycle tested: Toxicity	Cycle tested: Bioaccum-ulation	Cycle tested: Mussel	Initial TOC (%)	Initial total PCB (mg/kg)	Initial total PCB (mg/kg OC)	Range of total PCB (mg/kg OC)	Number of *Hyalella azteca* recovered	Number of *Lumbriculus variegatus* recovered
Control (WB)	X900033	--	--	--	--	--	--	33	A/B/I/L	A/B	B	1.16	.058	5	Control	10	12
TX10-01-P	X900016	3714325.18	576857.068	23-Aug-10	Core	0–30	89	16	B	B	B	0.16	.203	125	100–500	9	13
TX10-02-P	X900032	3714331.389	576857.019	23-Aug-10	Core	0–30	86	32	NT	NT	NT	0.16	.087	53	<100	9	11S
TX10-03-P	X900003	3714340.55	576851.747	23-Aug-10	Core	0–30	84	3	NT	NT	NT	0.17	.049	28	<100	10	10
TX20-01-P	X900028	3714009.202	598901.939	23-Aug-10	Core	0–30	87	28	B	A	NT	1.86	.645	35	<100	10	10
TX20-02-P	X900012	3714002.94	598896.804	23-Aug-10	Core	0–30	65	12	NT	NT	NT	0.49	.343	70	<100	10	14
TX20-03-P	X900024	3714005.859	598878.764	23-Aug-10	Core	0–30	77	24	B	B	NT	0.26	.202	77	<100	10	9S
TX30-01-P	X900025	3718585.158	609031.321	21-Aug-10	Core	30–60	85	25	A/I	A	B	2.59	26.3	1,015	500–1,000	8	6S
TX30-02-P	X900018	3718585.245	609039.022	21-Aug-10	Core	30–60	91	18	A/I(V)	NT	B	2.64	476.	18,030	>10,000	3	7S
TX30-03-P	X900007	3718579.088	609033.895	21-Aug-10	Core	30–60	93	7	A/I	NT	B	3.99	65.4	1,639	1,000–5,000	4	7
TX30-04-P	X900023	3718597.634	609036.283	21-Aug-10	Core	30–60	96	23	B	B	NT	2.18	5.58	256	100–500	10	8S
TX30-05-P	X900002	3718597.693	609041.479	21-Aug-10	Core	30–60	64	2	B/L	B	B	5.25	32.1	611	500–1,000	7	7
TX40-01-P	X900027	3718469.525	609151.136	20-Aug-10	Core	0–60	85	27	B/L	A	B	1.01	6.94	687	500–1,000	9	11
TX40-02-P	X900017	3718463.316	609151.206	20-Aug-10	Core	0–60	86	17	B/L	NT	B	1.09	43.5	3,991	1,000–5,000	10	7S
TX40-03-P	X900015	3718466.392	609148.666	20-Aug-10	Core	0–60	89	15	B/L	NT	NT	1.45	.82	57	<100	10	6A
TX40-04-P	X900001	3718469.497	609148.631	20-Aug-10	Core	0–60	92	1	A	A	B	1.88	27.	1,436	1,000–5,000	10	9
TX40-05-P	X900014	3718469.408	609140.837	20-Aug-10	Core	0–60	91	14	B	B	B	1.78	30.9	1,736	1,000–5,000	10	6
TX50-01-P	X900008	3718585.158	609031.321	21-Aug-10	Core	0–30	97	8	A	NT	B	2.76	320.	11,594	5,000–10,000	2	4S
TX50-02-P	X900019	3718585.245	609039.022	21-Aug-10	Core	0–30	85	19	A/I(C)	NT	B	2.59	437.	16,873	>10,000	2	7S
TX50-03-P	X900031	3718579.088	609033.895	21-Aug-10	Core	0–30	85	31	NT	NT	NT	2.73	447.	16,374	>10,000	6	2S
TX50-04-P	X900011	3718597.634	609036.283	21-Aug-10	Core	0–30	99	11	A/I	B	B	2.52	85.5	3,393	1,000–5,000	5	10
TX50-05-P	X900030	3718597.693	609041.479	21-Aug-10	Core	0–30	82	30	A/I	NT	B	2.30	204.	8,870	5,000–10,000	6	6
TX60-01-P	X900021	3718750.064	609227.102	19-Aug-10	Core	90–120	80	21	NT	NT	B	0.13	5.45	4,192	1,000–5,000	10	10
TX60-02-P	X900020	3718755.891	609222.674	19-Aug-10	Core	90–120	80	20	A/B/L	A	B	1.11	3.08	277	100–500	10	10
TX60-03-P	X900006	3718753.733	609227.895	19-Aug-10	Core	90–120	91	6	A/I	NT	B	1.33	59.9	4,504	1,000–5,000	9	9
TX60-04-P	X900013	3718753.674	609222.699	19-Aug-10	Core	90–120	92	13	B	A	B	1.28	14.2	1,109	1,000–5,000	6	12
TX60-05-P	X900005	3718750.856	609238.228	19-Aug-10	Core	90–120	74	5	NT	NT	NT	0.34	.049	14	<100	10	10
TXR1-01-P	X900026	3718243.149	613275.642	18-Aug-10	Grab	0–15	78	26	B	NT	B	0.26	.05	19	Reference	10	14
TXR1-02-P	X900009	3718240.044	613275.678	18-Aug-10	Grab	0–15	76	9	A	A	B	0.72	.046	6.4	Reference	10	17
TXR1-03-P	X900004	3718240.105	613280.875	18-Aug-10	Grab	0–15	71	4	B	NT	B	0.46	.049	11	Reference	10	12
TXR1-04-P	X900022	3718237.001	613280.911	18-Aug-10	Grab	0–15	77	22	B	NT	B	0.32	.048	15	Reference	10	13
TXR1-05-P	X900029	3718258.53	613272.862	18-Aug-10	Grab	0–15	74	29	B	B	B	0.70	.048	6.9	Reference	10	10
TXR1-06-P	X900010	3718267.552	613257.35	18-Aug-10	Grab	0–15	79	10	B	NT	B	0.69	.051	7.4	Reference	10	10

Site descriptions (see fig. C1–1 for maps of sites):

TRX1: Choccolocco Creek about 500 meters upstream from Morgan Road Bridge; collected along left bank within about a 10-meter radius of the designated sampling coordinates.

TX10: Choccolocco Creek near Jackson Shoals; collected along the lacustrine portion of the site (Lake Logan Martin) near an exposed island with about 50 meters of the designated sampling coordinates.

TX20: Choccolocco Creek near Jackson Shoals; collected on the inside portion of a river bend across the river from the designated sampling coordinates.

TX30: Choccolocco Creek about 125 meters upstream of confluence with Snow Creek; collected left, middle, right bank within about a 20-meters radius of the designated sampling coordinates.

TX40: Choccolocco Creek about 300 meters upstream of confluence with Snow Creek; collected left, middle, right bank within about a 15-meter radius of the designated sampling coordinates.

TX50: Choccolocco Creek about 125 meters upstream of confluence with Snow Creek; collected left, middle, right bank within about a 20-meter radius of the designated sampling coordinates.

TX60: Choccolocco Creek about 175 meters upstream of confluence with Snow Creek; collected along the left bank within about a 15-meter radius of the designated sampling coordinates.

Table C2–2. Summary of analytical methods, responsibilities, containers, volume requirements, preservation, and holding times for sediment samples.

[SPME, solid-phase microextraction; SVOC, semi-volatile organic contaminants; OC pesticide, organochlorine pesticides; PCB, polychlorinated biphenyls; PCDDs/DFs, polychlorinated dibenzo-*p*-dioxins and dibenzofurans; PAH, polycyclic aromatic hydrocarbon; TOC, total organic carbon; SEM, simultaneously extracted metals; AVS, acid volatile sulfide; %, percent; DO, dissolved oxygen; DOC, dissolved organic carbon; USEPA, United States Environmental Protection Agency; EDTA, ethylenediaminetetraacetic acid; --, no data; LDPE, low density polyethylene; HPDE, high density polyethylene; mL, milliliter; g, gram; °C, degree Celsius; HNO_3, nitric acid; SAOB, sulfide anti-oxidant buffer; d, day; h, hour]

Medium	Responsible laboratory	Analyte	Analytical method (or the equivalent)	Container material	Container volume (mL)	Minimum sample	Preservation method	Holding time
Sediment	ARCADIS	TR Metals	See ARCADIS (2010)	--	--	--	--	--
	ARCADIS	SVOCs, OC Pesticides	See ARCADIS (2010)	--	--	--	--	--
	ARCADIS	PCBs	See ARCADIS (2010)	--	--	--	--	--
	ARCADIS	PCDD/DFs	See ARCADIS (2010)	--	--	--	--	--
	ARCADIS	PAHs	See ARCADIS (2010)	--	--	--	--	--
	ARCADIS	TOC and grain size	See ARCADIS (2010)	--	--	--	--	--
	USGS	SEM	USEPA 376.3, 200.8	Amber glass	60	60 mL	4 °C	21 days
	USGS	AVS	USEPA 376.3, Brumbaugh and others (2011)	From same bottle listed above.	--	--	--	--
	ARCADIS	% Moisture	Dry weight determination	--	--	--	--	--
SPMEs	USACE	PCBs	SW846 Method 8082, Conder (2003)	Glass	1	0.1 mL	4 °C	28 days
Tissue	ARCADIS	PCBs	USEPA Method 1668A	Glass	5	0.4 g	4 °C	28 days
	ARCADIS	Lipids	Test America, Knoxville, SOP KNOX-OP-0019 Lotufo and others (2000)	Glass	Combined	0.1 g	4 °C	28 days
Pore water (peeper)	USGS	Dissolved Metals	Brumbaugh and May (2008)	LDPE vial	2.9	2.9 mL	0.16M HNO_3	6 months
Pore water (centrifuge)	USGS	Hydrogen Sulfide	Orion 94 16 Meter	Glass vial	20	10 mL	1+1 SAOB	7 days
	USGS	Ammonia	Orion EA940 Meter	Glass	125	125 mL	none	4 hours
	USGS	pH	Orion EA940 Meter	From same bottle listed above.	--	--	--	--
	USGS	Temperature	Orion 140 S-C-T Meter	From same bottle listed above.	--	--	--	--
	USGS	Hardness	EDTA Titration	From same bottle listed above.	--	--	--	--
	USGS	Alkalinity	Orion EA940 Meter	From same bottle listed above.	--	--	--	--
	USGS	Conductivity	Orion 140 S-C-T Meter	From same bottle listed above.	--	--	--	--
	USGS	DO	YSI 54a Meter & YSI 5739 Probe	From same bottle listed above.	--	--	--	--
Pore water	USGS	Major Cations	USEPA 200.7	LDPE	20	20 mL	0.16M HNO_3	6 months
	USGS	Major Anions	USEPA 300.0	HDPE	10	10 mL	4 °C	28 days
	USGS	DOC	USEPA 415.2	Amber glass	60	20 mL	4 °C	7 days
Overlying water (routine toxicity test monitoring)	USGS/USACE	Ammonia	Orion EA940 Meter	Glass	125	125 mL	none	4 hours
	USGS/USACE	Temperature	Orion 140 S-C-T Meter	From same bottle listed above.	--	--	--	--
	USGS/USACE	pH	Orion EA940 Meter	From same bottle listed above.	--	--	--	--
	USGS/USACE	DO	YSI 54a Meter & YSI 5739 Probe	From same bottle listed above.	--	--	--	--
	USGS/USACE	Hardness	EDTA Titration	From same bottle listed above.	--	--	--	--
	USGS/USACE	Alkalinity	Orion EA940 Meter	From same bottle listed above.	--	--	--	--
	USGS/USACE	Conductivity	Orion 140 S-C-T Meter	From same bottle listed above.	--	--	--	--

table A1–1 and A1–2). Results of these physical and chemical characteristics of the whole-sediment samples or pore-water samples are presented in appendix 1 and tables A1–3a to A1–3d). This chapter also provides a summary of the procedures used to select samples for testing in cycle 1a and in the cycle 1b toxicity testing with the amphipod, *Hyalella azteca,* and the midge, *Chironomus dilutus,* (chapter 4) and to select samples for testing in cycle 1a and in cycle 1b for sediment bioaccumulation testing with the oligochaete, *Lumbriculus variegatus* (chapter 3).

Briefly, physical characterization of samples of whole sediment included analyses of grain size, total organic carbon (TOC), and nutrients. Organic chemical characterization of samples of whole sediment included PCB homologs and select (13) PCB congeners, parent and alkylated polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides, and polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/PCDFs). The PCB aroclors analyzed included 1016, 1221, 1232, 1242, 1248, 1254, 1260, 1262, and 1268. Analyses of whole sediment also included total metals, simultaneously extracted metals (SEMs), and acid volatile sulfide (AVS). Chemical characterization of samples of pore water isolated from samples of whole sediment at the start of the sediment toxicity exposures or at the start of the sediment bioaccumulation exposures included metals, major cations, major anions, dissolved organic carbon, and additional water-quality characteristics. Concentrations of metals or PCBs in pore water during the sediment toxicity exposures or during sediment bioaccumulation exposures also were determined using peeper samples (for metals) or solid-phase microextraction (SPME) samplers (for PCBs).

Methods

Sediment Collection and Handling

A total of 32 samples of sediment were collected from the Anniston PCB Site in August 2010 (table C2–1). Because of the large number of samples and limited capacity of the laboratories, sediment toxicity testing and sediment bioaccumulation testing was done in two cycles (cycle 1a and cycle 1b). Results of 10-d range-finding toxicity tests with *H. azteca* and *L. variegatus* and initial characterization of TOC and total PCBs were used to select the samples for sediment toxicity and sediment bioaccumulation testing in cycle 1a (table C2–1). Summaries of cycle 1a toxicity data also were used to select samples for toxicity and bioaccumulation testing in cycle 1b (table C2–1).

Table C2–1 provides a summary of the sample identification numbers (field identification numbers and laboratory identification numbers), sampling coordinates, sampling dates, type of sampler used to collect the sediments, and depth of sample collected. Samples were collected from one reference site (n=6 from test site TXR1), and from six test sites (n=3 to 5 samples/test site: test sites TX10, TX20, TX30, TX40, TX50, and TX60; fig. C1–1). Samples for test site TX50 and test site TX30 were collected from a common set of cores and split based on the depth of the sediment (that is, TX50 samples were collected as composites from the upper parts of the cores at a depth of about 0 to 30 cm from the sediment surface and TX30 samples were collected as composites from lower parts of these same cores at a depth of about to 30 to 60 cm from the sediment surface).

Upon arrival at a sampling site, the field crew determined if site conditions allowed a sediment sample collection (provided there were no concerns regarding access, safety, and availability of fine-grained sediment). The field sampling crew identified the proposed sampling location in the data sheets along with description of the physical characteristics of the site. The location initially was identified with a hand-held global positioning system (GPS) unit to identify the location for collection of sediment samples within a site. A boat (if used) was positioned near the sampling location within a site. A GPS reading was then made near the location where each set of composited samples were collected within a site (table C2–1).

Samples of sediment were collected from the reference site using a posthole digger (grab sampler) and sediment samples from the test sites were collected using 10-centimeter (cm) Lexan® cores (core sampler) in accordance with procedures outline in ARCADIS (2010; see also the information summarized in table C2–1). Equipment used to collect sediment was decontaminated between samples by brushing to remove sediment particles, washing with soapy water and rinsing with site water between collections of each set of samples. The following is a description of the procedure used to collect the composite sediment cores for a sampling location within a site:

1. At each sampling location, the lower section of a Lexan® tube was placed on the top of the sediment and the depth of water was recorded.

2. The tube was driven into the sediment by hand until the targeted depth was achieved, or until refusal. The depth of penetration was recorded from the sediment surface.

3. The tube was driven several more centimeters into the substrate, using a stainless-steel core driver to improve recovery of soft sediment. This procedure was done to obtain a section at the bottom of the core that helped prevent the loose sediment from escaping from the bottom of the tube.

4. A cap was placed on the top end of the tube to create a vacuum to help prevent the sediment plug from escaping from the bottom of the tube.

5. The tube was slowly pulled from the sediment, twisting slightly as removed (if necessary).

6. The integrity and depth of the core was recorded. If the core within the tube was not suitably intact, the sediment within the tube was discarded and a new sample was collected.

7. Keeping the tube upright, the sediment was extruded to the target sample depth (table C2–1) into a 5-gallon high density polyethylene bucket.

8. The process was repeated until there was a minimum of 16 liters (L) of sediment in two to three 5-gallon buckets for that composite sample of sediment.

9. Pore water was included in the samples of sediment and care was made to ensure that overlying water (that is, surface water) was not included in the samples to the extent practical.

10. The buckets containing the samples of sediment were then transported to a local staging building and were stored at 4 degrees Celsius (°C) in the dark in a secured refrigeration truck.

11. Within 24 hours of sampling, the sediment samples were press sieved using a 2-millimeter (mm) stainless-steel or brass sieve in a processing area of the staging building. A minimal amount of site water was added to some of the sediment samples to facilitate the sieving process (that is, less than 1 L of water added to a bucket of sediment while sieving the sediment to less than 2 mm).

12. Sediments were pushed through the sieve by hand covered with powder-free, nitrile gloves. The weight of the material that passed through the 2-mm sieve and that of the material retained on the 2-mm sieve was determined using a hanging scale. The percentage weight of the less than 2-mm sieve fraction ranged from 64 to 99 percent by weight and was typically greater than 75 percent (table C2–1).

Once all of the sediments were collected and processed at the local staging building, the samples of sediment were delivered to U.S. Geological Survey, Columbia, Missouri (USGS–Columbia) laboratory using Federal Express Custom Critical refrigerated shipping. The sediments were logged in at USGS–Columbia laboratory and stored at 4 °C in the dark under chain of custody in a secured refrigeration unit. Subsamples of sediment in the 5-gallon buckets were combined in 7-gallon buckets and homogenized for about 5 minutes (min) using a hand-held drill and stainless steel auger (diameter 7.6 cm, overall length 38 cm, auger bit length 25.4 cm, Augers Unlimited, Coatesville, Pennsylvania; Ingersoll and others, 2008). Subsamples were then obtained for rapid turnaround analyses of aroclors and TOC by Test America, Knoxville, Tennessee under the direction of ARCADIS. The sediments were then returned to the refrigeration unit. Results of the analyses of aroclors and TOC were completed within 1 month and were used to select sediment samples for cycle 1a or cycle 1b toxicity testing (table C2–1). In addition, USGS–Columbia performed 10-d range-finding toxicity tests with *H. azteca* and *L. variegatus* to determine if samples were toxic or were avoided by these test organisms (1 replicate/sediment for each species; table C2–1).

Selection of Samples for Cycle 1a and Cycle 1b Toxicity and Bioaccumulation Testing

Results of the 10-d range-finding toxicity tests and the initial characterization of TOC and PCBs were used to select the samples for toxicity and bioaccumulation testing in cycle 1a. Summaries of cycle 1a toxicity data also were used to select samples for toxicity and bioaccumulation testing in cycle 1b. Samples of sediments selected for cycle 1a testing represented medium to high concentrations of total PCBs [based on dry weight (DW) concentrations or on concentrations normalized to TOC in sediment; chapter 1] with the goal to maximize the likelihood of observing moderate to severe toxicity in the cycle 1a samples. One control sediment, 1 reference sediment, and 10 test sediments were selected for cycle 1a toxicity testing and 1 control sediment, 1 reference sediment, and 6 test sediments were selected for cycle 1a bioaccumulation testing (table C2–1). The laboratory control sediment obtained from West Bearskin Lake in northeastern Minnesota (about 1.1 percent TOC) was run with each sediment toxicity test with each species (Ingersoll and others, 1998). Results of the cycle 1a toxicity tests demonstrated that moderate to severe toxicity was evident in the samples with elevated concentrations of PCBs (chapter 4). Therefore, samples of sediment for cycle 1b toxicity and bioaccumulation testing were selected to represent more of the remaining samples with low to moderate concentrations of PCBs. One control sediment, 5 reference sediments, and 10 test sediments were selected for cycle 1b toxicity testing and 1 control sediment, 1 reference sediment, and 6 test sediments were selected for cycle 1b bioaccumulation testing (table C2–1).

The samples of sediment for cycle 1a testing were subsampled at USGS–Columbia during the week of October 18, 2010, and were delivered to the U.S. Army Corps of Engineers, Engineer Research and Development Center, Vicksburg, Mississippi (USACE–Vicksburg) in a refrigerated truck following the same procedures used to deliver samples from Anniston PCB Site to Columbia. Samples of sediments for cycle 1b testing were subsampled at USGS–Columbia during the week of January 3, 2011, and were then delivered to USACE–Vicksburg. Additional samples were subsequently provided to USACE–Vicksburg for repeating cycle 1b bioaccumulation testing (chapter 3). Once at USACE–Vicksburg, samples of sediments were stored at 4 °C in the dark until the start of the toxicity or bioaccumulation testing (chapters 3 and 4). USGS–Columbia was the lead laboratory performing toxicity tests with *C. dilutus* in cycle 1a and in cycle 1b and USACE–Vicksburg was the lead laboratory performing toxicity tests with *H. azteca* and bioaccumulation tests with *L. variegatus* in cycle 1a and in cycle 1b (chapters 3 and 4).

In cycle 1a, one control sediment and five test sediments also were evaluated in interlaboratory toxicity testing of *H. azteca* by USGS–Columbia and *C. dilutus* by USACE–Vicksburg (table C2–1 and appendix 5). At the end of cycle 1b testing, USGS–Columbia also performed a study evaluating the sensitivity *C. dilutus* in toxicity tests started with 7-day (d)-old larvae (13-d exposures) compared to toxicity tests start with less than 24-hour (h)-old larvae (20-d exposures; appendix 6). One control sediment and five test sediments were evaluated in a study comparing relative life-stage sensitivity of *C. dilutus* (table C2–1).

The sediments evaluated in cycle 1b were stored for about 5 months before the start of cycle 1b sediment toxicity or before the start of cycle 1b sediment bioaccumulation testing. American Society for Testing and Materials International (2012a; 2012b) and U.S. Environmental Protection Agency (2000) recommend starting sediment toxicity or sediment bioaccumulation tests within about 2 months of collection of sediments from the field; however, the effect of storage time on sediments is dependent on the type of chemicals present in the sediment. Specifically, American Society for Testing and Materials International (2012a) and U.S. Environmental Protection Agency (2000) state that samples of sediment containing stable compounds (such as PCBs) or samples of sediment that exhibit moderate to high toxicity typically do not vary appreciably in toxicity in relation to storage duration. Hence, long-term storage (for example, greater than 2 months) of sediments before the start of sediment exposures is acceptable for evaluating contaminants such as PCBs in field-collected sediments (American Society for Testing and Materials International, 2012a; U.S. Environmental Protection Agency, 2000). American Society for Testing and Materials International (2012a) and U.S. Environmental Protection Agency (2000) also recommend additional characterizations of sediment to evaluate possible effects of storage on chemicals of interest. To address the potential effect of sediment storage, sample 20 also was selected for retesting in cycle 1b using *C. dilutus* (results following).

Based on the guidance provided by American Society for Testing and Materials International (2012a) and U.S. Environmental Protection Agency (2000), the physical and chemical characteristics of the sediment samples were determined at the start of cycle 1a and cycle 1b testing. Specifically, during the week of October 18, 2010, for cycle 1a testing and during the week of January 3, 2011, for cycle 1b testing, USGS–Columbia subsampled sediments for characterization of physical and chemical characteristics of whole sediment and for characterization of pore water by centrifugation. The sediments were subsampled about 14 days before the start of the sediment exposures (about day minus 14) that was about 7 days before sediments were placed in exposure chambers (about day minus 7). Exposures of test organisms were started (day 0) at USGS–Columbia and at USACE–Vicksburg during the week of November, 1, 2010, for cycle 1a testing and during the week of January 17, 2011, for cycle 1b testing. Additionally, sample 20 was tested in cycle 1a and retested in cycle 1b with *C. dilutus* by USGS–Columbia to determine repeatability of effects observed across storage time between the start of cycle 1a and the start of cycle 1b (chapter 4).

Each sediment sample was re-homogenized with a stainless steel auger about 7 days before the start of the sediment exposures (that is day minus 7) at USGS–Columbia or at USACE–Vicksburg (during the week of October 25, 2010, for cycle 1a testing and during the week of January 17, 2011, for cycle 1b testing). Test organisms were placed in exposure chambers (day 0) during the week of November 1, 2010, for cycle 1a testing and during the week of January 17, 2011, for cycle 1b testing. From about day minus 7 to day 0, the exposure chambers containing sediment and overlying water were maintained at 23 °C without renewal of overlying water to allow sediments to more efficiently equilibrate in the exposure chambers (chapters 3 and 4; Ingersoll and others, 2008).

Separate replicate chemistry chambers containing sediment and test organisms were included with each sediment toxicity treatment to sample sediments for SEMs and AVSs, and to sample pore-water metals and major cations in pore water with peeper samplers during the exposure (Ingersoll and others, 2008). Peeper samplers were placed in these replicate chemistry chambers on day 14 of the exposures. These replicate chemistry chambers were then sampled on day 21 for SEM, AVS, and water from the peeper samplers (table C2–2, see below for more detail on SEM, AVS, and peeper sampling). Solid-phase microextraction fibers were placed in additional replicate chemistry chambers containing sediment and test organisms on day 0 of each sediment toxicity and sediment bioaccumulation treatment and sampled on day 28 of exposures to measure pore-water concentration of PCBs (table C2–2, see chapter 3 for more detail on SPME sampling). The replicate chemistry chambers containing sediment and test organisms were maintained following the same procedures as the replicate chambers used to determine sediment bioaccumulation by test organism (chapter 3) or sediment toxicity to test organisms (chapter 4).

The following sections describe the methods used to determine the physical and chemical characteristics of the whole-sediment samples and the chemical characteristics of pore water associated with the whole-sediment samples.

Grain Size and Total Organic Carbon Analyses of Whole Sediment

Analyses of grain size and TOC of sediments were done under the direction of ARCADIS (ARCADIS, 2011). Methods outlined in American Society for Testing and Materials International (2012c) were used to determine grain-size distribution using a series of sieves. Grain-size distribution of the sediment samples was described using the soil classification system outlined in American Society for Testing and Materials International (2012d, 2012e). Total organic carbon was determined using the Lloyd Kahn Method (U.S. Environmental Protection Agency, 1988).

Chemical Characterization of Whole Sediment

Organic Analyses

Organic analyses of whole-sediment samples performed by Test America under the direction of ARCADIS included the following: (1) PCB aroclors, homologs and select congeners; (2) parent and alkylated PAHs; (3) organochlorine pesticides; and, (4) PCDDs/PCDFs. Specific methods followed U.S. Environmental Protection Agency SW–846 (2011) and specific procedures are outlined in ARCADIS (2008) and in U.S. Environmental Protection Agency (2012a–e). PCBs were analyzed in sediment and tissue samples by ARCADIS and SPME was analyzed by USACE–Vicksburg. Different PCB analyses are shown in table C2–3. PCB total homolog groups were analyzed using U.S. Environmental Protection Agency (USEPA) method 680 by low resolution mass spectrometry in selective ion mode (LR/MS/SIM). The reporting limit in the current study for each homolog group was 3 micrograms per kilogram (µg/kg). PCB aroclors (1016, 1221, 1232, 1242, 1248, 1254, 1260, and 1268) were analyzed by USEPA method 8082 with a reporting limit of 33–67 µg/kg. A subset of 13 PCB congeners (77, 81, 105, 114, 118, 123, 126, 153, 156, 157, 167, 169, 189) were analyzed by USEPA method 8082 with a reporting limit of 1.5–3 µg/kg. Dioxins were analyzed following USEPA method 8290 with a reporting limit of 4–20 nanograms per kilogram (ng/kg) depending on the congener. Volatile and semivolatile compounds were analyzed by USEPA methods 8260B and 8270C, respectively. Pesticides were analyzed by USEPA methods 8081A, 8270C, or 8141A.

Inorganic Analyses

The analyses of whole-sediment samples, completed by following the direction of ARCADIS, included total concentrations of 16 metals or metalloids on the USEPA target analyte list (antimony, arsenic, barium, beryllium, cadmium, chromium, cobalt, copper, lead, mercury, nickel, selenium, silver, thallium, vanadium, and zinc) and most of the "matrix" elements (aluminum, calcium, iron, magnesium, manganese, potassium, and sodium; ARCADIS, 2011; table C2–2).

The analyses of whole-sediment samples done by USGS–Columbia included AVS and five SEMs—cadmium, copper, lead, nickel, and zinc (U.S. Environmental Protection Agency, 2005; table C2–2). Specific analysis procedures followed those of Brumbaugh and Arms (1996) and Brumbaugh and others (2011). Briefly, from each 60-mL sediment subsample that was collected from the test chamber, a 5-gram (g) wet sample was transferred to a nitrogen-purged, 250-mL round bottom flask containing a tetrafluoroethylene (TFE)-coated stir bar. Fifty mL of de-oxygenated, deionized water was added and the flask was sealed using a ground-glass tapered fitting connected to a three-way TFE valve connected to a nitrogen purge line. From the outlet of the valve a glass bubbling tube was connected and the end of the tube was positioned into the bottom of a 50-mL glass centrifuge tube filled with sulfide anti-oxidant buffer (SAOB). After briefly purging the flask and sample with nitrogen, the gas flow was momentarily halted and 50 mL of de-oxygenated, 1 molar (M) hydrochloric acid (HCl) was added by a three-way valve. The sample was then gently stirred for 1 hour at a constant nitrogen flow of 60 mL/min with the gas bleed tube outlet submerged in the SAOB trapping solution. The 1M HCl extract was allowed to settle for 10–15 min and a 20-mL part of this extract was filtered through a 0.45 micrometers (µm), polyethersulfone membrane and transferred to an acid-cleaned, polyethylene bottle for analysis. Concentrations of the SEMs were determined on the sediment extract using inductively coupled plasma-mass spectrometry (ICP-MS) following USEPA method 6020A (U.S. Environmental Protection Agency, 2007a). Extracts were diluted at least 20-fold with 1 percent [volume per volume (v/v)] nitric acid before analysis to minimize potential interferences caused by the HCl matrix (May and others, 1997). The AVS collected within each SAOB trap was measured within 1 week of preparation using an ion-selective electrode (Allen and others, 1991).

Table C2–3. Summary of polychlorinated biphenyl analysis laboratory and methods for sediment, tissue, and solid-phase microextraction.

[*, 13 polychlorinated biphenyls including, PCB077, PCB081, PCB105, PCB114, PCB118, PCB123, PCB126, PCB153, PCB156, PCB157, PCB167, PCB169, PCB189]

Sample	Sediment	Tissue	Solid-phase microextraction
Analytical laboratory	ARCADIS–Test America	ARCADIS–Test America	USACE–Vicksburg
Analysis	Homolog analysis	Homolog analysis	Congener analysis (209) summed to calculate homolog group concentrations
	Selected congener (13 total)*	Selected congener (13 total)*	
	Aroclor analysis		

Chemical Characterization of Pore Water

Sampling of Pore Water by Centrifugation

Pore water was sampled in two ways, depending on the analyte. Samples for general water quality, major cations, major anions, and DOC, were collected by centrifugation of whole sediment at 5,200 revolutions per minute (7000 times relative centrifugal force) for 15 min at 4 °C at the start of the toxicity and bioaccumulation exposures. Sub-samples for major cations, major anions, sulfide, or DOC were drawn separately (20 mL each) and each was filtered through a polypropylene cartridge containing a 0.45-µm pore-size, polyethersulfone membrane. Samples for major anions were collected in a polypropylene vial and were stored at 4 °C for as many as 28 days before analysis. Samples for major cations were collected in an acid-rinsed polyethylene bottle and were acidified to 1 percent (v/v) with sub-boiled nitric acid. Samples for sulfide were immediately mixed 1+1 with SAOB in a glass vial and stored at 4 °C for as many as 7 days. Samples for DOC were collected in an amber glass bottle and were acidified to a pH of less than 2 with 4.5 normal sulfuric acid and stored at 4 °C for as many as 28 days in accordance with U.S. Environmental Protection Agency (1983). The remainder of each centrifugate was decanted into a clean glass beaker for immediate analysis (within 30 min) of selected water-quality characteristics, including alkalinity, hardness, ammonia, conductivity, and pH.

Centrifuged pore-water samples were analyzed for general water-quality characteristics including pH, alkalinity, hardness, conductivity, ammonia, and hydrogen sulfide, using standard methods (American Public Health Association, 2005). Analysis methods and targets for selected measures of accuracy and precision for these analytes are summarized in appendix 1. Analysis for major cations (calcium, magnesium, potassium, sodium, iron, manganese, and strontium) was done by inductively-coupled plasma-atomic emission spectroscopy (ICP-AES) according to USEPA method 200.7 (U.S. Environmental Protection Agency, 1994) by LET Labs (Columbia, Missouri). Analysis for major anions (fluoride, chloride, bromide, nitrite+nitrate, and sulfate) was done by ion chromatography according to USEPA method 9056a (U.S. Environmental Protection Agency, 2007b). Samples were analyzed for DOC by ultraviolet-promoted persulfate oxidation followed by acidification and detection of carbon dioxide in a manner similar to USEPA Method 415.2 (U.S. Environmental Protection Agency, 1983).

Sampling of Pore Water with Peepers

Concentrations of trace metals in pore water were sampled from each test sediment in the chemistry replicate exposure chambers during whole-sediment toxicity testing with a small dialysis chamber ("peeper"). Peeper samplers were fabricated from acid-cleaned, 2.9-mL polyethylene cylindrical vials, each filled with deoxygenated, deionized water and fitted with a 0.45-µm pore size polyethersulfone membrane on one end. Four sets of peeper samplers (one set for each testing laboratory and each testing cycle) were prepared at USGS–Columbia, each within 4 days of deployment. For each set, several extra peeper samplers were prepared to serve as blanks. Peeper samplers were stored in an acid-cleaned polyethylene bottle filled with deoxygenated, high-purity water. To minimize trace metal background in the peeper samplers during storage, one extra peeper was included in each bottle that was filled with a metal chelating resin (Chelex-100™) in the sodium form. On day 14 of each toxicity test, a peeper was carefully inserted into the sediment of one replicate chemistry test chamber for each sediment tested (a replicate that contained test organisms, with food and overlying water provided daily; chapter 4). Using a micro spatula to create a small trench and to backfill, the peeper was inserted to a depth of about 1 cm. The trench and back end of the peeper were situated against the chamber wall such that the membrane end of the peeper was oriented facing the greatest possible volume of undisturbed sediment within the chamber. On day 21 of each toxicity exposure, all chambers containing peeper samplers were removed and transported to the respective analytical laboratories at USGS–Columbia or at USACE–Vicksburg for processing. Each peeper was removed with plastic forceps and then rinsed with a stream of deionized water until no visible particles remained on the exterior. The peeper was carefully opened with particle-free, gloved hands and the contents transferred to an acid-cleaned polyethylene bottle using an acid-rinsed, disposable polyethylene micropipette. The sample was then diluted gravimetrically to a volume of 29 mL with 1.1 percent (v/v) high-purity nitric acid to produce a dilution factor of 10 and a final acid matrix of 1 percent nitric acid for analysis.

Analysis of peeper contents was done using a 62-element semiquantitative analysis method using ICP-MS, as described by Brumbaugh and May (2008). Uncertainty limits for the semiquantitative method are reported by the manufacturer to be plus or minus 30 to 50 percent, depending on the element and daily instrumental conditions. However, except at concentrations near detection limits, USGS–Columbia historically has obtained uncertainty limits of about plus or minus 15 percent for most determinations done in this manner. Uncertainty is generally greatest for lighter elements; furthermore, at low concentrations the results for arsenic, chromium, and iron are susceptible to positive interferences (high bias) caused by sample constituents. Targets for selected measures of accuracy and precision for the COPC metals and metalloids are summarized in appendix 1, table A1–1. Reporting limits for this method are conservative estimates (rounded up to reduce the probability of reporting false positives that might result from random, low-level laboratory contamination) based on historical averages. Calibration was done with a National Institute of Standards and Technology traceable reference solution to which five elements were added for the rare earth region of the mass spectral range. Internal standards used to correct for instrument drift and matrix-induced ionization effects were

scandium, rhodium, and bismuth; each was added on-line to produce effective concentrations of 10 micrograms per liter (µg/L).

Solid-Phase Microextraction

Polydimethylsiloxane (PDMS) SPMEs were used to estimate pore-water concentrations of PCBs as passive samplers in each sediment that was evaluated in bioaccumulation testing (chapter 3) or in each sediment that was evaluated in toxicity testing (chapter 4). The SPME samplers were placed in separate replicate sediment bioaccumulation test chambers or sediment toxicity test chambers for 28 days to measure the bioavailable pore-water fraction of PCBs. See chapter 3 for full details on methods and data analysis used to deploy, sample, and analyze PCBs that accumulated in the SPME fibers and methods used to estimate pore-water concentrations of PCBs in the bioaccumulation sediment exposures or toxicity sediment exposures. Results of the estimated pore-water concentrations of PCBs also are discussed in chapter 3 (in relation to sediment bioaccumulation) and in chapter 5 (in relation to sediment toxicity).

Results and Discussion

Physical Characterization of Whole Sediment

Appendix 1, table A1–3a provides a summary of the data for physical characteristics measured in the whole-sediment samples. Briefly, the average (and range) for the physical characteristics of the sediments, all in percent, were as follows: solids, 64.4 (46.2–74.8); TOC, 1.5 (0.22–3.99); clay, 19.7 (0.0–41.1); silt, 28.5 (3.2–54.3); and sand, 51.1 (9.6–97.1).

Chemical Characterization of Whole Sediment

Organics

Appendix 1, table A1–3a provides a summary of the data for concentrations of organic contaminants in the whole-sediment samples. Analysis of sediments for organic contaminants was performed by Test America and reported by ARCADIS (2011). The PCB data were reported as aroclors, congeners, and homologs. The dominant aroclor was 1242 (median = 17,000 µg/kg DW; maximum = 350,000 µg/kg) followed by aroclor 1260, 1254, and 1268. The median concentration for total PCB aroclors was 27,650 µg/kg and the maximum concentration was 476,000 µg/kg. Normalizing these sediment concentrations to organic carbon (OC) resulted in median total PCB aroclor of 13,250 µg/kg OC and a maximum of 180,000 µg/kg OC. Aroclor PCB was not detected in any of the reference or control samples. Based on the median homolog concentration, sediments were dominated, in decreasing order, by the dichloro-, trichloro-, monochloro-, and tetrachlorobiphenyl homolog groups. General trends indicated sediments with a higher concentration of total PCBs had a greater proportion of monochloro-, dichloro-, trichloro-, and tetrachlorobiphenyl homolog groups. In contrast, sediments with lower concentrations of total PCBs were dominated by penta-, hexa-, and heptachlorobiphenyl. The probable effect concentration quotient (PEC-Q) for total PCBs was calculated using the total homolog concentration and dividing by the PEC for total PCBs (expressed as PEC-Q_{TPCBs}; MacDonald and others, 2000; see also chapter 4). A total of 21 sediment samples exceeded the PEC-Q_{TPCBs} of 1.0, and an additional 5 samples had a PEC-Q_{TPCB} between 0.1 and 1. Individual PCB congeners 153 and 118 were the highest detected congeners with concentrations as high as 5,900 and 3,200 µg/kg, respectively. The PCB congeners 77, 81, 114, 157, and 169 were not detected in any sample, but the detection limits for these congeners were very high in some of the samples (for example, greater than 500 ng/g; appendix 1, table A1–3a).

Other organic compounds that were detected in the sediments include organochlorine pesticides, dioxins, and PAHs. The chlorinated pesticides chlordane, dichlorodiphenyltrichloroethane (DDT) [including dichlorodiphenyldichloroethane (DDD), dichlorodiphenyldichloroethylene (DDE), DDT], and hexachlorocyclohexane (HCH) were detected in test sediments. When these compounds were detected, there was proportionally more HCH than total DDT and chlordane. One reference sample (sediment 09) contained minimal amounts of total DDT and HCH (less than 10 µg/kg). A total of three samples exceeded a PEC-Q of 1.0 for chlorinated pesticides and 12 samples exceeded a PEC-Q of 0.1 (appendix 1, table A1–3a).

Dioxin and furan compounds were detected in all sediment samples. Toxicity equivalents (TEQ) were calculated using fish toxic equivalency factors reported in Van den Berg and others (1998) and ranged from 0.15 to 189 ng/kg. The median value for dioxins was 6.4 ng/kg; well within the range of background conditions summarized by U.S. Environmental Protection Agency (2007c) that ranged from 0.21 to 22.9 ng/kg. However, a total of 10 samples exceeded these background levels ranging as high as 348 ng/kg (appendix 1, table A1–3a).

Concentrations of individual PAHs were generally low and total PAH median values ranged from 10,500 µg/kg to 83,300 µg/kg. A total of four sediments exceeded a PEC-Q for total PAH of 1.0 (appendix 1, table A1–3a). Seven sediments exceeded a $\sum$ESB-TU_{FCV} of 1.0 (the chronic sum equilibrium-partitioning sediment benchmark toxic unit). The $\sum$ESB-TU_{FCV} was calculated based on a final chronic value for *H. azteca* (U.S. Environmental Protection Agency, 2003; Ingersoll and others, 2009).

In general, the highest concentrations of PCBs were associated with the highest concentrations of PAHs, dioxins, and organochlorine pesticides. Specifically, sediments 08, 18, and 19 exceeded PEC-Qs of 1.0 for all organic classes of contaminants. In general the sediment samples that had elevated concentrations of dioxins also paired with the sediments having the highest concentrations of PCBs.

Total Metals

Appendix 1, table A1–3a provides a summary of the data for concentrations of total metals in the whole-sediment samples. Among the results for total metals provided by ARCADIS (2011), concentrations of only two were remarkable—mercury and lead. Antimony, thallium, selenium, and silver were at or below detection limits in all samples. Other metal concentrations, including barium, beryllium, cadmium, copper, nickel, vanadium and zinc were about 2–3 times greater on average in cycle 1a samples as compared with cycle 1b samples, but none of those concentrations were particularly elevated. Compared to sediment PECs (MacDonald and others, 2000), three of the cycle 1a sediments had lead concentrations higher than the PEC value of 128 µg lead/g DW, and 10 of the 11 cycle 1a sediments and 6 of the 16 cycle 1b sediments had mercury concentrations higher than that PEC value of 1.1 µg mercury/g. The highest total lead concentrations were in sediments 08, 18, and 19 (153, 137, and 188 µg/g DW, respectively) and these same three sediments had among the highest mercury concentrations (14, 31, and 40 µg/g DW, respectively [some values are rounded in the text for clarity]). Sediment 30 also contained a high concentration of mercury (22 µg/g) and had the fourth highest concentration of lead (116 µg/g). Following those four sediments, the next highest concentration of mercury was in sediment 06 (12.9 µg/g), and the next highest concentration of lead was in sediment 11 (71 µg/g). The five sediments assumed to represent reference conditions (chapter 5) had lead concentrations that ranged from 4.0 to 5.8 µg/g. Six cycle 1b sediments also contained relatively high concentrations of mercury. These included sediments 02, 13, 14, 17, 20, and 27 that contained mercury concentrations of 3.2, 8.2, 7.9, 5.9, 2.0, and 2.0 µg/g, respectively. Futhermore, only seven Anniston PCB Site sediments (one from cycle 1a and six from cycle 1b) had mercury concentrations less than 0.1 µg/g. In contrast, the five sediments assumed to represent reference conditions (chapter 4) had mercury concentrations that ranged from 0.016 to 0.027 µg/g. Finally, the West Bearskin Lake control sediment (appendix 1, table A1–3a and chapter 4) contained only about 0.02 µg/g; whereas, the maximum total mercury concentration among 17 stream sediments (less than 2-mm particle size) collected from a wide geographic distribution in the United States was reported as only 0.09 µg/g DW (Horowitz and Elrick, 1987). Based on these comparisons, many of the Anniston PCB Site sediments would be classified as highly contaminated with mercury. See chapter 5 for additional evaluations of relations between sediment toxicity and concentrations of mercury in the Anniston PCB Site sediments.

Acid Volatile Sulfide and Simultaneously Extracted Metals

Appendix 1, table A1–3a provides a summary of the data for concentrations of AVS and SEM measured in the whole-sediment samples. More detailed summaries on the measurements of SEM and AVS done by USGS–Columbia are provided in appendix 1, table A1–7. Results for quality-control samples associated with these analyses are summarized in appendix 1, tables A1–10, A1–11, A1–12, and A1–13. The AVS concentrations of Anniston PCB Site sediments in general would be classified as low to moderate, and except for one sample (sediment 16) that did not have measureable AVS [less than 0.01 micromole per gram (µmol/g)], the range of concentrations was relatively narrow. On average, cycle 1a samples had AVS concentrations that were about twice those of cycle 1b samples. The difference in AVS between cycles is likely an accurate reflection of real differences between the sample groups, and was not because of a longer storage time for cycle 1b samples. Notably mean TOC concentrations of each of the two cycles followed the same pattern as AVS, that is consistent with reports that TOC and AVS often co-vary in freshwater sediments (Besser and others, 2011). For example, mean AVS concentration for cycle 1a samples was 1.25 µmol/g (range = 0.46 to 2.74) and mean TOC was 2.1 percent; whereas, mean AVS concentration was 0.59 µmol/g (range = less than 0.01 to 1.86) and mean TOC was 1.0 percent for cycle 1b samples. Concentrations of SEMs (copper, nickel, zinc, cadmium, and lead) followed the same pattern as AVS—concentrations were relatively low and were on average about 2–3 times greater in cycle 1a samples than in cycle 1b samples. Few samples had any SEM concentration near the respective PECs. For example, the maximum SEM concentrations (µg/g DW) in Anniston PCB Site sediments compared to the respective PEC values (in parentheses) were as follows: cadmium, 0.55 (5.0); copper, 17 (149); nickel,13.8 (49); lead, 146 (128); and zinc, 125 (460). Thus, among these five metals only lead was at a concentration near or above the respective PEC value. This was true for three Anniston PCB Site sediments: sediments 19, 08, and 18, had mean SEM lead values of 138, 122, and 111 µg/g (DW), respectively. In addition, sediment 30 had the next highest SEM lead concentration (mean = 78 µg/g DW). Total lead concentrations were highest in these same four sediments (188, 153, 137, and 116 µg/g DW, respectively; ARCADIS, 2011), values that corresponded closely with the slightly lower SEM lead concentrations measured by USGS–Columbia in these same sediments. As noted previously, these four sediments were the same ones having the highest total mercury concentrations.

Calculation of values of SEM minus AVS (SEM-AVS, molar basis), and SEM-AVS normalized to the fraction of sediment organic carbon (SEM-AVS/f_{OC}; appendix 1, table A1–7) indicate low bioavailability and low probability of risk from these five metals (U.S. Environmental Protection Agency, 2005). The sediments with the four highest positive SEM-AVS values were the same four having the highest lead concentrations (sediments 08, 18, 19, and 30). However, mean values of SEM AVS/f_{OC} for those ranged from only 56 to 73, and according to USEPA guidance, a sediment having a value of SEM-AVS/f_{OC} of less than 130 should pose low risk of adverse biological effects from these five metals (U.S. Environmental Protection Agency, 2005). Agreement for measurements of

AVS and SEM (all that were analyzed at USGS–Columbia) between samples processed at USGS–Columbia and those processed at USACE–Vicksburg was excellent. The lone exception was the measurement for SEM nickel in sediment 20 that had values of 1.41 µg/g at USGS–Columbia and 13.8 µg/g at USACE–Vicksburg.

Chemical Characterization of Pore Water: General Water Quality, Inorganics and Dissolved Organic Carbon

Appendix 1, table A1–3a provides a summary of general water quality, major cations, major anions, dissolved organic carbon (DOC), and hydrogen sulfide measured in the pore-water samples isolated by centrifugation at the start of the toxicity exposures and at the start of the bioaccumulation exposures. Means and ranges (in parentheses) of concentrations (mg/L) of major cations in the Anniston PCB site sediment pore-water samples (excluding the West Bearskin Lake control sediment) were as follows: calcium, 41.6 (11.6–86.5); magnesium, 17.1 (2.1–35.5); potassium, 3.1 (0.1–10.8); sodium, 4.5 (2.4–10.0); iron, 13.3 (0.0–36.1); and manganese, 4.3 (0.0–17.5). Analogous values (in mg/L) for DOC were 8.8 (0.4–81.8); for chloride, 7.3 (2.5–45.3); and for sulfate, 16.0 (1.1–70.0). Sulfide and nitrate were at or below detection limits in all pore-water samples; fluoride was detected only at low concentrations.

Pore-water metals and other element concentrations measured with ICP-MS semiquantitative scan in peeper samples during each of the toxicity or bioaccumulation exposures are provided in appendix 1, table A1–5 (cycle 1a) and in table A1–6 (cycle 1b; see also appendix 1, tables A1–3b, A1–3c and A1–3d). Results for quality-control samples associated with these analyses are summarized in appendix 1, tables A1–8 and A1–9. There was good agreement between samples processed at USGS–Columbia and at USACE–Vicksburg for cycle 1a samples, but sample handling and processing background contamination issues were evident for cycle 1b samples. Compared to cycle 1a peeper blanks (processed at USGS–Columbia or at USACE–Vicksburg) or cycle 1b peeper blanks processed at USGS–Columbia, peeper blanks (and evidently some samples) processed at USACE–Vicksburg during cycle 1b tests were elevated by factors of more than 10-fold for some metals, including iron, aluminum, nickel, copper, tin, barium, and lead. The reason for this is unclear, but the fact that each of the three blanks processed at USACE–Vicksburg had similar concentrations for most of these metals indicates that there was a constant source of contamination. Possible sources include the nitric acid that was used for dilution at USACE–Vicksburg, the shipping container prepared at USGS–Columbia, or the batch of Chelex-100™ used for that one set of peepers. Peeper blanks from all tests contained elevated concentrations of sodium (5, 6 or 16 mg/L; appendix 1, tables A1–5 and A1–6), that presumably originated from the Chelex-100™ (sodium form) used to maintain low concentrations of trace metals in the surrounding water during shipment and storage.

Considering blank contamination and excluding obvious outliers (described below), no sediments consistently had substantially elevated concentrations of metals of potential concern in pore water sampled with peepers. There were two substantial outliers: single values for chromium (290 µg/L; Sediment 25; *C. dilutus* test, USACE–Vicksburg) and nickel (30 µg/L; sediment 18; *C. dilutus* test, USGS–Columbia). The values for those metals were substantially less in corresponding peeper samplers from each of three replicate samples of those two sediments (means of 7 and 3 µg/L, respectively; appendix 1, table A1–5). Excluding those two outliers, the maximum concentration of chromium was 7.9 µg/L and the maximum for nickel was 6.0 µg/L. The maximum concentrations for cadmium and zinc were 0.6 (sediment 08) and 58 µg/L (sediment 30), respectively; however, those values also were markedly higher than the mean values for corresponding replicate results. Concentrations of zinc were consistently about 15–25 µg/L in blanks prepared at the USGS–Columbia and at the USACE–Vicksburg laboratories for cycle 1a, and those prepared at USGS–Columbia for cycle 1b. These zinc concentrations are similar to, or less than those that USGS–Columbia has obtained for peeper samplers with previous studies. Accounting for these blank concentrations, actual concentrations of zinc in pore waters of these sediments were probably at most about 30–40 µg/L. Cycle 1b peeper samples processed at USACE–Vicksburg are presumed to be biased high for some elements (particularly copper, nickel, lead, and zinc), as evidenced by blank concentrations and comparison of sample concentrations with results for samples processed at USGS–Columbia. Applying blank corrections to the USACE–Vicksburg cycle 1b results improved consistency with the USGS–Columbia cycle 1b values in many instances, but variability was generally too great to allow for confidence in those values. To a large extent, variability among the trace metals in peeper samplers can be attributed to the relatively low concentrations involved. Relative variability was much lower for the major cations (calcium, potassium, magnesium, manganese, and iron). Accordingly, if only peeper samples processed at USGS–Columbia are considered for assessment of trace metals, the maximum copper concentration was only 1.1 µg/L and the maximum lead concentration was only 0.7 µg/L. Arsenic concentrations were typically between 10 and 20 µg/L for most samples; however, those results must be viewed with caution because of the semiquantitative analysis mode of the ICP-MS tends to produce artificially high results for arsenic in that concentration range.

Concentrations of metals in peeper samples were relatively low in all samples, but cycle 1a samples tended to have higher concentrations as compared with cycle 1b samples (based only on samples processed at USGS–Columbia). This finding was not particularly surprising because all but one of the cycle 1a samples were selected to represent highly contaminated locations (based on concentrations of PCBs); whereas, the cycle 1b samples were obtained primarily from reference locations or locations with lower concentrations of

PCBs. Metals, including barium, chromium, nickel, copper, zinc, cadmium, tin, and lead, were about 2–3 times greater on average in samples from cycle 1a as compared with cycle 1b. Notably, this same trend was observed for many total metals and the SEM concentrations in cycle 1a samples (as discussed in previous sections of this chapter). Perhaps the most notable difference between peeper samples of the two test cycles was for barium—cycle 1a samples averaged about 330 µg/L; whereas, cycle 1b samples averaged about 92 µg/L. Total barium concentrations in sediments (ARCADIS, 2011) were correspondingly much higher in cycle 1a samples, especially sediments 08, 18, 19, and 30.

Concentrations of major cations in peeper samples were more similar between samples of each test cycle (based only on samples processed at USGS–Columbia); however, centrifuged and filtered pore-water samples tended to have higher concentrations than those pore-water samples collected with peepers, especially calcium, magnesium, and manganese. For example, in centrifuged samples calcium averaged 42 mg/L, magnesium about 17 mg/L, and manganese about 4 mg/L; whereas, in peepers, the concentrations averaged about 20 mg/L, 6 mg/L, and 2 mg/L, respectively. In contrast, concentrations of iron, potassium, strontium, and sodium (blank corrected) in peeper samples were only slightly lower than those of centrifuged samples. The reason for the larger differences between centrifuged samples and peeper samplers for calcium, magnesium, and manganese is unclear. One possibility is that those three cations were fractionally associated with (complexed by) dissolved organic matter in some sediment pore waters. If so, such molecular complexes might not readily passively dialyze across the membrane of the peeper, but could be made to pass through a similar membrane when applying moderate pressure, as was done when centrifuged samples were filtered. Alternately, concentrations of some of the major ions or metals might have been partially depleted in the localized volume of pore water surrounding the peepers, resulting in somewhat lower concentrations being measured as compared with centrifuged samples. Other possible factors include the following: (a) peeper samplers were not fully equilibrated or (b) centrifugation disrupted the sediment/pore-water equilibrium distribution of those three elements.

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Evaluation of Bioaccumulation in the Oligochaete, *Lumbriculus variegatus*, Exposed to Sediments from the Anniston PCB Site

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Chapter 3 of

Evaluation of Toxicity to the Amphipod, *Hyalella azteca*, and to the Midge, *Chironomus dilutus*; and Bioaccumulation by the Oligochaete, *Lumbriculus variegatus*, with Exposure to PCB-Contaminated Sediments from Anniston, Alabama

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Evaluation of Bioaccumulation in the Oligochaete, *Lumbriculus variegatus*, Exposed to Sediments from the Anniston PCB Site

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Abstract

Polychlorinated biphenyls (PCBs) in the Anniston, Alabama area were released from the operations of the former Monsanto Corporation's PCB manufacturing plant resulting in sediment contamination by PCBs in Choccolocco Creek and its floodplain. An integrated, multi-agency research team was assembled to assess bioavailability and toxicity of PCBs in sediments collected from the Anniston, Alabama area in August 2010. As part of this study, a total of 32 sediment samples were collected from selected locations within the study area to support chemical characterization and whole-sediment toxicity or bioaccumulation testing and for toxicity testing (chapters 2 and 5). The bioavailability and bioaccumulation of PCBs in 14 sediment samples were investigated using solid-phase microextraction (SPME) passive samplers and the 28-day *Lumbriculus variegatus* whole-sediment bioaccumulation exposures. Tissue residues predicted using SPME-derived pore-water data accurately predicted body residues in sediment-exposed oligochaetes and provide information regarding the bioavailability of PCBs in these sediments. In general the accumulation of PCBs consistently was predicted through the use of organic carbon normalization and equilibrium partitioning. The observed differences in bioavailability of PCB homolog groups corresponded to the resultant relative concentrations of homologs accumulated in tissues in oligochaetes. As part of this assessment homolog specific biota sediment accumulation factor (BSAF) values were developed that could be applied across the larger site to predict tissue levels of PCBs.

Introduction

Assessing the potential ecological effects of polychlorinated biphenyls (PCBs) requires an assessment of bioaccumulation because of the propensity of PCBs to partition from solid phase sediments to lipids in organism tissues. In the current study, the bioavailability and potential for uptake of PCBs was evaluated with the oligochaete, *Lumbriculus variegatus* in 28-day (-d) whole-sediment laboratory exposures. Bioavailability and bioaccumulation assessments often are performed using whole-sediment laboratory bioaccumulation exposures to determine the potential for bioavailability of these hydrophobic compounds (American Society for Testing and Materials International, 2012; U.S. Environmental Protection Agency, 2000a). Laboratory bioaccumulation exposures typically provide reasonable estimates of field bioaccumulation (Burkhard and others, 2012; Ingersoll and others, 2003; Beckingham and Ghosh, 2010).

Net bioaccumulation may be affected by sediment characteristics such as the sediment organic carbon content. The interaction of the physicochemical characteristics of chemicals (for example, log K_{ow}; octanol-water partitioning coefficient) with sediment (for example, organic carbon content) can reduce, sometimes substantially, the bioavailability and toxicity of hydrophobic organic contaminants (U.S. Environmental Protection Agency, 2000b). Evaluating the bioaccumulation and toxicity of contaminated sediments based dry weight (DW) concentrations in sediment may result in inaccuracies because of poor concentration-response relations (for example, Paine and others, 1996).

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Freely dissolved pore-water concentrations of hydrophobic organic contaminants have been determined to be useful indicators of bioavailability (for example, You and others, 2006; Lu and others, 2011). Several techniques have been developed to facilitate and improve the quantification of the freely dissolved pore-water concentrations of hydrophobic contaminants in sediment pore water, including the use passive samplers (Gschwend and others, 2011). Among the available passive samplers, polydimethylsiloxane (PDMS) coated solid-phase microextraction (SPME) fibers were selected for this study because their configuration of a thin annular layer of sorbent coating on a small-diameter silica core provides a high surface area to volume ratio (relatively fast contaminant uptake kinetics) in a configuration that can be inserted easily into sediments during bioaccumulation and toxicity studies. Solid-phase microextraction is a partition-based, solvent-free, negligible-depletion extraction technique used to measure freely dissolved organic chemicals (Van der Wal and others, 2004). Application of the SPME technique included direct insertion into the sediment to allow equilibration with the sediment-pore-water system (for example, Mayer and others, 2000; Conder and others, 2003; You and others, 2007; Lu and others, 2011). The SPMEs were used in the current study to provide a measure of chemical activity in the whole-sediment phase as altered by the various modifying factors affecting bioavailability, and hence, bioavailability and toxicity, in exposures to whole sediments.

This chapter addresses three objectives in understanding bioavailability of PCBs in Anniston, Alabama sediments: (1) to determine the bioavailability of PCBs in Anniston, Alabama sediments multiple measures including SPME and bioaccumulation were used. As described above, SPME directly measures pore water and establishes a mechanistic linkage between PCBs in sediment and PCBs available for an organism to accumulate in tissues; (2) pore-water concentration of PCBs was determined for use as a direct measure of bioavailability fraction for the purposes of understanding and refining relations between sediment chemistry (chapter 2) and sediment toxicity tests performed with the amphipod, *Hyalella azteca,* and the midge, *Chironomus dilutus* (chapters 4 and 5); and, (3) bioaccumulation data were compared to values derived from the literature to determine the confidence to which equilibrium partitioning can be used to predict bioavailability and bioaccumulation from the Anniston PCB Site sediments.

Methods

Lumbriculus variegatus Bioaccumulation Testing

Lumbriculus variegatus 28-d bioaccumulation exposures to Anniston PCB Site sediments were performed in basic accordance with methods outlined in American Society for Testing and Materials International (2012), U.S. Environmental Protection Agency (2000a). A summary of the test conditions used is provided in appendix 2, table A2–1 to A2–3). Chapter 2 provides a summary of how sediments were collected, processed, and characterized. Briefly, exposures were performed in 2-liter (L) beakers with a sediment volume of 600 milliliters (mL) and an overlying water volume of 1,400 mL. Five replicate chambers were tested for each sediment. Testing was performed in an environmental chamber at 23 degrees Celsius (°C) and a 16:8 light:dark cycle. Sediments and overlying water were added to beakers 6 to 9 days before the addition of *L. variegatus* to allow for equilibration. An 80 percent water change of overlying water was done 2 days before organism addition and then overlying water ammonia concentrations were measured. Thereafter, 80 percent water exchanges were performed three times weekly on Monday, Wednesday, and Friday. The source of overlying water was dechlorinated Vicksburg, MS tap water, dechlorinated using activated carbon filtration (appendix 3, table A3–7). Hardness, alkalinity, pH, conductivity, and ammonia were measured in overlying water at the start and end of the exposures. Dissolved oxygen and conductivity were measured weekly, and temperature was measured daily. At the beginning and end of the test, ammonia did not exceed 2.0 milligrams per liter (mg/L) in any test sediments. Water-quality characteristics were similar across Anniston PCB Site sediments (appendix 2, table A2–4 and A2–5): mean temperature 23 °C (range 20.1–24.8), dissolved oxygen 7.7 mg/L (6.0–8.7), pH 8.2 (7.2–8.7), alkalinity 85.6 mg/L as calcium carbonate ($CaCO_3$, 20–150), hardness 86.7 mg/L as $CaCO_3$ (60–112), conductivity 300 microsiemens (µS) (180–460), and total ammonia 0.7 mg/L (less than 1 to 2).

A total of 2.66 grams [(g) wet weight (WW)] was the target mass of oligochaetes added at the start of the 28-d bioaccumulation exposures. This target was derived using a 2.0 g target oligochaete tissue weight allowing for an extra 33 percent to account for excess mass of water in the aliquot of oligochaetes used to load each replicate beaker according to guidance (American Society for Testing and Materials International, 2012; Brunson and others, 1998). Five replicates of about 2.66 g of *L. variegatus* tissue also were sampled at the start of the exposures and frozen for later analysis of background contaminant concentration and lipid content. *Lumbriculus variegatus* were obtained from the commercial supplier Aquatic Research Organisms (Hampton, New Hampshire). The *L. variegatus* were not fed during testing, and beakers were aerated with trickle flow aeration (about 1–2 bubbles per second). The duration of testing was 28 days. On day 28, *L. variegatus* were recovered by sieving from the sediment with 425-micrometer (µm) sieves, and then oligochaetes were manually removed from detritus. *L. variegatus* were allowed to purge their gut contents in clean water overnight before weighing and freezing. Because of the large number of samples tested in each cycle, addition and recovery of organisms was divided across 2 days. The tissue samples were shipped on dry ice by overnight carrier

to Test America (Knoxville, Tennessee) for analysis of tissue PCB residues and lipid concentration. Results of the tissue analyses and lipid analyses are provided in appendix 1, table A1–3e.

Solid-Phase Microextraction Passive Samplers

Polydimethylsiloxane (PDMS) solid-phase microextraction fibers (SPME) were used as passive samplers in each sediment evaluated in bioaccumulation testing to estimate pore-water concentrations of PCBs in separate replicate bioaccumulation beakers (You and others, 2006; Lu and others, 2011). In addition, SPMEs were used in the same manner in each sediment evaluated in toxicity testing with *H. azteca* and *C. dilutus* (chapter 4).

The SPME fibers consisting of a glass core (230-µm diameter) with a 10 µm-thick PDMS coating (Model SPC210/230R) were purchased from Fiberguide Industries (www.fiberguide.com). At USACE-Vicksburg, fibers were cut into 2.5-centimeters (cm) pieces using a double-bladed, stainless steel razor blade apparatus. Four fiber pieces (10-cm total length) of SPME fiber were placed in a protective 5-cm by 4-cm 100-µm stainless steel mesh envelope (Model 165 Mesh T316 Stainless from TWP Inc., *www.twpinc.com*) and inserted into the sediment using a method developed by Conder and others (2003).

The total volume of PDMS coating per 10 cm length was 0.69 microliters (µL). The use of a mesh envelope provided a means to safely handle the fragile fibers when deploying into and retrieving from sediment. The 100-µm openings in the stainless mesh were large enough to allow free passage of pore water and fine sediment particles for intimate contact with the fiber, but were minimal enough to retain the small diameter fibers and keep test organisms from being inadvertently removed from the sediment upon SPME envelope removal. To secure fibers in the envelope, the edges of the stainless steel mesh were folded and firmly pressed on all edges. Because fibers are brittle, the fibers were manipulated with care and removed from the envelopes using plastic forceps. Once closed, the envelopes containing the fibers were rinsed with hexane followed by distilled or ultrapure water, and allowed to dry overnight in a fume hood at room temperature. The envelopes were then wrapped in paper towels for mechanical protection and, placed in sealable plastic bags. Fibers were then packaged and shipped by overnight carrier to USGS–Columbia *(H. azteca* or *C. dilutus* exposures; chapter 4) or stored at room temperature for later use at USACE–Vicksburg in sediment bioaccumulation exposures or in sediment toxicity exposures (chapter 4).

On day 0 of the exposures (the day that test organisms were added to sediment), mesh envelopes containing SPMEs were inserted into the sediment such that the envelope containing the SPME fiber were located below the sediment surface at about mid-depth in the layer of sediment, and near the center of the chemistry beaker.

The SPME fibers were retrieved after 28-d exposure to sediment beakers from toxicity or bioaccumulation tests. Envelopes were removed from the sediment, rinsed with distilled or ultrapure water, and opened for the removal of fibers. Fibers were removed carefully from the envelope using plastic forceps, rinsed with distilled or ultrapure water, and placed on lint-free paper (for example, a Kimwipe; kimberly-clark.com) for blotting. The number of intact fiber segments was recorded as well as the length of broken segments. The dry fiber from each passive sampler beaker was transferred to a 0.1 mL conical glass insert placed inside a high performance liquid chromatography (HPLC) autosampling vial and capped tightly with a Teflon-lined screw cap. The top of each vial was wrapped around the cap and neck of the vial in self-sealing Parafilm for a tight seal. Vials were placed on a HPLC vial rack and secured in place with multiple layers of Parafilm and placed in plastic bags for analysis by the USACE–Vicksburg chemistry laboratory. The SPMEs generated in toxicity tests performed by USGS–Columbia also were shipped overnight to USACE–Vicksburg for analysis of PCBs.

Calculation of Pore-Water Concentration from Passive Sampler

Pore-water concentrations were estimated by matrix-SPME involving the measurement of the PCB concentration on SPME fibers inserted into the sediment. Pore-water concentration (C_{pw}) is calculated from the fiber concentration (C_f; mass of contaminant absorbed by fiber/volume of PDMS) and the fiber/water (f/w) partition coefficient (C_f; volume of water/volume of PDMS) as indicated by the following equation.

$$C_{pw} = C_f / K_{f/w} \quad (1)$$

A linear correlation between log $K_{f/w}$ and log K_{ow} generated by Mayer and others (2000) and K_{ow} values for PCBs from Hawker and Connell (1988) were used to derive fiber-water partition coefficients for PCB congeners.

$$\text{Log } K_{f/w} = 1.03 \log K_{ow} - 0.938 \quad (2)$$

In this study, octanol-water partition coefficients (K_{ow}), used as lipid-water partition coefficient ($K_{lip\text{-}w}$) or bioconcentration factors, were multiplied by the SPME-derived pore-water concentrations (C_{pw}) to predict the lipid-normalized accumulation on individual PCB congener concentration in oligochaetes ($Ct_{predicted}$) as previously used (Lu and others, 2011).

$$Ct_{predicted} = K_{ow} \text{ x } C_{pw} \quad (3)$$

The use of K_{ow} as an estimate of the partitioning of organic contaminants from water to organism lipid phases was introduced by Mackay (1982).

Chemical Analysis of Sediment, Tissues, and Solid-Phase Microextraction Fibers

Sediment and tissues were analyzed for 13 PCB congeners and PCB homolog groups by Test America. The SPME samples were analyzed using the full list of 209 PCB congeners by the USACE–Vicksburg Chemistry Laboratory (table C2–3). Methods for analyses of sediment and tissue are summarized in chapter 2 and in detail by ARCADIS (2010). Lipid analysis was done using a colorimetric method following Test America standard operating procedure LM-OP-Lipids that is based on the U.S. Environmental Protection Agency (USEPA) Region 4 method ASB P100. These methods are available from Test America and described in the data report by ARCADIS (2010). Results of the sediment chemistry are summarized in appendix 1, table A1–3a. Results of the PCB tissue residue data and lipid concentration data are provided in appendix 1, table A1–3e and in appendix 2.

The SPME were analyzed by adding 100 microliters of ultrapure hexane to 0.1- mL conical glass insert placed inside a HPLC autosampling vial before analysis by gas chromatography electron capture detector (GC-ECD) following USEPA method 8082 (U.S. Environmental Protection Agency, 1986). Analyses of PCBs were performed with an Agilent 6890 series gas chromatograph equipped with a GC-ECD and HP-5 type 30-m capillary column (internal diameter of 0.32 millimeters (mm) and film thickness 0.25 mm). Polychlorinated biphenyl congeners were identified based on the retention times of the corresponding peaks in the standard. A second column was used to confirm individual congener. Congeners were selected for quantification and reporting based on good agreement between the two columns, suggesting little or no interference such as co-eluting peaks for these congeners. Most PCB congeners were within acceptable limits for Lab Control Sample recoveries, Matrix Spike (MS) and Matrix Spike Duplicate (MSD) recoveries and Relative Percent Differences (RPD). The spike recoveries were calculated without subtraction of PCBs in the sample before spiking with the recovery test PCBs. The reported amount reflects the PCBs in the sample itself plus the spiked PCBs. Thus recoveries of several PCBs in the MS/MSD appear to be outside of acceptable ranges because of the high concentration of the compounds in the sample in relation to the low concentration of the spike. These data were flagged in the analytical chemistry report and included in the analysis without further correction.

Results and Discussion

Bioaccumulation Testing

The whole-sediment bioaccumulation exposures were performed for 14 sediments collected from the Anniston PCB Site (see chapter 2 for criteria used to select these sediments). Testing was performed in two cycles, cycle 1a and cycle 1b, because of the large number of sediments to be tested (table C2–1). Within each cycle the addition and recovery of test animals was split over 2 days. Sediments tested in cycle 1a included sediment 01, 9, 13, 20, 25, 27, and 28. Cycle 1a bioaccumulation testing was done between November 3, 2010, and December 2, 2010. Sediments tested in cycle 1b included sediment 02, 11, 14, 16, 23, 24, and 29 (table C2–1). Originally, cycle 1b bioaccumulation testing was done between February 2 and March 11. However, during the recovery of animals the incorrect water was used during the depuration phase resulting in mortality of the organisms. Because these organisms were compromised, cycle 1b bioaccumulation retesting was done between March 30, 2011, and April 29, 2011. The control sediment (sediment 33) was tested in cycle 1a and in cycle 1b.

Sediments were selected for bioaccumulation testing (1) based on the results of 10-d toxicity screening testing performed with *Lumbriculus variegatus* and *Hyalella azteca* (table C2–1) and (2) based on screening of concentrations of PCBs and total organic carbon in the sediment samples (table C2–1). Concentration of PCBs in tissue for each of the test sediments was predicted using equilibrium partitioning and compared to analytical reporting limits. Sediment selection criteria included sites where PCBs were expected to accumulate in tissues at concentrations detected by existing analytical methods and below concentrations that would cause adverse effects (that is, decreased tissue mass, decreased mortality). Results from the initial toxicity screening were used to identify and exclude samples where avoidance behavior or toxicity was observed in the *L. variegatus* or *H. azteca* tests. The median total PCB concentration of sediment samples selected for bioaccumulation testing was 721 milligrams per kilogram (mg/kg) organic carbon and ranged in total PCB concentration from 2.5 to 4,521 mg/kg organic carbon. Sediments tested in cycle 1a tended to have higher concentrations of PCBs compared to the sediments tested in cycle 1b. The median total PCB concentration was 792 mg/kg OC (milligrams per kilogram normalized to organic carbon) in cycle 1a and 431 mg/kg OC in cycle 1b.

Bioaccumulation Test Performance

A subsample of *L. variegatus* was analyzed at the start of the bioaccumulation exposures for PCB residues and for lipid concentrations. Concentrations of PCB congeners were low; the highest concentration was for PCB congener 153 (0.74 µg/kg WW) and for PCB congener 118 (0.4 µg/kg, WW). The other five congeners were less than 0.1 µg/kg (WW; appendix 1, table A1–3e). Total PCBs, based on homolog group concentrations was 7.8 µg/kg (WW) in tissues at the start of cycle 1a testing and was 6.9 µg/kg (WW) in tissues at the start of cycle 1b testing. Mean lipid concentration in oligochaetes was 1.56 percent (WW) at the start of cycle 1a testing and was 1.6 percent (WW) at the start of cycle 1b testing. These values are similar to values of 11 studies reported in the USACE Lipid Database with a median value of 1.02 percent (range: 0.55–1.5 percent) WW (Jeff Steevens, U.S. Army Corps of Engineers,Vicksburg, Miss., unpub. data, 2013). During the bioaccumulation testing

no observations of avoidance or lethargy were noted that might affect overall bioaccumulation of PCBs by the organisms.

Total tissue biomass recovery at the end of the 28-d exposures is provided in appendix 2, table A2–6. The targeted amount of tissue for chemical analysis was 1.0 g to allow for each replicate analysis. In preliminary data collected in an initial toxicity screen of the sediments there was mortality observed for several of the sediments (table C2–1). Because of this reduced survival and expected loss of biomass 2 g of oligochaetes were added per replicate. According to American Society for Testing and Materials International (2012) guidance the ratio of total organic carbon (TOC) in sediment to the dry weight of *L. variegatus* added should be considered when determining the amount of sediment and mass of *L. variegatus* worms to be used, although this is not a requirement. Section A8.3.2 of the American Society for Testing and Materials International (2012) guidance states: "To minimize the depletion of sediment contaminants, the ratio of TOC in sediment to dry weight of organisms *should* be no less than about 50:1" (emphasis added). The ratio of TOC to dry weight organism has not affected BSAF values as low as 27:1 (Van Geest and others, 2011); however, the study did not determine a ratio that affected BSAF values. An analysis of the relation between OC normalized to oligochaete dry weight and the BSAF values suggests organism loading did not affect the BSAF values and the chemical was not limiting uptake in the exposure chamber (fig. C3–1). In cycle 1a, a minimum of 1 g of tissue was collected and then stopped because this was twice the amount required for analysis. Several replicates in cycle 1a (sediments 01, 20, 25, and 27) did not have adequate tissue mass (55 to 464 mg/replicate) for chemical or lipid analysis and were pooled with other replicates within that sediment treatment to reach a total of 0.5 g for analysis. In cycle 1b, organisms were collected until no other organisms could be determined in the sediment. The median total biomass in replicates from cycle 1b was 1.3 g and ranged from 0.8 to 1.8 g. Therefore, no pooling of replicates was required in cycle 1b.

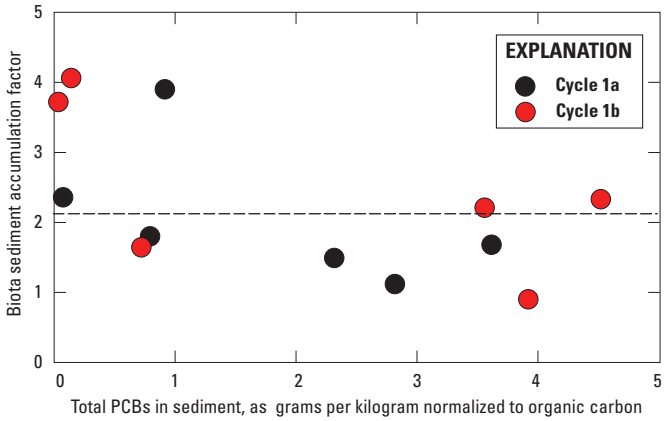

Figure C3–1. Relation between the total organic carbon in sediment divided by the dry weight of *Lumbriculus variegatus* to biota sediment accumulation factor for total polychlorinated biphenyls (PCBs).

Total lipid concentration in oligochaetes at the end of the bioaccumulation exposures (1.2–1.3 percent; WW) was slightly lower than the lipid concentration at the start of the exposures (1.6 percent; WW). However, this is often expected because of the lack of feeding in the bioaccumulation exposures. Generally the variability across treatments was low. Lipid content in cycle 1a ranged from 1.3 to 2.9 percent with a median of 1.6 percent lipid. Organisms exposed to sediments 25 and 27 had limited tissue available for analysis, as a result of a skewed result, and had higher lipid content. Lipid content in cycle 1b ranged from 1.3 to 1.8 percent with a median of 1.5 percent lipid.

Bioaccumulation of Polychlorinated Biphenyl Homologs

Accumulation of total PCBs in tissues (fig. C3–2) was calculated as a sum of PCB homolog groups. Oligochaetes exposed to control sediments (Sample 33) accumulated low concentrations of PCBs ranging from 22 to 382 µg/kg (WW). Organisms exposed to the two reference sediments accumulated total PCBs to concentrations similar to control sediments. Total PCBs was 262 µg/kg (WW) and 32.8 µg/kg (WW) in organisms exposed to reference sediments 09 and 29, respectively. The median concentration of total PCBs in oligochaetes exposed to Anniston PCB Site test sediments was 35,300 µg/kg (WW) and ranged from 32.8 to 137,000 µg/kg (WW). After lipid normalization the concentration of total PCBs ranged from 128,000 to 10,538,000 µg/kg lipid (appendix 1, table A1–3e).

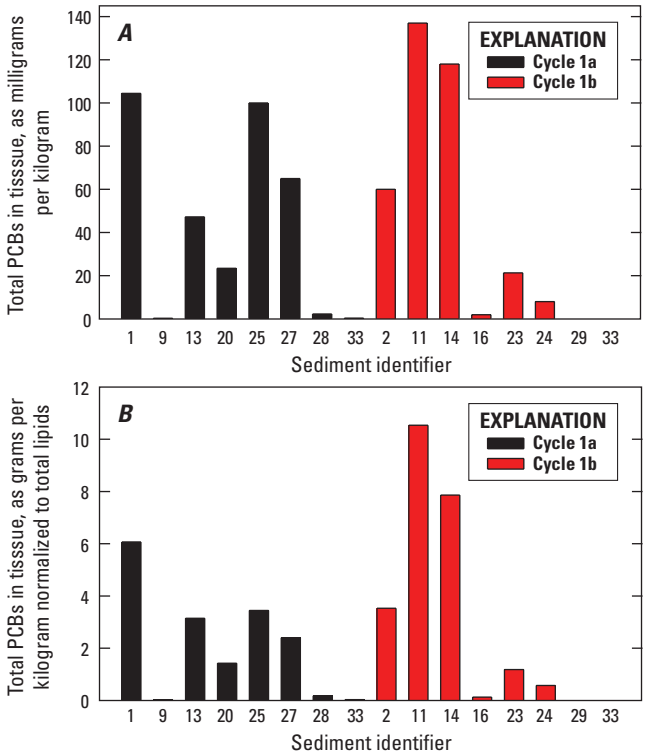

Figure C3–2. Total polychlorinated biphenyls (PCBs) in tissue for each sample site tested in cycle 1a and cycle 1b shown as *A*, milligrams PCB per kilogram wet weight and *B*, milligrams PCB per kilogram lipid.

The distribution of PCB homolog groups in oligochaetes was affected by total PCB concentration in the sediments (fig. C3–3*A*). Mono- and dichlorobiphenyls represented only a small fraction of the total PCB concentration in low-PCB sediments [PCBs less than 0.1 grams per kilogram; (g/kg) OC]; whereas mono- and dichlorobiphenyls were the highest fraction of the total PCB concentration in high-PCB sediments (PCBs greater than 0.1 g/kg OC). The distribution of PCB homolog groups in tissues (fig. C3–3*B*) was relatively uniform across a wide range of total PCB body residues. Tri- and tetrachlorobiphenyls were the dominant group in the tissues, with the mono- and dichlorobiphenyls contributing much less to the total tissue burden compared to their prevalence in the contribution to the sediments total concentration.

The distribution of PCB homologs in sediment is likely a function of the depth that the sediment was collected (table C2–1). Surface sediments had a greater proportion of more highly chlorinated PCBs likely because of the partitioning of lower K_{ow} PCB homologs into surface water. The

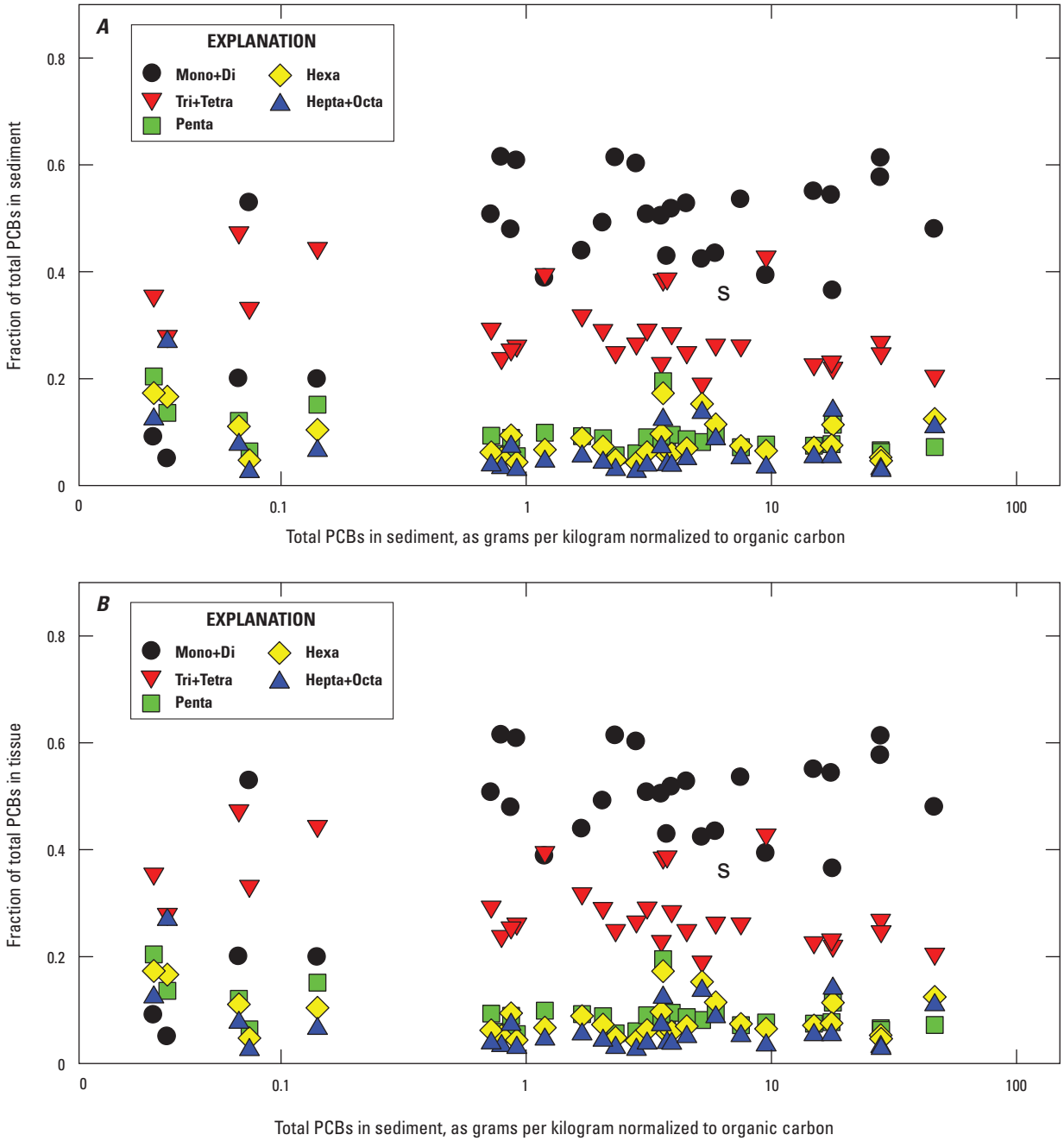

Figure C3–3. Polychlorinated biphenyl (PCB) homolog group, monochrobiphenyl (mono), dichrobiphenyl (di), trichrobiphenyl (tri), tetrachrobiphenyl (tetra), hexachrobiphenyl (hexa), heptachrobiphenyl (hetpa), and octachrobiphenyl (octa), as a fraction of total PCBs in *A*, sediment samples at the start of the exposures, and *B*, the oligochaetes *Lumbriculus variegatus* at the end of the 28-day sediment exposures.

PCBs with a higher degree of chlorination (for example, trichlorobiphenyls and higher homolog groups) are more strongly associated with the sediment particulate fraction. Across all sites, the mono-, di-, and tri-chlorinated homologs dominated the sediments (fig. C3–4). However the overall mean fraction of PCB homolog accumulated in oligochaetes were dominated by the tetra, penta, and hexa homolog groups (fig. C3–4).

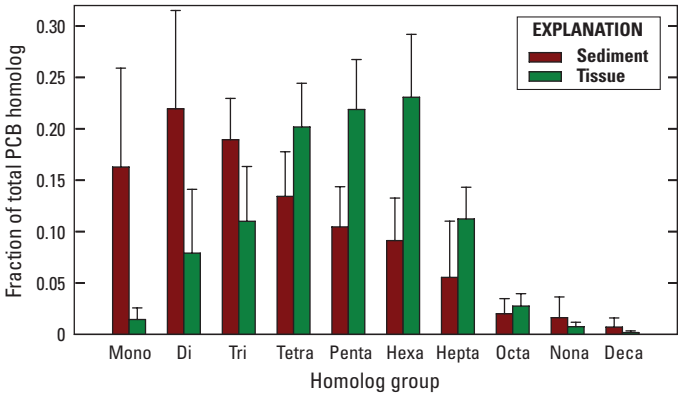

Figure C3–4. Overall mean fraction of polychlorinated biphenyl (PCB) homolog group, monochlorobiphenyl (mono), dichlorobiphenyl (di), trichlorobiphenyl (tri), tetrachlorobiphenyl (tetra), hexachlorobiphenyl (hexa), heptachrobiphenyl (hetpa), and octachrobiphenyl (octa), in sediment and oligochaete tissue.

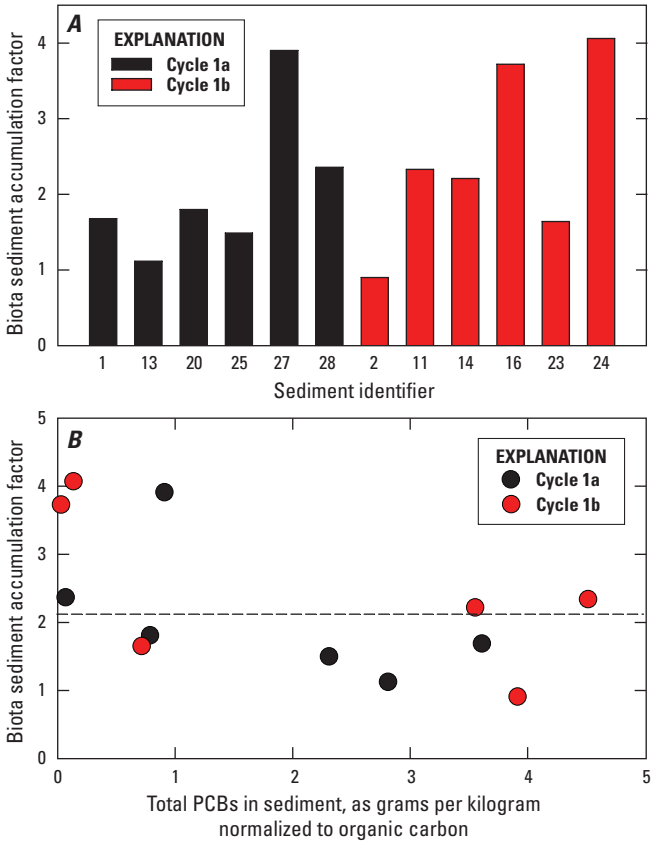

Figure C3–5. Mean total polychlorinated biphenyl (PCB) biota sediment accumulation factors (BSAF) for the oligochaete *Lumbriculus variegatus* exposed to Anniston site sediments. The BSAF values are shown for *A*, each sediment sample and *B*, compared to PCB normalized to organic carbon in sediment.

Biota Sediment Accumulation Factors

Biota sediment accumulation factors (BSAF) values for total PCBs calculated for oligochaetes exposed for 28 days to Anniston PCB Site sediments ranged from 0.90 to 4.06 (table C3–1; fig. C3–5), with a mean of 2.20 (plus or minus 0.99, 1 standard deviation). There did not appear to be a difference in BSAF values based on PCB concentrations in sediment or testing cycle, except for the high BSAF values associated with sample 16 and 24 that have relatively low concentrations of total PCBs (fig. C3–5). In sample 16 and 24 the low concentrations that approach detection limits may affect the calculated BSAF value. The range of BSAF values calculated for Anniston PCB Site sediments was similar to those observed for when *L. variegatus* were exposed in the laboratory

Table C3–1. Biota sediment accumulation factor values for total polychlorinated biphenyl (PCB) and for the homolog groups monochlorobiphenyls (mono), dichlorobiphenyls (di), trichlorobiphenyls (tri), tetrachlorobiphenyls (tetra), pentachlorobiphenyls (penta), and hexachlorobiphenyls (hexa).

[--, no data]

Cycle	Sediment	Total PCB		Mono	Di	Tri	Tetra	Penta	Hexa
		Mean	Standard deviation	Mean	Mean	Mean	Mean	Mean	Mean
1a	1	1.7	.2	.29	.8	1.	2.8	3.8	4.5
1a	13	1.1	.1	.17	.5	.8	1.8	2.5	2.9
1a	20	1.8	.5	.25	.9	1.5	2.9	3.9	4.8
1a	25	1.5	--	.15	1.1	1.7	3.	3.1	3.6
1a	27	3.9	3.2	.22	1.1	2.1	4.7	5.	5.7
1a	28	2.4	.4	.31	1.	.7	2.6	3.1	3.5
1b	2	.9	.1	.07	.3	.9	1.8	2.3	2.9
1b	11	2.4	2.4	.07	.3	1.3	5.1	6.6	8.7
1b	14	2.3	.2	.04	.2	1.	4.4	5.7	7.2
1b	16	3.7	.8	.14	.5	.6	3.7	4.9	4.8
1b	23	1.7	.4	.1	.5	1.2	2.6	4.1	6.
1b	24	4.3	1.5	.12	.5	.9	3.	7.9	10.5

to field-collected sediments in studies by Trimble and others (2008) and Van Geest and others (2011), but was lower than those reported by Beckingham and Ghosh (2010).

The BSAF values calculated for homolog groups varied widely from 0.07 to 10.5 (table C3–1). Mean BSAF values across sediments were lowest for monochlorobiphenyls and highest for hexachlorobiphenyls and increased with increasing chlorination (fig. C3–6). Because hydrophobicity, expressed log K_{ow}, increases with increasing chlorination (Hawker and Connell 1988), this increasing trend indicates that the bioaccumulation potential increased with increasing hydrophobicity from mono- to hexachlorobiphenyls. Landrum and others (2001) also reported a strong relation between K_{ow} and BSAF for PCBs in the amphipods *Diporeia* spp. collected in Lake Michigan sediment. According to Landrum and others (2001) a strong relation between BSAF values and hydrophobicity suggest that the mechanism for accumulation is not simply passive partitioning as suggested by the equilibrium partitioning theory (Di Toro and others, 1991). The equilibrium partitioning theory predictions suggest that the BSAF value should be a constant value invariant with log K_{ow} and in the range of 1.7 (McFarland, 1984; McFarland and Clarke, 1989). In this study, most homolog-group-specific BSAF values were either substantially lower or substantially higher than would be expected from equilibrium partitioning theory. However, when You and others (2007) investigated the bioavailability of PCBs from sediment collected from Crab Orchard Lake in Marion, Illinois to *L. variegatus*, BSAF values for specific congeners ranged from 1.09 to 2.65 and no apparent relation with degree of chlorination, and therefore hydrophobicity, from PCB congener 44 to PCB congener 170.

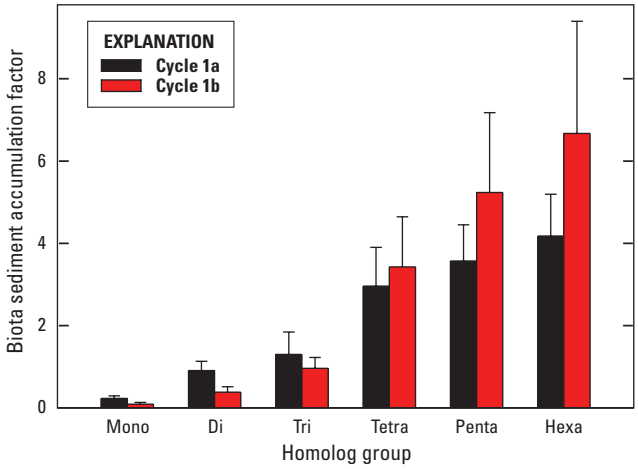

Figure C3–6. Mean polychlorinated biphenyl (PCB) biota sediment accumulation factors (BSAF) for the oligochaete *Lumbriculus variegatus* exposed to Anniston site sediments from cycle 1a and cycle 1b. The BSAF values are shown for the homolog groups monochlorobiphenyls (mono), dichlorobiphenyls (di), trichlorobiphenyls (tri) , tetrachlorobiphenyls (tetra), pentachlorobiphenyls (penta), and hexachlorobiphenyls (hexa).

Estimated Polychlorinated Biphenyl in Pore Water

Pore-water concentration of PCB congeners and total PCBs were successfully estimated for all sediments used in bioaccumulation testing with *L. variegatus* as well as in sediments used in toxicity testing with *H. azteca* and *C. dilutus* (chapter 4, appendix 3, and appendix 5). The method used in this study of directly exposing SPME fibers into the sediment matrix (matrix-SPME) has been used by several investigators (You and others 2006, 2007; Trimble and others, 2008; Brennan and others, 2009). No significant linear relation between sediment (organic carbon-normalized) and pore-water PCB concentrations were evident (fig. C3–7). PCB concentrations in pore water apparently approached saturation at sediment PCB concentrations in the range of 18.0 g/kg OC and higher. Relative to sediment concentrations, pore-water concentrations were overall lower in cycle 1b.

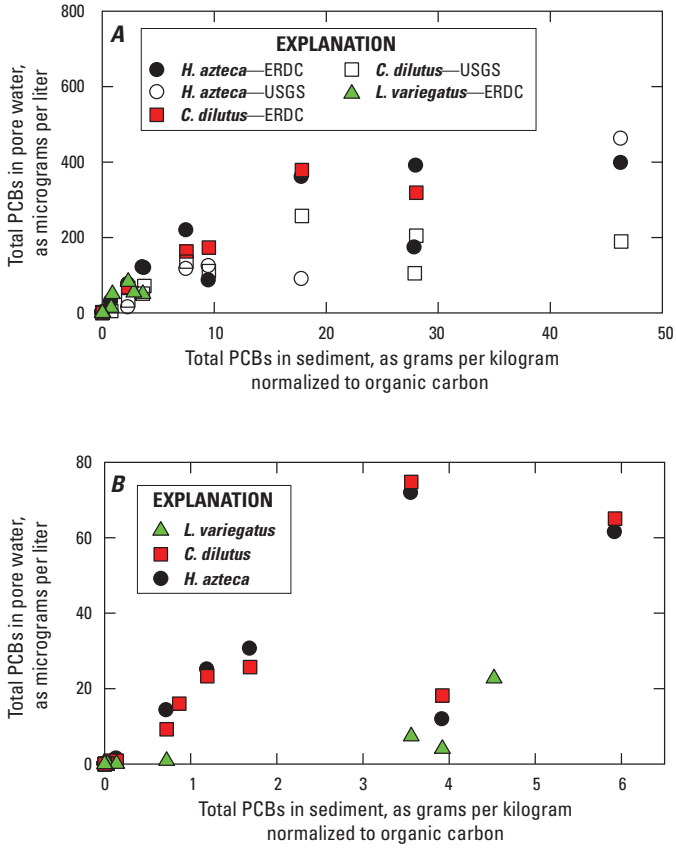

Figure C3–7. Total polychlorinated biphenyls (PCBs) in pore water determined from SPME for the amphipod *Hyalella azteca* (*H. azteca*), the midge *Chironomus dilutus* (*C. dilutus*), and the oligochaete *Lumbriculus variegatus* (*L. variegatus*) in whole-sediment exposures for each sediment site compared to PCBs in sediment as milligrams per kilogram normalized to organic carbon for *A*, cycle 1a and *B*, cycle 1b. [ERDC, ??; USGS, U.S. Geological Survey]

Pore-water concentrations in cycle 1b were remarkably lower for the *L. variegatus* exposures than for *H. azteca* and *C. dilutus* exposures (table C3–1 and fig. C3–7). This observation is not unexpected because oligochaete differentially modify the sediments during exposure compared to *H. azteca* or *C. dilutus*. To further illustrate differences in pore-water concentration among tests, total PCBs in pore water for the midge, *C. dilutus* and the oligochaete, *L. variegatus* were expressed as a fraction of the concentration measured in exposures using *H. azteca* in select sediments from cycle 1a and cycle 1b (fig. C3–8). For the same sediment, total PCB concentrations in pore water were typically highest for *H. azteca* exposures and lowest for *L. variegatus*. Variability in pore-water PCB concentrations among tests was attributed at least partially to differences in species-specific test-organism/sediment interactions and test design.

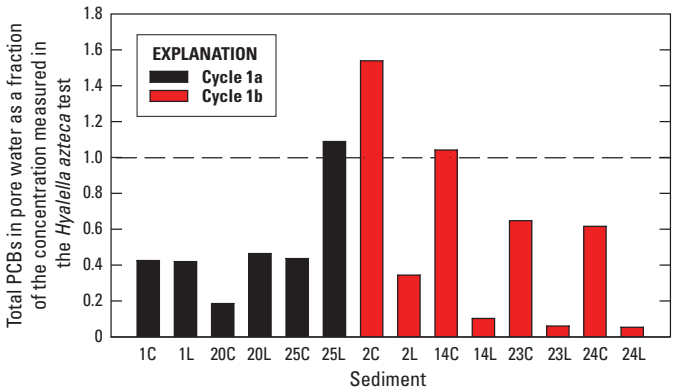

Figure C3–8. Total polychlorinated biphenyls (PCBs) in pore water measured in whole-sediment exposures for select sediments from cycle 1a and cycle 1b for the midge *Chironomus dilutus* and the oligochaete *Lumbriculus variegatus* expressed as a fraction of the concentration measured in exposures using *Hyalella azteca*. Abbreviation on x-axis are for sample number and *Chironomus dilutus* bioassay (#C) and sample number and *Lumbriculus variegatus* bioassay (#L).

The concentration of a subset of PCB congeners was measured in the whole sediment and in the pore water using passive samplers. Site specific organic carbon-water partitioning coefficient, expressed on a log10 basis (log K_{oc}), was calculated for PCB congeners 118 and 153 using data from the *H. azteca* exposures performed at USACE–Vicksburg (figs. C3–3 through C3–9). For PCB congener 118, log K_{oc} values ranged from 5.9 to 7.1, with a mean of 6.62 and a median of 6.67. For PCB congener 153, log K_{oc} values ranged from 6.56 to 7.56, with a mean of 7.04 and a median of 7.0. The site-specific log K_{oc} values were similar to the model-predicted values (Di Toro and others, 1991) of 6.63 and 6.8 for PCB congener 118 and PCB congener 153, respectively, using K_{ow} values from Hawker and Connell (1988).

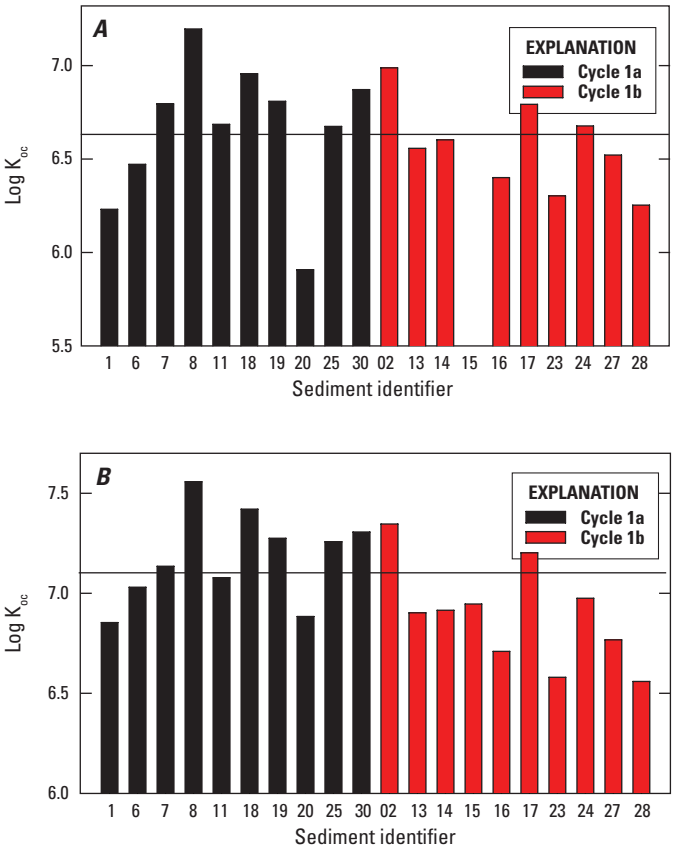

Figure C3–9. Organic carbon partition coefficients (log K_{oc}) for polychlorinated biphenyl (PCB) congener *A*, 118 and *B*, 153 for each sample site. Model predicted values (Di Toro and others, 1991) using K_{ow} values from Hawker and Connell (1988) are shown with horizontal lines.

Prediction of Bioaccumulation

A significant linear relation was evident between dry weight sediment concentrations of total PCBs and lipid-normalized total PCBs whole-body residue (fig. C3–10*A*). Despite a broad range of sediment total organic carbon (TOC ranged from 0.3 to 4.5 percent), normalizing sediment concentration by TOC caused only a minor improvement on the sediment/tissue relation (fig. C3–10*B*). Tissue residues were accurately predicted using SPME-derived pore-water data (fig. C3–10*C*). However, the relation between measured and pore-water-predicted tissue concentration was not as strong as the relation between bioaccumulation and PCBs in sediment organic carbon. The slope of the relation between measured bioaccumulation and bioaccumulation predicted from pore-water concentrations was higher for cycle 1 b compared to cycle 1a.

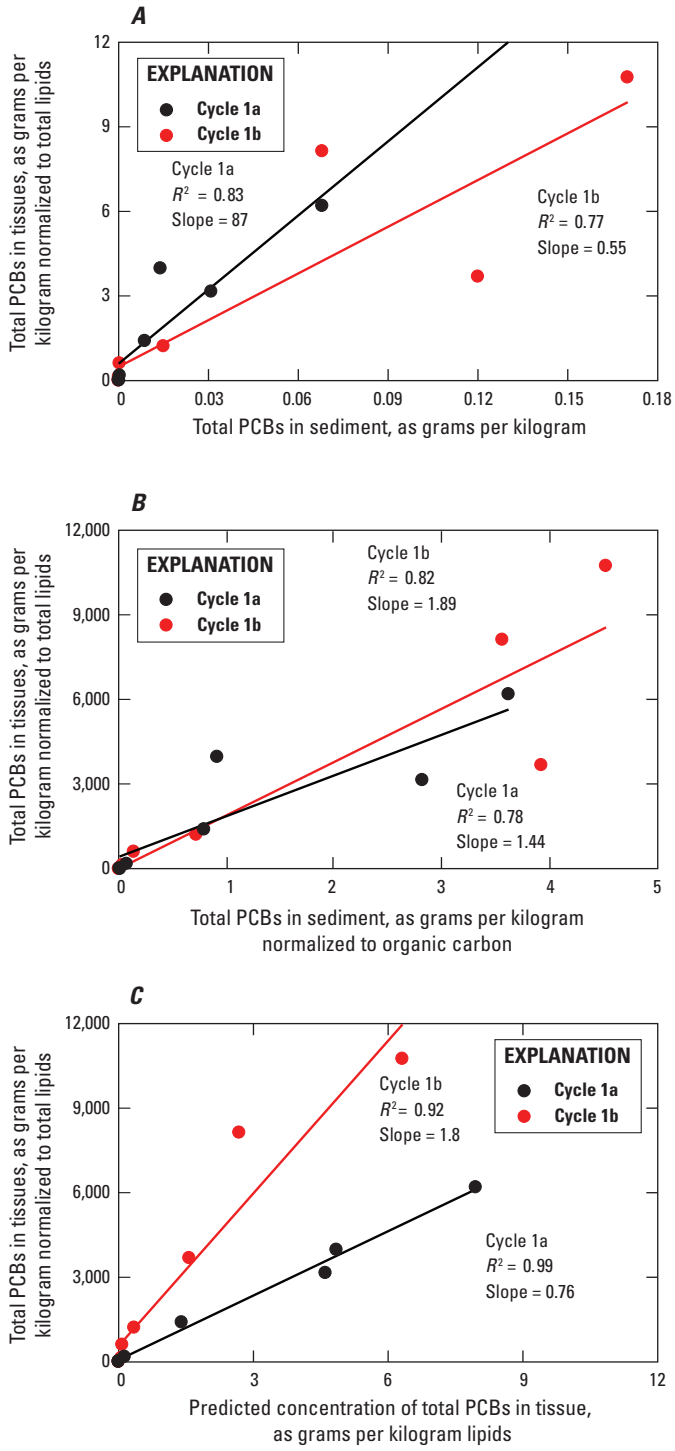

Figure C3-10. Relation between total polychlorinated biphenyl (PCB) concentrations in *A*, sediment (expressed as dry weight), *B*, sediment organic carbon, or *C*, tissue (predicted concentrations) to measured total PCB concentrations in *Lumbriculus variegatus* tissue at the end of the 28-day exposures.

The matrix-SPME method accurately predicted bioaccumulation of PCBs and other hydrophobic organic compounds from sediments in the studies by Kraaij and others (2003), You and others (2007), Trimble and others (2008), and Lu and others (2011). Although the route of uptake for the strongly hydrophobic compounds in deposit-feeding oligochaetes is expected to be through ingestion (Lu and others, 2004), the pore-water concentration seems to be a reliable indicator of bioaccumulation in the organism, with a lipid-water partition coefficient approximately equal to the octanol-water partition coefficient (Lu and others, 2011).

Summary

In general the accumulation of PCBs from sediment into oligochaete tissues was consistent with equilibrium partitioning predictions. Using these data, site specific BSAF values were derived and can be applied to predict bioaccumulation in Anniston PCB Site areas with data for only sediment concentrations of PCBs and TOC. Pore-water samplers (SPME), used to estimate sediment PCBs in pore water, in these pore-water estimates accurately predicted lipid-based concentrations of PCB in oligochaetes. These predicted values were independent of concentration and similar to measured values across sediment treatments. This evaluation also suggests that the equilibrium model using literature K_{oc} values is predictive of bioavailability of PCBs in Anniston PCB Site sediments. Strong concurrence of equilibrium-based measures, including bioaccumulation of total PCBs, pore-water sampling with SPME, and concurrence of K_{oc}, suggests these endpoints can be used as indicators of bioavailability of PCBs in sediments. Therefore, tissue concentrations and pore-water concentrations may be used as an additional line of evidence to further understand the bioavailability and toxicity responses in the whole-sediment toxicity tests done with *H. azteca* and *C. dilutus* (chapters 4 and 5, and appendix 5).

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Toxicity Testing with the Amphipod, *Hyalella azteca*, and with the Midge, *Chironomus dilutus*

By Jacob K. Stanley, Nile E. Kemble, J. Daniel Farrar, Christopher G. Ingersoll, Donald D. MacDonald, Jeffery A. Steevens, James L. Kunz, and Jesse A. Sinclair

Chapter 4 of

Evaluation of Toxicity to the Amphipod, *Hyalella azteca*, and to the Midge, *Chironomus dilutus*; and Bioaccumulation by the Oligochaete, *Lumbriculus variegatus*, with Exposure to PCB-Contaminated Sediments from Anniston, Alabama

Edited by Christopher G. Ingersoll, Jeffery A. Steevens, and Donald D. MacDonald

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Toxicity Testing with the Amphipod, *Hyalella azteca*, and with the Midge, *Chironomus dilutus*

By Jacob K. Stanley[1], Nile E. Kemble[2], J. Daniel Farrar[1], Christopher G. Ingersoll[2], Donald D. MacDonald[3], Jeffery A. Steevens[1], James L. Kunz[2], and Jesse A. Sinclair[3]

Abstract

Long-term reproduction sediment toxicity testing with the amphipod *Hyalella azteca* and the midge *Chironomus dilutus* was done in basic accordance with U.S. Environmental Protection Agency (2000) and American Society for Testing and Materials International (2012) standard methods in two cycles of testing (cycle 1a and cycle 1b) on sediment from Operable Unit 4 of the Anniston PCB Site. The whole-sediment toxicity tests met all of the established American Society for Testing and Materials International (2012) and U.S. Environmental Protection Agency (2000) test acceptability criteria, and intralaboratory control responses between the two cycles were similar. Samples were designated toxic or not toxic based on a reference-envelope approach. Relative endpoint sensitivity was assessed by graphing control-normalized data points against each other in pairs and assessing the number of data points that exceeded a 20-percent difference from the line of unity as well as through the assessment of the number of test sediments classified as toxic by falling below the response of the lower distribution of the reference-envelope for each toxicity endpoint. This analysis of endpoint responsiveness demonstrated that the most responsive *H. azteca* endpoints were day 42 survival normalized young per female and day 28 biomass and that the most responsive *C. dilutus* endpoints were adult biomass and percent adult emergence. Overall, between the two species, the most responsive endpoint assessed for these two species was *H. azteca* survival-normalized young per female (67 percent of the samples classified as toxic).

Introduction

Long-term reproduction sediment toxicity testing with *Hyalella azteca* and *Chironomus dilutus* was done by U.S. Army Corps of Engineers Engineer Research and Development Center, Vicksburg, Mississippi (USACE–Vicksburg) and U.S. Geological Survey Columbia Environmental Research Center, Columbia, Missouri (USGS–Columbia) on sediments from Operable Unit 4 of the Anniston PCB Site to characterize relations between sediment chemistry and sediment toxicity. The focus of this chapter is to describe toxicity testing methods and results, the meeting of test acceptability criteria, the use of the reference-envelope approach to designate samples toxic or not toxic, and relative endpoint sensitivity within and across species. Methods for selecting and collecting sediments for physical and chemical characterization of sediments are described in chapter 2. Relations between sediment chemistry and sediment toxicity are described in chapter 5. USACE–Vicksburg served as the primary *H. azteca* testing laboratory; whereas, USGS–Columbia served as the primary *C. dilutus* testing laboratory. Two cycles of *C. dilutus* and *H. azteca* intralaboratory testing were done, cycle 1a and cycle 1b (table C2–1). An interlaboratory comparison study also was done with cycle 1a samples by having each of the two toxicity testing laboratories test six sediments with their nonprimary species (appendix 5). Results of a study designed to evaluate the effect of the starting age of *C. dilutus* with exposure to Anniston PCB Site sediments are described in appendix 6.

Methods

Long-term reproduction sediment toxicity testing with *H. azteca* and *C. dilutus* was done in basic accordance with methods outlined in American Society for Testing and Materials International (2012), U.S. Environmental Protection Agency (2000), and Ingersoll and others (2008; appendix 3, table A3–1 to A3–6). Appendix 3, tables A3–7 to A3–27 provide a summary of the toxicity data and water-quality data. A description of how each of the *H. azteca* and

[1] U.S. Army Corps of Engineers, Vicksburg, Mississippi.

[2] U.S. Geological Survey, Columbia, Missouri.

[3] MacDonald Environmental Sciences Ltd., Nanaimo, British Columbia.

C. dilutus endpoints was calculated is provided in appendix 3, table A3–28. Control-adjusted responses of *H. azteca* and *C. dilutus* to each test sediment are provided in appendix 3, tables A3–29 and A3–30. Exposures were done in 300 milliliters (mL) high-form lipless beakers with a sediment volume of 100 mL and an overlying water volume of 175 mL. The beakers were fitted with holes or notches covered by stainless steel mesh screen to allow for daily flow-through water renewal. Testing was done at 23 degrees Celsius (°C) using a 16:8 light:dark cycle. Sediments and overlying water were added to beakers 5 to 8 days before the addition of organisms to allow for equilibration (chapter 2). A laboratory control sediment (West Bearskin Lake sediment, 1.1 percent total organic carbon; Ingersoll and others, 1998) was run with each sediment toxicity test with each species. An acute reference toxicity test with sodium chloride was done according to methods outlined in appendix 3, table A3–6 to provide an indicator of organism health at the time of testing.

Overlying water additions were started the day before organisms were added to test beakers containing sediment. Water was added using automated water renewal systems at the rate of two volume additions per day. The source of overlying water in the testing done at USACE–Vicksburg was dechlorinated Vicksburg, Mississippi tap water, dechlorinated using activated carbon filtration. The source of water in the testing done at USGS–Columbia was well water diluted with deionized water to a hardness of about 100 milligrams per liter as calcium carbonate (mg/L as $CaCO_3$), alkalinity 85 mg/L (as $CaCO_3$), and pH about 8.0. A comparison of the water-quality condition of the water used in each of the respective laboratories, as well as a comparison to Choccolocco Creek site water including measurements of major ions is provided in appendix 3, table A3–7. Water hardness and other water conditions can affect toxicity of metals and the overall performance of organisms in a sediment toxicity test. Choccolocco Creek surface water has a hardness of 80 to 90 mg/L as $CaCO_3$. Laboratory water at the USACE–Vicksburg was 73 mg/L as $CaCO_3$ and is similar to the Choccolocco Creek water. The well water at the USGS–Columbia is about 300 mg/L as $CaCO_3$; therefore the USGS Columbia well water was diluted with deionized water to a hardness of 100 mg/L as $CaCO_3$. These two laboratory waters were determined to be similar to Choccolocco Creek and were used for all sediment toxicity testing and sediment bioaccumulation testing (chapter 3).

Hyalella azteca Toxicity Testing Methods

Hyalella azteca exposures were 42 days in duration. Twenty *H. azteca* were archived at the start of the exposures for length measurement. Length was measured using a Leica MZ12 dissection microscope (Leica Microsystems, Inc., Buffalo Grove, Illinois) fitted with a Leica DFC425 digital camera and Image-Pro Plus (Media Cybernetics, Bethesda, Maryland) image analysis software. Twelve replicate beakers were tested for each sediment. In addition, one solid-phase microextraction (SPME) beaker for estimating pore-water concentrations of PCBs and one peeper beaker for sampling pore-water metals were tested per treatment (chapter 2). Ten approximately 7-d-old organisms (determined by size by sieve according to U.S. Environmental Protection Agency, 2000) were added to each beaker. Organisms were fed 1.0 mL of a yeast, Cerophyl, and trout chow (YCT) food mixture daily from days 0 to 28 and 2.0 mL of YCT daily on days 28 to 42. The decision to increase the addition of YCT from day 28 to 42 was based on studies performed by USGS–Columbia demonstrating improved weight and reproduction of *H. azteca* in water or sediment exposures with an increase in the ration of YCT (Chris Ingersoll, U.S. Geological Survey, Columbia, Mo., unpub. data, 2010). On day 28, *H. azteca* from 4 of the 12 replicates were recovered by sieving the sediment and the length of each surviving organism was measured followed by an assessment of dry weight on all of the organisms recovered from each replicate combined. Mean dry weight per individual and replicate total biomass, an endpoint that in effect is a combination of survival and dry weight endpoints, were determined. Dry weight was determined after drying overnight in a 60 °C oven. The *H. azteca* from the remaining eight replicates were sieved from the sediment with 425-µm sieves (at USACE–Vicksburg) or with 500-µm sieves (at USGS–Columbia), counted, and placed into beakers containing water and no sediment for assessment of reproduction on days 35 and 42. A 5-cm x 5-cm piece of Nitex screen (at USACE–Vicksburg) or a thin layer of sand (5 mL/beaker at USGS–Columbia) was added to the beakers from day 28 to 42 as a substrate for the organisms. On day 35, adults and young were removed from beakers and counted, and the adults were returned to the beaker. On day 42, young and adults were counted, and adults were archived for later assessment of sex, and weight analysis. The additional chemistry replicates were treated the same as the toxicity replicates (addition of sediment, water, test organisms, and food). The peeper samplers were placed into the sediment on day 14 of the exposures and sampled on day 21 of the exposures, and the SPMEs were placed into sediment on day 0 of the exposures and sampled on day 28 of the exposures (chapter 2). Appendix 3 provides detailed tables and figures summarizing the intralaboratory sediment toxicity data described in the current chapter and the interlaboratory sediment toxicity data described in appendix 5. Further details about *H. azteca* testing characteristics and scheduling of the toxicity tests are provided in appendix 3, tables A3–1 and A3–2. Further detail on procedures used to calculate each endpoint are provide in appendix 3, table A3–28.

Chironomus dilutus Toxicity Testing Methods

The decision was made to perform the *C. dilutus* toxicity tests starting with 7-d-old larvae rather than starting tests with less than 24-h-old larvae [as is described in U.S. Environmental Protection Agency (2000) and in American Society for Testing and Materials International (2012)]. This decision

was based on previously observed problems with control acceptability with tests started with less than 24-h-old larvae at USGS–Columbia, USACE–Vicksburg, and at other laboratories. Specifically, poor survival of *C. dilutus* larvae has been observed at day 20 of the sediment exposures before midge pupate and emerge as adults. The low or variable survival of tests started with midge less than 24-h-old in control sediment may result from difficulties in handling these young larvae. Before the start of the definitive sediment toxicity testing, USGS–Columbia and USACE–Vicksburg performed a study to evaluate midge emergence in three control sediments and observed better performance of *C. dilutus* in exposures started with about 7-d-old larvae compared to exposures started with less than 24-h-old larvae (appendix 6), Chris Ingersoll, U.S. Geological Survey, Columbia, Mo., and Jeff Steevens, U.S. Army Corps of Engineers, Vicksburg, Miss.; unpub. data, 2010). Also, a *C. dilutus* age comparison study was done after the completion of cycle 1b midge sediment toxicity testing to evaluate the relative sensitivity of *C. dilutus* in sediment toxicity tests started with 7-d-old larvae (cycle 1c) compared to the sensitivity of tests started with less than 24-h-old larvae (appendix 6). This modification to the method is consistent with the guidance provided in U.S. Environmental Protection Agency (2000) and American Society for Testing and Materials International (2012). Specifically, American Society for Testing and Materials International (2012) states in section A7.1.2 that the standard "describes general guidance for performing a long-term sediment toxicity test with *C. dilutus* that can be used to evaluate sublethal effects of contaminants associated with sediment. More definitive methods may be described in future versions of this standard after additional laboratories have successfully used the method."

Sixteen replicate beakers were tested with *C. dilutus* for each sediment: four for assessment of day 13 survival, weight (ash free dry weight; AFDW) and biomass [determined by sieving sediment on day 13 with 425-µm sieves (at USACE–Vicksburg) or with 500-µm sieves (at USGS–Columbia)], eight for assessment of survival, emergence (percent emergence and time to emergence), and reproduction (number of egg cases, number of eggs per case, percent of eggs hatched, total young produced, average young per replicate, and adult biomass) and four auxiliary male beakers (appendix 3, tables A3–1 and A3–3). Adult biomass was estimated for each replicate beaker as the product of control-adjusted percent emergence of adults and control-adjusted day 13 weight (expressed as a percentage of the mean control response). This estimate of adult biomass assumes average weight of emerging adults was proportional to average weight of larvae on day 13. Auxiliary male beakers are needed because it is typical for males to begin emerging 4 to 7 days before females (U.S. Environmental Protection Agency, 2000; American Society for Testing and Materials International, 2012). Therefore, in order to increase the likelihood of having males available to pair with females during the prime emergence period for each treatment, auxiliary male beakers are started with organisms 3 days after the initial 12 beakers are loaded with organisms. In addition, one separate replicate SPME beaker for estimating pore-water concentrations of PCBs and one separate replicate peeper beaker for measuring pore-water metals was tested per treatment (chapter 2). These additional chemistry replicates were treated the same as the toxicity replicates (addition of sediment, water, test organisms, and food). The peeper samplers were placed into the sediment on day 14 of the exposures and sampled on day 21 of the exposures, and the SPMEs were placed into sediment on day 0 of the exposures and sampled on day 28 of the exposures (chapter 2). *Chironomus dilutus* were fed a suspension of Tetrafin goldfish food. The *C. dilutus* exposures in cycle 1a or in cycle 1b were ended after 7 days of no emergence observed from the control sediment. Further details about *C. dilutus* testing conditions and scheduling of the toxicity tests are provided in appendix 3, tables A3–1 and A3–3. Further detail on procedures used to calculate each endpoint are provide in appendix 3, table A3–28.

Reference Envelope Development and Control-Adjusted Responses

A reference-envelope approach was used to classify sediments from the Anniston PCB Site as toxic or not toxic (Hunt and others, 2001; Ingersoll and others, 2009; Besser and others, 2009; Wang and others, 2013; Kemble and others 2013). Chapter 5 provides a summary of the concentration-response models that were developed using the sediment chemistry (appendix 1, table A1–3a) and sediment toxicity data based on comparisons to response of test organisms in reference sediments. The reference-envelope approach is a procedure for assessing sediment toxicity that was developed to overcome the limitations associated with the use of control sediments for this purpose, including accounting for differences in the noncontaminant characteristics of test sediments and for overcoming the low statistical power associated with comparing many test results to a single control sediment with physical characteristic that may differ from site sediments. Application of this approach necessitates identification of reference sediment samples for each toxicity test endpoint that is evaluated. That is, all of the sediment samples in cycle 1a or in cycle 1b testing that met the selection criteria were considered to be candidate reference sediment samples.

Candidate reference sediment samples were evaluated using chemical criteria and biological criteria. As a first step, sediment samples with chemical characteristics representative of reference conditions were identified (that is, samples substantially free of contamination). As a second step, sediment samples that met survival or weight test acceptability requirements of a control sediment were identified. Specifically, reference sediment samples were identified using the following criteria.

1. Chemical criteria:

    a. Mean probable effect concentration quotient (PEC-Q) less than 0.1. Based on combined

measures of mean metal PEC-Q (PEC-Q_{METALs}), PEC-Q for total polycyclic aromatic hydrocarbons (PEC-Q_{TPAH}), PEC-Q for total PCBs (PEC-Q_{TPCB}), and mean PEC-Q for organochlorine pesticides (PEC-$Q_{OCPESTs}$) (MacDonald and others, 2000; Ingersoll and others, 2001, 2009; appendix 1, table A1–3a)

b. PEC-Q_{TPAH} less than 0.1

c. PEC-Q_{TPCB} less than 0.1

d. Mean PEC-$Q_{OCPESTs}$ less than 0.1

e. Mean PEC-Q_{METALs} less than 0.1

f. Chronic sum equilibrium-partitioning sediment benchmark toxic units ($\sum$ESB-TU_{FCV}; based on a final chronic value for *H. azteca* and 34 parent and alkylated PAHs) less than 0.1 (U.S. Environmental Protection Agency, 2003; Ingersoll and others, 2009; appendix 1, table A1–3a)

g. Sum Simultaneous Extracted Metals and Acid Volatile Sulfate normalized to fraction organic carbon [($\sum$SEM-AVS)/f_{OC}] less than 130 micromole per gram [(µmol/g) appendix 1, tables A1–3b, A1–3c, and A1–3d]

2. Biological criteria:

a. Biological criterion 2a—Response of test organisms in the control sediment meets American Society for Testing and Materials International (2012) and U.S. Environmental Protection Agency (2000) test acceptability requirements (appendix 3, tables A3–1, A3–4, A3–5, A3–22, and A3–26):

i. Greater than or equal to 80 percent survival of amphipod

ii. Greater than or equal to 70 percent survival and greater than or equal to 0.48 mg/individual AFDW for midge

b. Biological criterion 2b—Response of test organisms in reference sediment meets American Society for Testing and Materials International (2012) and U.S. Environmental Protection Agency (2000) test acceptability requirements (appendix 3, tables A3–1, A3–4, A3–5, A3–22, and A3–26):

i. Greater than or equal to 80 percent survival of amphipod

ii. Greater than or equal to 70 percent survival and Greater than or equal to 0.48 mg/individual AFDW of midge

3. If ANOVA for an endpoint within a batch of samples is not significant (p greater than 0.05), data for that endpoint was not be further evaluated (Besser and others, 2009)

4. Interpretation of chemical or biological criteria to establish a sediment as an acceptable reference sample

a. If 1 or more of chemical criterion not met, data for that sample was not used in reference-envelope calculation

b. If 1 or more biological of the American Society for Testing and Materials International (2012) or U.S. Environmental Protection Agency (2000) test acceptability requirements not met, data for that species was not used in reference-envelope calculation (Biological criterion 2b)

5. A sediment sample was classified as toxic for an endpoint if the mean response of the test organism for that endpoint is less than the lowest mean for the reference samples

6. A sediment sample was identified as highly toxic if the mean response of the test organism for that endpoint is 10 percent less than the lowest mean for the reference samples

Sediment samples that met the chemical criteria and biological criteria were included in the reference pool of reference sediments. The reference sediment samples were selected independently for each of the laboratory toxicity tests. If the American Society for Testing and Materials International (2012) and U.S. Environmental Protection Agency (2000) test acceptability criteria were not met for all of the endpoints for a species, the toxicity test results for that sediment sample were not considered in the calculation of the reference envelope for any of the endpoints for that species. In total, of the six samples initially categorized as reference sediments before the start of sediment collection (sediments 04, 9, 10, 22, 26, and 29; table C2–1), five of these sediment met the chemical and biological criteria for inclusion in the reference envelopes for the toxicity test endpoints. One of the six reference sediments (sediment 04) was excluded as a reference sediment for *H. azteca* testing and for *C. dilutus* testing because of slightly elevated sediment PAH concentrations (appendix 1, table A1–3a).

Control Normalization of the Toxicity Test Response Data

Sediment chemistry and sediment toxicity data were evaluated to support the development of concentration-response models for selected chemicals of potential concern (COPCs) and for COPC mixtures (chapter 5). Because these data were generated in different batches of samples within the

same laboratory (that is, cycle 1a and cycle 1b), there was a need to normalize the data in a manner that made the toxicity test results more comparable across different batches and across laboratories (appendix 5). More specifically, normalization of responses of test organisms to the response in the control sediment is intended to account for variability in the test response data because of organism health, test procedures, test conditions, and the local physical characteristics of the sediments (when using reference normalization). Specifically, toxicity test response data for each endpoint within a batch of samples were normalized to the mean response observed in the control treatment within that batch (Ingersoll and others, 2008, appendix 3, tables A3–29 and A3–30).

A complete listing of the physical characteristics [that is, grain size and total organic carbon (TOC)] of sediment samples in the control sediment and in the reference samples for the Anniston PCB Site are presented in appendix 1, table A1–3a. In summary, the control sediment was made up of 1.1 percent TOC and a grain size distribution of 37 percent fines and 63 percent sand. Mean TOC in the five reference sediments was 0.498 percent with a range of 0.303 percent to 0.718 percent. The mean grain size composition in the reference sediments was 16.8 percent fines (range: 11.3 to 26.6 percent) and 83.2 percent sand (range: 73.4 to 88.7 percent). Reference samples for cycle 1a and cycle 1b were collected from the same area (that is, within about 50 m). These data emphasize the importance of accounting for TOC and grain size within the study area as a whole and within each of the reaches identified (fig. C1–1).

Development of Reference Envelope

Following the identification of reference sediment samples, the range of the biological responses in these samples was determined for each toxicity test and for each toxicity endpoint measured. In this study, the reference envelope was defined as the range of biological responses that encompassed 100 percent of the response data for the reference sediment samples. Accordingly, the lower limit of the reference envelope was calculated as the minimum control-adjusted response value for each toxicity test and endpoint, using the data for the reference sediment samples that were selected for each toxicity test (Besser and others, 2009; Moran and others, 2012; MacDonald and others, 2012; Kemble and others, 2013; Wang and others 2013). The sediment chemistry data for the stations that had the minimum value for the reference envelope for each toxicity test endpoint are presented in appendix 4, table A4–1 and table A4–2.

Designation of Samples as Toxic to *Hyalella azteca* or to *Chironomus dilutus*

The reference envelope was considered to define the normal range of responses associated with exposure of toxicity test organisms to relatively uncontaminated sediment samples. Sediment samples with effect values lower than the lower limit of the normal range of control-adjusted responses for the reference samples (that is, lower than the minimum value) were designated as toxic for the endpoint under consideration. The sediment samples also were designated as toxic or not toxic based on the results of multiple endpoints for each toxicity test (that is, survival, weight, biomass, or reproduction of *H. azteca* and survival, weight, biomass, emergence, or reproduction of *C. dilutus*). Finally, sediment samples were designated as toxic or not toxic based on the results obtained from any of the toxicity test endpoints. The toxicity designations that were assigned to each of the sediment samples that were included in the project database are listed in tables C4–1 and C4–2.

Whereas, classification of sediment samples as toxic or not toxic provides important information for assessing sediment quality conditions, additional information on the magnitude of toxicity can contribute to such evaluations. For this reason, toxic sediment samples were further classified to identify moderately toxic and highly toxic sediment samples (chapter 5). Highly toxic (HT) sediment samples were identified based on a greater than 10 percent reduction in survival, weight, biomass, emergence, or reproduction relative to the lower limit of the reference envelope (MacDonald and others, 2002, 2012; tables C4–1 and C4–2). Moderately toxic (MT) sediment samples were identified based on survival, weight, biomass, emergence, or reproduction that fell less than 10 percent below the lower limit of the reference envelope.

The toxicity designations for individual endpoints and multiple endpoints provide information in chapter 5 for interpreting relations between sediment toxicity and sediment chemistry for samples collected from the Anniston PCB Site. Specifically, these toxicity designations support the development of concentration-response models (CRMs) for individual COPCs and COPC mixtures in samples from the Anniston PCB Site. Furthermore, this information is required in chapter 5 for evaluating the reliability and predictive ability of the various sediment toxicity thresholds (TT) that are derived to support assessment of whole-sediment chemistry data or porewater chemistry data.

Results and Discussion

Water Quality

Measured water quality values and water bath temperature recordings for *H. azteca* and *C. dilutus* are provided in appendix 3, tables A3–8 through A3–14. All water quality characteristics measured were within their acceptable range (U.S. Environmental Protection Agency, 2000; American Society for Testing and Materials International, 2012).

Table C4–1. Summary of the toxicity of sediment samples from the Anniston PCB Site to the amphipod *Hyalella azteca*.

[ID, identifier; PCB, polychlorinated biphenyls; µg/kg, micrograms per kilogram; NT, not toxic (within the reference envelope); MT, moderately toxic (within 10 percent of the reference envelope); HT, highly toxic (> 10 percent below the reference envelope); mm, millimeter; NA, not applicable; mg, milligram; %, percent; The percentage and number of samples classified as not toxic, moderately toxic, and highly toxic for a given endpoint are shown at the bottom of the table. The percentage and number of endpoints included in the table classified as not toxic, moderately toxic, and highly toxic for a given sample are shown at the right of the table; * Bolded results represent sediment samples classified as toxic.]

Station ID	Sample ID	Cycle	ΣPCB (µg/kg)[1]	Day 28 percent survival	Day 28 growth (length; mm)	Day 28 growth (weight; mm)	Day 28 biomass (mg)	Day 35 percent survival	Day 35 reproduction (number of young per sediment)	Day 42 percent survival	Day 42 growth (length; mm)	Day 42 growth (weight; mg)
TXR1-04-P	X900022	1b	< 32	NT	NT	NT	NT	NT	NT	NT	NT	NT
TXR1-06-P	X900010	1b	< 33	NT	NT	NT	NT	NT	NT	NT	NT	NT
TXR1-01-P	X900026	1b	< 33	NT	NT	NT	NT	NT	NT	NT	NT	NT
TXR1-05-P	X900029	1b	< 33	NT	NT	NT	NT	NT	NT	NT	NT	NT
TXR1-02-P	X900009	1a	62.	NT	NT	NT	NT	NT	NT	NT	NT	NT
TX10-01-P	X900016	1b	91.2	**MT***	**MT**	**HT**	**HT**	NT	**HT**	**MT**	**MT**	NT
TX20-01-P	X900028	1b	220	NT	NT	**MT**	**HT**	NT	NT	NT	NT	NT
TX20-03-P	X900024	1b	310	NT	NT	NT	**MT**	NT	NT	NT	NT	NT
TX40-03-P	X900015	1b	990	**MT**	NT	NT	NT	**MT**	**HT**	**MT**	NT	NT
TX60-02-P	X900020	1a	8,800	**MT**	NT	NT	**MT**	NT	NT	NT	NT	NT
TX40-01-P	X900027	1b	13,000	**MT**	NT	NT	**MT**	**MT**	NT	**MT**	NT	NT
TX30-04-P	X900023	1b	15,000	NT	NT	**HT**	**HT**	**MT**	**HT**	**MT**	NT	NT
TX60-04-P	X900013	1b	25,000	**MT**	NT	**MT**	**HT**	**MT**	**HT**	**MT**	**MT**	NT
TX30-01-P	X900025	1a	60,000	NT	**MT**	**MT**	**MT**	NT	**HT**	NT	NT	NT
TX40-02-P	X900017	1b	64,000	NT	NT	NT	NT	NT	**HT**	NT	NT	NT
TX40-04-P	X900001	1a	68,000	NT	NT	NT	**HT**	NT	NT	NT	NT	**HT**
TX40-05-P	X900014	1b	68,000	**MT**	NT	**MT**	**HT**	NT	**HT**	NT	**MT**	NT
TX60-03-P	X900006	1a	100,000	**HT**	NT	NT	**HT**	**HT**	**HT**	**HT**	NT	NT
TX30-05-P	X900002	1b	120,000	**HT**	NT	NT	**HT**	**HT**	**HT**	**HT**	NT	NT
TX30-03-P	X900007	1a	150,000	**HT**	**MT**	**HT**	**HT**	**HT**	**HT**	**HT**	NT	**MT**
TX50-04-P	X900011	1a	240,000	NT	NT	NT	**HT**	NT	**HT**	NT	NT	NT
TX50-05-P	X900030	1a	410,000	**HT**	NT	NT	**HT**	**HT**	**HT**	**HT**	NT	NT
TX30-02-P	X900018	1a	740,000	**HT**	NA	NT	**HT**	**HT**	**HT**	**HT**	NT	NT
TX50-01-P	X900008	1a	770,000	**HT**	NT	NT	**HT**	**HT**	**HT**	**HT**	NT	NT
TX50-02-P	X900019	1a	1,200,000	**HT**	NA	NT	**HT**	**HT**	**HT**	**HT**	NT	NT
Not Toxic				48% (12 of 25)	87%(20 of 23)	72% (18 of 25)	28% (7 of 25)	56% (14 of 25)	40% (10 of 25)	52% (13 of 25)	88% (22 of 25)	92% (23 of 25)
Moderately Toxic				24% (6 of 25)	13% (3 of 23)	16% (4 of 25)	16% (4 of 25)	16% (4 of 25)	0% (0 of 25)	20% (5 of 25)	12% (3 of 25)	4% (1 of 25)
Highly Toxic				28% (7 of 25)	0% (0 of 23)	12% (3 of 25)	56% (14 of 25)	28% (7 of 25)	60% (15 of 25)	28% (7 of 25)	0% (0 of 25)	4% (1 of 25)
Toxic (Moderately and Highly Toxic)				52% (13 of 25)	13% (3 of 23)	28% (7 of 25)	72% (18 of 25)	44% (11 of 25)	60% (15 of 25)	48% (12 of 25)	12% (3 of 25)	8% (2 of 25)

Table C4–1. Summary of the toxicity of sediment samples from the Anniston PCB Site to the amphipod Hyalella azteca.—Continued

[ID, identifier; PCB, polychlorinated biphenyls; µg/kg, micrograms per kilogram; NT, not toxic (within the reference envelope); MT, moderately toxic (within 10 percent of the reference envelope); HT, highly toxic (> 10 percent below the reference envelope); mm, millimeter; NA, not applicable; mg, milligram; %, percent; The percentage and number of samples classified as not toxic, moderately toxic, and highly toxic for a given endpoint are shown at the bottom of the table. The percentage and number of endpoints included in the table classified as not toxic, moderately toxic, and highly toxic for a given sample are shown at the right of the table; * Bolded results represent sediment samples classified as toxic.]

Station ID	Sample ID	Cycle	ΣPCB (µg/kg)[1]	Day 42 biomass (mg)	Total number of young produced	Number of young per female	Day 42 reproduction (young per female; normalized to survival)	Not toxic	Moderately toxic	Highly toxic	Toxic (moderately and highly toxic)
TXR1-04-P	X900022	1b	< 32	NT	NT	NT	NT	100% (13 of 13)	0% (0 of 13)	0% (0 of 13)	0% (0 of 13)
TXR1-06-P	X900010	1b	< 33	NT	NT	NT	NT	100% (13 of 13)	0% (0 of 13)	0% (0 of 13)	0% (0 of 13)
TXR1-01-P	X900026	1b	< 33	NT	NT	NT	NT	100% (13 of 13)	0% (0 of 13)	0% (0 of 13)	0% (0 of 13)
TXR1-05-P	X900029	1b	< 33	NT	NT	NT	NT	100% (13 of 13)	0% (0 of 13)	0% (0 of 13)	0% (0 of 13)
TXR1-02-P	X900009	1a	62.	NT	NT	NT	NT	100% (13 of 13)	0% (0 of 13)	0% (0 of 13)	0% (0 of 13)
TX10-01-P	X900016	1b	91.2	**HT**	**HT**	**HT**	**HT**	15% (2 of 13)	31% (4 of13)	54% (7 of 13)	85% (11 of 13)
TX20-01-P	X900028	1b	220.	NT	NT	NT	NT	85% (11 of 13)	8% (1 of 13)	8% (1 of 13)	15% (2 of 13)
TX20-03-P	X900024	1b	310.	NT	NT	NT	NT	92% (12 of 13)	8% (1 of 13)	0% (0 of 13)	8% (1 of 13)
TX40-03-P	X900015	1b	990.	NT	NT	NT	**HT**	62% (8 of 13)	23% (3 of 13)	15% (2 of 13)	38% (5 of 13)
TX60-02-P	X900020	1a	8,800.	**MT**	NT	**HT**	**HT**	62% (8 of 13)	23% (3 of 13)	15% (2 of 13)	38% (5 of 13)
TX40-01-P	X900027	1b	13,000.	NT	NT	NT	NT	69% (9 of 13)	31% (4 of13)	0% (0 of 13)	31% (4 of13)
TX30-04-P	X900023	1b	15,000.	NT	NT	**HT**	**HT**	46% (6 of 13)	15% (2 of 13)	38% (5 of 13)	54% (7 of 13)
TX60-04-P	X900013	1b	25,000.	**HT**	**HT**	**HT**	**HT**	15% (2 of 13)	38% (5 of 13)	46% (6 of 13)	85% (11 of 13)
TX30-01-P	X900025	1a	60,000.	NT	**HT**	**HT**	**HT**	46% (6 of 13)	23% (3 of 13)	31% (4 of13)	54% (7 of 13)
TX40-02-P	X900017	1b	64,000.	NT	**HT**	**HT**	**HT**	69% (9 of 13)	0% (0 of 13)	31% (4 of13)	31% (4 of13)
TX40-04-P	X900001	1a	68,000.	**HT**	**MT**	**HT**	**HT**	54% (7 of 13)	8% (1 of 13)	38% (5 of 13)	46% (6 of 13)
TX40-05-P	X900014	1b	68,000.	NT	**HT**	**HT**	**HT**	38% (5 of 13)	23% (3 of 13)	38% (5 of 13)	62% (8 of 13)
TX60-03-P	X900006	1a	100,000.	**HT**	**HT**	**HT**	**HT**	31% (4 of13)	0% (0 of 13)	69% (9 of 13)	69% (9 of 13)
TX30-05-P	X900002	1b	120,000.	**HT**	**HT**	**HT**	**HT**	31% (4 of13)	0% (0 of 13)	69% (9 of 13)	69% (9 of 13)
TX30-03-P	X900007	1a	150,000.	**HT**	**HT**	**HT**	**HT**	8% (1 of 13)	15% (2 of 13)	77% (10 of 13)	92% (12 of 13)
TX50-04-P	X900011	1a	240,000.	NT	**HT**	**HT**	**HT**	62% (8 of 13)	0% (0 of 13)	38% (5 of 13)	38% (5 of 13)
TX50-05-P	X900030	1a	410,000.	**HT**	**HT**	**HT**	**HT**	31% (4 of13)	0% (0 of 13)	69% (9 of 13)	69% (9 of 13)
TX30-02-P	X900018	1a	740,000.	**HT**	**HT**	NA	NA	30% (3 of 10)	0% (0 of 10)	70% (7 of 10)	70% (7 of 10)
TX50-01-P	X900008	1a	770,000.	**HT**	**HT**	**HT**	**HT**	31% (4 of13)	0% (0 of 13)	69% (9 of 13)	69% (9 of 13)
TX50-02-P	X900019	1a	1,200,000.	**HT**	**HT**	**HT**	**HT**	25% (3 of 12)	0% (0 of 12)	75% (9 of 12)	75% (9 of 12)
Not Toxic				56% (14 of 25)	44% (11 of 25)	38% (9 of 24)	33% (8 of 24)				
Moderately Toxic				4% (1 of 25)	4% (1 of 25)	0% (0 of 24)	0% (0 of 24)				
Highly Toxic				40% (10 of 25)	52% (13 of 25)	63% (15 of 24)	67% (16 of 24)				
Toxic (Moderately and Highly Toxic)				44% (11 of 25)	56% (14 of 25)	63% (15 of 24)	67% (16 of 24)				

[1]ΣPCBs are calculated as the sum of each of the 10 homolog groups.

Table C4–2. Summary of the toxicity of sediment samples from the Anniston PCB Site to the amphipod *Chironomus dilutus*.

[ID, identifier; PCB, polychlorinated biphenyls; µg/kg, micrograms per kilogram; NT, not toxic (within the reference envelope); MT, moderately toxic (within 10 percent of the reference envelope); HT, highly toxic (> 10 percent below the reference envelope); mg, milligram; NA, not applicable; %, percent; The percentage and number of samples classified as not toxic, moderately toxic, and highly toxic for a given endpoint are shown at the bottom of the table. The percentage and number of endpoints included in the table classified as not toxic, moderately toxic, and highly toxic for a given sample are shown at the right of the table; * Bolded results represent sediment samples classified as toxic.]

Station ID	Sample ID	Cycle	ΣPCB (µg/kg)[1]	Day 13 percent survival	Day 13 growth (weight; mg)	Day 13 biomass (mg)	Percent emergence	Median emergence time (days)	Adult biomass	Adult time to death (days)	Number of egg cases
TXR1-04-P	X900022	1b	< 32	NT	NT	NT	NT	NT	NT	NT	NT
TXR1-06-P	X900010	1b	< 33	NT	NT	NT	NT	NT	NT	NT	NT
TXR1-01-P	X900026	1b	< 33	NT	NT	NT	NT	NT	NT	NT	NT
TXR1-05-P	X900029	1b	< 33	NT	NT	NT	NT	NT	NT	NT	NT
TXR1-02-P	X900009	1a	62	NT	NT	NT	NT	NT	NT	NT	NT
TX10-01-P	X900016	1b	91.2	**MT***	NT	**MT**	**MT**	NT	**MT**	NT	NT
TX20-01-P	X900028	1b	220	NT	NT	NT	NT	NT	NT	NT	NT
TX20-03-P	X900024	1b	310	NT	NT	NT	NT	NT	NT	NT	MT
TX40-03-P	X900015	1b	990	**MT**	**HT**	**HT**	**HT**	NT	**HT**	NT	NT
TX60-02-P	X900020	1b	6,000	NT	**HT**	**MT**	NT	NT	**HT**	NT	NT
TX60-02-P	X900020	1a	8,800	**MT**	**MT**	**MT**	**HT**	NT	**HT**	**HT**	**HT**
TX40-01-P	X900027	1b	13,000	NT	**HT**	**HT**	**HT**	NT	**HT**	NT	NT
TX30-04-P	X900023	1b	15,000	NT	**MT**	NT	NT	NT	**MT**	NT	NT
TX60-04-P	X900013	1b	25,000	**MT**	NT	NT	**HT**	NT	**HT**	NT	NT
TX30-01-P	X900025	1a	60,000	NT	**HT**	**HT**	**HT**	NT	**HT**	**HT**	**HT**
TX40-02-P	X900017	1b	64,000	**HT**	NT	**MT**	**HT**	NT	NT	NT	**HT**
TX40-04-P	X900001	1a	68,000	NT	**HT**	**HT**	**HT**	NT	**HT**	**MT**	NT
TX40-05-P	X900014	1b	68,000	**HT**	NT	**MT**	**HT**	NT	NT	NT	NT
TX60-03-P	X900006	1a	100,000	NT	**HT**	**HT**	**HT**	NT	**HT**	**HT**	**HT**
TX30-05-P	X900002	1b	120,000	**MT**	**HT**	**HT**	**HT**	NT	**HT**	**HT**	**HT**
TX30-03-P	X900007	1a	150,000	NT	**HT**	**HT**	**HT**	NT	**HT**	**HT**	**HT**
TX50-04-P	X900011	1a	240,000	NT	**HT**	**HT**	**MT**	NT	**HT**	**HT**	NT
TX50-05-P	X900030	1a	410,000	**HT**	**HT**	**HT**	**HT**	NT	**HT**	**MT**	**HT**
TX30-02-P	X900018	1a	740,000	**HT**	**HT**	**HT**	**HT**	NT	**HT**	**HT**	**HT**
TX50-01-P	X900008	1a	770,000	**HT**	**HT**	**HT**	**HT**	NT	**HT**	**HT**	**HT**
TX50-02-P	X900019	1a	1,200,000	**HT**	**HT**	**HT**	**HT**	NT	**HT**	**HT**	**HT**
Not Toxic				58% (15 of 26)	42% (11 of 26)	35% (9 of 26)	35% (9 of 26)	100% (26 of 26)	35% (9 of 26)	58% (15 of 26)	58% (15 of 26)
Moderately Toxic				19% (5 of 26)	8% (2 of 26)	19% (5 of 26)	8% (2 of 26)	0% (0 of 26)	8% (2 of 26)	8% (2 of 26)	4% (1 of 26)
Highly Toxic				23% (6 of 26)	50% (13 of 26)	46% (12 of 26)	58% (15 of 26)	0% (0 of 26)	58% (15 of 26)	35% (9 of 26)	38% (10 of 26)
Toxic (Moderately and Highly Toxic)				42% (11 of 26)	58% (15 of 26)	65% (17 of 26)	65% (17 of 26)	0% (0 of 26)	65% (17 of 26)	42% (11 of 26)	42% (11 of 26)

Table C4–2. Summary of the toxicity of sediment samples from the Anniston PCB Site to the amphipod *Chironomus dilutus*.—Continued

[ID, identifier; PCB, polychlorinated biphenyls; µg/kg, micrograms per kilogram; NT, not toxic (within the reference envelope); MT, moderately toxic (within 10 percent of the reference envelope); HT, highly toxic (> 10 percent below the reference envelope); mg, milligram; NA, not applicable; %, percent; The percentage and number of samples classified as not toxic, moderately toxic, and highly toxic for a given endpoint are shown at the bottom of the table. The percentage and number of endpoints included in the table classified as not toxic, moderately toxic, and highly toxic for a given sample are shown at the right of the table; * Bolded results represent sediment samples classified as toxic.]

Station ID	Sample ID	Cycle	ΣPCB (µg/kg)[1]	Number of eggs per case	Percent hatched	Total number of young produced	Average number of young produced per replicate	Not toxic	Moderately toxic	Highly toxic	Toxic (moderately and highly toxic)
TXR1-04-P	X900022	1b	< 32	NT	NT	NT	NT	100% (12 of 12)	0% (0 of 12)	0% (0 of 12)	0% (0 of 12)
TXR1-06-P	X900010	1b	< 33	NT	NT	NT	NT	100% (12 of 12)	0% (0 of 12)	0% (0 of 12)	0% (0 of 12)
TXR1-01-P	X900026	1b	< 33	NT	NT	NT	NT	100% (12 of 12)	0% (0 of 12)	0% (0 of 12)	0% (0 of 12)
TXR1-05-P	X900029	1b	< 33	NT	NT	NT	NT	100% (12 of 12)	0% (0 of 12)	0% (0 of 12)	0% (0 of 12)
TXR1-02-P	X900009	1a	62	NT	NT	NT	NT	100% (12 of 12)	0% (0 of 12)	0% (0 of 12)	0% (0 of 12)
TX10-01-P	X900016	1b	91.2	NT	NT	NT	NT	67% (8 of 12)	33% (4 of 12)	0% (0 of 12)	33% (4 of 12)
TX20-01-P	X900028	1b	220	NT	NT	NT	NT	100% (12 of 12)	0% (0 of 12)	0% (0 of 12)	0% (0 of 12)
TX20-03-P	X900024	1b	310	NT	NT	NT	NT	92% (11 of 12)	8% (1 of 12)	0% (0 of 12)	8% (1 of 12)
TX40-03-P	X900015	1b	990	NT	NT	NT	NT	58% (7 of 12)	8% (1 of 12)	33% (4 of 12)	42% (5 of 12)
TX60-02-P	X900020	1b	6,000	NT	NT	NT	NT	75% (9 of 12)	8% (1 of 12)	17% (2 of 12)	25% (3 of 12)
TX60-02-P	X900020	1a	8,800	NT	NT	NT	NT	42% (5 of 12)	25% (3 of 12)	33% (4 of 12)	58% (7 of 12)
TX40-01-P	X900027	1b	13,000	NT	NT	NT	NT	67% (8 of 12)	0% (0 of 12)	33% (4 of 12)	33% (4 of 12)
TX30-04-P	X900023	1b	15,000	NT	NT	NT	NT	83% (10 of 12)	17% (2 of 12)	0% (0 of 12)	17% (2 of 12)
TX60-04-P	X900013	1b	25,000	NT	NT	NT	NT	75% (9 of 12)	8% (1 of 12)	17% (2 of 12)	25% (3 of 12)
TX30-01-P	X900025	1a	60,000	NT	NT	**HT**	NT	42% (5 of 12)	0% (0 of 12)	58% (7 of 12)	58% (7 of 12)
TX40-02-P	X900017	1b	64,000	NT	NT	NT	NT	67% (8 of 12)	8% (1 of 12)	25% (3 of 12)	33% (4 of 12)
TX40-04-P	X900001	1a	68,000	NT	NT	NT	NT	58% (7 of 12)	8% (1 of 12)	33% (4 of 12)	42% (5 of 12)
TX40-05-P	X900014	1b	68,000	NT	NT	NT	NT	75% (9 of 12)	8% (1 of 12)	17% (2 of 12)	25% (3 of 12)
TX60-03-P	X900006	1a	100,000	NT	NA	NA	**HT**	30% (3 of 10)	0% (0 of 10)	70% (7 of 10)	70% (7 of 10)
TX30-05-P	X900002	1b	120,000	NT	NT	**HT**	**HT**	25% (3 of 12)	8% (1 of 12)	67% (8 of 12)	75% (9 of 12)
TX30-03-P	X900007	1a	150,000	NT	NT	NT	NT	50% (6 of 12)	0% (0 of 12)	50% (6 of 12)	50% (6 of 12)
TX50-04-P	X900011	1a	240,000	NT	NT	NT	NT	58% (7 of 12)	8% (1 of 12)	33% (4 of 12)	42% (5 of 12)
TX50-05-P	X900030	1a	410,000	NT	NT	NT	NT	42% (5 of 12)	8% (1 of 12)	50% (6 of 12)	58% (7 of 12)
TX30-02-P	X900018	1a	740,000	NA	NA	NA	**HT**	11% (1 of 9)	0% (0 of 9)	89% (8 of 9)	89% (8 of 9)
TX50-01-P	X900008	1a	770,000	NT	NT	**HT**	NT	33% (4 of 12)	0% (0 of 12)	67% (8 of 12)	67% (8 of 12)
TX50-02-P	X900019	1a	1,200,000	NA	NA	NA	**HT**	11% (1 of 9)	0% (0 of 9)	89% (8 of 9)	89% (8 of 9)
Not Toxic				100% (24 of 24)	100% (24 of 24)	87% (20 of 23)	85% (22 of 26)				
Moderately Toxic				0% (0 of 24)	0% (0 of 24)	0% (0 of 23)	0% (0 of 26)				
Highly Toxic				0% (0 of 24)	0% (0 of 24)	13% (3 of 23)	15% (4 of 26)				
Toxic (Moderately and Highly Toxic)				0% (0 of 24)	0% (0 of 24)	13% (3 of 23)	15% (4 of 26)				

[1]ΣPCBs are calculated as the sum of each of the 10 homolog groups

Test Acceptability and Relative Control Response between Cycle 1a and Cycle 1b Testing

All of the established test acceptability criteria (appendix 3, tables A3–1, A3–4, and A3–5), including control survival, for *H. azteca* and *C. dilutus* in intralaboratory testing were met. Similarly, test acceptability criteria in interlaboratory testing also were met (appendix 5. Acute sodium chloride reference toxicity testing with *H. azteca* and *C. dilutus* generated results similar to historic median lethal effect concentrations (LC_{50}s) for these species at USACE–Vicksburg and USGS–Columbia.

In *H. azteca* testing, intralaboratory variability in control survival at USACE–Vicksburg between cycle 1a and cycle 1b was low (appendix 3, tables A3–20 and A3–22). Day 42 control survival was 92.5 percent in cycle 1a and 93.8 percent in cycle 1b. There was relatively more intralaboratory variability in control day 42 mean individual dry weight between cycle 1a (0.42 mg/individual) and cycle 1b (0.28 mg/individual). When survival and weight were considered together as biomass, this difference between cycle 1a and cycle 1b was only reduced slightly: 3.83 mg in cycle 1a and 2.62 mg in cycle 1b. In addition to the *H. azteca* in the controls in cycle 1a being somewhat larger than those from cycle 1b, the *H. azteca* in cycle 1a also exhibited higher reproduction. Mean day 42 young per female was 4.2 in cycle 1a and 2.1 in cycle 1b. Because control survival was similar between cycle 1a and cycle 1b, normalizing day 42 reproduction to survival did not reduce intralaboratory variability between cycle 1a and cycle 1b (3.8 and 1.9 young per female, respectively).

The reason behind the differences in size and reproduction between the *H. azteca* in the control sediment at the end of testing in cycle 1a and cycle 1b is unknown. Starting mean individual lengths (cycle 1a = 2.14 millimeters (mm)/individual; cycle 1b = 2.00 mm/individual) were similar, although the cycle 1a organisms were slightly larger at the start of the tests. At day 28 the control organisms from cycle 1a were larger in terms of length (cycle 1a = 4.06 mm/individual; cycle 1b = 3.77 mm/individual), but not individual dry weight (cycle 1a = 0.23 mg/individual; cycle 1b = 0.24 mg/individual) or biomass (cycle 1a = 2.21 mg; cycle 1b = 2.33 mg).

Intralaboratory variability in mean control survival at USGS–Columbia between cycle 1a (93.8 percent) and cycle 1b (95.8 percent) in *C. dilutus* was low as was the intralaboratory variability in individual ash-free dry weight (cycle 1a = 0.93 mg/individual; cycle 1b = 0.96 mg/individual), day 13 total ash-free biomass (cycle 1a = 10.45 mg; cycle 1b = 11.03 mg), and adult biomass (cycle 1a = 8.06 mg; cycle 1b = 7.47 mg; appendix 3, tables A3–23 and A3–26). Mean percent emergence also was similar in the controls from cycle 1a (77.1 percent) and cycle 1b (67.7 percent). Reproductive endpoints, that are inherently more variable than survival, weight, and biomass, also were similar between the controls of cycle 1a and cycle 1b with slightly more reproductive output in cycle 1b (number of egg cases produced: cycle 1a = 4.1 and cycle 1b = 4.4; number of eggs per egg case: cycle 1a = 853.6 and cycle 1b = 1,031.2; percent hatch: cycle 1a = 90.9 percent and cycle 1b = 89.6 percent; average young produced per replicate: cycle 1a = 769.5 and cycle 1b = 927.8).

Sediment 20 was tested in cycle 1a and retested in cycle 1b with *C. dilutus* by USGS–Columbia to determine repeatability of effects observed across storage time between the start of cycle 1a and the start of cycle 1b. Percent survival was high in both cycles of testing (cycle 1a = 85.4 percent; cycle 1b = 97.9 percent) and mean total biomass at day 13 was similar in both cycles (cycle 1a = 9.61 mg; cycle 1b = 10.12 mg). Percent emergence and adult biomass also were similar between cycles of testing (for example, percent emergence in cycle 1a = 54.2 percent in cycle 1b = 62.5 percent). Whereas the mean number of egg cases produced in cycle 1b (3.8) was greater than that for cycle 1a (1.6), the mean number of eggs produced per egg case was similar (cycle 1a = 982.2; cycle 1b = 992.2) as was hatching percentage (cycle 1a = 98.4 percent; cycle 1b = 93.6 percent). Because of the overall greater number of egg cases produced in cycle 1b, the mean total number of young produced also was higher in cycle 1b (3,461.6, 84.7 percent of the control response) relative to cycle 1a (1,610.8, 52.3 percent of the control response). However, variance also was high in the number of egg cases and total number of young endpoints for the other sediments tested. For example, see the interlaboratory comparison for *C. dilutus* illustrated in appendix 5, fig. A5–15 and fig. A5–16.

In summary, some differences between control responses in cycle 1a and cycle 1b were noted; however, these differences were deemed to be minor, and thus, no strong batch effects were noted between cycle 1a and cycle 1b for intralaboratory testing with *H. azteca* or *C. dilutus*. In addition, it is important to note that all endpoints were normalized to their respective control responses before data analysis and interpretation, further decreasing the minor differences in control responses between cycles of testing (appendix 3, tables A3–29 and A3–30). Overall, other than some inherently more variable *C. dilutus* reproductive endpoints, *C. dilutus* response to sediment 20 was similar between testing of this sediment in cycle 1a and cycle 1b indicating storage time between testing cycles likely had little effect on *C. dilutus* response to this sediment.

Toxicity Summaries

Complete summaries of *H. azteca* toxicity testing results are provided in appendix 3, tables A3–15 through A3–22. Complete summaries of *C. dilutus* toxicity testing results are provided in appendix 3, table A3–23 through table A3–26. Cycle 1a sediments were chosen to represent sediments with high to moderate PCB concentrations. Cycle 1b sediments were chosen to represent sediments with moderate to low PCB concentrations (table C2–1). Therefore, as expected, mean responses of *H. azteca* and *C. dilutus* generally varied more widely in

cycle 1a testing than in cycle 1b testing. Below are summaries of the range of mean *H. azteca* and *C. dilutus* responses for each cycle of testing (excluding control responses) as well as a description of the responses of each organism in the reference sediment in comparison to the control.

In some instances *H. azteca* response data were excluded or estimated because of absence of endpoint information. Specifically, data for reproduction of *H. azteca* were excluded where young were recovered but no females were present at day 42 (appendix 3, table A3–29). Because of no survival at day 28 in sediment 18 replicate beakers that were destructively sampled for measuring day 28 weight, day 28 biomass of *H. azteca* was estimated based on the ratio of day 42 biomass to day 28 control biomass. Where the number of *H. azteca* used to determine number of males and females on day 42 in a replicate beaker was less than the number of *H. azteca* recovered from that replicate beaker on day 42, the number of females in that replicate beaker was estimated by multiplying the percent females in that replicate by the total number of organisms recovered in that replicate.

Hyalella azteca—Cycle 1a (appendix 3, table A3–22)

In *H. azteca* testing in cycle 1a by USACE–Vicksburg, mean day 28 survival ranged from 5.0 percent (sediment 19) to 93.3 percent (sediment 09), and mean day 42 survival ranged from 5.0 percent (sediment 18) to 98.8 percent (sediment 09). Mean day 28 biomass ranged from 0.09 mg (sediment 19) to 2.30 (sediment 09). Mean day 42 dry weight ranged from 0.30 mg/individual (sediment 01) to 1.32 mg/individual (sediment 18), and mean day 42 biomass ranged from 0.35 mg (sediment 19) to 3.42 mg (sediment 25). The phenomenon of low survival but high dry weight (for example, sediment 18) can be explained by increased food availability to relatively few surviving organisms. This is an example of why replicate total biomass, an endpoint that integrates survival and weight responses, can sometimes provide a better measure of toxic responses in a treatment. Reproduction (mean day 42 young per female) varied from 0.0 (sediment 19) to 4.6 (sediment 09). In order to account for combined effects on reproduction and survival, the 42-d reproduction data (young per female) were normalized to 42-d survival. Reproduction normalized to day 42 survival might be decreased by a decrease in reproduction, by a decrease in survival, or by a combined decrease in reproduction and survival. When this reproductive measure was normalized to day 42 survival, responses varied from 0.0 (sediments 08 and 19) to 4.5 (sediment 09).

Hyalella azteca—Cycle 1b (appendix 3, table A3–22)

In *H. azteca* testing in cycle 1b by USACE–Vicksburg, mean day 28 survival ranged from 33.3 percent (sediment 02) to 94.2 percent sediment (10), and mean day 42 survival ranged from 27.5 percent (sediment 02) to 91.3 percent (sediment 10). Mean day 28 biomass ranged from 0.87 mg (sediment 02) to 2.71 (sediment 10). Mean day 42 dry weight ranged from 0.25 mg/individual (sediment 13) to 0.50 mg/individual (sediment 02), and mean day 42 biomass ranged from 1.25 mg (sediment 02) to 2.91 mg (sediment 10). Reproduction (mean day 42 young per female) varied from 0.4 (sediment 16) to 4.0 (sediment 04). When this reproductive measure was normalized to day 42 survival, responses varied from 0.3 (sediments 13 and 16) to 3.6 (sediments 04 and 10).

Hyalella azteca—Responses in Reference Sediments in Comparison to the Control (appendix 3, table A3–22)

In samples evaluated by USACE–Vicksburg, *H. azteca* day 28 survival in the reference sediments ranged from 88.8 percent (sediment 22) to 97.4 percent (sediment 10) of the control response, and day 42 survival ranged from 84.0 percent (sediment 22) to 106.8 percent (sediment 09) of the control response. Day 28 biomass ranged from 86.6 percent (sediment 26) to 116.2 percent (sediment 10) of the control response, and day 28 dry weight ranged from 86.7 percent (sediment 29) to 125.4 percent (sediment 09) of the control response. Day 42 biomass and weight ranged from 82.3 percent (sediment 22) to 110.8 percent (sediment 10) and 80.4 percent (sediment 09) to 113.3 percent (sediment 29) of the control response, respectively. Total number of young produced ranged from 64.3 percent (sediment 22) to 134.5 percent (sediment 10), number of young per female ranged from 110.9 percent (sediment 09) to 189.8 percent (sediment 10), and survival normalized young per female ranged from 99.7 percent (sediment 26) to 189.3 percent (sediment 10) of the control response.

Chironomus dilutus—Cycle 1a (appendix 3, table A3–26)

In *C. dilutus* testing in cycle 1a by USGS–Columbia, mean day 13 survival ranged from 20.8 percent (sediment 18) to 100.0 percent (sediment 06). Mean day 13 biomass ranged from 0.45 mg (sediment 18) to 11.99 mg (sediment 09), and mean weight ranged from 0.20 mg/individual (sediment 18) to 1.02 mg/individual (sediment 09). Mean adult biomass ranged from 0.0744 mg (sediment 18) to 8.55 mg (sediment 09). Mean percent emergence ranged from 3.1 percent (sediments 18 and 19) to 69.8 percent (sediment 09). Mean number of egg cases ranged from 0.0 (sediments 18 and 19) to 2.4 (sediment 11); mean number of eggs per egg case ranged from 810.2 (sediment 1) to 1,227.5 (sediment 30); and mean percent hatch ranged from 0.0 percent (sediment 06) to 98.4 percent (sediment 20). Mean total number of young produced ranged from 0.0 (sediments 06, 18, and 19) to 2,076.6 (sediment 30), and mean average number of young produced per replicate ranged from 0.0 (sediments 06, 18, and 19) to 988.6 (sediment 30).

Chironomus dilutus—Cycle 1b (appendix 3, table A3–26)

In *C. dilutus* testing in cycle 1b by USGS–Columbia, mean day 13 survival ranged from 64.6 percent (sediment 14) to 100.0 percent (sediments 10 and 28). Mean day 13 biomass ranged from 8.01 mg (sediment 27) to 15.41 mg (sediment 28), and mean weight ranged from 0.72 mg/individual (sediment 27) to 1.48 mg/individual (sediment 14). Mean adult biomass ranged from 2.52 mg (sediment 02) to 10.7 mg (sediment 04). Mean percent emergence ranged from 24.0 percent (sediment 02) to 79.2 percent (sediment 26). Mean number of egg cases ranged from 0.8 (sediment 02) to 4.8 (sediment 26); mean number of eggs per egg case ranged from 808.8 (sediment 10) to 1,291.9 (sediment 28); and mean percent hatch ranged from 66.0 percent (sediment 10) to 98.2 percent (sediment 13). Mean total number of young produced ranged from 615.1 (sediment 02) to 4,609.8 (sediment 04), and mean average number of young per replicate ranged from 396.1 (sediment 02) to 1,103.6 (sediment 28).

Chironomus dilutus—Responses in Reference Sediments in Comparison to the Control (appendix 3, table A3–26)

In samples evaluated by USGS–Columbia, *C. dilutus* day 13 survival in the reference sediments ranged from 97.8 percent (sediment 22) to 104.4 percent (sediment 09) of the control response. Day 13 biomass ranged from 101.2 percent (sediment 22) to 131.7 percent of the control response (sediment 10), and adult biomass ranged from 99.5 percent (sediment 09) to 136 percent (sediment 04) of the control response. Day 13 weight ranged from 104.1 percent (sediment 22) to 125.8 percent (sediment 10) of the control response. Adult emergence ranged from 89.2 percent (sediment 10) to 116.9 percent (sediment 26) of the control response. Number of egg cases ranged from 51.5 percent (sediment 09) to 108.6 percent (sediment 26), number of eggs per egg case ranged from 78.4 percent (sediment 10) to 96.2 percent (sediment 09), and percent hatch ranged from 73.6 percent (sediment 10) to 101.8 percent (sediment 26) of the control response. Total number of young produced ranged from 41.4 percent (sediment 10) to 107.8 percent (sediment 26), and average number of young produced per replicate ranged from 49.0 percent (sediment 10) to 97.3 percent of the control response (sediment 09).

Relative Endpoint Responsiveness

One of our data interpretation goals was to assess whether additional useful information was gained from the use of extended life cycle *H. azteca* and *C. dilutus* whole-sediment toxicity tests that included reproduction endpoints. Pairs of intra and interspecies endpoints in the intralaboratory toxicity tests were plotted against each other after expressed as a percentage of the control response (appendix 3, figs. A3–1 to A3–25). A line of unity was added representing equal response between the two endpoints and this line was bracketed with blue lines representing a 20-percent difference above or below the line of unity as a visualization tool to aid in the comparison between endpoints. Data points above this line of unity indicate that the endpoint on the x-axis was more responsive; whereas, data points below this line of unity indicate that the endpoint on the y-axis was more responsive. Vertical and horizontal lines on these graphs represent the lower distribution of the threshold reference-envelope response for the variable on the x and y axes. Open symbols represent reference sediments and closed symbols represent test sediments in these graphs.

Hyalella azteca Responsiveness

In order to assess if duration of exposure affected the responsiveness of the *H. azteca* survival endpoint, percent survival on day 28, 35, and 42 was compared in appendix 3, figs. A3–1, A3–5, and A3–7. Because in each of these comparisons data points cluster tightly around the line of unity and were typically within 20 percent of the line of unity, there is no apparent difference in sensitivity of the survival endpoint at day 28, 35, and 42. Using the reference-envelope approach described above, the percentage of sediments identified as toxic at day 28, 35, and 42 were 52, 44, and 48 percent, respectively (table C4–1). Thus, extending the exposure duration from 28 to 42 days did not yield increased responsiveness of the survival endpoint for *H. azteca*.

Day 28 *H. azteca* survival was more responsive than day 28 dry weight (appendix 3, fig. A3–1*C*), but slightly less responsive than day 28 biomass (appendix 3, fig. A3–1*D*). Day 28 weight and biomass classified 28 percent and 72 percent of sediments as toxic, respectively, in comparison to the 52 percent classified as toxic by day 28 survival (table C4–1). Day 42 *H. azteca* survival was more responsive than day 42 dry weight (appendix 3, fig. A3–7*I*), but day 42 survival was similar in responsiveness to day 42 biomass (appendix 3, fig. A3–7*J*). Day 42 survival classified 48 percent of sediments as toxic, and day 42 biomass classified 44 percent as toxic (table C4–1). Day 42 dry weight classified only 8 percent as toxic.

Next, a comparison was made between day 42 *H. azteca* reproductive endpoints. Relative responsiveness was as follows: (least responsive) total number of young produced (56 percent of sediments classified as toxic) less than number of young per female (63 percent of sediments classified as toxic) less than survival normalized young per female (67 percent of sediments classified as toxic) (most responsive) (appendix 3, figs. A3–13*K* and A3–13*L*). Survival normalized young per female was calculated as the sum total of young produced on day 35 and 42 divided by the number of surviving females at day 42 (appendix 3, table A3–29). This value is then multiplied by percent survival at day 42 for the estimate of reproduction normalized to survival. The most sensitive day 42 reproductive endpoint, survival normalized young per female, was then compared to the most sensitive day 28 sublethal endpoint, day 28 biomass (appendix 3, fig. A3–4*M*). Day 28

biomass (classified 72 percent of sediments as toxic) was comparable in sensitivity yet slightly more sensitive than survival normalized young per female (classified 67 percent of sediments as toxic; table C4–1). However, a stronger gradient in the most toxic samples was observed for the survival normalized young per female (0 to 40 percent of control) compared to day 28 biomass for these same samples (25 to 90 percent of control; appendix 3, fig. A3–13*D*); therefore, survival normalized young per female was deemed to be the most responsive *H. azteca* endpoint.

Chironomus dilutus Responsiveness

Of the three day 13 *C. dilutus* endpoints, survival, weight, and biomass, day 13 biomass was the most responsive (table C4–2*;* appendix 3, figs. A3–16*A* and A3–16*B*). The day 13 biomass endpoint classified 65 percent of sediments as toxic; whereas, day 13 survival and weight classified 42 percent and 58 percent of sediments as toxic, respectively (table C4–2). The most responsive of all of the emergence and reproductive endpoints was percent adult emergence and adult biomass (appendix 3, figs. A3–17 and A3–25). Percent adult emergence and adult biomass (classified 65 percent of sediments as toxic) were more sensitive than number of egg cases (42 percent of sediments classified as toxic), total number of young produced (13 percent of sediments classified as toxic), and average number of young produced per replicate (15 percent of sediments classified as toxic; table C4–2). Number of eggs per case, percent of eggs hatched, and median emergence time of adults all did not classify any samples as toxic. The most sensitive of the day 13 endpoints, day 13 biomass, was then compared with the most responsive of the endpoints from the adult emergence and reproductive phase of testing. Day 13 biomass and percent adult emergence endpoints were comparable in responsiveness, both classifying 65 percent of sediments as toxic. However, percent adult emergence or adult biomass could be considered slightly more responsive than day 13 biomass as the percent adult emergence or adult biomass classified a higher percentage of sediments as highly toxic (58 percent) compared to day 13 biomass (46 percent; table C4–2). Moreover, a higher percentage of samples were below the line of unity for percent adult emergence or adult biomass compared to day 13 biomass, indicating that emergence or adult biomass was more responsive than day 13 biomass (appendix 3, figs. A3–16*D* and A3–16*L*). Therefore, additional information was gained by extending the *C. dilutus* test past 13 days to assess adult emergence, reproductive endpoints, and adult biomass.

Interspecies Responsiveness

A comparison of the most responsive *H. azteca* endpoint, survival normalized young per female, and one of the most responsive *C. dilutus* endpoints, adult biomass, (fig. C4–1, tables C4–1 and C4–2) reveals that *H. azteca* survival-normalized young per female is the most responsive endpoint measured in the current study (*H. azteca* survival normalized young per female classified 67 percent of sediments as toxic and *C. dilutus* adult biomass classified 65 percent of sediments as toxic).

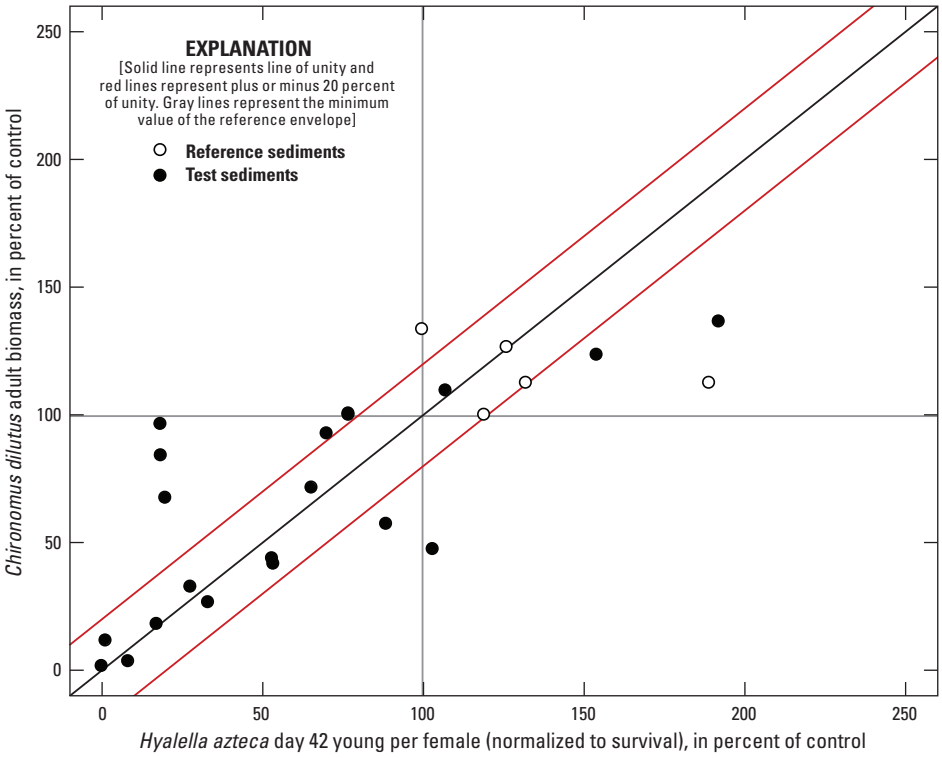

Figure C4–1. Relative endpoint sensitivity of the most responsive *Hyalella azteca* endpoint, survival-normalized young per female, and most responsive *Chironomus dilutus* endpoint, adult biomass.

Summary

The whole-sediment toxicity tests done with *H. azteca* and *C. dilutus* met all of U.S. Environmental Protection Agency (2000) and American Society for Testing and Materials International (2012) test acceptability criteria, and intra-laboratory control responses between the two cycles were similar. The results of this study also demonstrated that certain refinements to the toxicity testing methods (such as improving nutrition of test organisms and starting the long-term midge test with 7-d old larvae) can enhance the likelihood of running a successful test and reduce variability in the results. Mean responses of *H. azteca* and *C. dilutus* generally varied more widely in cycle 1a than in cycle 1b, as cycle 1a sediments were chosen to represent relatively higher PCB concentrations. An analysis of endpoint responsiveness demonstrated that the most responsive *H. azteca* endpoints were day 28 biomass and day 42 survival normalized young per female. The most responsive *C. dilutus* endpoints were day 13 biomass, percent adult emergence, and adult biomass. Overall, between the two species, the most responsive endpoint assessed for these two species was *H. azteca* survival-normalized young per female (67 percent of the samples classified as toxic). Chapter 5 of this report discusses endpoint response in relation to sediment chemistry. The toxicity designations that were assigned to each of the sediment samples that were included in the project database are listed in table C4–1 and table C4–2.

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Evaluation of Relations Between Sediment Toxicity and Sediment Chemistry at the Anniston PCB Site

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Chapter 5 of

Evaluation of Toxicity to the Amphipod, *Hyalella azteca*, and to the Midge, *Chironomus dilutus*; and Bioaccumulation by the Oligochaete, *Lumbriculus variegatus*, with Exposure to PCB-Contaminated Sediments from Anniston, Alabama

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Evaluation of Relations Between Sediment Toxicity and Sediment Chemistry at the Anniston PCB Site

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Abstract

The Anniston PCB Site is located in northeastern Alabama. Environmental concerns focus primarily on PCBs released from 1935 to 1971 from production waste, spills, effluent discharges, releases from landfills, and stormwater runoff. The U.S. Environmental Protection Agency, Solutia/Pharmacia, and Department of Interior are evaluating risks to ecological receptors associated with exposure to PCBs and other chemicals of potential concern (COPCs) in environmental media at the site. As part of this investigation, a total of 32 sediment samples were collected from the study area by ARCADIS (under contract to Solutia/Pharmacia) to support chemical characterization and whole-sediment toxicity and bioaccumulation testing needed to support a Comprehensive Environmental Response, Compensation, and Liability Act Remedial Investigation/Feasibility Study. Of these 32 samples, 26 samples were selected for toxicity testing based on concentrations of total PCBs and total organic carbon (TOC; that is, to provide a broad gradient of total PCB concentrations and relatively consistent levels of TOC in sediments tested).Two toxicity tests were performed including life-cycle whole-sediment toxicity tests with the midge, *Chironomus dilutus* (endpoints: survival, weight, biomass, emergence, and reproduction) and a 42-day (d) whole-sediment toxicity tests with the amphipod, *Hyalella azteca* (endpoints: survival, weight, biomass, and reproduction). Matching sediment chemistry and sediment toxicity data were used to develop site-specific concentration-response models and toxicity thresholds for PCBs, other COPCs, or various COPC mixtures using empirically based sediment quality guidelines (SQGs; for example, probable effect concentrations; PECs) and mechanistically-based SQGs (for example, equilibrium partitioning). The reliability of site-specific toxicity thresholds for PCBs and other COPCs were then evaluated to identify the most reliable basis for assessing risks to sediment-dwelling organisms at the site. The results of this study indicate that PCBs are the primary COPCs affecting benthic invertebrates at this site and that the toxicity threshold low risk (TT_{LR}) for total PCBs was 499 micrograms per kilogram (µg/kg) dry weight for amphipod reproduction and 1,140 µg/kg for midge adult biomass. The reliability of these toxicity thresholds for PCBs was high, with correct classification rates ranging from 85 to 92 percent.

Introduction

Evaluations of sediment quality conditions at the Anniston PCB Site historically have been performed primarily using sediment chemistry data. Such data frequently are interpreted using sediment quality guidelines (SQGs) that were derived to support regional or national assessments. However, the results of studies performed at other sites (for example, Indiana Harbor, Indiana; Calcasieu Estuary, Louisiana) indicate that generic SQGs can overestimate or underestimate toxicity to sediment-dwelling organisms (MacDonald and others, 2002a, 2002b, 2003; Ingersoll and others, 2001, 2009; Wang and others, 2013). Hence, the site-specific calibration of such SQGs has been identified as one of the key steps in the overall sediment quality assessment process (Ingersoll and others, 2005; Douglas and others, 2005; Word and others, 2005).

To support the development of site-specific SQGs [termed site-specific sediment toxicity thresholds (TTs) in this study] for the Anniston PCB Site, matching sediment chemistry and sediment toxicity data were generated using sediment samples collected during the Phase 2 Sediment Sampling Program (chapter 2). More specifically, large-volume sediment samples [about 30 liters (L) of sediment, sieved to less than 2 millimeters (mm)] were collected at a total of 32 locations in Operable Unit-4 (OU-4; Choccolocco

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Creek; table C2–1), as identified in the Field Sampling Plan that was developed by ARCADIS (2010). Of these sediment samples, six were collected at one station (fig. C1–1) and are considered to reflect reference conditions (as defined by chemical selection criteria and biological selection criteria described in chapter 4 and in U.S. Environmental Protection Agency, 2000 and American Society for Testing and Materials International, 2012 and presented in appendix 4, tables A4–1 and A4–2). Based on the results of chemical analysis of these samples (that is, measurements of aroclor and total organic carbon (TOC) in the sediment; appendix 1, table A1–3a), 26 of these samples were selected by U.S. Environmental Protection Agency (USEPA) and ARCADIS for further chemical characterization and sediment toxicity testing (table C2–1). Detailed characterization of these samples included analyses of total metals, simultaneously extracted metals (SEM), acid volatile sulfides (AVS), polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), organochlorine pesticides, semivolatile compounds, TOC, and grain size in whole sediment and dissolved metals, major ions and conventional variables in pore water (chapter 2). The concentrations of PCBs in pore water also were estimated based on the results of analysis of solid-phase microextraction fibers (chapter 3) in each sediment used to perform each of the toxicity tests (chapter 4). Because PCBs are known to affect reproduction in many vertebrate organisms (for example, Eisler, 1996), long-term toxicity tests were performed with the midge *Chironomus dilutus* [48- to 54-day (d) exposures] and with the amphipod *Hyalella azteca* (42-d exposures) to determine if PCBs affect the survival, weight, or reproduction in these invertebrates (chapter 4). A reference-envelope approach was used to designate sediment samples from the Anniston PCB Site as toxic or not toxic (Besser and others 2009; Hunt and others, 2001; Kemble and others, 2013; MacDonald and others, 2010, 2012; Wang and others 2013; chapter 4).

This chapter describes the relations between sediment chemistry and sediment toxicity for pairs of selected chemicals of potential concern (COPCs) or COPC mixtures and selected sediment toxicity test endpoints (for example, total PCBs and 10-d midge survival). In addition, the TTs that were derived for selected COPCs or COPC mixtures, using the concentration-response models (CRMs), are presented. Because adult biomass and percent adult emergence of *C. dilutus* and reproduction of *H. azteca* were among the most sensitive and responsive toxicity test endpoints, these endpoints were selected for detailed CRM and TT derivation (chapter 4). The CRMs and TTs for other, more commonly reported endpoints are presented for comparison (for example day 13 biomass of *C. dilutus* and day 28 biomass of *H. azteca*). Finally, the results of the evaluations of TT reliability are presented in this chapter.

Methods

This study was performed to evaluate and interpret the matching sediment toxicity and sediment chemistry data that were collected during the Phase 2 Sediment Sampling Program of the Anniston PCB Site (chapters 2 and 4). A step-wise process was used to evaluate relations between sediment chemistry and sediment toxicity in the Anniston PCB Site sediments, to develop and evaluate the reliability of the site-specific sediment TTs. This process consisted of six main steps:

1. Identification of the COPCs and COPC mixtures that were unlikely to cause or substantially contribute to sediment toxicity;
2. Identification of the COPCs or COPC mixtures that were significantly correlated with the toxicity test endpoints [based on results of Spearman's rank correlation analyses (Zar, 1999), Spearman's rank correlation coefficient (r_s) greater than 0.4; p less than 0.005];
3. Development of CRMs for selected COPCs/COPC mixtures and toxicity test endpoint pairs;
4. Derivation of site-specific sediment TTs for selected COPCs/COPC mixtures and toxicity test endpoint pairs;
5. Evaluation of the reliability of the site-specific sediment TTs for selected COPCs/COPC mixtures and toxicity test endpoint pairs; and,
6. Evaluation of the comparability of the site-specific sediment TTs for selected COPCs or COPC mixtures.

Identification of Priority Chemicals of Potential Concern or Priority Chemical of Potential Concern Mixtures

Matching sediment chemistry and sediment toxicity data were obtained for 26 sediment samples collected in the vicinity of the Anniston PCB Site. See chapter 2 and ARCADIS (2010) for more information on the rationale for selecting these sampling locations. For each of these sediment samples, the concentrations of more than 70 COPCs were measured (appendix 1, table A1–3a). In addition, information on 22 toxicity endpoints is available to evaluate the toxicity of these sediment samples to *H. azteca* or *C. dilutus* (chapter 4 and appendix 3, table A3–28). Two analyses of the data were performed to help focus the development of CRMs for individual COPCs/COPC mixtures and toxicity test endpoint combinations that would be most relevant for TT derivation.

As a first step in the analysis, a screening-level evaluation was performed to identify the COPCs and COPC mixtures that were unlikely to cause or substantially contribute to sediment toxicity. More specifically, the concentrations of each analyte that were measured in sediment samples from the site were compared to toxicity screening values (TSVs) summarized in table C5–1 (at the back of the chapter), for whole sediment and in table C5–2 (at the back of the chapter) for pore water. For whole-sediment chemistry, the TSVs were based on threshold effect concentrations (TECs; MacDonald and others, 2000a; or analogous TEC-type values summarized in table C5–1). For pore-water chemistry, the TSVs were based on State water quality standards (WQSs; Alabama Department of Environmental Management, 2011; table C5–2). The COPCs with concentrations below the TECs in any of the whole-sediment samples or below the State WQSs in any of the pore-water samples were considered to pose a low risk to benthic invertebrates and other aquatic organisms. Such COPCs were not considered in the CRM development process. In addition, the COPC concentrations were estimated to be zero when results were reported as less than the detection limit and; samples that were nondetects with detection limits above the TSV were not evaluated. Those analytes for which no TT existed were identified as uncertain COPCs. These COPCs were carried forward into the subsequent steps of the CRM development process.

In the second step of the analysis, potential relations between the concentrations of COPCs and the responses of toxicity test organisms were identified by performing Spearman's rank correlation analysis on the toxicity and chemistry data. Any COPCs or COPC mixtures that did not exhibit a significant correlation (r_s less than 0.4; p greater than 0.005) with one or more of the toxicity test endpoints were eliminated from further consideration. In this way, the results of the two step data analyses provided a basis for identifying the COPCs and COPC mixtures that were most likely to be causing or substantially contributing to sediment toxicity.

Development of Quantitative Relations between Sediment Chemistry and Sediment Toxicity

In the third step of the analysis, CRMs were developed for each of the COPCs and COPC mixtures in sediment that were retained following the analyses described above (step 1: screening sediment chemistry relative to TSVs; and, step 2: Spearman's rank correlation analyses between sediment chemistry and sediment toxicity). More specifically, the site-specific sediment chemistry and sediment toxicity data were used to develop CRMs for selected COPCs and COPC mixtures based on the magnitude of toxicity to *H. azteca* (that is, control-adjusted survival, weight, biomass, or reproduction) and *C. dilutus* (that is, control-adjusted 13-d survival, weight, biomass or adult emergence, biomass, or reproduction; chapter 4). The 28-d biomass of *H. azteca* or the 13-d biomass of *C. dilutus* was calculated as the product of the survival and weight endpoints (chapter 4 and appendix 3, table A3–28). The reproduction endpoint for *H. azteca* was calculated as the product of 42-d reproduction (young/female) and 42-d survival. This reproduction endpoint provides an overall estimate of reproductive output of amphipods within each replicate. The biomass of emergent adult *C. dilutus* was calculated as the product of 13-d weight and percent adult emergence. This estimate of biomass of emergent *C. dilutus* assumes that 13-d weight of larvae would be proportional to the weight of resulting adults from that replicate (chapter 4). Development of the CRMs involved plotting the COPC concentration data against the corresponding response data and determining the dependence of the toxicity test response data (dependent variables) on the COPC concentration data (independent variables) as described in MacDonald and others (2002b, 2003, 2005a, 2005b, 2009, 2010, 2012). The CRMs were generally developed using a log-logistic CRM (Seefeldt and others, 1995; MacDonald and others, 2010) using the following equation:

$$f(x) = \frac{a}{1 + \left(\frac{x}{EC_{50}}\right)^{b}} \tag{1}$$

where

a = upper limit of the response (asymptote);
EC_{50} = Estimated median effect concentration; and,
b = Slope at the estimated median effect concentration.

The median effect concentration in the above model provides an estimate of the COPC concentration where a 50-percent effect is predicted to be observed (for example, 50-percent decline in survival relative to the upper limit and the baseline). In some cases, linear regression models fit the data better than the log-logistic models and, hence, were used to describe the relation between concentration and response. The distribution of responses for each of the endpoints was tested for normality before CRM development. All of the relations were described using the R environment for statistical computing and graphics (R Development Core Team, 2013).

Development of Site-Specific Sediment Toxicity Thresholds for Selected Chemicals of Potential Concern or Chemical of Potential Concern Mixtures

In the fourth step of the analysis, site-specific sediment TTs were established for selected COPCs or COPC mixtures and sediment toxicity endpoints, based on the site-specific CRMs derived from sediment chemistry and sediment toxicity data for *H. azteca* and *C. dilutus*. These COPCs and COPC mixtures were selected based on the coefficients of determination (R^2) and associated level of significance ($\alpha = 0.05$) that were calculated for the regression equations that described the relation. Chemicals of potential concern and COPC mixtures were selected for TT derivation if R^2 greater than 0.4

and p less than 0.05. Results at other sites suggests that TTs derived for COPCs or COPC mixtures that exhibited such correlations with endpoints such as survival or biomass of *H. azteca* or *C. dilutus* were likely to be the most reliable TTs (that is, such TTs accurately predict toxicity based on chemical concentration; MacDonald and others, 2002a, 2009; Ingersoll and others, 2001, 2009).

The TTs for sediment were established for selected COPCs and COPC mixtures using the CRMs generated using data on the survival, weight, biomass, emergence, or reproduction of *H. azteca* or *C. dilutus*. Various procedures have been used to derive numerical TTs for benthic invertebrates (for details, see MacDonald and others, 2002b, 2004, 2009; Field and others, 2002; Wenning and others, 2005). In this study, two TTs were calculated for each COPC-endpoint pair, including a low-risk TT (that is, TT_{LR}) and a high-risk TT (that is, TT_{HR}). The TT_{LR} values were calculated by determining the concentrations of COPCs or COPC mixtures that corresponded to the response rates at the lower limit of the reference envelope (Besser and others, 2009; Kemble and others, 2013; MacDonald and others, 2012; Wang and others 2013; chapter 4). By comparison, the TT_{HR} values were calculated by determining the concentrations of COPCs or COPC mixtures that corresponded to the response rates at 10 percent below the lower limit of the reference envelope (MacDonald and others, 2010, 2012). These TTs were estimated using the regression equations that were developed for the corresponding CRMs.

Evaluation of the Site-Specific Toxicity Thresholds for Sediment

In the fifth step in the analysis, the reliability of the TTs was evaluated for selected COPC/COPC mixtures and toxicity test endpoint pairs. The principal objective of this chapter is to establish TTs that can be used to assess risks to benthic invertebrates associated with exposure to contaminated sediments within the Anniston PCB Site. As such, the TTs developed for each of the selected COPCs and COPC mixtures were evaluated to support selection of TTs for assessing risks to benthic invertebrates and potentially, other aquatic receptors throughout the Anniston PCB Site. The goal of this study was to characterize relations between sediment chemistry and sediment toxicity and relations between sediment chemistry and sediment bioaccumulation in samples of sediments collected from the Anniston PCB Site in Alabama (chapter 1). The goal of this study was not to determine the extent of sediment contamination or sediment toxicity across the Anniston PCB Site. Hence, the test sites or samples collected from within a test site were not selected to represent the spatial extent of sediment contamination across the Anniston PCB Site. Additional studies may be required to determine the spatial extent of sediment contamination and sediment toxicity at the Anniston PCB Site (chapter 1).

The evaluation of reliability provides a basis for assessing the ability of the site-specific sediment TTs to correctly classify sediment samples as toxic or not toxic, using the same data that were applied to derive the TT. In the first step of the process, the TTs were used to classify the 26 sediment samples evaluated in the sediment toxicity tests from the Anniston PCB Site into two categories (that is, predicted toxic or predicted not toxic to the test organisms) based on the concentrations of individual COPCs or COPC mixtures in the sediment samples (chapter 4). More specifically, samples with concentrations of the selected COPC or COPC mixture that exceeded the TT were predicted to be toxic; whereas, those with chemical concentrations less than the corresponding TT were predicted to be not toxic. The accuracy of these predictions was then evaluated by determining the proportion of samples within each group of samples (that is, predicted toxic and predicted not toxic) that were actually toxic to the test organisms, based on comparisons of the results of the sediment toxicity tests to the response of test organisms in reference sediments. Specifically, a sediment sample was classified as toxic for an endpoint if the mean response of the test organism for that endpoint was less than the lowest mean response of test organisms in the reference sediments (chapter 4). Toxicity test results were available for five of the six reference sediment samples obtained from Choccolocco Creek upstream from the Solutia/Pharmacia facility. These reference samples were selected by Solutia/Pharmacia (ARCADIS, 2010) because the samples were located proximal to the site and because the sediments at these locations had low concentrations of COPCs and physical characteristics similar to those at the site (table C2–1 and appendix 1, table A1–3a). For the reliability calculation, the frequency of toxicity above and below the TT was determined using data on the toxicity test endpoint and test organism used to derive the TT.

Criteria for evaluating the reliability of the TTs used the procedures that had been established previously for evaluating TTs at sites such as the Calcasieu Estuary in Louisiana; Indiana Harbor in Indiana; the Tri-State Mining District in Missouri, Oklahoma, and Kansas; and the Upper Columbia River in Washington. More specifically, a TT was considered to be reliable if the incidence of toxicity (IOT) was less than 20 percent below the TT, if the IOT was greater than 50 percent above the TT, and if the overall correct classification rate was greater than or equal to 80 percent (MacDonald and others, 2002b, 2003, 2005a, 2005b, 2009, 2010, 2012). The TTs that met these criteria were considered to provide a reliable basis for classifying sediment samples as toxic or not toxic (that is, the overall classification error rate would be no greater than 20 percent). Such TTs also minimize the potential for false negative errors (that is, Type II error rate would be less than 20 percent) and for identifying sediment samples that would be toxic, more likely than not (that is, Type I error rate would be less than 50 percent).

In the sixth and final step in the analysis, the comparability of the TTs was evaluated for selected COPCs or COPC mixtures to (1) sediment quality guidelines (SQGs) based on empirically based approaches, (2) SQGs based on equilibrium partitioning approaches, and (3) results of published spike-sediment toxicity tests with COPCs.

Results and Discussion

The matching sediment chemistry data (chapter 2) and sediment toxicity data (chapter 4) generated in conjunction with the Phase 2 Sediment Sampling Program were reviewed and evaluated to determine their applicability to the TT-derivation process. The results of this evaluation indicated that the toxicity and chemistry data for the 26 site sediment samples generated in 2010 and 2011 met the performance criteria for measurement data that were established for the project [ARCADIS, 2010; that is, performance criteria sediment chemistry (chapter 2)] and performance criteria for toxicity tests (chapter 4; as outlined in American Society for Testing and Materials International, 2012 and U.S. Environmental Protection Agency, 2000). Therefore, all of the sediment chemistry and sediment toxicity data collected during the study were used to generate the site-specific sediment TTs for selected COPCs or COPC mixtures and selected toxicity test endpoints.

Selection of Chemicals of Potential Concern and Chemical of Potential Concern Mixtures for Concentration-Response Model Development

Several analyses were performed to help focus the development of CRMs on the COPC toxicity endpoint combinations that would be most relevant for TTs derivation. First, examination of the sediment chemistry data indicated that 29 substances were not detected in any of the sediment samples from the Anniston PCB Site and, hence, were not considered in the CRM development process (appendix 1, table A1–3a and appendix 4, table A4–3 to table A4–7). Screening of the data for the remainder of the COPCs revealed that various pore-water metals, individual PAH or PAH mixtures, PCB homologs or PCB mixtures, organochlorine pesticides or organochlorine pesticide mixtures, polychlorinated dibenzo-*p*-dioxins/polychlorinated dibenzofurans (PCDDs/PCDFs), and mean probable effect concentration-quotients (PEC-Qs) were observed in one or more samples at concentrations in excess of the selected TSV in whole sediment or in pore water (table C5–1 and table C5–2). See chapters 2 and 4 for a description of how the PEC-Qs, $(\sum\text{SEM-AVS})/f_{OC}$, and $\sum\text{ESB-TU}_{FCV}$, were calculated. In all such calculations, less than detection limit values were treated as 0, unless the detection limit was above the PEC. In those latter cases, the data were not used in the calculations.

Second, the results of Spearman's rank correlation analysis indicated that the following 73 COPCs or COPC mixtures were significantly correlated (r_s greater than 0.4; p less than 0.005; appendix 4, tables A4–3 to table A4–7) with one of the selected toxicity test endpoints:

- 10 metals;
- 17 individual PAH or PAH mixtures;
- 19 PCB homolog groups or PCB mixtures;
- 9 organochlorine pesticides or organochlorine pesticide mixtures;
- 17 PCDD/PCDF congeners; and,
- Mean PEC-Q.

Total organic carbon and grain size also were significantly negatively correlated with many of the toxicity test endpoints for midge and amphipods (appendix 4, tables A4–3 and A4–4). These results indicate that many of the COPCs are associated with the fine fraction of the sediment matrix and that COPCs represented a substantial proportion of the organic carbon (OC) in many sediment samples.

Development of Concentration-Response Models

A total of 73 COPCs or COPC mixtures met the selection criteria and were considered for developing CRMs. This list of COPCs or COPC mixtures was further refined using PEC-type TSVs for whole sediment (table C5–1) or TSVs based on chronic water quality criteria for pore water (table C5–2) to focus CRM development activities. More specifically, based on exceedance of PEC-type TSVs, as many as 24 COPCs or COPC mixtures per toxicity test endpoint and media type were selected for CRM development, including 10 metals (barium, chromium, copper, lead, manganese, mercury, nickel, silver, vanadium, and zinc), 3 PAH mixtures, 17 PCB mixtures, mean PEC-Q for organochlorine pesticides ($\text{PEC-Q}_{OCPESTs}$), and overall mean PEC-Q (based on contributions of metals, PAHs, organochlorine pesticides, and PCBs). A PEC is defined as a concentration of COPC(s) in sediment above which adverse effects on sediment-dwelling organisms are expected to occur more often than not (MacDonald and others, 2000a). Hence, use of PEC-type values to screen COPCs or COPC mixtures for CRM development provides a basis for identifying the COPCs that may be causing or contributing to sediment toxicity (Ingersoll and others, 2001, 2002, 2005).

Data for 22 toxicity test endpoints were examined to select the endpoints that would be targeted for CRM development. Based on the results of the Spearman's rank-correlation analyses (appendix 4, tables A4–3 to table A4–7) and based on the results of analyses presented in chapter 4, it was determined that percent emergence of adult *C. dilutus*, biomass of adult *C. dilutus*, and reproduction of *H. azteca* normalized to

42-d survival represent the most responsive endpoints evaluated in this study (chapter 4). Therefore, percent emergence of adult *C. dilutus*, biomass of adult *C. dilutus*, and 42-d reproduction of *H. azteca* were selected for CRM development. Models also were developed for several more traditional endpoints (for example, day 28 *H. azteca* or day 13 *C. dilutus* biomass) to provide a basis for comparison with the selected models. In advance of CRM development, the distribution of responses for each of the endpoints was tested for normality using the Shapiro-Wilk test (Zar, 1999); all of the data were normally distributed. Therefore, none of the underlying datasets were transformed before CRM development. Overall, CRMs were developed for 69 COPC/COPC mixtures and toxicity test endpoint pairs using the matching whole-sediment chemistry and sediment toxicity data for the Anniston PCB Site (table C5–3, at the back of the chapter). For percent emergence of adult *C. dilutus* and biomass of adult *C. dilutus*, significant relations between COPC whole-sediment concentration and response were observed for all 24 COPCs and COPC mixtures considered, with R^2 ranging from 0.49 to 0.76 (table C5–3). The highest R^2-values were observed for the various measures of PCB concentrations or metal (that is, lead and mercury) concentrations; whereas, the lower R^2-values typically were observed for the PAH-based concentrations (table C5–3). The CRMs generated for 21 COPCs and COPC mixtures based whole-sediment chemistry and on reproduction of *H. azteca* were significant, with R^2-values ranging from 0.52 to 0.72 (table C5–3). Again, the R^2-values observed for measures of whole-sediment total PCB concentration were among the highest for reproduction of *H. azteca* (fig. C5–1 and fig. C5–2, at the back of the chapter), for emergence of adult *C. dilutus* (fig. C5–3 and fig. C5–4, at the back of the chapter), and for biomass of adult *C. dilutus* (fig. C5–5 and fig. C5–6, at the back of the chapter). Example CRMs and TTs for comparison are provided for day 28 biomass of *H. azteca* (fig. C5–7 and fig. C5–8, at the back of the chapter) and for day 13 biomass of *C. dilutus* (fig. C5–9 and fig. C5–10, at the back of the chapter).

In addition, CRMs were developed for 32 COPC/COPC mixtures and toxicity test endpoint pairs using the PCB pore-water chemistry (table C5–4, at the back of the chapter). The pore-water concentrations of PCBs were estimated using solid-phase microextraction (SPME) fibers (chapter 2). For percent emergence of adult *C. dilutus* or biomass of adult *C. dilutus*, significant relations between COPC pore-water concentration and response were observed for 10 to 11 COPCs or COPC mixtures, with CRM R^2 values ranging from 0.54 to 0.73 (table C5–4). The CRMs generated for 11 COPCs or COPC pore-water mixtures based on reproduction of *H. azteca* were significant, with CRM R^2-values ranging from 0.43 to 0.62 (table C5–4). CRMs developed for the pore-water metals that were identified based on Spearman's rank correlation analyses either did not meet the required criteria for TT development or the developed TTs were well below ambient water quality criteria and were not presented.

Derivation of Sediment Toxicity Thresholds for Selected Chemicals of Potential Concern or Chemical of Potential Concern Mixtures

Two TTs were developed for each of the selected COPC/COPC mixtures and toxicity test endpoint pairs. The low-risk TTs were determined by calculating the concentration of each COPC or COPC mixture that corresponded to a lower limit of the control-adjusted response of the toxicity test organisms exposed to reference sediment samples. Therefore, such response rates (that is, those consistent with the reference envelope) are likely to be associated with conditions that would support healthy benthic invertebrate communities at substantially uncontaminated reference sites. The high-risk TTs were derived by calculating the concentrations of COPCs or COPC mixtures that corresponded to greater than a 10 percent increase in the magnitude of toxicity relative to the lower limit of the reference envelope (that is, control-adjusted response; MacDonald and others, 2002b, 2010, 2012). The TT_{LR} and TT_{HR} are considered to provide a basis for identifying the concentrations of COPCs or COPC mixtures that pose low risks (less than TT_{LR}), moderate risks (between the TT_{LR} and TT_{HR}), and high risks (that is, greater than TT_{HR}) to the benthic invertebrate community (MacDonald and others, 2010, 2012). Although the procedures for deriving such TTs have been revised in recent years (MacDonald and others, 2010, 2012), the method used in this investigation is equivalent to the procedures that were described by MacDonald and others (2002b).

In total, TT_{LR}s and TT_{HR}s were derived for 69 COPC/COPC mixtures and toxicity test endpoint pairs using whole-sediment chemistry and sediment toxicity data from the Anniston PCB Site (table C5–3). Specifically, TTs for whole sediment based on reproduction of *H. azteca*, percent emergence of adult *C. dilutus*, or biomass of adult *C. dilutus* were derived for 2 metals, 3 PAH mixtures, 17 PCB homolog groups or mixtures, organochlorine pesticides, and mean PEC-Q (table C5–3).

For pore-water chemistry, TTs were derived for as many as 11 PCB concentration metrics and 3 toxicity test endpoints (table C5–4). The TT_{LR}s and TT_{HR}s that were derived for 32 COPC/COPC mixtures and toxicity test endpoint pairs using the pore-water chemistry data are presented in table C5–4.

Relative Sensitivity of Sediment Toxicity Thresholds for Total Polychlorinated Biphenyls Based on Short-Term Biomass Endpoints Compared to Long-Term Endpoints

The reproduction endpoint for *H. azteca* provided lower TTs compared to the day 28 biomass endpoint for *H. azteca*. Example TT_{LR}s and TT_{HR}s for total PCBs (that is, sum of homologs) based on whole-sediment chemistry are provided for day 28 biomass of *H. azteca* (fig. C5–7 and fig. C5–8). The TTs based on dry weight concentrations of total PCBs

for day 28 biomass of *H. azteca* were a factor of 10 to 24 greater than the comparable TTs for reproduction of *H. azteca* (fig C5–1 and fig. C5–7). The TTs based OC normalized concentrations of total PCBs for day 28 biomass of *H. azteca* were a factor of 3.6 to 8.1 greater than the comparable TTs for reproduction of *H. azteca* (fig. C5–2 and fig. C5–8). These results are consistent with the results of the relative endpoint sensitivity analysis presented in chapter 4.

The emergence or biomass endpoints for adult *C. dilutus* provided lower TTs compared to the day 13 biomass endpoint for *C. dilutus*. Example TT_{LR}s and TT_{HR}s for total PCBs based on whole-sediment chemistry are provided for day 13 biomass of *C. dilutus* (fig. C5–9 and fig. C5–10). The TTs based on dry weight concentrations of total PCBs for day 13 biomass of *C. dilutus* were a factor of 5.0 to 8.0 greater than the comparable TTs for emergence of adult *C. dilutus* (fig. C5–3 and fig. C5–9). The TTs based on dry weight concentrations of total PCBs for day 13 biomass of *C. dilutus* were a factor of 11 to 13 greater than the comparable TTs for biomass of adult *C. dilutus* (fig. C5–5 and fig. C5–9). The TTs based on OC normalized concentrations of total PCBs for day 13 biomass of *C. dilutus* were a factor of 3.3 to 4.6 greater than the comparable TTs for emergence of adult *C. dilutus* (fig. C5–4 and fig. C5–10). The TTs based on OC normalized concentrations of total PCBs for day 13 biomass of *C. dilutus* were a factor of 6.0 to 6.9 greater than the comparable TTs for biomass of adult *C. dilutus* (fig. C5–6 and fig. C5–10). Again, these results are consistent with the results of the relative endpoint sensitivity analysis presented in chapter 4.

Uncertainty Around Variability Observed in the Sediment Toxicity Data

Variability in toxicity results can affect the slope and intercepts for a regression analysis of a dataset. Ultimately, this becomes important as site-specific TTs are identified using CRM (for example, fig. C5–1 to fig. C5–10). Therefore, careful examination of toxicity endpoints is critical when determining relations between concentrations of COPCs in sediments and the results of sediment toxicity tests.

Sediment 16 (table C2–1) was identified for additional analysis because reproductive effects were observed on *H. azteca* with relatively low concentrations of COPCs in the sediment (appendix 3, table A3–22). Day 28 survival of *H. azteca* was slightly lower (84.2 percent) for sediment 16 compared to control and reference sediments. This result was affected by low survival of *H. azteca* in two replicates (survival of 40 and 60 percent; appendix 3, table A3–15). However, reproduction of *H. azteca* in sediment 16 was lower than control and other reference sediments with similar concentrations of COPCs (control-adjusted reproduction normalized to survival was 18.3 percent in sediment 16; appendix 3, table A3–22). In contrast, effects on survival or sublethal endpoints of *C. dilutus* were not observed with exposure to sediment 16 (appendix 3, table A3–26).

Further examination of sediment 16 did not reveal any specific causes for the reproductive response of *H. azteca*. Field collection notes did not reveal any remarkable information. Sediment 16 was composed of sand, silt, and clay, and had an orange color that was not characteristic of other sediments (appendix 1, table A1–3a) that would suggest a rich iron content and oxidized conditions. Metal, OC, AVS, and organic chemicals in sample 16 were all relatively low or not detected and were comparable to reference sediments (appendix 1, table A1–3a). Pore-water chemistry revealed corresponding low concentrations of metals although specific essential ions appeared to be high enough to sustain *H. azteca* (appendix 1, table A1–3b). These results do not identify any specific cause for the observed toxicity of sediment 16 to *H. azteca*. It is possible that another unmeasured stressor in sediment 16 could have affected *H. azteca*; however, it should be noted the same stressor might be present in other Anniston PCB Site sediments where effects were observed, but the toxicity was associated primarily with the concentrations of PCBs or other measured contaminants.

Reliability of the Site-Specific Sediment Toxicity Thresholds

The principal objective of this chapter is to establish site-specific sediment TTs that can be used to assess risks to benthic invertebrates associated with exposure to sediments located within the Anniston PCB Site. As such, the TTs developed for each of the selected COPCs or COPC mixtures were evaluated to support selection of TTs for assessing risks to benthic invertebrates throughout the study area. The evaluation of reliability provides a basis for assessing the ability of the site-specific sediment TTs to correctly classify sediment samples as toxic or not toxic, using the same data that were applied to derive the TT.

Low-risk and high-risk TTs were developed for 24 COPCs or COPC mixtures based on percent emergence of adult *C. dilutus*. All of the TTs were evaluated to determine if these thresholds would provide a reliable basis for classifying sediment samples from the Anniston PCB Site as toxic or not toxic (table C5–5, at the back of the chapter). These results demonstrate that the TT_{LR}s for 20 of the 24 COPCs or COPC mixtures were classified as reliable. A TT was considered to be reliable in the following conditions: (1) if the IOT below the TT was less than or equal to 20 percent; (2) if the IOT above the TT was greater than 50 percent; and, (3) if the overall correct classification rate was greater than or equal to 80 percent (table C5–5). Similarly, 12 of the 24 TT_{HR}s that were established based on percent emergence of adult *C. dilutus* were classified as reliable (table C5–5). Among the TTs for percent emergence of adult *C. dilutus* that were evaluated, the TT_{LR}s for lead, various PCB metrics, organochlorine pesticides, and mean PEC-Q were the most reliable. Overall, correct classification rates ranged from

85 to 89 percent for all of these TTs (n = 20). The TTs for the three PAH values (total PAHs, PAH-Qs, ∑ESB-TUs) were less reliable, suggesting that PAH concentrations do not provide the best predictors of sediment toxicity based on percent emergence of adult *C. dilutus* (that is, PAHs are likely not causing or substantially contributing to the toxicity observed on emergence of adult *C. dilutus*). In addition, the TTs for total PAHs are substantially lower than the PEC for total PAHs, suggesting that COPCs other than PAHs are driving toxicity to benthic invertebrates.

The data on the biomass of adult *C. dilutus* also were used to derive low-risk and high-risk TTs for 24 COPCs or COPC mixtures. These TTs were evaluated to determine their reliability (that is, ability to correctly classify sediment samples from the Anniston PCB Site as toxic or not toxic; table C5–6, at the back of the chapter). These results demonstrate that the TT_{LR}s for 19 of the 24 COPCs or COPC mixtures were classified as reliable. Similarly, 20 of the 24 TT_{HR}s that were established based on biomass of adult *C. dilutus* were classified as reliable. Among the TTs for biomass of adult *C. dilutus* that were evaluated, the TT_{LR}s and TT_{HR}s for lead, the various PCB metrics, organochlorine pesticides, and mean PEC-Q were the most reliable. Overall, correct classification rates ranged from 85 to 89 percent for all of these TTs. The TTs for the three PAH values (total PAHs, PAH-Qs, ∑ESB-TUs) were less reliable, suggesting that PAH concentrations do not provide the best predictors of sediment toxicity based on biomass of adult *C. dilutus* (that is, PAHs are not causing or substantially contributing to the toxicity observed on biomass of adult *C. dilutus*).

For the *H. azteca* reproduction data, low-risk and high-risk TTs were developed for 21 COPCs or COPC mixtures (table C5–7, at the back of the chapter). All of these TTs were evaluated to determine if they would provide a reliable basis for classifying sediment samples from the Anniston PCB Site as toxic or not toxic (table C5–7). These results demonstrate that the TT_{LR}s for 16 of the 21 COPCs or COPC mixtures were classified as reliable. By comparison, 17 of the 21 TT_{HR}s that were established based on reproduction of *H. azteca* were classified as reliable. The TT_{LR}s and TT_{HR}s for lead, the various PCB metrics, and mean PEC-Q were the most reliable among the TTs for reproduction of *H. azteca* that were evaluated. Correct classification rates ranged from 88 to 96 percent for TT_{LR}s (n = 16) and from 84 to 92 percent for the TT_{HR}s (n = 17). The TTs for the two indicators of PAH contamination (total PAHs, PAH-Qs) were less reliable, suggesting that PAH concentrations do not provide the best predictors of sediment toxicity based on reproduction of *H. azteca* (that is, PAHs are not causing or substantially contributing to the toxicity observed on reproduction of *H. azteca*). The TTs for organochlorine pesticides were less than 0.1 when expressed as mean $PEC\text{-}Q_{OCPESTs}$. Accordingly, organochlorine pesticide concentrations were well below the concentrations expected to cause toxicity to *H. azteca*. At this site, organochlorine pesticide concentrations appear to be correlated with the concentrations of other COPCs that were causing toxicity.

The PCB pore-water TTs were evaluated to determine their reliability (tables C5–8 to C5–10, at the back of the chapter). The results of this evaluation indicated that six of the TT_{LR}s based on percent emergence of adult *C. dilutus* were classified as reliable, with overall correct classification rates ranging from 85 to 96 percent (table C5–8). The TT_{LR} of 0.005 µg/L for dichlorobiphenyls had the highest reliability of the 11 pore-water TTs that were evaluated. The five TT_{HR}s classified as reliable generally exhibited slightly lower reliability compared to the corresponding TT_{LR}s (that is, reliability ranged from 85 to 89 percent; table C5–8; hence, PCBs are likely causing or substantially contributing to the toxicity observed on emergence of adult *C. dilutus*).

Eight of the pore-water TT_{LR}s based on biomass of adult *C. dilutus* were classified as reliable. Overall correct classification rates ranged from 81 to 89 percent for the eight reliable TT_{LR}s that were derived for PCBs; hence, PCBs are likely causing or substantially contributing to the toxicity observed on biomass of adult *C. dilutus*.

The pore-water TTs for PCBs based on reproduction of *H. azteca* tended to have intermediate reliability (table C5–10) compared to the pore-water TTs for *C. dilutus* (tables C5–8 and C5–9). For the nine reliable TT_{LR}s, overall correct classification rates ranged from 84 to 92 percent, with the TT_{LR} of 0.000131 micrograms per liter (µg/L) for total heptachlorobiphenyls having the highest reliability (table C5–10). Overall correct classification rates were similar for the five reliable TT_{HR}s based on reproduction of *H. azteca*; however, there was a higher incidence of toxicity below the TT_{HR}s compared to below the TT_{LR}s for most of the COPCs (that is, ranging from 12 to 36 percent; table C5–10; hence, PCBs are likely causing or substantially contributing to the toxicity observed on reproduction of *H. azteca*).

In general, the 69 TTs for whole sediment and 32 TTs for pore water, derived using data on percent emergence of adult *C. dilutus*, biomass of adult *C. dilutus*, or reproduction of *H. azteca*, provide reliable tools for evaluating the toxicity of sediments from the Anniston PCB Site. The TT_{LR} for total PCBs (homologs) was 499 micrograms per kilogram (µg/kg) dry weight for amphipod reproduction and 1,140 µg/kg for midge adult biomass. The TT_{LR} for lead was 10.3 milligrams per kilogram (mg/kg) dry weight for amphipod reproduction and 9.48 mg/kg for midge adult biomass. These TT_{LR}s had low rates of false negative errors (0 to 20 percent of the samples below the TT were classified as toxic), low rates of false positive errors (6 to 15 percent of the samples above the TT were classified as not toxic), and high rates of correct classification (that is, 85 to 92 percent).

Comparability of the Site-Specific Sediment Toxicity Thresholds to Published Sediment Quality Guidelines

The results of the reliability evaluation provide a basis for determining if the TTs derived in this study can be used to accurately classify sediment samples from the study area as toxic or not toxic. Whereas, the TTs derived for many of the COPCs or COPC mixtures were reliable, it also is instructive to compare the site-specific sediment TTs for those that have been developed using alternate methods or alternate data. In this analysis, the comparability of the site-specific sediment TTs for PCBs or other COPCs were compared to (1) empirically based SQGs, (2) equilibrium-partitioning based SQGs, and (3) the results of spiked-sediment toxicity tests. More specifically, the comparability of the TTs for PCBs (homologs) derived from data on *H. azteca* 42-d reproduction (499–1,180 µg/kg DW and 659–1,320 µg/kg at 1 percent OC) (figs. C5–1 and C5–2), adult emergence of *C. dilutus* (1,870–7,110 µg/kg DW and 2,000–5,950 µg/kg at 1 percent OC) (figs. C5–3 and C5–4), and biomass of adult *C. dilutus* (1,140–3,370 µg/kg DW and 1,340–3,240 µg/kg at 1 percent OC) (figs. C5–5 and C5–6) were compared to empirically based SQGs, to equilibrium-partitioning based SQGs, and to TTs based on results of spiked-sediment toxicity tests.

Agreement of Site-Specific Sediment Toxicity Thresholds with Empirically Based Sediment Quality Guidelines

Consensus-based SQGs have been derived for total PCBs and other COPCs to support assessments of sediment quality conditions in freshwater and marine ecosystems. More specifically, MacDonald and others (2000a, 2000b) compiled numerical SQGs from numerous sources that had been developed using empirical and theoretical approaches. Each of the underlying SQGs were classified into three general categories, in accordance with their original narrative intents and used to derive three empirically based sediment effect concentrations (SECs) for total PCBs including a threshold effect concentration, a mid-range effect concentration, and an extreme effect concentration (MacDonald and others, 2000b, table C5–11, at the back of the chapter,). These consensus-based SECs were derived because they estimate the central tendency of the published SQGs and, thus, reconcile the guidance values that have been derived using various approaches. The site-specific sediment TTs for PCBs derived for the Anniston PCB Site are generally similar to the consensus-based SQGs in MacDonald and others (2000b). More specifically the TT_{LR} values generated in this study are similar to the consensus-based mid-range effect concentration (400 µg/kg DW developed by MacDonald and others, 2000b). In addition, the TT_{HR} values for the Anniston PCB Site are generally similar to the consensus-based extreme effect concentration (1,700 µg/kg DW developed by MacDonald and others, 2000b). As the consensus-based SECs provide a unifying synthesis of existing SQGs, reflect causal rather than correlative effects, and accurately predict sediment toxicity in PCB-contaminated sediments (MacDonald and others, 2000b), the site-specific sediment TTs derived in this study also represent the concentrations of PCBs that are sufficient to likely cause adverse effects on sediment-dwelling organisms.

In contrast to PCBs, the levels of metals, PAHs, and organochlorine pesticides were typically less than PECs in sediments from the Anniston PCB Site (MacDonald and others, 2000a). Mean PEC-Qs for metals never exceeded 1.0 in any of the sediment samples used to derive TTs. In addition, mean PEC-Qs were less than 1.0 in all but four PAH samples and three organochlorine pesticide samples. Similarly ∑ESB-TUs were less than 1.0 in all but eight of the sediment samples collected at the Anniston PCB Site. By comparison, mean PEC-Qs for PCBs exceeded 1.0 in 17 of 26 sediment samples from the site, with PEC-Qs for PCBs of as much as 1,780 calculated for these sediment samples. These results indicate that PCBs likely caused most of the toxicity to benthic invertebrates exposed to the Anniston PCB Site sediments, with metals, PAHs, and organochlorine pesticides contributing only minimally to the toxicity observed in these sediments.

Agreement of the Site-Specific Sediment Toxicity Thresholds with Equilibrium-Partitioning Based Sediment Quality Guidelines

The equilibrium-partitioning approach provides a theoretical basis for identifying chronic TT for sediment-associated PCBs. Using this approach, the New York State Department of Environmental Conservation (1999) has developed chronic SQGs for PCBs that are intended to protect freshwater and saltwater benthic aquatic life. These guidelines indicate that the threshold for chronic toxicity of total PCBs in freshwater sediments is 193 µg/kg DW at 1 percent OC. An equilibrium-partitioning based SQG of 70 µg/kg DW at 1 percent OC also has been derived to support the evaluation of sediment quality conditions at freshwater locations in the United States (Bolton and others, 1985, table C5–11). Together, these equilibrium-partitioning based SQGs suggest that chronic effects on sediment-dwelling organisms are likely at total PCB concentrations in excess of 70 to 193 µg/kg DW. Fuchsman and others (2006) estimated equilibrium-partitioning based sediment quality benchmarks of 2,100 to 15,000 µg/kg DW at 1 percent OC for aroclor 1242, 1248, and 1254, which are the predominant PCB formulations at the Anniston PCB Site. All of the site-specific sediment TTs for PCBs, expressed on an OC-normalized basis, fall within the range of benchmarks generated using the equilibrium partitioning approach (table C5–11).

Agreement of Site-Specific Sediment Toxicity Thresholds to Results of Spiked-Sediment Toxicity Tests

Dose-response data for toxicity tests performed with COPCs spiked into sediment provide a basis for identifying the concentrations of sediment-associated contaminants that would be sufficient to cause sediment toxicity (Wenning and others, 2005). Whereas no information was located on the toxicity of spiked-sediment toxicity tests with total PCBs, data from five spiked-sediment toxicity tests that evaluated formulated mixtures of PCBs provide relevant information for evaluating the consensus-based SECs for total PCBs; McLeese and Metcalfe, 1980; Polikarpov and others, 1983; Plesha and others, 1988; Swartz and others, 1988). The results of these spiked-sediment toxicity studies indicate that PCBs are acutely toxic to sediment-dwelling organisms in 10-d lethality tests (at concentrations ranging from greater than 780 to 251,000 µg/kg DW). A median lethal concentration of 8,800 µg/kg DW was reported for the amphipod, *Rhepoxynius abronius,* when PCBs (aroclor 1254) alone were tested (Plesha and others, 1988). U.S. Environmental Protection Agency (1980) reported an acute-to-chronic ratio of 11 for the freshwater amphipod, *Gammarus pseudolimnaeus*, based on the results of toxicity tests performed with waterborne PCBs (endpoints: survival and reproduction). This acute-to-chronic ratio is much lower than the acute-to-chronic ratios (27 to 58) calculated from the results of toxicity tests performed on the copepod, *Microarthridion littorale* (endpoints: survival and reproduction; DiPinto and others, 1993). Application of the empirically derived acute-to-chronic ratio for the freshwater amphipod to the 10-d median lethal effect concentration (LC_{50}) for the marine amphipod suggests that PCBs, when present alone in sediments, are likely to cause chronic toxicity to amphipods at concentrations of about 800 µg/kg DW (that is, 8,800 µg/kg DW divided by 11 = 800 µg/kg DW; table C5–11).

Spiked-sediment toxicity tests, performed under controlled laboratory conditions, can be used to determine lethal or effective concentrations of a wide range of chemical substances. However, such response thresholds could underestimate the ecological effects in the field because of the presence of contaminant mixtures in sediments (Swartz, 1999). As such, sediments containing mixtures of contaminants could be more toxic than sediments that contain PCBs alone.

To evaluate the possible interactive effects of PCBs when PCBs are present with other contaminants, several investigators have performed spiked-sediment toxicity tests with mixtures of contaminants. The results of these studies indicate that sediments tend to be more toxic when the sediments contain mixtures of contaminants (that is, PCBs and other substances). For example, Plesha and others (1988) reported acute toxicity to amphipods (*R. abronius*) in sediments that contained several chlorinated hydrocarbons and aroclor 1254 (at concentrations well below the acute LC_{50}; that is, 1,000 µg/kg DW). Similarly, sediments that contained 2,100 µg/kg DW of aroclor 1254 and fluoranthene were acutely toxic to amphipods (*R. abronius*; Plesha and others, 1988). These data indicate that PCB-contaminated sediments are more toxic when they also contain other common contaminants (such as PAHs; Plesha and others, 1988). Considering the relation between the acute LC_{50} for PCBs (that is, 8,800 µg/kg DW) and the concentration of PCBs in acutely toxic sediments that contained aroclor 1254 and fluoranthene (2,100 µg/kg DW, giving a ratio of 4.2), it is likely that PCBs would contribute to sediment toxicity at concentrations below the chronic TT of 800 µg/kg DW (estimated from Swartz and others, 1988), when PCBs are in mixtures with other contaminants. Based on the results of this review, the TTs derived in this study are comparable to the chronic TTs that have been estimated for PCBs using the results of spiked-sediment toxicity tests. Comparability between the TTs for PCBs derived usi ng different species of benthic invertebrates increases confidence that the TTs derived in this study can be used to accurately evaluate sediment toxicity (table C5–11).

In contrast to the results for PCBs, the concentrations of metals, PAHs, or organochlorine pesticides never exceeded median lethal concentrations reported from the results of spiked-sediment toxicity tests. For example, Swartz and others (1988) reported an LC_{50} of mercury of 13,100 µg/kg in 10-d toxicity tests with amphipods, *R. abronius*; this level was never exceeded in sediments evaluated in this study. Similarly, the levels of fluoranthene in the site sediments evaluated in this study never exceed the LC_{50}s of 3,300 to 10,500 µg/kg that were reported for amphipods, *R. abronius* (Swartz and others, 1988; 1989).

Nebeker and others (1989) reported LC_{50}s of 11,000 to 49,700 µg/kg (3,700 to 4,700 µg/kg at 1 percent OC) for total DDT for the amphipod, *H. azteca*, that are substantially higher than the levels of total DDTs that have been measured in the sediment samples evaluated to derive the site-specific TTs. Collectively, these results indicate that PCBs, rather than metals, PAHs, or organochlorine pesticides, are the principal contaminants causing toxicity in the sediments that were evaluated in this study.

Summary

Sediment chemistry, pore-water chemistry, and sediment toxicity data were compiled for as many as 26 sediment samples from the Anniston polychlorinated biphenyl (PCB) Site. All of the samples were evaluated to determine if they qualified as reference sediment samples (chapter 4). Those samples that met the chemical selection criteria and biological selection criteria were identified as reference samples and used to develop the reference envelope for each toxicity test endpoint (chapter 4). Because interbatch variability in control performance was observed for some of the toxicity test endpoints, all of the response data were control-normalized before performing subsequent data analyses (chapter 4 and appendix 3, tables A3–29 and A3–30).

The reference-envelope approach was used to identify the sediment samples that were toxic to benthic invertebrates. This procedure involved identification of reference sediment samples, normalizing the toxicity data to reflect control responses, developing a reference envelope for each toxicity test endpoint, and designating each sediment sample as toxic or not toxic for each toxicity test endpoint, for each species, and for all species combined. These results demonstrated that percent emergence of adult *Chironomus dilutus*, biomass of adult *C. dilutus*, and reproduction of *Hyalella azteca* normalized to survival were among the most responsive endpoints that were evaluated (chapter 4); therefore, these endpoints were selected for concentration-response model (CRM) development.

A step-wise process was used to evaluate relations between sediment chemistry and sediment toxicity for the Anniston PCB Site, to develop site-specific sediment toxicity thresholds (TTs), and to evaluate the reliability of the resultant TTs. This process consisted of six main steps:

1. Screening-level evaluation performed to identify the chemicals of potential concern (COPCs) and COPC mixtures that were unlikely to cause or substantially contribute to sediment toxicity;

2. Identification of the COPCs or COPC mixtures that were significantly correlated with the toxicity test endpoints (based on results of Spearman's rank correlation analyses, r_s greater than 0.4; p less than 0.005);

3. Development of CRMs for selected COPCs/COPC mixtures andtoxicity test endpoint pairs;

4. Derivation of site-specific sediment TTs for selected COPC/COPC mixtures and toxicity test endpoint pairs;

5. Evaluation of the reliability of the site-specific sediment TTs for selected COPC/COPC mixtures and toxicity test endpoint pairs; and,

6. Evaluation of the comparability of the site-specific sediment TTs for selected COPCs or COPC mixtures.

First, a series of analyses were performed to identify the COPCs and COPC mixtures that were most likely to be correlated with the responses of toxicity test organisms (for example, an evaluation of sediment chemistry based on the frequency of detection and comparisons to conservative toxicity screening values).

Second, potential relations between the concentrations of COPCs and the responses of toxicity test organisms were identified by performing Spearman's rank correlation analysis on the underlying data. The results of these analyses indicated that the relations between concentration and response tended to be strongest for PCBs, certain metals (that is, lead and mercury), polycyclic aromatic hydrocarbons (PAHs), organochlorine pesticides, and mean probable effect concentration-quotients (PEC-Qs).

Third, CRMs were developed for each of the COPCs and COPC mixtures in sediment that were retained following these initial analyses. The CRMs were then examined to identify the COPC/COPC mixtures and toxicity test endpoint pairs that would be most relevant for development of site-specific sediment TTs (that is, R^2 greater than 0.4; p less than 0.05). Overall, 69 COPC/COPC mixtures and toxicity test endpoint pairs were selected for deriving TTs for whole sediment. In addition, 32 COPC or COPC mixture pairs for pore water were selected for deriving TTs for pore water.

Fourth, two types of TTs, including low-risk or high-risk TTs (TT_{LR}s and TT_{HR}s), were developed using the CRMs for 69 COPC/COPC mixtures and toxicity test endpoint pairs for whole sediment and 32 COPC/COPC mixtures and toxicity test endpoint pairs for pore water. The TT_{LR}s were established as the concentrations of COPCs or COPC mixtures that corresponded to the lower limit of the reference envelope for the selected toxicity test endpoint. The TT_{HR}s were established as the concentrations of COPCs or COPC mixtures that corresponded to a 10-percent reduction in survival, weight, biomass, emergence, or reproduction, compared to the lower limit of the reference envelope. The reliability of the resultant TTs were then evaluated using sediment chemistry and sediment toxicity data from the Anniston PCB Site. Toxicity thresholds were considered to be reliable and predictive of sediment toxicity if the incidence of toxicity (IOT) below the TT was less than 20 percent, IOT above the TT was greater than 50 percent, and the rate of correct classification of sediment samples as toxic and not toxic was greater than or equal to 80 percent.

Fifth, the results of this evaluation indicated that most of the site-specific TTs for whole sediment provide a reliable basis for identifying toxic and not toxic sediment samples at the Anniston PCB Site (that is, for correctly classifying the sediment samples used to derive the TTs as toxic or not toxic, for the endpoint used to derive the TTs). Among the 69 TTs for sediment, the TT_{LR} for total PCBs was 499 micrograms per kilogram (µg/kg) dry weight (DW) for amphipod reproduction and 1,140 µg/kg for midge adult biomass. The TT_{LR} for lead was 10.3 milligrams per kilogram (mg/kg) DW for amphipod reproduction and 9.48 mg/kg for midge adult biomass. These TT_{LR}s had low rates of false negative errors (0 to 20 percent of the samples below the TT were classified as toxic), low rates of false positive errors (6 to 15 percent of the samples above the TT were classified as not toxic), and high rates of correct classification (85 to 94 percent). Of the 32 TTs for pore water that were evaluated, the TT_{LR} of 0.00536 µg/L for total dichlorobiphenyls, based on emergence of adult *C. dilutus* had the highest reliability.

Finally, the site-specific TTs for PCBs and other COPCs derived in this study also were compared to empirically based sediment quality guidelines (SQGs), to equilibrium-partitioning based SQGs, and to the results of spiked-sediment toxicity tests. The results of this evaluation indicated that the site-specific sediment TTs for PCBs were comparable to the consensus-based SQGs that were derived for PCBs. In addition, the site-specific sediment TTs for PCBs are within the range of

SQGs derived using the equilibrium partitioning approach. The site-specific sediment TTs for PCBs also are consistent with the chronic TTs that have been estimated for benthic invertebrates using the results of spiked-sediment toxicity tests. As the site-specific sediment TTs for PCBs are consistent with the results of empirically based SQGs, equilibrium-partitioning based SQGs, and sediment-spiking studies, these site-specific sediment TTs likely represent the concentrations of PCBs that are sufficient to cause toxicity to benthic invertebrates (as opposed to simply being correlated with adverse effects on the survival, weight, or reproduction of benthic invertebrates). Importantly, such site-specific sediment TTs have been demonstrated to accurately classify sediment samples as toxic or not toxic to benthic invertebrates at the Anniston PCB Site. In contrast, the TTs for metals, PAHs and organochlorine pesticides were generally lower than the consensus-based SQGs (that is, PECs), and median lethal effect concentrations (LC_{50}s) generated in spiked-sediment toxicity tests, indicating that these COPCs are likely not the main contributors to the observed toxicity of the sediments from the site that were evaluated in this study. The reproduction endpoint for *H. azteca* provided lower TTs compared to the day 28 biomass endpoint for *H. azteca* and the emergence or biomass endpoints for adult *C. dilutus* provided lower TTs compared to the day 13 biomass endpoint for *C. dilutus*. Therefore, the site-specific sediment TTs developed for PCBs in this study based on the reproductive endpoint for *H. azteca* and based on the biomass endpoint for adult *C. dilutus* are recommended for evaluating the effects on benthic invertebrates associated with exposure to PCB contaminated sediments at the Anniston PCB Site.

The results of this study also demonstrate the utility of additional endpoints in assessments of sediment toxicity. For both species, adverse effects on reproduction or biomass were observed at substantially lower concentrations of COPCs than was the case for survival or weight. As benthic invertebrates are typically exposed to sediment-associated COPCs for extended periods of time in aquatic ecosystems and the long-term viability of their populations and the ecosystem services (for example, decomposition of detritus, food source for higher trophic levels) that they provide are dependent upon maintaining adequate biomass and reproduction, these additional endpoints are relevant to the sediment quality assessment process. The results of this study also demonstrated that certain refinements to the toxicity testing methods (such as improving nutrition of test organisms and starting the long-term midge test with 7-d old larvae) can enhance the likelihood of running a successful test and reduce variability in the results.

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Figures and Tables

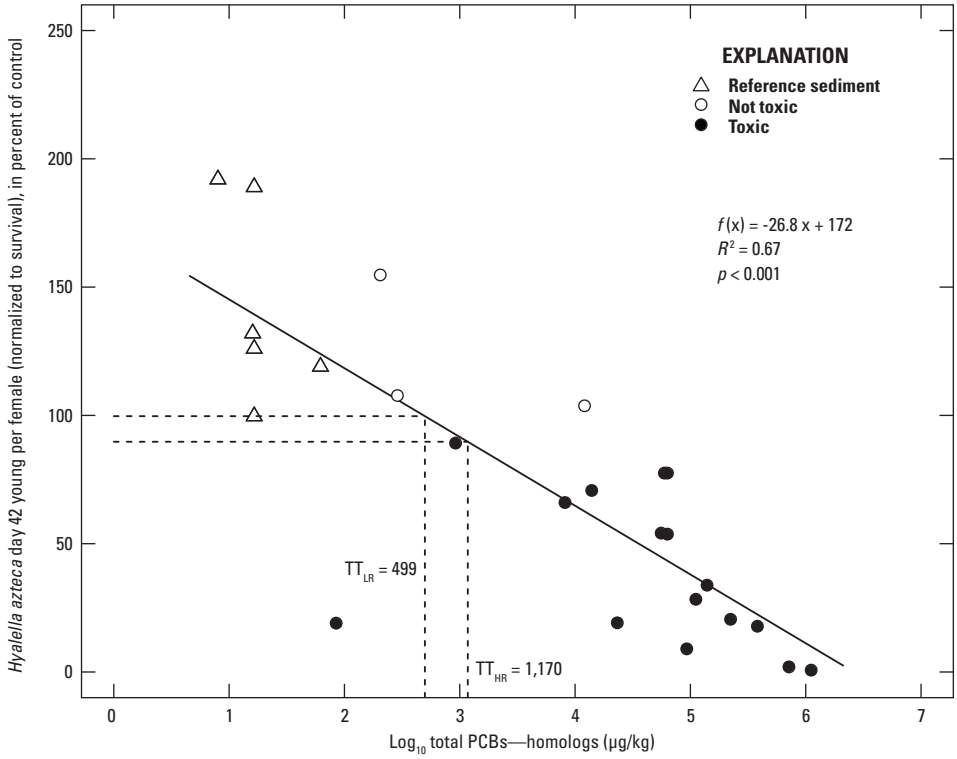

Figure C5–1. Concentration–response model for total polychlorinated biphenyls (PCBs; µg/kg DW) and *Hyalella azteca* reproduction (young per female normalized to percent survival). [µg/kg, microgram per kilogram; DW, dry weight; TT_{LR}, low-risk toxicity threshold; TT_{HR}, high-risk toxicity threshold]

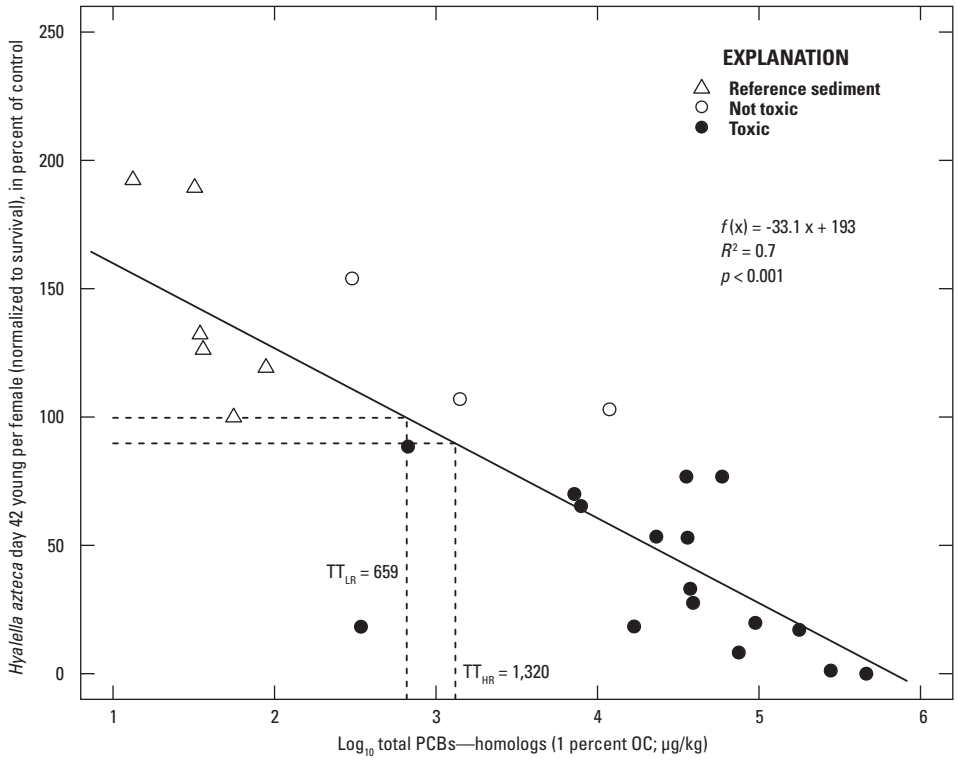

Figure C5–2. Concentration–response model for total polychlorinated biphenyls (PCBs; at 1 percent OC; µg/kg DW) and *Hyalella azteca* reproduction (young per female normalized to percent survival). [OC, organic carbon; µg/kg, microgram per kilogram; DW, dry weight; TT_{LR}, low-risk toxicity threshold; TT_{HR}, high-risk toxicity threshold]

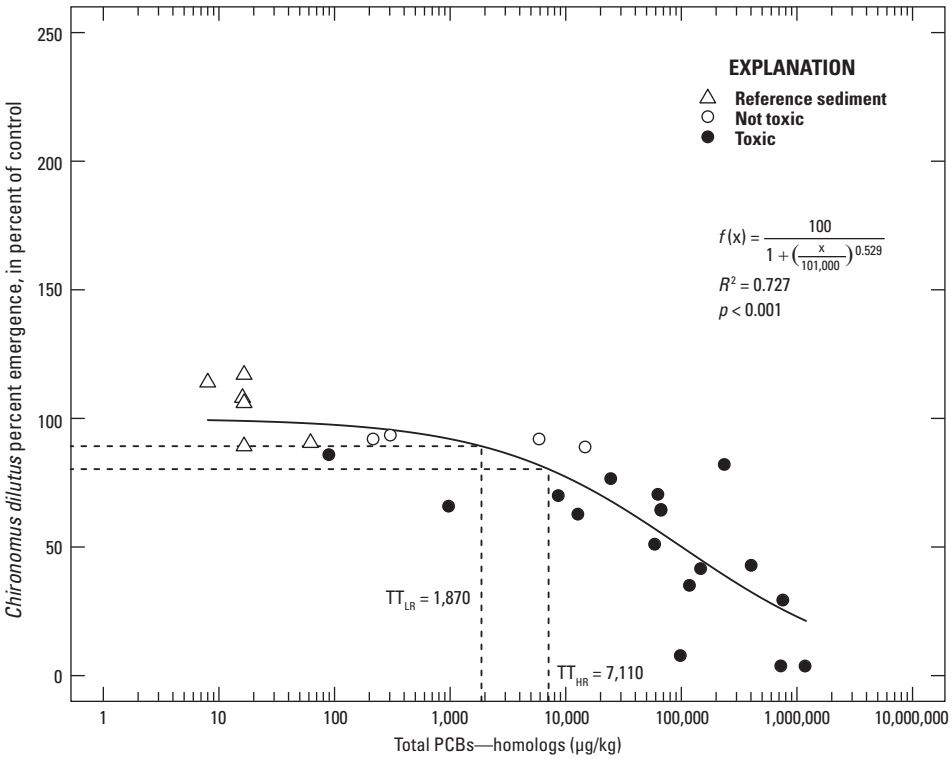

Figure C5–3. Concentration–response model for total polychlorinated biphenyls (PCBs; µg/kg DW) and *Chironomus dilutus* percent emergence of adults. [µg/kg, micrograms per kilogram; DW, dry weight; TT_{LR}, low-risk toxicity threshold; TT_{HR}, high-risk toxicity threshold]

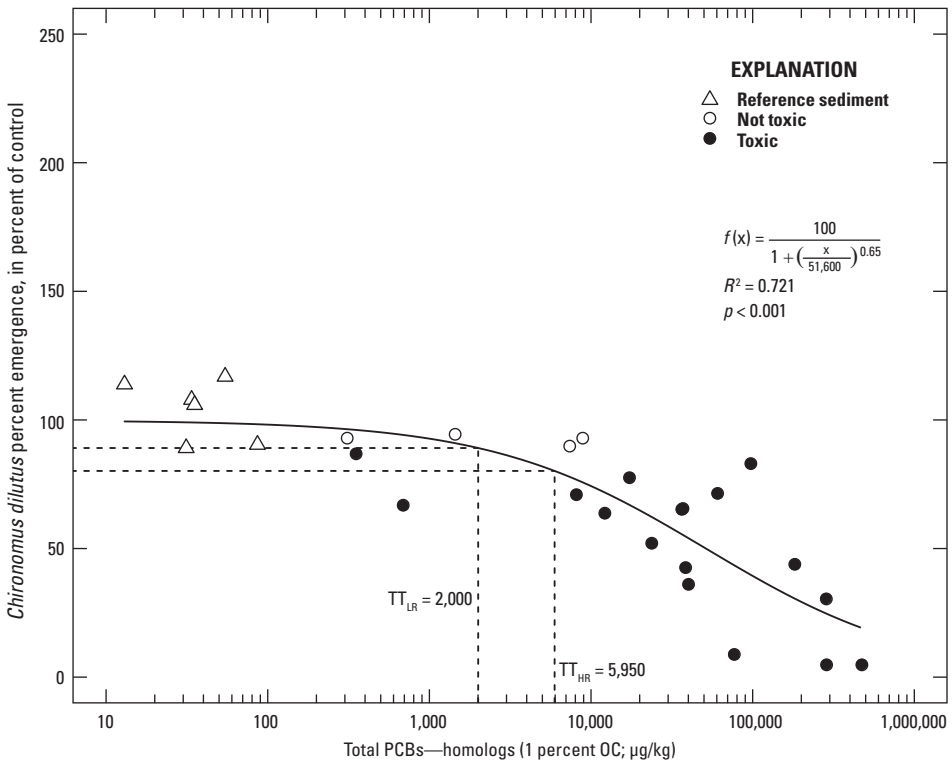

Figure C5–4. Concentration–response model for total polychlorinated biphenyls (PCBs; at 1 percent OC; µg/kg DW) and *Chironomus dilutus* percent emergence of adults. [OC, organic carbon; µg/kg, micrograms per kilogram; DW, dry weight; TT_{LR}, low-risk toxicity threshold; TT_{HR}, high-risk toxicity threshold]

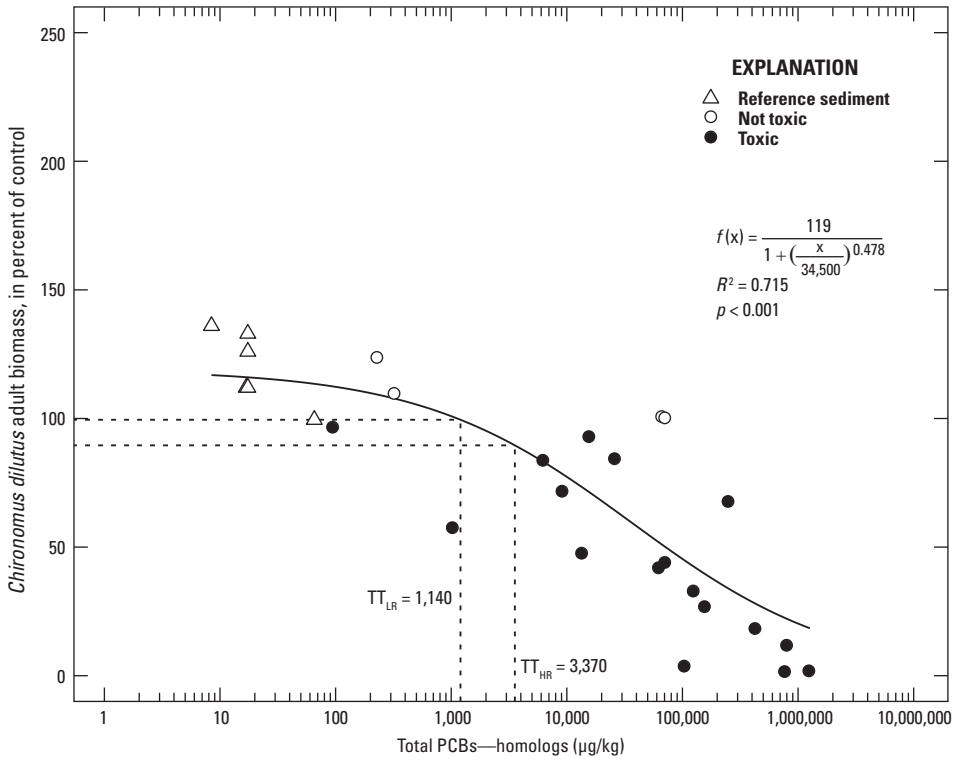

Figure C5–5. Concentration–response model for total polychlorinated biphenyls (PCBs; µg/kg DW) and *Chironomus dilutus* adult biomass. [µg/kg, micrograms per kilogram; DW, dry weight; TT_{LR}, low-risk toxicity threshold; TT_{HR}, high-risk toxicity threshold]

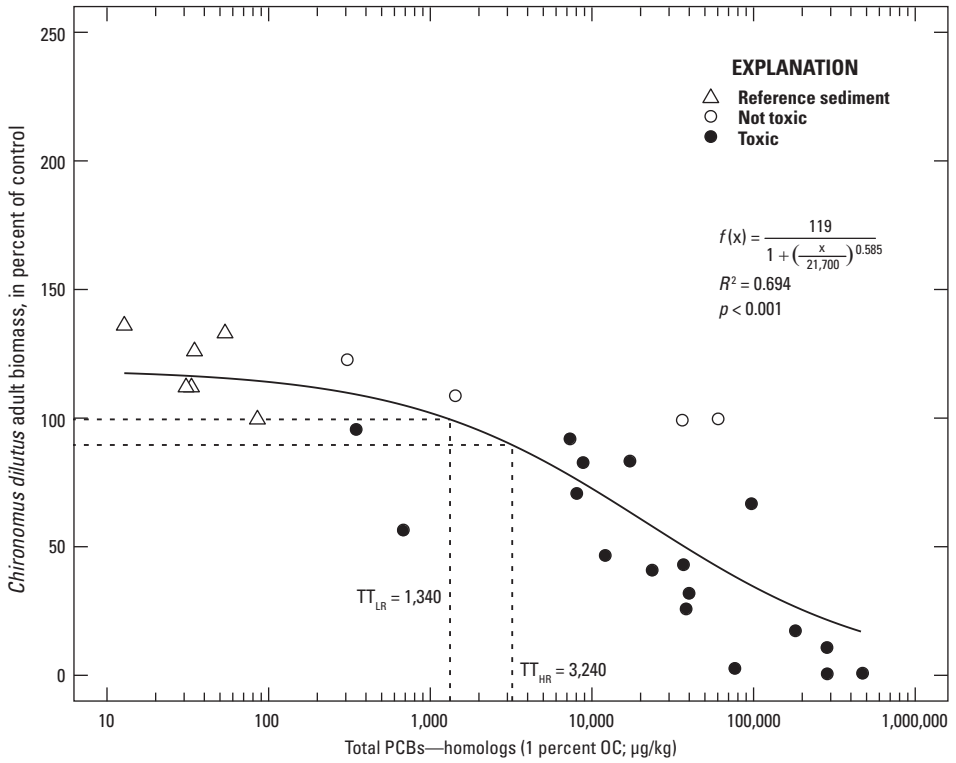

Figure C5–6. Concentration–response model for total polychlorinated biphenyls (PCBs; at 1 percent OC; µg/kg DW) and *Chironomus dilutus* adult biomass. [OC, organic carbon; µg/kg, micrograms per kilogram; DW, dry weight; TT_{LR}, low-risk toxicity threshold; TT_{HR}, high-risk toxicity threshold]

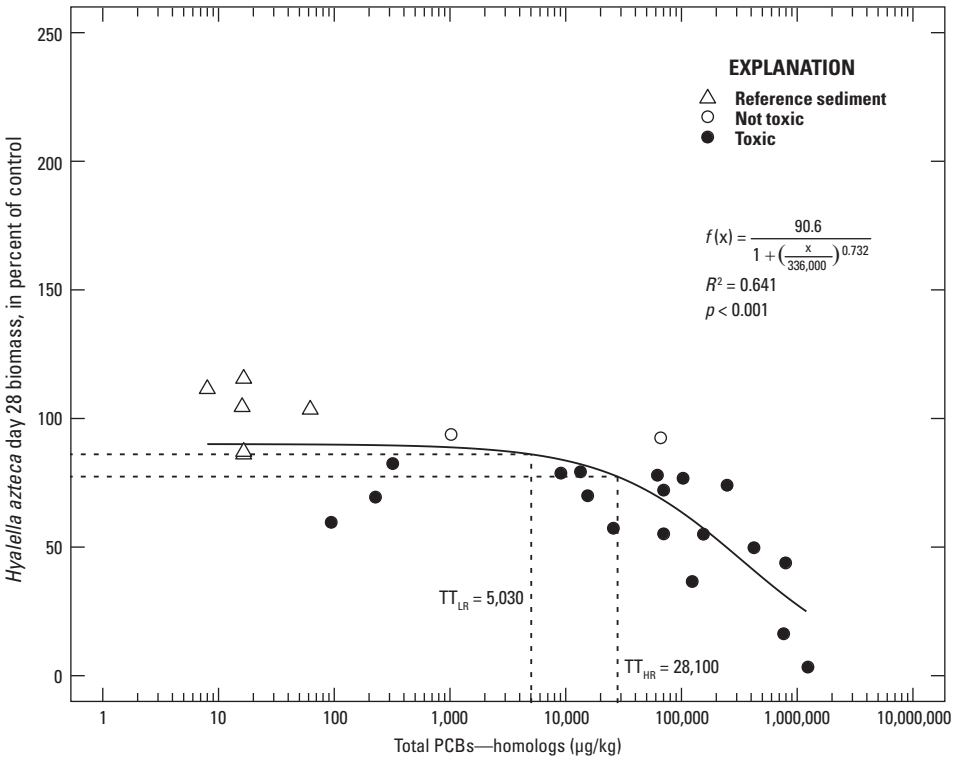

Figure C5–7. Concentration–response model for total polychlorinated biphenyls (PCBs; µg/kg DW) and *Hyalella azteca* day 28 biomass. [µg/kg, micrograms per kilogram; DW, dry weight; TT_{LR}, low-risk toxicity threshold; TT_{HR}, high-risk toxicity threshold]

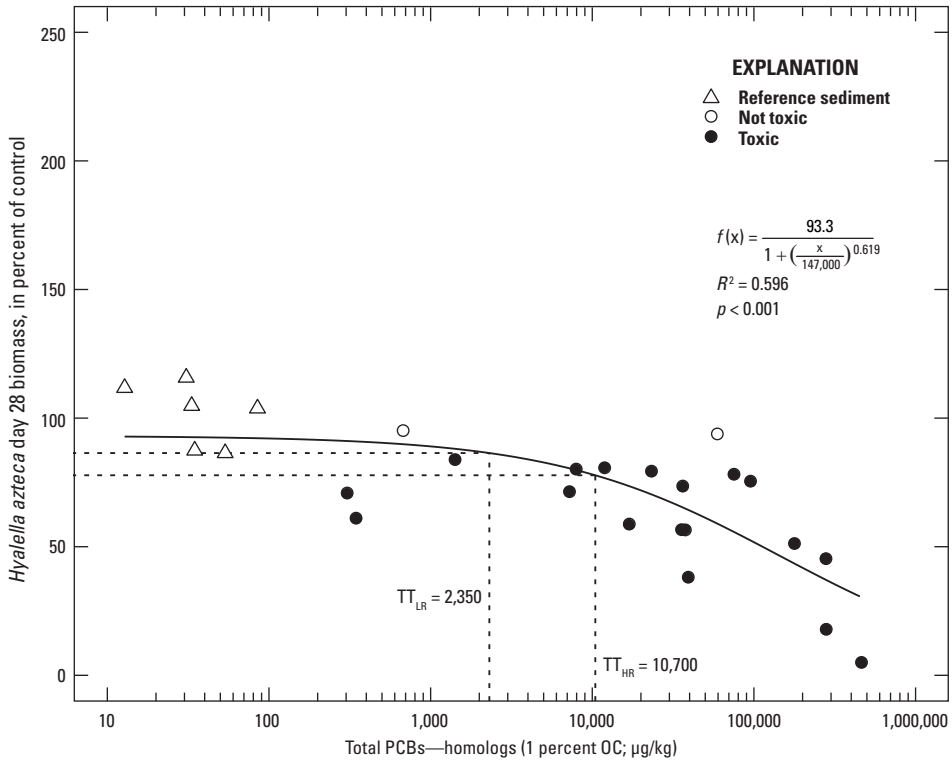

Figure C5–8. Concentration–response model for total polychlorinated biphenyls (PCBs; at 1 percent OC; µg/kg DW) and *Hyalella azteca* day 28 biomass. [OC, organic carbon; µg/kg, micrograms per kilogram; DW, dry weight; TT_{LR}, low-risk toxicity threshold; TT_{HR}, high-risk toxicity threshold]

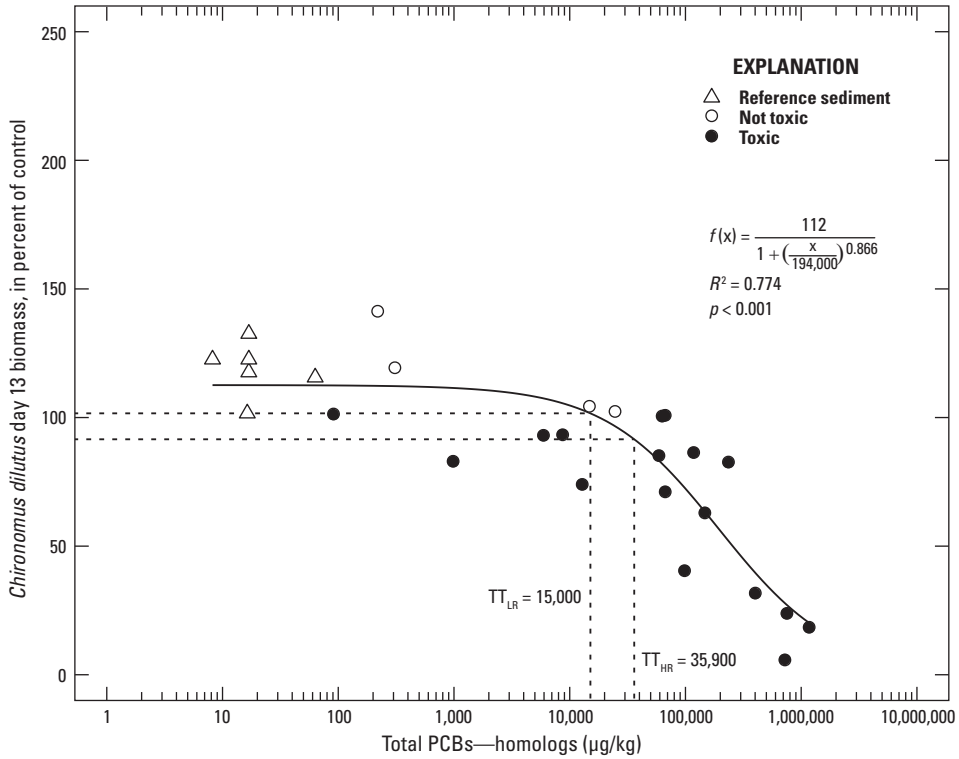

Figure C5–9. Concentration–response model for total polychlorinated biphenyls (PCBs; µg/kg DW) and *Chironomus dilutus* day 13 biomass. [µg/kg, micrograms per kilogram; DW, dry weight; TT_{LR}, low-risk toxicity threshold; TT_{HR}, high-risk toxicity threshold]

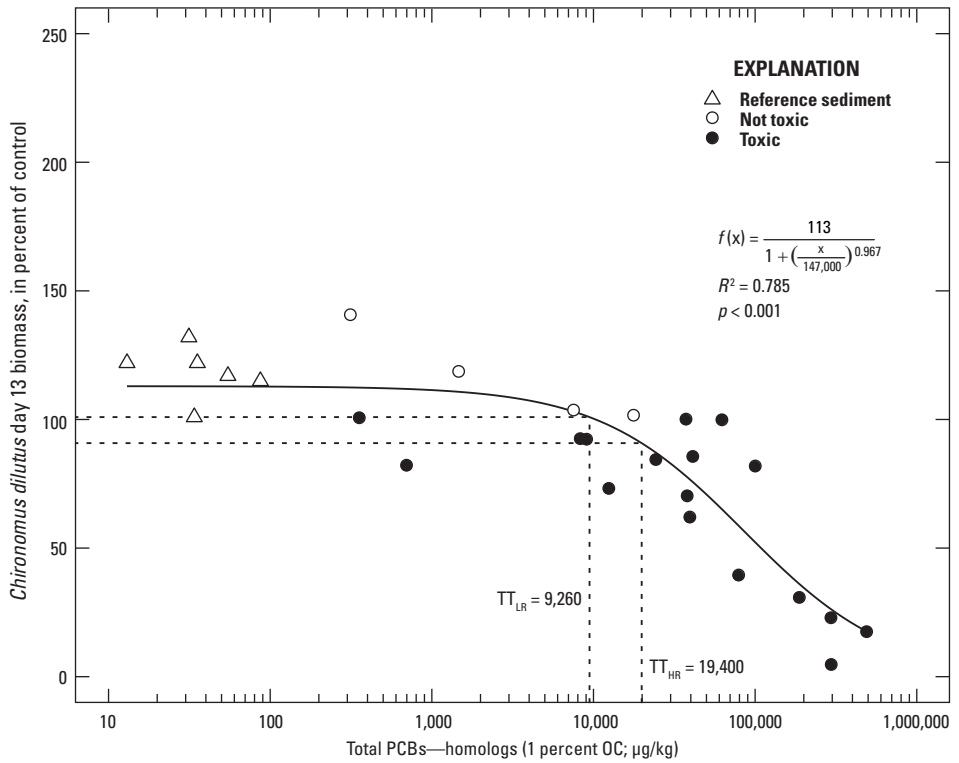

Figure C5–10. Concentration–response model for total polychlorinated biphenyls (PCBs; at 1 percent OC; µg/kg DW) and *Chironomus dilutus* day 13 biomass. [OC, organic carbon; µg/kg, micrograms per kilogram; DW, dry weight; TT_{LR}, low-risk toxicity threshold; TT_{HR}, high-risk toxicity threshold]

Table C5–1. Selected toxicity screening values and toxicity reference values for assessing sediment quality conditions for the Anniston PCB Site.

[∑SEM-AVS)/f_{OC}, simultaneously extracted metal and acid volatile sulfide AVS normalized to the fraction organic carbon; AVS, acid volatile sulfides; f_{OC}, fraction organic carbon; LMW, low molecular weight; PAH, polycyclic aromatic hydrocarbon; HMW, high molecular weight; mg/kg, milligram per kilogram; PCB, polychlorinated biphenyl; DDD, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethane; DDE, 1,1-dichloro-2,2-bis(p-chlorophenyl)ethylene; DDT, 1,1,1-trichloro-2,2-bis(p-chlorophenyl)ethane; TSV, toxicity screening value; TEC, threshold effects concentration; LEL, lowest effect level; ERL, effects range low; NOEC, no observed effect concentration; ISQG, Interim sediment quality guideline; TEL, threshold-effects level; mg/kg, milligram per kilogram; DW, dry weight; µmol/g, micromole per gram; USEPA, U.S. Environmental Protection Agency; CCME, Canadian Council of Ministers of the Environment; PEC, probable effect concentration; TRV, toxicity reference value; SEL, severe effect level; ERM, effects range medium; µg/kg, microgram per kilogram; OEC, observed effect concentration; PEL, probable effects level; CCC, continuous chronic concentration; NA, not applicable]

Chemical of potential concern	TSV type	Selected TEC-type value	Reference	TRV type	Selected PEC-type value	Reference
Arsenic, total	TEC	9.79	MacDonald and others (2000a)	PEC	33	MacDonald and others (2000a).
Cadmium, total	TEC	0.99	MacDonald and others (2000a)	PEC	4.98	MacDonald and others (2000a).
Chromium, total	TEC	43.4	MacDonald and others (2000a)	PEC	111	MacDonald and others (2000a).
Copper, total	TEC	31.6	MacDonald and others (2000a)	PEC	149	MacDonald and others (2000a).
Lead, total	TEC	35.8	MacDonald and others (2000a)	PEC	128	MacDonald and others (2000a).
Manganese, total	LEL	460	Persaud and others (1993)	SEL	1,100	Persaud and others (1993).
Mercury, total	TEC	0.18	MacDonald and others (2000a)	PEC	1.06	MacDonald and others (2000a).
Nickel, total	TEC	22.7	MacDonald and others (2000a)	PEC	48.6	MacDonald and others (2000a).
Silver, total	ERL	1	Long and others (1995)	ERM	3.7	Long and others (1995).
Zinc, total	TEC	121	MacDonald and others (2000a)	PEC	459	MacDonald and others (2000a).
Simultaneously extracted metals (SEM; µmol/g DW)						
(∑SEM-AVS)/f_{OC}	NOEC	130	USEPA (2005)	OEC	3,000	USEPA (2005).
Polycyclic aromatic hydrocarbons (PAHs; µg/kg DW)						
2-Methylnaphthalene	ISQG	20.2	CCME (2002)	PEL	201	CCME (2002).
Acenaphthene	ISQG	6.71	CCME (2002)	PEL	88.9	CCME (2002).
Acenaphthylene	ISQG	5.87	CCME (2002)	PEL	128	CCME (2002).
Anthracene	TEC	57.2	MacDonald and others (2000a)	PEC	845	MacDonald and others (2000a).
Fluorene	TEC	77.4	MacDonald and others (2000a)	PEC	536	MacDonald and others (2000a).
Naphthalene	TEC	176	MacDonald and others (2000a)	PEC	561	MacDonald and others (2000a).
Phenanthrene	TEC	204	MacDonald and others (2000a)	PEC	1,170	MacDonald and others (2000a).
Benz(*a*)anthracene	TEC	108	MacDonald and others (1996)	PEC	1,050	MacDonald and others (2000a).
Benzo(*a*)pyrene	TEC	150	MacDonald and others (2000a)	PEC	1,450	MacDonald and others (2000a).
Chrysene	TEC	166	MacDonald and others (2000a)	PEC	1,290	MacDonald and others (2000a).
Dibenz(*a,h*)anthracene	TEC	33	MacDonald and others (2000a)	PEC	135	CCME (2002)
Fluoranthene	TEC	423	MacDonald and others (2000a)	PEC	2,230	MacDonald and others (2000a).
Pyrene	TEC	195	MacDonald and others (2000a)	PEC	1,520	MacDonald and others (2000a).
Total LMW-PAHs	TEL	312	MacDonald and others (1996)	PEL	1,442	MacDonald and others (1996).
Total HMW-PAHs	TEL	655	MacDonald and others (1996)	PEL	6,676	MacDonald and others (1996).
Total PAHs	TEC	1,610	MacDonald and others (2000a)	PEC	22,800	MacDonald and others (2000a).
Polychlorinated biphenyls (PCBs; µg/kg DW)						
Total PCBs	TEC	40	MacDonald and others (2000b)	PEC	676	MacDonald and others (2000a).
Organochlorine pesticides (µg/kg DW)						
Chlordane (cis & trans)	TEC	17.6	MacDonald and others (2000a)	PEC	3.24	MacDonald and others (2000a).
Dieldrin	TEC	61.8	MacDonald and others (2000a)	PEC	1.9	MacDonald and others (2000a).
Sum DDDs (*o,p′* + *p,p′*)	TEC	28	MacDonald and others (2000a)	PEC	4.88	MacDonald and others (2000a).
Sum DDEs (*o,p′* + *p,p′*)	TEC	31.3	MacDonald and others (2000a)	PEC	3.16	MacDonald and others (2000a).
Sum DDTs (*o,p′* + *p,p′*)	TEC	62.9	MacDonald and others (2000a)	PEC	4.16	MacDonald and others (2000a).
Total DDTs (total Sum DDD, DDE, DDT)	TEC	572	MacDonald and others (2000a)	PEC	5.28	MacDonald and others (2000a).
Endrin	TEC	207	MacDonald and others (2000a)	PEC	2.22	MacDonald and others (2000a).
Heptachlor epoxide	TEC	2.47	MacDonald and others (2000a)	PEC	2.47	MacDonald and others (2000a).

Table C5–2. Selected toxicity screening values for assessing pore-water chemistry at the Anniston PCB Site.

[PCB, polychlorinated biphenyl; TSV, toxicity screening value; mg/L, milligram per liter; µg/L, microgram per liter; e, base of the natural logarithm; ln, natural logarithm]

Chemical of potential concern	Selected TSV value	TSV type	Reference
Metals (mg/L)			
Arsenic, total	0.15	Chronic criteria	Alabama Department of Environmental Management (2011).
Cadmium, total	0.00023	Chronic criteria[1]	Alabama Department of Environmental Management (2011).
Chromium, total	0.011	Chronic criteria	Alabama Department of Environmental Management (2011).
Copper, total	0.0081	Chronic criteria[2]	Alabama Department of Environmental Management (2011).
Lead, total	0.0022	Chronic criteria[3]	Alabama Department of Environmental Management (2011).
Mercury, total	0.000012	Chronic criteria	Alabama Department of Environmental Management (2011).
Nickel, total	0.047	Chronic criteria[4]	Alabama Department of Environmental Management (2011).
Zinc, total	0.11	Chronic criteria[5]	Alabama Department of Environmental Management (2011).
Polychlorinated biphenyls (PCBs; µg/L)			
Total PCBs	0.014	Chronic criteria	Alabama Department of Environmental Management (2011).
Semivolatile compounds (µg/L)			
Pentachlorophenol	13.1	Chronic criteria[6]	Alabama Department of Environmental Management (2011).

[1]Chonic criteria for cadmium calculated with the following formula, and an averaged surface water hardness value for Choccolocco Creek (89 mg/L): Conc. (µg/L) = (e(0.7409[ln(hardness in mg/L as $CaCO_3$)]-4.719))(CF); conversion factor (CF) = 1.101672-[ln(hardness)(0.041838)].

[2]Chronic criteria for copper calculated with the following formula, and an averaged surface water hardness value for Choccolocco Creek (89 mg/L): Conc. (µg/L) = conc. (µg/L) = (e(0.8545[ln(hardness in mg/L as $CaCO_3$)]-1.702))(CF); conversion factor (CF) = 0.960.

[3]Chronic criteria for lead calculated with the following formula, and an averaged surface water hardness value for Choccolocco Creek (89 mg/L): Conc. (µg/L) = conc. (µg/L) = (e(1.273[ln(hardness in mg/L as $CaCO_3$)]-4.705))(CF); conversion factor (CF) = 1.46203-[ln(hardness)(0.145712)].

[4]Chronic criteria for nickel calculated with the following formula, and an averaged surface water hardness value for Choccolocco Creek (89 mg/L): Conc. (µg/L = conc. (µg/L) = (e(0.8460[ln(hardness in mg/L as $CaCO_3$)]+0.0584))(CF); conversion factor (CF) = 0.997.

[5]Chronic criteria for zinc calculated with the following formula, and an averaged surface water hardness value for Choccolocco Creek (89 mg/L): Conc. (µg/L) = (e(0.8473[ln(hardness in mg/L as $CaCO_3$)]+0.884))(CF); conversion factor (CF) = 0.986.

[6]Chronic criteria for pentachlorophenol calculated with the following formula, and an averaged pH value for Choccolocco Creek (pH = 7.7). Conc. (µg/L) = e[1.005(pH)-5.134].

Table C5–3. Summary of the concentration-response models derived for sediment based on magnitude of toxicity to *Hyalella azteca* in 42-day whole-sediment toxicity tests and *Chironomus dilutus* in life-cycle whole-sediment toxicity tests. The toxicity thresholds derived using these regression equations also are presented.

[COPC, chemical of potential concern; PCB, polychlorinated biphenyl; %, percent; OC, organic carbon; PAH, polycyclic aromatic hydrocarbon; PEC-Q, probable effect concentration quotient; TPAH, total polycyclic aromatic hydrocarbon; TPCB, total polychlorinated biphenyl; OCPEST, organochlorine pesticides; ESB-TUFCV, equilibrium partitioning sediment benchmarks-toxic units/final chronic value; TT, toxicity threshold; LR, low risk; HR, high risk; d, day; µg/kg, microgram per kilogram; mg/kg, milligram per kilogram]

Toxicity test endpoint used to develop the relation/COPC/COPC mixture	Model type	Regression equation	R^2	*p*-value	TT_{LR}	TT_{HR}
Basis for TT_{LR}/TT_{HR} values: *Hyalella azteca* 28-d biomass						
Polychlorinated biphenyls (PCBs; homologs; µg/kg)						
Total PCBs (homologs)	log-logistic	$f(x) = 90.6/[1+(x/336000)^{0.732}]$	0.641	< 0.001	5,030	28,100
Total PCBs (homologs; 1% OC)	log-logistic	$f(x) = 93.3/[1+(x/147000)^{0.619}]$	0.596	< 0.001	2,350	10,700
Basis for TT_{LR}/TT_{HR} values: *Hyalella azteca* 42-d reproduction (normalized to survival)						
Metals (mg/kg)						
Lead, total	log-logistic	$f(x) = 192/[1+(x/11.1)^{0.987}]$	0.653	< 0.001	10.3	12.7
Mercury, total	log-logistic	$f(x) = 140/[1+(x/1.52)^{0.631}]$	0.605	< 0.001	0.362	0.607
Polycyclic aromatic hydrocarbons (PAHs; µg/kg)						
Total PAHs	log-logistic	$f(x) = 115/[1+(x/2950)^{1.38}]$	0.523	< 0.001	759	1,180
Polychlorinated biphenyls (PCBs; homologs; µg/kg)						
Total monochlorobiphenyls	log-logistic	$f(x) = 131/[1+(x/3120)^{0.453}]$	0.624	< 0.001	242	562
Total dichlorobiphenyls	log-logistic	$f(x) = 131/[1+(x/4640)^{0.505}]$	0.629	< 0.001	468	997
Total trichlorobiphenyl	log-logistic	$f(x) = 145/[1+(x/1690)^{0.442}]$	0.632	< 0.001	284	565
Total tetrachlorobiphenyl	log-logistic	$f(x) = 143/[1+(x/1220)^{0.484}]$	0.615	< 0.001	218	415
Total pentachlorobiphenyl	log-logistic	$f(x) = 141/[1+(x/1090)^{0.522}]$	0.622	< 0.001	201	373
Total hexachlorobiphenyls	log-logistic	$f(x) = 167/[1+(x/314)^{0.404}]$	0.63	< 0.001	119	217
Total heptachlorobiphenyls	log-logistic	$f(x) = 167/[1+(x/172)^{0.43}]$	0.621	< 0.001	69	121
Σ Mono- and di- chlorobiphenyls	log-logistic	$f(x) = 131/[1+(x/7790)^{0.481}]$	0.627	< 0.001	701	1,550
Σ Mono-, di-, and tri- chlorobiphenyls	log-logistic	$f(x) = 138/[1+(x/7710)^{0.44}]$	0.631	< 0.001	876	1,880
Σ Tetra- and penta- chlorobiphenyls	log-logistic	$f(x) = 142/[1+(x/2330)^{0.501}]$	0.618	< 0.001	421	792
Σ Tri-, tetra-, and penta- chlorobiphenyls	log-logistic	$f(x) = 142/[1+(x/4310)^{0.482}]$	0.625	< 0.001	728	1,400
Total PCBs (homologs)	linear	$f(x) = -26.8x + 172$	0.67	< 0.001	499	1,180
Total PCBs (homologs; 1% OC)	linear	$f(x) = -33.1x + 193$	0.7	< 0.001	659	1,320
Total PCBs (aroclors)	log-logistic	$f(x) = 149/[1+(x/4770)^{0.5}]$	0.715	< 0.001	1,170	2,080
Mixture models (no units)						
$PEC\text{-}Q_{TPAH}$	log-logistic	$f(x) = 115/[1+(x/0.129)^{1.38}]$	0.523	< 0.001	0.033	0.052
$PEC\text{-}Q_{TPCB}$	log-logistic	$f(x) = 229/[1+(x/0.172)^{0.233}]$	0.649	< 0.001	0.525	1.13
Mean PEC-Q	log-logistic	$f(x) = 157/[1+(x/2.44)^{0.379}]$	0.635	< 0.001	0.566	1.14
Mean $PEC\text{-}Q_{OCPEST}$	log-logistic	$f(x) = 159/[1+(x/0.108)^{0.949}]$	0.643	< 0.001	0.063	0.082

Table C5–3. Summary of the concentration-response models derived for sediment based on magnitude of toxicity to *Hyalella azteca* in 42-day whole-sediment toxicity tests and *Chironomus dilutus* in life-cycle whole-sediment toxicity tests. The toxicity thresholds derived using these regression equations also are presented.—Continued

[COPC, chemical of potential concern; PCB, polychlorinated biphenyl; %, percent; OC, organic carbon; PAH, polycyclic aromatic hydrocarbon; PEC-Q, probable effect concentration quotient; TPAH, total polycyclic aromatic hydrocarbon; TPCB, total polychlorinated biphenyl; OCPEST, organochlorine pesticides; ESB-TUFCV, equilibrium partitioning sediment benchmarks-toxic units/final chronic value; TT, toxicity threshold; LR, low risk; HR, high risk; d, day; µg/kg, microgram per kilogram; mg/kg, milligram per kilogram]

Toxicity test endpoint used to develop the relation/COPC/COPC mixture	Model type	Regression equation	R^2	*p*-value	TT_{LR}	TT_{HR}
Basis for TT_{LR}/TT_{HR} values: *Chironomus dilutus* percent emergence						
Metals (mg/kg)						
Lead, total	log-logistic	$f(x) = 128/[1+(x/27.4)^{0.794}]$	0.715	< 0.001	9.6	14.2
Mercury, total	log-logistic	$f(x) = 99.4/[1+(x/7.75)^{0.793}]$	0.702	< 0.001	0.503	1.27
Polycyclic aromatic hydrocarbons (PAHs; µg/kg)						
Total PAHs	log-logistic	$f(x) = 99.6/[1+(x/6480)^{0.686}]$	0.668	< 0.001	283	813
$\Sigma ESB\text{-}TU_{FCV}$	log-logistic	$f(x) = 97.3/[1+(x/1.91)^{0.8}]$	0.553	< 0.001	0.095	0.275
Polychlorinated biphenyls (PCBs; homologs; µg/kg)						
Total monochlorobiphenyls	log-logistic	$f(x) = 98.7/[1+(x/25100)^{0.503}]$	0.707	< 0.001	292	1,340
Total dichlorobiphenyls	log-logistic	$f(x) = 99.2/[1+(x/30100)^{0.527}]$	0.72	< 0.001	473	1,940
Total trichlorobiphenyl	log-logistic	$f(x) = 101/[1+(x/17500)^{0.539}]$	0.728	< 0.001	410	1,420
Total tetrachlorobiphenyl	log-logistic	$f(x) = 102/[1+(x/9380)^{0.533}]$	0.712	< 0.001	246	807
Total pentachlorobiphenyl	log-logistic	$f(x) = 99.5/[1+(x/7800)^{0.635}]$	0.725	< 0.001	260	821
Total hexachlorobiphenyls	log-logistic	$f(x) = 98.1/[1+(x/7430)^{0.702}]$	0.74	< 0.001	279	871
Total heptachlorobiphenyls	log-logistic	$f(x) = 98.3/[1+(x/3520)^{0.703}]$	0.738	< 0.001	137	420
Total octachlorobiphenyls	log-logistic	$f(x) = 97.8/[1+(x/1310)^{0.743}]$	0.749	< 0.001	56.2	169
Total nonchlorobiphenyls	log-logistic	$f(x) = 98.2/[1+(x/485)^{0.794}]$	0.757	< 0.001	27	73.4
Decachlorobiphenyl	log-logistic	$f(x) = 106/[1+(x/104)^{0.617}]$	0.714	< 0.001	6.95	16.4
Σ Mono- and di- chlorobiphenyls	log-logistic	$f(x) = 99/[1+(x/55600)^{0.519}]$	0.716	< 0.001	789	3,360
Σ Mono-, di-, and tri- chlorobiphenyls	log-logistic	$f(x) = 99.7/[1+(x/72300)^{0.521}]$	0.72	< 0.001	1,190	4,740
Σ Tri-, tetra-, and penta- chlorobiphenyls	log-logistic	$f(x) = 101/[1+(x/35000)^{0.56}]$	0.724	< 0.001	945	3,120
Σ Tetra- and penta- chlorobiphenyls	log-logistic	$f(x) = 101/[1+(x/17400)^{0.578}]$	0.719	< 0.001	526	1,670
Total PCBs (homologs)	log-logistic	$f(x) = 100/[1+(x/101000)^{0.529}]$	0.727	< 0.001	1,870	7,110
Total PCBs (homologs; 1% OC)	log-logistic	$f(x) = 100/[1+(x/51600)^{0.65}]$	0.721	< 0.001	2,000	5,950
Mixture models (no units)						
$PEC\text{-}Q_{TPAH}$	log-logistic	$f(x) = 99.6/[1+(x/0.284)^{0.686}]$	0.668	< 0.001	0.0124	0.0356
$PEC\text{-}Q_{TPCB}$	log-logistic	$f(x) = 100/[1+(x/149)^{0.529}]$	0.727	< 0.001	2.75	10.5
Mean PEC-Q	log-logistic	$f(x) = 100/[1+(x/49.5)^{0.541}]$	0.725	< 0.001	0.999	3.7
Mean $PEC\text{-}Q_{OCPEST}$	log-logistic	$f(x) = 133/[1+(x/0.174)^{0.56}]$	0.719	< 0.001	0.0489	0.0821
Basis for TT_{LR}/TT_{HR} values: *Chironomus dilutus* 13-d biomass						
Polychlorinated biphenyls (PCBs; homologs; µg/kg)						
Total PCBs (homologs)	log-logistic	$f(x) = 112/[1+(x/194000)^{0.866}]$	0.774	< 0.001	15,000	35,900
Total PCBs (homologs; 1% OC)	log-logistic	$f(x) = 113/[1+(x/83800)^{0.967}]$	0.785	< 0.001	9,260	19,400
Total PCBs (aroclors)	log-logistic	$f(x) = 103/[1+(x/105000)^{1.19}]$	0.777	< 0.001	3,890	19,300

Table C5–3. Summary of the concentration-response models derived for sediment based on magnitude of toxicity to *Hyalella azteca* in 42-day whole-sediment toxicity tests and *Chironomus dilutus* in life-cycle whole-sediment toxicity tests. The toxicity thresholds derived using these regression equations also are presented.—Continued

[COPC, chemical of potential concern; PCB, polychlorinated biphenyl; %, percent; OC, organic carbon; PAH, polycyclic aromatic hydrocarbon; PEC-Q, probable effect concentration quotient; TPAH, total polycyclic aromatic hydrocarbon; TPCB, total polychlorinated biphenyl; OCPEST, organochlorine pesticides; ESB-TUFCV, equilibrium partitioning sediment benchmarks-toxic units/final chronic value; TT, toxicity threshold; LR, low risk; HR, high risk; d, day; µg/kg, microgram per kilogram; mg/kg, milligram per kilogram]

Toxicity test endpoint used to develop the relation/COPC/COPC mixture	Model type	Regression equation	R^2	*p*-value	TT_{LR}	TT_{HR}
Basis for TT_{LR}/TT_{HR} values: *Chironomus dilutus* adult biomass						
Metals (mg/kg)						
Lead, total	log-logistic	$f(x) = 143/[1+(x/21.5)^{1.01}]$	0.725	< 0.001	9.48	12.9
Mercury, total	log-logistic	$f(x) = 118/[1+(x/3.67)^{0.681}]$	0.651	< 0.001	0.31	0.681
Polycyclic aromatic hydrocarbons (PAHs; µg/kg)						
Total PAHs	log-logistic	$f(x) = 105/[1+(x/4,630)^{1.11}]$	0.644	< 0.001	341	951
$\Sigma ESB\text{-}TU_{FCV}$	log-logistic	$f(x) = 103/[1+(x/1.52)^{1.04}]$	0.487	< 0.001	0.0608	0.246
Polychlorinated biphenyls (PCBs; homologs; µg/kg)						
Total monochlorobiphenyls	log-logistic	$f(x) = 115/[1+(x/8,770)^{0.463}]$	0.697	< 0.001	158	579
Total dichlorobiphenyls	log-logistic	$f(x) = 116/[1+(x/11,200)^{0.501}]$	0.712	< 0.001	310	982
Total trichlorobiphenyls	linear	$f(x) = -24.7x + 149$	0.73	< 0.001	101	255
Total tetrachlorobiphenyls	log-logistic	$f(x) = 120/[1+(x/3,710)^{0.54}]$	0.713	< 0.001	199	503
Total pentachlorobiphenyls	log-logistic	$f(x) = 119/[1+(x/3,010)^{0.560}]$	0.697	< 0.001	164	413
Total hexachlorobiphenyls	log-logistic	$f(x) = 123/[1+(x/2,170)^{0.503}]$	0.686	< 0.001	123	306
Total heptachlorobiphenyls	log-logistic	$f(x) = 127/[1+(x/871)^{0.484}]$	0.675	< 0.001	61.1	144
Total octachlorobiphenyls	log-logistic	$f(x) = 129/[1+(x/294)^{0.477}]$	0.677	< 0.001	23	52.7
Total nonachlorobiphenyls	log-logistic	$f(x) = 126/[1+(x/152)^{0.576}]$	0.696	< 0.001	15.3	31.9
Decachlorobiphenyl	log-logistic	$f(x) = 147/[1+(x/20.7)^{0.481}]$	0.677	< 0.001	4.45	8.23
Σ Mono- and di- chlorobiphenyls	linear	$f(x) = -20.0x + 138$	0.71	< 0.001	84.1	265
Σ Mono-, di-, and tri- chlorobiphenyls	log-logistic	$f(x) = 117/[1+(x/25,900)^{0.491}]$	0.714	< 0.001	752	2,330
Σ Tetra- and penta- chlorobiphenyls	log-logistic	$f(x) = 119/[1+(x/6,880)^{0.554}]$	0.708	< 0.001	363	924
Σ Tri-, tetra-, and penta- chlorobiphenyls	log-logistic	$f(x) = 119/[1+(x/13,700)^{0.544}]$	0.717	< 0.001	685	1,770
Total PCBs (homologs)	log-logistic	$f(x) = 119/[1+(x/34,500)^{0.478}]$	0.715	< 0.001	1,140	3,370
Total PCBs (homologs; 1% OC)	log-logistic	$f(x) = 119/[1+(x/21,700)^{0.585}]$	0.694	< 0.001	1,340	3,240
Mixture models (no units)						
$PEC\text{-}Q_{TPAH}$	log-logistic	$f(x) = 105/[1+(x/0.203)^{1.11}]$	0.643	< 0.001	0.015	0.0417
$PEC\text{-}Q_{TPCB}$	log-logistic	$f(x) = 119/[1+(x/51.1)^{0.478}]$	0.714	< 0.001	1.69	4.99
Mean PEC-Q	log-logistic	$f(x) = 119/[1+(x/19)^{0.529}]$	0.709	< 0.001	0.873	2.32
Mean $PEC\text{-}Q_{OCPEST}$	log-logistic	$f(x) = 227/[1+(x/0.0281)_{0.53}]$	0.72	< 0.001	0.0449	0.0631

Table C5–4. Summary of the concentration-response models derived for pore water based on magnitude of toxicity to *Hyalella azteca* in 42-day whole-sediment toxicity tests and *Chironomus dilutus* in life-cycle whole-sediment toxicity tests. The toxicity thresholds derived using these regression equations are also presented.

[COPC, chemical of potential concern; PCB, polychlorinated biphenyl; TT, toxicity threshold; LR, low risk; HR, high risk; µg/L, microgram per liter]

Toxicity test endpoint used to develop the relation/COPC/COPC mixture	Model type	Regression equation	R^2	*p*-value	TT_{LR}	TT_{HR}
Basis for TT_{LR}/TT_{HR} values: *Hyalella azteca* 42-d reproduction (normalized to survival)						
Polychlorinated biphenyls (PCBs; homologs; µg/L)						
Total monochlorobiphenyls	log-logistic	$f(x) = 122/[1+(x/9.01)^{0.494}]$	0.579	< 0.001	0.435	1.14
Total dichlorobiphenyls	log-logistic	$f(x) = 139/[1+(x/0.112)^{0.254}]$	0.622	< 0.001	0.00287	0.0106
Total trichlorobiphenyls	log-logistic	$f(x) = 144/[1+(x/0.876)^{0.35}]$	0.618	< 0.001	0.0863	0.208
Total pentachlorobiphenyls	log-logistic	$f(x) = 145/[1+(x/0.0388)^{0.453}]$	0.619	< 0.001	0.0068	0.0133
Total heptachlorobiphenyls	log-logistic	$f(x) = 145/[1+(x/0.000808)^{0.433}]$	0.569	< 0.001	0.000131	0.000264
Total octachlorobiphenyls	log-logistic	$f(x) = 116/[1+(x/0.000202)^{1.03}]$	0.538	< 0.001	0.0000348	0.0000613
Total nonachlorobiphenyls	log-logistic	$f(x) = 116/[1+(x/0.0000245)^{2.15}]$	0.433	< 0.001	0.0000106	0.0000138
Σ Mono- and di- chlorobiphenyls	log-logistic	$f(x) = 117/[1+(x/19.5)^{0.583}]$	0.564	< 0.001	0.967	2.53
Σ Mono-, di-, and tri- chlorobiphenyls	log-logistic	$f(x) = 130/[1+(x/7.67)^{0.34}]$	0.581	< 0.001	0.231	0.727
Σ Tri-, tetra-, and penta- chlorobiphenyls	log-logistic	$f(x) = 135/[1+(x/0.385)^{0.453}]$	0.555	< 0.001	0.0389	0.085
Σ Tetra- and penta- chlorobiphenyls	log-logistic	$f(x) = 133/[1+(x/2.17)^{0.402}]$	0.57	< 0.001	0.142	0.354
Basis for TT_{LR}/TT_{HR} values: *Chironomus dilutus* percent emergence						
Polychlorinated biphenyls (PCBs; homologs; µg/L)						
Total monochlorobiphenyls	log-logistic	$f(x) = 93/[1+(x/67.2)^{0.859}]$	0.707	< 0.001	1.71	7.87
Total dichlorobiphenyls	log-logistic	$f(x) = 98.4/[1+(x/6.64)^{0.319}]$	0.698	< 0.001	0.00536	0.0625
Total trichlorobiphenyls	log-logistic	$f(x) = 96.2/[1+(x/15.2)^{0.97}]$	0.688	< 0.001	1.1	2.87
Total pentachlorobiphenyls	log-logistic	$f(x) = 101/[1+(x/0.32)^{0.717}]$	0.677	< 0.001	0.0191	0.0484
Total hexachlorobiphenyls	log-logistic	$f(x) = 91/[1+(x/0.0895)^{0.729}]$	0.63	< 0.001	0.000423	0.00565
Total heptachlorobiphenyls	log-logistic	$f(x) = 98/[1+(x/0.00894)^{0.712}]$	0.633	< 0.001	0.00346	0.00107
Total octachlorobiphenyls	log-logistic	$f(x) = 91.6/[1+(x/0.000733)^{0.861}]$	0.644	< 0.001	0.000011	0.0000753
Σ Mono- and di- chlorobiphenyls	log-logistic	$f(x) = 93/[1+(x/53.4)^{0.722}]$	0.642	< 0.001	0.675	4.16
Σ Tri-, tetra-, and penta- chlorobiphenyls	log-logistic	$f(x) = 101/[1+(x/0.848)^{0.471}]$	0.656	< 0.001	0.0116	0.0478
Σ Tetra- and penta- chlorobiphenyls	log-logistic	$f(x) = 95.1/[1+(x/14.5)^{0.981}]$	0.63	< 0.001	0.91	2.59
Total PCBs (homologs)	log-logistic	$f(x) = 95/[1+(x/98.4)^{0.874}]$	0.719	< 0.001	4.31	14.1
Basis for TT_{LR}/TT_{HR} values: *Chironomus dilutus* adult biomass						
Polychlorinated biphenyls (PCBs; homologs; µg/L)						
Total monochlorobiphenyls	log-logistic	$f(x) = 106/[1+(x/23.2)^{0.708}]$	0.611	< 0.001	0.492	2.12
Total dichlorobiphenyls	linear	$f(x) = -12.1x + 62$	0.54	< 0.001	0.000796	0.00529
Total trichlorobiphenyls	log-logistic	$f(x) = 116/[1+(x/5.62)^{0.653}]$	0.638	< 0.001	0.359	0.868
Total tetrachlorobiphenyls	linear	$f(x) = -16.8x + 31.3$	0.73	< 0.001	0.0000872	0.000341
Total pentachlorobiphenyls	log-logistic	$f(x) = 118/[1+(x/0.13)^{0.783}]$	0.649	< 0.001	0.0152	0.0301
Total heptachlorobiphenyls	log-logistic	$f(x) = 139/[1+(x/0.000716)^{0.37}]$	0.603	< 0.001	0.000059	0.000144
Σ Mono- and di- chlorobiphenyls	log-logistic	$f(x) = 109/[1+(x/22.2)^{0.609}]$	0.645	< 0.001	0.469	1.81
Σ Mono-, di-, and tri- chlorobiphenyls	log-logistic	$f(x) = 115/[1+(x/21.8)^{0.519}]$	0.661	< 0.001	0.606	1.93
Σ Tetra- and penta- chlorobiphenyls	log-logistic	$f(x) = 116/[1+(x/6.47)^{0.685}]$	0.648	< 0.001	0.47	1.09
Total PCBs (homologs)	log-logistic	$f(x) = 117/[1+(x/24.7)^{0.529}]$	0.652	< 0.001	0.924	2.64

Table C5–5. Reliability of the sediment toxicity thresholds that were derived based on the results of life-cycle whole-sediment toxicity tests with *Chironomus dilutus* (endpoint: percent emergence of adults).

[ESB-TU_{FCV}, equilibrium partitioning sediment benchmarks-toxic units/final chronic value; PCB, polychlorinated biphenyl; %, percent; OC, organic carbon; PEC-Q, probable effect concentration quotient; TPAH, total polycyclic aromatic hydrocarbon; TPCB, total polychlorinated biphenyl; OCPEST, organochlorine pesticides; *n*, number of samples; TT, toxicity threshold; LR, low risk; HR, high risk; mg/kg, milligram per kilogram; µg, microgram; PAH, polycyclic aromatic hydrocarbon; ND, no data; Bolded values represent reliable TTs. TT's were considered to be reliable if the incidence of toxicity was ≤20% below the TT, if the incidence of toxicity was >50% above the TT, and if the overall correct classification rate was >80%]

Chemical of potential concern	*n*	TT_{LR}	TT_{HR}	Percent incidence of toxicity (number of samples in parentheses)						
				$<TT_{LR}$	$\geq TT_{LR}$	Correct classification rate for TT_{LR}	TT_{LR}-TT_{HR}	$\leq TT_{HR}$	$>TT_{HR}$	Correct classification rate for TT_{HR}
Metals (mg/kg)										
Lead, total	27	9.6	14.2	**0 (0/7)**	**85 (17/20)**	**89**	50 (1/2)	**11 (1/9)**	**89 (16/18)**	**89**
Mercury, total	27	0.503	1.27	22 (2/9)	83 (15/18)	81	0 (0/2)	**18 (2/11)**	**94 (15/16)**	**89**
Polycyclic aromatic hydrocarbons (PAHs; µg/kg)										
Total PAHs	27	283	813	29 (2/7)	75 (15/20)	74	50 (2/4)	36 (4/11)	81 (13/16)	74
Σ$ESB\text{-}TU_{FCV}$	27	0.0952	0.275	29 (2/7)	75 (15/20)	74	50 (2/4)	36 (4/11)	81 (13/16)	74
Polychlorinated biphenyls (PCBs; homologs; µg/kg)										
Total monochlorobiphenyls	27	292	1,340	**20 (2/10)**	**88 (15/17)**	**85**	0 (0/1)	**18 (2/11)**	**94 (15/16)**	**89**
Total dichlorobiphenyls	27	473	1,940	**20 (2/10)**	**88 (15/17)**	**85**	0 (0/1)	**18 (2/11)**	**94 (15/16)**	**89**
Total trichlorobiphenyls	27	410	1,420	**20 (2/10)**	**88 (15/17)**	**85**	0 (0/1)	**18 (2/11)**	**94 (15/16)**	**89**
Total tetrachlorobiphenyls	27	246	807	**20 (2/10)**	**88 (15/17)**	**85**	50 (1/2)	25 (3/12)	93 (14/15)	85
Total pentachlorobiphenyls	27	260	821	**20 (2/10)**	**88 (15/17)**	**85**	50 (1/2)	25 (3/12)	93 (14/15)	85
Total hexachlorobiphenyls	27	279	871	**20 (2/10)**	**88 (15/17)**	**85**	50 (1/2)	25 (3/12)	93 (14/15)	85
Total heptachlorobiphenyls	27	137	420	**20 (2/10)**	**88 (15/17)**	**85**	33 (1/3)	23 (3/13)	100 (14/14)	89
Total octachlorobiphenyls	27	56.2	169	**20 (2/10)**	**88 (15/17)**	**85**	67 (2/3)	31 (4/13)	93 (13/14)	81
Total nonachlorobiphenyls	27	27	73.4	**20 (2/10)**	**88 (15/17)**	**85**	67 (2/3)	31 (4/13)	93 (13/14)	81
Decachlorobiphenyl	27	6.95	16.4	**11 (1/9)**	**89 (16/18)**	**89**	67 (2/3)	25 (3/12)	93 (14/15)	85
Total PCBs (homologs)	27	1,870	7,110	**20 (2/10)**	**88 (15/17)**	**85**	0 (0/1)	**18 (2/11)**	**94 (15/16)**	**89**
Total PCBs (homologs; 1% OC)	27	2,000	5,950	**20 (2/10)**	**88 (15/17)**	**85**	ND	**20 (2/10)**	**88 (15/17)**	**85**
Σ Mono- and di- chlorobiphenyls	27	789	3,360	**20 (2/10)**	**88 (15/17)**	**85**	0 (0/1)	**18 (2/11)**	**94 (15/16)**	**89**
Σ Mono-, di-, and tri- chlorobiphenyls	27	1,190	4,740	**20 (2/10)**	**88 (15/17)**	**85**	0 (0/1)	**18 (2/11)**	**94 (15/16)**	**89**
Σ Tetra- and penta- chlorobiphenyls	27	526	1,670	**20 (2/10)**	**88 (15/17)**	**85**	50 (1/2)	25 (3/12)	93 (14/15)	85
Σ Tri-, tetra-, and penta- chlorobiphenyls	27	945	3,120	**20 (2/10)**	**88 (15/17)**	**85**	50 (1/2)	25 (3/12)	93 (14/15)	85
Mixture models (no units)										
$PEC\text{-}Q_{TPAH}$	27	0.0124	0.0356	29 (2/7)	75 (15/20)	74	50 (2/4)	36 (4/11)	81 (13/16)	74
$PEC\text{-}Q_{TPCB}$	27	2.75	10.5	**20 (2/10)**	**88 (15/17)**	**85**	0 (0/1)	**18 (2/11)**	**94 (15/16)**	**89**
Mean PEC-Q	27	0.999	3.7	**20 (2/10)**	**88 (15/17)**	**85**	0 (0/1)	**18 (2/11)**	**94 (15/16)**	**89**
Mean $PEC\text{-}Q_{OCPEST}$	27	0.0489	0.0821	**11 (1/9)**	**89 (16/18)**	**89**	100 (1/1)	**20 (2/10)**	**88 (15/17)**	**85**

Table C5–6. Reliability of the sediment toxicity thresholds that were derived based on the results of life-cycle whole-sediment toxicity tests with *Chironomus dilutus* (endpoint: adult biomass).

[ESB-TU_{FCV}, equilibrium partitioning sediment benchmarks-toxic units/final chronic value; PCB, polychlorinated biphenyl; %, percent; OC, organic carbon; PEC-Q, probable effect concentration quotient; TPAH, total polycyclic aromatic hydrocarbon; TPCB, total polychlorinated biphenyl; OCPEST, organochlorine pesticides; *n*, number of samples; TT, toxicity threshold; LR, low risk; HR, high risk; mg/kg, milligram per kilogram; µg, microgram; PAH, polycyclic aromatic hydrocarbon; ND, no data; Bolded values represent reliable TTs. TT's were considered to be reliable if the incidence of toxicity was ≤20% below the TT, if the incidence of toxicity was >50% above the TT, and if the overall correct classification rate was >80%]

Chemical of potential concern	*n*	TT_{LR}	TT_{HR}	Percent incidence of toxicity (number of samples in parentheses)						
				$<TT_{LR}$	$\geq TT_{LR}$	Correct classification rate for TT_{LR}	TT_{LR}-TT_{HR}	$\leq TT_{HR}$	$>TT_{HR}$	Correct classification rate for TT_{HR}
Metals (mg/kg)										
Lead, total	27	9.48	12.9	**0 (0/7)**	**85 (17/20)**	**89**	50 (1/2)	**11 (1/9)**	**89 (16/18)**	**89**
Mercury, total	27	0.31	0.681	25 (2/8)	79 (15/19)	78	50 (1/2)	30 (3/10)	82 (14/17)	78
Polycyclic aromatic hydrocarbons (PAHs; µg/kg)										
Total PAHs	27	341	951	29 (2/7)	75 (15/20)	74	60 (3/5)	42 (5/12)	80 (12/15)	70
Σ$ESB\text{-}TU_{FCV}$	27	0.0608	0.246	29 (2/7)	75 (15/20)	74	100 (3/3)	50 (5/10)	71 (12/17)	63
Polychlorinated biphenyls (PCBs; homologs; µg/kg)										
Total monochlorobiphenyls	27	158	579	**20 (2/10)**	**88 (15/17)**	**85**	No Data	**20 (2/10)**	**88 (15/17)**	**85**
Total dichlorobiphenyls	27	310	982	**20 (2/10)**	**88 (15/17)**	**85**	No Data	**20 (2/10)**	**88 (15/17)**	**85**
Total trichlorobiphenyls	27	101	255	**11 (1/9)**	**89 (16/18)**	**89**	No Data	**11 (1/9)**	**89 (16/18)**	**89**
Total tetrachlorobiphenyls	27	199	503	**20 (2/10)**	**88 (15/17)**	**85**	No Data	**20 (2/10)**	**88 (15/17)**	**85**
Total pentachlorobiphenyls	27	164	413	**20 (2/10)**	**88 (15/17)**	**85**	No Data	**20 (2/10)**	**88 (15/17)**	**85**
Total hexachlorobiphenyls	27	123	306	**20 (2/10)**	**88 (15/17)**	**85**	No Data	**20 (2/10)**	**88 (15/17)**	**85**
Total heptachlorobiphenyls	27	61.1	144.	**20 (2/10)**	**88 (15/17)**	**85**	No Data	**20 (2/10)**	**88 (15/17)**	**85**
Total octachlorobiphenyls	27	23	52.7	**11 (1/9)**	**89 (16/18)**	**89**	100 (1/1)	**20 (2/10)**	**88 (15/17)**	**85**
Total nonachlorobiphenyls	27	15.3	31.9	**20 (2/10)**	**88 (15/17)**	**85**	No Data	**20 (2/10)**	**88 (15/17)**	**85**
Decachlorobiphenyl	27	4.45	8.23	**11 (1/9)**	**89 (16/18)**	**89**	100 (1/1)	**20 (2/10)**	**88 (15/17)**	**85**
Total PCBs (homologs)	27	1,140	3,370	**20 (2/10)**	**88 (15/17)**	**85**	No Data	**20 (2/10)**	**88 (15/17)**	**85**
Total PCBs (homologs; 1% OC)	27	1,340	3,240	22 (2/9)	83 (15/18)	81	0 (0/1)	**20 (2/10)**	**88 (15/17)**	**85**
Σ Mono- and di- chlorobiphenyls	27	84.1	265	**11 (1/9)**	**89 (16/18)**	**89**	100 (1/1)	**20 (2/10)**	**88 (15/17)**	**85**
Σ Mono-, di-, and tri- chlorobiphenyls	27	752	2,330	**20 (2/10)**	**88 (15/17)**	**85**	No Data	**20 (2/10)**	**88 (15/17)**	**85**
Σ Tetra- and penta- chlorobiphenyls	27	363	924	**20 (2/10)**	**88 (15/17)**	**85**	No Data	**20 (2/10)**	**88 (15/17)**	**85**
Σ Tri-, tetra-, and penta- chlorobiphenyls	27	685	1,770	**20 (2/10)**	**88 (15/17)**	**85**	No Data	**20 (2/10)**	**88 (15/17)**	**85**
Mixture models (no units)										
$PEC\text{-}Q_{TPAH}$	27	0.015	0.0417	29 (2/7)	75 (15/20)	74	60 (3/5)	42 (5/12)	80 (12/15)	70
$PEC\text{-}Q_{TPCB}$	27	1.69	4.99	**20 (2/10)**	**88 (15/17)**	**85**	No Data	**20 (2/10)**	**88 (15/17)**	**85**
Mean PEC-Q	27	0.873	2.32	**20 (2/10)**	**88 (15/17)**	**85**	No Data	**20 (2/10)**	**88 (15/17)**	**85**
Mean $PEC\text{-}Q_{OCPEST}$	27	0.0449	0.0631	**11 (1/9)**	**89 (16/18)**	**89**	No Data	**11 (1/9)**	**89 (16/18)**	**89**

Table C5–7. Reliability of the sediment toxicity thresholds that were derived based on the results of 42-day whole-sediment toxicity tests with *Hyalella azteca* (endpoint: reproduction; young per female normalized to percent survival).

[PAH, polycyclic aromatic hydrocarbon; PCB, polychlorinated biphenyl; %, percent; OC, organic carbon; PEC-Q, probable effect concentration quotient; TPAH, total polycyclic aromatic hydrocarbon; TPCB, total polychlorinated biphenyl; OCPEST, organochlorine pesticides; *n*, number of samples; TT, toxicity threshold; LR, low risk; HR, high risk; mg/kg, milligram per kilogram; µg, microgram; ND, no data; Bolded values represent reliable TTs. TT's were considered to be reliable if the incidence of toxicity was ≤20% below the TT, if the incidence of toxicity was >50% above the TT, and if the overall correct classification rate was >80%]

Chemical of potential concern	*n*	TT_{LR}	TT_{HR}	Percent incidence of toxicity (number of samples in parentheses)						
				$<TT_{LR}$	$\geq TT_{LR}$	Correct classification rate for TT_{LR}	TT_{LR}-TT_{HR}	$\leq TT_{HR}$	$>TT_{HR}$	Correct classification rate for TT_{HR}
Metals (mg/kg)										
Lead, total	25	10.3	12.7	**0 (0/8)**	**94 (16/17)**	**96**	100 (1/1)	**11 (1/9)**	**94 (15/16)**	**92**
Mercury, total	25	0.362	0.607	22 (2/9)	88 (14/16)	84	100 (1/1)	30 (3/10)	87 (13/15)	80
Polycyclic aromatic hydrocarbons (PAHs; µg/kg)										
Total PAHs	25	759	1,180	30 (3/10)	87 (13/15)	80	33 (1/3)	31 (4/13)	100 (12/12)	84
Polychlorinated biphenyls (PCBs; homologs; µg/kg)										
Total monochlorobiphenyls	25	242	562	**20 (2/10)**	**93 (14/15)**	**88**	ND	**20 (2/10)**	**93 (14/15)**	**88**
Total dichlorobiphenyls	25	468	997	**20 (2/10)**	**93 (14/15)**	**88**	ND	**20 (2/10)**	**93 (14/15)**	**88**
Total trichlorobiphenyls	25	284	565	**20 (2/10)**	**93 (14/15)**	**88**	ND	**20 (2/10)**	**93 (14/15)**	**88**
Total tetrachlorobiphenyls	25	218	415	**20 (2/10)**	**93 (14/15)**	**88**	ND	**20 (2/10)**	**93 (14/15)**	**88**
Total pentachlorobiphenyls	25	201	373	**20 (2/10)**	**93 (14/15)**	**88**	ND	**20 (2/10)**	**93 (14/15)**	**88**
Total hexachlorobiphenyls	25	119	217	**20 (2/10)**	**93 (14/15)**	**88**	ND	**20 (2/10)**	**93 (14/15)**	**88**
Total heptachlorobiphenyls	25	69	121	**20 (2/10)**	**93 (14/15)**	**88**	ND	**20 (2/10)**	**93 (14/15)**	**88**
Σ Mono- and di- chlorobiphenyls	25	701	1,550	**20 (2/10)**	**93 (14/15)**	**88**	ND	**20 (2/10)**	**93 (14/15)**	**88**
Σ Mono-, di-, and tri- chlorobiphenyls	25	876	1,880	**20 (2/10)**	**93 (14/15)**	**88**	ND	**20 (2/10)**	**93 (14/15)**	**88**
Σ Tetra- and penta- chlorobiphenyls	25	421	792	**20 (2/10)**	**93 (14/15)**	**88**	ND	**20 (2/10)**	**93 (14/15)**	**88**
Σ Tri-, tetra-, and penta- chlorobiphenyls	25	728	1,400	**20 (2/10)**	**93 (14/15)**	**88**	ND	**20 (2/10)**	**93 (14/15)**	**88**
Total PCBs (homologs)	25	499	1,180	**11 (1/9)**	**94 (15/16)**	**92**	100 (1/1)	**20 (2/10)**	**93 (14/15)**	**88**
Total PCBs (homologs; 1% OC)	25	659	1,320	**12 (1/8)**	**88 (15/17)**	**88**	100 (1/1)	**22 (2/9)**	**88 (14/16)**	**84**
Total PCBs (aroclors)	18	1,170	2,080	33 (1/3)	93 (14/15)	89	ND	33 (1/3)	93 (14/15)	89
Mixture models (no units)										
$PEC\text{-}Q_{TPAH}$	25	0.0332	0.0515	30 (3/10)	87 (13/15)	80	33 (1/3)	31 (4/13)	100 (12/12)	84
$PEC\text{-}Q_{TPCB}$	25	0.525	1.13	**11 (1/9)**	**94 (15/16)**	**92**	ND	**11 (1/9)**	**94 (15/16)**	**92**
Mean PEC-Q	25	0.566	1.14	**20 (2/10)**	**93 (14/15)**	**88**	ND	**20 (2/10)**	**93 (14/15)**	**88**
Mean $PEC\text{-}Q_{OCPEST}$	25	0.0625	0.0822	57 (12/21)	100 (4/4)	52	ND	**20 (2/10)**	**93 (14/15)**	**88**

Table C5–8. Reliability of the pore-water toxicity thresholds that were derived based on the results of life-cycle whole-sediment toxicity tests *Chironomus dilutus* (endpoint: percent emergence of adults).

[PCB, polychlorinated biphenyl; *n*, number of samples; TT, toxicity threshold; LR, low risk; HR, high risk; µg/L, microgram per liter; ND, no data; Bolded values represent reliable TTs. TT's were considered to be reliable if the incidence of toxicity was ≤20% below the TT, if the incidence of toxicity was >50% above the TT, and if the overall correct classification rate was >80%]

Chemical of potential concern	*n*	TT_{LR}	TT_{HR}	Percent incidence of toxicity (number of samples in parentheses)						
				$<TT_{LR}$	$\geq TT_{LR}$	Correct classification rate for TT_{LR}	TT_{LR}-TT_{HR}	$\leq TT_{HR}$	$>TT_{HR}$	Correct classification rate for TT_{HR}
Polychlorinated biphenyls (PCBs; homologs; µg/L)										
Total monochlorobiphenyls	27	1.71	7.87	**20 (2/10)**	**88 (15/17)**	**85**	75 (3/4)	36 (5/14)	92 (12/13)	78
Total dichlorobiphenyls	27	0.00536	0.0625	**9 (1/11)**	**100 (16/16)**	**96**	100 (2/2)	23 (3/13)	100 (14/14)	89
Total trichlorobiphenyls	27	1.1	2.87	**20 (2/10)**	**88 (15/17)**	**85**	0 (0/1)	**18 (2/11)**	**94 (15/16)**	**89**
Total pentachlorobiphenyls	27	0.0191	0.0484	**20 (2/10)**	**88 (15/17)**	**85**	ND	**20 (2/10)**	**88 (15/17)**	**85**
Total hexachlorobiphenyls	27	0.000423	0.00565	29 (4/14)	100 (13/13)	85	ND	29 (4/14)	100 (13/13)	85
Total heptachlorobiphenyls	27	0.00346	0.00107	33 (5/15)	100 (12/12)	81	ND	**18 (2/11)**	**94 (15/16)**	**89**
Total octachlorobiphenyls	27	0.000011	0.0000753	29 (4/14)	100 (13/13)	85	ND	29 (4/14)	100 (13/13)	85
Σ Mono- and di- chlorobiphenyls	27	0.675	4.16	**20 (2/10)**	**88 (15/17)**	**85**	100 (1/1)	27 (3/11)	88 (14/16)	81
Σ Tetra- and penta- chlorobiphenyls	27	0.91	2.59	**20 (2/10)**	**88 (15/17)**	**85**	0 (0/1)	**18 (2/11)**	**94 (15/16)**	**89**
Σ Tri-, tetra-, and penta- chlorobiphenyls	27	0.0116	0.0478	**12 (1/8)**	**84 (16/19)**	**85**	50 (1/2)	**20 (2/10)**	**88 (15/17)**	**85**
Total PCBs (homologs)	27	4.31	14.1	**20 (2/10)**	**88 (15/17)**	**85**	67 (2/3)	31 (4/13)	93 (13/14)	81

Table C5–9. Reliability of the pore-water toxicity thresholds that were derived based on the results of life-cycle whole-sediment toxicity tests with *Chironomus dilutus* (endpoint: adult biomass).

[PCB, polychlorinated biphenyl; *n*, number of samples; TT, toxicity threshold; LR, low risk; HR, high risk; µg/L, micrograms per liter; ND, no data; Bolded values represent reliable TTs. TT's were considered to be reliable if the incidence of toxicity was ≤20% below the TT, if the incidence of toxicity was >50% above the TT, and if the overall correct classification rate was >80%]

Chemical of potential concern	*n*	TT_{LR}	TT_{HR}	Percent incidence of toxicity (number of samples in parentheses)						
				$<TT_{LR}$	$\geq TT_{LR}$	Correct classification rate for TT_{LR}	TT_{LR}-TT_{HR}	$\leq TT_{HR}$	$>TT_{HR}$	Correct classification rate for TT_{HR}
Polychlorinated biphenyls (PCBs; homologs; µg/L)										
Total monochlorobiphenyls	27	0.492	2.12	27 (3/11)	88 (14/16)	81	ND	27 (3/11)	88 (14/16)	81
Total dichlorobiphenyls	27	0.000796	0.00529	**14 (1/7)**	**80 (16/20)**	**81**	ND	**14 (1/7)**	**80 (16/20)**	**81**
Total trichlorobiphenyls	27	0.359	0.868	22 (2/9)	83 (15/18)	81	0 (0/1)	**20 (2/10)**	**88 (15/17)**	**85**
Total tetrachlorobiphenyls	27	0.0000872	0.000341	**14 (1/7)**	**80 (16/20)**	**81**	33 (1/3)	20 (2/10)	88 (15/17)	85
Total pentachlorobiphenyls	27	0.0152	0.0301	**12 (1/8)**	**84 (16/19)**	**85**	50 (1/2)	**20 (2/10)**	**88 (15/17)**	**85**
Total heptachlorobiphenyls	27	0.000059	0.000144	**11 (1/9)**	**89 (16/18)**	**89**	ND	**11 (1/9)**	**89 (16/18)**	**89**
Σ Mono- and di- chlorobiphenyls	27	0.469	1.81	**20 (2/10)**	**88 (15/17)**	**85**	ND	**20 (2/10)**	**88 (15/17)**	**85**
Σ Mono-, di-, and tri- chlorobiphenyls	27	0.606	1.93	**20 (2/10)**	**88 (15/17)**	**85**	ND	**20 (2/10)**	**88 (15/17)**	**85**
Σ Tetra- and penta- chlorobiphenyls	27	0.47	1.09	**20 (2/10)**	**88 (15/17)**	**85**	ND	**20 (2/10)**	**88 (15/17)**	**85**
Total PCBs (homologs)	27	0.924	2.64	**20 (2/10)**	**88 (15/17)**	**85**	ND	**20 (2/10)**	**88 (15/17)**	**85**

Table C5–10. Reliability of the pore-water toxicity thresholds that were derived based on the results of 42-day whole-sediment toxicity tests with *Hyalella azteca* (endpoint: reproduction; young per female; normalized to percent survival).

[*n*, number of samples; TT, toxicity threshold; LR, low risk; HR, high risk; µg/L, microgram per liter; ND, no data; Bolded values represent reliable TTs. TT's were considered to be reliable if the incidence of toxicity was ≤20% below the TT, if the incidence of toxicity was >50% above the TT, and if the overall correct classification rate was >80%]

Chemical of potential concern	*n*	TT_{LR}	TT_{HR}	Percent incidence of toxicity (number of samples in parentheses)						
				$<TT_{LR}$	$\geq TT_{LR}$	Correct classification rate for TT_{LR}	TT_{LR}-TT_{HR}	$\leq TT_{HR}$	$>TT_{HR}$	Correct classification rate for TT_{HR}
Polychlorinated biphenyls (PCBs; homologs; µg/L)										
Total monochlorobiphenyls	25	0.435	1.14	**20 (2/10)**	**93 (14/15)**	**88**	ND	**20 (2/10)**	**93 (14/15)**	**88**
Total dichlorobiphenyls	25	0.00287	0.0106	**20 (2/10)**	**93 (14/15)**	**88**	0 (0/1)	**18 (2/11)**	**100 (14/14)**	**92**
Total trichlorobiphenyls	25	0.0863	0.208	**12 (1/8)**	**88 (15/17)**	**88**	ND	**12 (1/8)**	**88 (15/17)**	**88**
Total pentachlorobiphenyls	25	0.0068	0.0133	**14 (1/7)**	**83 (15/18)**	**84**	0 (0/1)	**12 (1/8)**	**88 (15/17)**	**88**
Total heptachlorobiphenyls	25	0.000131	0.000264	**11 (1/9)**	**94 (15/16)**	**92**	100 (1/1)	**20 (2/10)**	**93 (14/15)**	**88**
Total octachlorobiphenyls	25	0.0000348	0.0000613	31 (4/13)	100 (12/12)	84	ND	31 (4/13)	100 (12/12)	84
Total nonachlorobiphenyls	25	0.0000106	0.0000138	36 (5/14)	100 (11/11)	8	ND	36 (5/14)	100 (11/11)	8
Σ Mono- and di- chlorobiphenyls	25	0.967	2.53	**20 (2/10)**	**93 (14/15)**	**88**	ND	**20 (2/10)**	**93 (14/15)**	**88**
Σ Mono-, di-, and tri- chlorobiphenyls	25	0.231	0.727	**12 (1/8)**	**88 (15/17)**	**88**	100 (1/1)	22 (2/9)	88 (14/16)	84
Σ Tetra- and penta- chlorobiphenyls	25	0.142	0.354	**0 (0/6)**	**84 (16/19)**	**88**	67 (2/3)	22 (2/9)	88 (14/16)	84
Σ Tri-, tetra-, and penta- chlorobiphenyls	25	0.0389	0.085	**0 (0/6)**	**84 (16/19)**	**88**	67 (2/3)	22 (2/9)	88 (14/16)	84

Table C5–11. Comparability of site-specific toxicity thresholds to published toxicity thresholds for polychlorinated biphenyls.

[LC_{50}, lethal concentration affecting 50 percent of the population; LOEC, lowest-observed effect concentration; LD_{50}, lethal dose affecting 50 percent of the population; PCB, polychlorinated biphenyl; DW, dry weight; NA, not available; µg/kg, microgram per kilogram; OC, organic carbon]

Approach for deriving toxicity threshold	Chemical of potential concern		Reference
	Total PCBs (homologs) (µg/kg DW)	Total PCBs (homologs) (µg/kg at 1 percent OC)	
Reference envelope approach			
Hyalella azteca 42-day reproduction	499–1,180	659–1,320	This study.
Chironomus dilutus adult emergence	1,870–7,110	2,000–5,950	This study.
Chironomus dilutus adult biomass	1,140–3,370	1,340–3,240	This study.
Empirically based sediment quality guidelines			
Threshold effect concentration for freshwater organisms	40	40	MacDonald and others (2000b).
Mid-range effect concentration for freshwater organisms	400	400	MacDonald and others (2000b).
Probable effect concentration for freshwater organisms	1,700	1,700	MacDonald and others (2000b).
Equilibrium-partitioning based sediment quality guidelines			
Sediment quality guideline for freshwater organisms	70	70	Bolton and others (1985).
Sediment quality guideline for freshwater organisms	193	193	New York State Department of Environmental Conservation (1999).
Lowest sediment quality benchmark	NA	2,100–15,000	Fuchsman and others (2006).
Spiked-sediment toxicity testing			
Rhepoxynius abronius 10-day LC_{50}	8,800	NA	Swartz and others (1988).
Rhepoxynius abronius LOEC[1]	800	NA	Swartz and others (1988); U.S. Environmental Protection Agency (1980).
Rhepoxynius abronius 10-day LC_{50} (Aroclor 1254/fluoranthene)	2,100	NA	Swartz and others (1988; 1989).
Microarthridium littorale 4-day LD_{50}-male	NA	30,000	DiPinto and others (1993).
Microarthridium littorale 12-day reproduction	NA	1,000	DiPinto and others (1993).

[1]Based on application of an acute to chronic ratio of 11 to the reported LC_{50} of 8,800 µg/kg DW.

Appendixes 1–6

Appendix 1. Sediment Chemistry Data

Appendix 1 contains sediment chemistry data tables in an Excel spreadsheet (which includes the tables along with a list of tables and abbreviations). The Excel file is available at *http://pubs.usgs.gov/sir/2013/5125*.

Table A1–1. Chemicals of potential concern, methods of analysis and associated target quality criteria for analyses performed at the U.S. Geological Survey–Columbia laboratory and by the U.S. Army Corps of Engineers–Vicksburg laboratory.

Table A1–2. Volume of whole sediment needed for each sample evaluated in cycle 1a and cycle 1b toxicity and bioaccumulation testing of Anniston PCB Site sediment samples.

Table A1–3a. Summary of chemistry in whole sediment and centrifuged pore water collected from the Anniston PCB Site.

Table A1–3b. Summary of chemistry measured in *Hyalella azteca* exposures during toxicity testing of whole sediment collected from the Anniston PCB Site.

Table A1–3c. Summary of chemistry measured in *Chironomus dilutus* exposures during toxicity testing of whole sediment collected from the Anniston PCB Site.

Table A1–3d. Summary of chemistry measured in *Lumbriculus variegatus* exposures during toxicity testing of whole sediment collected from the Anniston PCB Site.

Table A1–3e. Summary of chemistry measured in *Lumbriculus variegatus* tissue during bioaccumulation testing of whole sediment collected from the Anniston PCB Site.

Table A1–3f. Summary of chemistry measured in *Lampsilis siloquoidea* exposures during toxicity testing of whole sediment collected from the Anniston PCB Site.

Table A1–4. Concentrations of selected constituents in centrifuged/filtered pore waters from sediment samples obtained from Anniston PCB Site.

Table A1–5. Elemental concentrations in pore waters of Anniston PCB Site sediments evaluated during cycle 1a toxicity testing.

Table A1–6. Elemental concentrations in pore waters of Anniston PCB Site sediments evaluated during cycle 1b toxicity testing.

Table A1–7. Acid volatile sulfide and simultaneously extracted metals of Anniston PCB Site sediments evaluated during toxicity testing.

Table A1–8. Accuracy (as percent recovery) of results for an independent check sample analyzed during semiquantitative measurements of elements in pore water.

Table A1–9. Precision of replicate analyses of a standard reference water during semiquantitative measurements of elements in pore water.

Table A1–10. Measured values for acid-volatile sulfide and simultaneously extracted metals in a standard reference sediment.

Table A1–11. Precision of duplicate preparation and analyses for acid volatile sulfide and simultaneously extracted metals of four Anniston PCB Site test sediments.

Table A1–12. Spike recoveries for acid volatile sulfide and simultaneously extracted metals added during analyses of Anniston PCB Site test sediments.

Table A1–13. Blank equivalent concentrations of acid volatile sulfide and simultaneously extracted metals measured during analyses of Anniston PCB Site test sediments.

Appendix 2. Bioaccumulation Data

Appendix 2 contains bioaccumulation data tables in an Excel spreadsheet (which includes the tables along with a list of tables). The Excel file is available at *http://pubs.usgs.gov/sir/2013/5125*.

Table A2–1. Recommended test conditions for conducting a 28-day sediment bioaccumulation test with *Lumbriculus variegatus*.

Table A2–2. General activity schedule for conducting a 28-day sediment bioaccumulation test with *Lumbriculus variegatus*.

Table A2–3. Test acceptability requirements for a 28-day sediment bioaccumulation test with *Lumbriculus variegatus*.

Table A2–4. *Lumbriculus variegatus* overlying water quality data.

Table A2–5. *Lumbriculus variegatus* overlying water quality summary data.

Table A2–6. *Lumbriculus variegatus* tissue mass added at test initiation (day 0) and recovered at test termination (day 28).

Table A2–7. Sediment biota-sediment-accumulation factor values for each polychlroinated biphenyl homolog group.

Appendix 3. Toxicity Data

Appendix 3 contains toxicity data figures in a PDF file and tables in an Excel spreadsheet (which includes the tables along with a list of tables).

Figures

The PDF file is available at *http://pubs.usgs.gov/sir/2013/5125*.

Figure A3–1. Graphs showing *Hyalella azteca* day 28 survival (all values are expressed as percent of control).

Figure A3–2. Graphs showing *Hyalella azteca* day 28 length (all values are expressed as percent of control).

Figure A3–3. Graphs showing *Hyalella azteca* day 28 weight (all values are expressed as percent of control).

Figure A3–4. Graphs showing *Hyalella azteca* day 28 biomass (all values are expressed as percent of control).

Figure A3–5. Graphs showing *Hyalella azteca* day 35 survival (all values are expressed as percent of control).

Figure A3–6. Graphs showing *Hyalella azteca* day 35 reproduction (number of young per sediment) (all values are expressed as percent of control).

Figure A3–7. Graphs showing *Hyalella azteca* day 42 survival (all values are expressed as percent of control).

Figure A3–8. Graphs showing *Hyalella azteca* day 42 length (all values are expressed as percent of control).

Figure A3–9. Graphs showing *Hyalella azteca* day 42 weight (all values are expressed as percent of control).

Figure A3–10. Graphs showing *Hyalella azteca* day 42 biomass (all values are expressed as percent of control).

Figure A3–11. Graphs showing *Hyalella azteca* day 42 total number of young (all values are expressed as percent of control).

Figure A3–12. Graphs showing *Hyalella azteca* day 42 young per female (all values are expressed as percent of control).

Figure A3–13. Graphs showing *Hyalella azteca* day 42 young per female (normalized to survival) (all values are expressed as percent of control).

Figure A3–14. Graphs showing *Chironomus dilutus* day 13 survival (all values are expressed as percent of control).

Figure A3–15. Graphs showing *Chironomus dilutus* day 13 ash-free dry weight (all values are expressed as percent of control).

Figure A3–16. Graphs showing *Chironomus dilutus* day 13 biomass (all values are expressed as percent of control).

Figure A3–17. Graphs showing *Chironomus dilutus* percent emergence (all values are expressed as percent of control).

Figure A3–18. Graphs showing *Chironomus dilutus* median emergence time (all values are expressed as percent of control).

Figure A3–19. Graphs showing *Chironomus dilutus* adult time-to-death (all values are expressed as percent of control).

Figure A3–20. Graphs showing *Chironomus dilutus* number of egg cases (all values are expressed as percent of control).

Figure A3–21. Graphs showing *Chironomus dilutus* eggs per case (all values are expressed as percent of control).

Figure A3–22. Graphs showing *Chironomus dilutus* percent hatched (all values are expressed as percent of control).

Figure A3–23. Graphs showing *Chironomus dilutus* total number of young (all values are expressed as percent of control).

Figure A3–24. Graphs showing *Chironomus dilutus* average number of young per replicate (all values are expressed as percent of control).

Figure A3–25. Graphs showing *Chironomus dilutus* adult biomass (all values are expressed as percent of control).

Tables

The Excel file is available at *http://pubs.usgs.gov/sir/2013/5125*.

Table A3–1. Test conditions for conducting a long-term sediment toxicity test with the amphipod *Hyalella azteca* or the midge *Chironomus dilutus*.

Table A3–2. General activity schedule for conducting a long-term sediment toxicity test with *Hyalella azteca*.

Table A3–3. General activity schedule for conducting a long-term sediment toxicity test with *Chironomus dilutus*.

Table A3–4. Test acceptability requirements for a long-term sediment toxicity test with *Hyalella azteca*.

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Table A3–6. Summary of test conditions for conducting reference toxicant tests.

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Table A3–8. *Hyalella azteca* overlying water quality data.

Table A3–9. *Hyalella azteca* combined water quality data summary.

Table A3–10. U.S. Geological Survey–Columbia cycle 1a *Hyalella azteca* water bath temperature measurements.

Table A3–11. *Chironomus dilutus* overlying water quality data.

Table A3–12. *Chironomus dilutus* combined data summary.

Table A3–13. U.S. Geological Survey–Columbia cycle 1a *Chironomus dilutus* water bath temperature measurements.

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Table A3–15. *Hyalella azteca* day 28 survival data.

Table A3–16. *Hyalella azteca* day 28 biomass data.

Table A3–17. *Hyalella azteca* day 28 length data.

Table A3–18. *Hyalella azteca* day 42 biomass data.

Table A3–19. *Hyalella azteca* day 35 survival and reproduction data.

Table A3–20. *Hyalella azteca* day 42 survival and reproduction data.

Table A3–21. *Hyalella azteca* day 42 length data.

Table A3–22. *Hyalella azteca* combined data summary.

Table A3–23. *Chironomus dilutus* survival data.

Table A3–24. *Chironomus dilutus* biomass data.

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Table A3–26. Anniston *Chironomus dilutus* combined endpoint data summary.

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Table A3–28. Endpoint calculation methods for *Hyalella azteca* and *Chironomus dilutus* chronic tests.

Table A3–29. Summary of control-adjusted response observed in *Hyalella azteca* exposures during toxicity testing of whole sediment collected from the Anniston PCB Site.

Table A3–30. Summary of control-adjusted response observed in *Chironomus dilutus* exposures during toxicity testing of whole sediment collected from the Anniston PCB Site.

Appendix 4. Data Summaries Illustrating Relations between Sediment Toxicity and Sediment Chemistry

Appendix 4 contains data summary tables in an Excel spreadsheet (which includes the tables along with a list of tables). The Excel file is available at *http://pubs.usgs.gov/sir/2013/5125*.

Table A4–1. Summary of chemistry for selected chemicals of potential concern in whole sediment collected from reference stations from the Anniston PCB Site.

Table A4–2. Summary of the results of sediment toxicity tests performed on the reference samples that were selected for the Anniston PCB Site.

Table A4–3. Spearman's rank correlations between whole-sediment chemistry and *Hyalella azteca* response in sediments from the Anniston PCB Site.

Table A4–4. Spearman's rank correlations between whole-sediment chemistry and *Chironomus dilutus* response in sediments from the Anniston PCB Site.

Table A4–5. Spearman's rank correlations between pore-water chemistry and *Hyalella azteca* response in sediments from the Anniston PCB Site.

Table A4–6. Spearman's rank correlations between pore-water chemistry and *Chironomus dilutus* response in sediments from the Anniston PCB Site.

Table A4–7. Summary of the correlation of chemicals of potential concern in sediments collected from the Anniston PCB Site.

Appendix 5. Interlaboratory Sediment Toxicity Testing with the Amphipod, *Hyalella azteca*, and with the Midge, *Chironomus dilutus*

By Christopher G. Ingersoll[1], William G. Brumbaugh[1], Jacob K. Stanley[2], and Jessie A. Sinclair[3]

Introduction

A study was done to evaluate the interlaboratory variability in long-term reproduction sediment toxicity tests with the amphipod, *Hyalella azteca*, and the midge, *Chironomus dilutus*, exposed to Anniston PCB Site sediments. Only a limited number of laboratories had the demonstrated capacity to perform long-term reproduction sediment toxicity tests with *H. azteca* or *C. dilutus* with the number of samples required for the project (table C2–1). Hence, ARCADIS contracted with U.S. Geological Survey Columbia Environmental Research Center, Columbia, Missouri (USGS–Columbia) and U.S. Army Corps of Engineers Engineer Research and Development Center, Vicksburg, Mississippi (USACE–Vicksburg) to perform these long-term reproduction sediment toxicity tests. USGS–Columbia and USACE–Vicksburg participated in the study to increase the number of sediments that could be concurrently tested. USGS–Columbia was the lead laboratory for performing toxicity tests with *C. dilutus* and USACE–Vicksburg was the lead laboratory for performing toxicity tests with *H. azteca* and bioaccumulation tests with *L. variegatus* (chapter 3 and chapter 4).

In cycle 1a, one control sediment and five test sediments were selected to be evaluated in interlaboratory toxicity testing of *H. azteca* by USGS–Columbia and in interlaboratory toxicity testing of *C. dilutus* by USACE–Vicksburg. In cycle 1a, five additional Anniston PCB Site sediments were tested with *H. azteca* by USACE–Vicksburg and five additional Anniston PCB Site sediments were tested with *C. dilutus* by USGS–Columbia (in intralaboratory testing; chapter 4). Samples of sediments selected for cycle 1a testing represented medium to high concentrations of total PCBs (based on dry weight concentrations or on concentrations normalized to total organic carbon in sediment) with the goal of observing moderate to severe toxicity in the cycle 1a samples (table C2–1).

Methods

The sediments evaluated by USGS–Columbia in the *H. azteca* interlaboratory toxicity testing were sediments 06, 11, 19, 25, 30, and the West Bearskin Lake control sediment (sediment 33; table C2–1). The sediments evaluated by USACE–Vicksburg in the *C. dilutus* interlaboratory toxicity testing were sediments 06, 11, 18, 25, 30, and the West Bearskin Lake control sediment (table C2–1). USGS–Columbia mistakenly tested sediment 19 rather than sediment 18 in the interlaboratory testing with *H. azteca*. Fortunately, the physical and chemical characteristics of sediments 18 and 19 were quite similar (table C2–1) given that these two samples were collected from the same site but at different depths (sediment 19 from a depth of 0 to 30 cm and sediment 18 from a depth of 30 to 60 cm; table C2–1). Methods used to perform the long-term whole-sediment toxicity tests with *H. azteca* and *C. dilutus* are summarized in chapter 4 and in appendix 3, tables A3–1 to A3–7.

In the August 2008, USGS–Columbia and USACE–Vicksburg performed a preliminary study to evaluate the methods and conditions for performing the long-term sediment toxicity tests with *C. dilutus* using three control sediments (included West Bearskin Lake control sediment used in the current study; USGS–Columbia Study Outline 08–20–25). Methods used to perform this study were the same as the methods outlined in chapter 4 and in appendix 3, tables A3–1 to A3–7. The response of *C. dilutus* in this preliminary study in the West Bearskin Lake control sediment met American Society for Testing and Materials International (2012) and U.S. Environmental Protection Agency (2000) test acceptability requirements for performing long-term testing, so West Bearskin Lake sediment was selected as a control sediment for the definitive toxicity testing in the current study (chapter 4).

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The water-quality characteristics of the overlying water delivered to the exposure chambers in the 2008 study and in the definitive testing in the current study were selected to be relatively consistent between the two laboratories (for example, 100 milligrams per liter (mg/L) hardness as calcium carbonate ($CaCO_3$) at USGS–Columbia and 73 mg/L hardness as $CaCO_3$ at USACE–Vicksburg; appendix 3, table A3–7). The same taxonomic strains of the test organisms were used to perform the toxicity tests by USGS–Columbia and USACE–Vicksburg in the preliminary 2008 sediment testing of *C. dilutus* and in the definitive testing done with both species in the current study (chapter 4).

In the definitive study, subsamples of sediments for testing were provided to USACE–Vicksburg and were processed and tested during the same week in cycle 1a testing and in cycle 1b testing (see chapter 2 for additional details). In all of the interlaboratory testing samples (and in all of the intralaboratory testing samples; chapter 4), separate replicate chemistry chambers containing sediment and test organisms were included with each sediment toxicity treatment for sampling sediments for simultaneously extract metals (SEM), acid volatile sulfides (AVS) and for sampling pore-water metals and major cations in pore water with peeper samplers during the exposure. Peeper samplers were placed in these replicate chemistry chambers on day 14 of the exposures. These replicate chemistry chambers were then sampled on day 21 for SEM, AVS, and peeper samplers (see table C2–2 for more detail on SEM, AVS, and peeper sampling). In all of the interlaboratory testing samples, solid-phase microextraction (SPME) fibers were placed in additional chemistry chambers containing sediment and test organisms on day 0 of each sediment toxicity treatment to sample PCBs on day 28 of exposures (see table C2–2 for more detail on SPME sampling). The replicate chemistry chambers contained sediment and test organisms and were maintained following the same procedures as the replicate chambers used to determine sediment toxicity to test organisms (chapter 4).

Results and Discussion

Interlaboratory Toxicity Testing with *Hyalella azteca*

Control responses of *H. azteca* in the interlaboratory testing (and in the intralaboratory testing; chapter 4) met U.S. Environmental Protection Agency (2010) and American Society for Testing and Materials International (2012) test acceptability requirements (appendix 3, table A3–22). Mean starting weight of *H. azteca* was 0.05 mg/individual at USACE–Vicksburg and was 0.03 mg/individual at USGS–Columbia (appendix 3, table A3–22). Mean control survival of *H. azteca* was greater than 98 percent at day 28 and was greater than 92 percent at day 42 at USACE–Vicksburg and USGS–Columbia (appendix 3, table A3–22 and figs. A5–1 and A5–4). Mean weight and biomass of *H. azteca* in the control at day 28 and at day 42 were higher at USGS–Columbia (weight: 0.36 and 0.63 mg/individual and biomass: 3.44 and 5.97 mg/treatment) compared to USACE–Vicksburg (weight: 0.23 and 0.42 mg/individual and biomass: 2.21 and 3.83 mg/treatment; appendix 3, table A3–22 and figs. A5–2, A5–3, A5–5, and A5–6). Mean young/female and mean young/female normalized to survival of *H. azteca* at day 42 in the control also were higher at USGS–Columbia (for example, young/female normalized to survival: 8.1) compared to USACE–Vicksburg (mean young/female normalized to survival of 3.8; appendix 3, table A3–22 and figs. A5–7 and A5–8). Hence, the USGS–Columbia *H. azteca* in the control that averaged about twice the weight of the USACE–Vicksburg *H. azteca* by the end of the exposures produced on average about twice the number of young/female.

Figures A5–1 to A5–8 illustrate relations in responses of *H. azteca* in the interlaboratory toxicity testing with the five samples of Anniston PCB Site sediments. Mean survival of *H. azteca* at day 28 and day 42 in the Anniston PCB Site sediments was relatively consistent between USACE–Vicksburg and USGS–Columbia, but tended to be lower in sediments 19 or 30 tested by USACE–Vicksburg (figs. A5–1 and A5–4). Mean weight of H. azteca at day 28 and day 42 tended to be higher in sediments tested by USACE–Vicksburg (for example, sediments 19 and 30; figs. A5–2 and A5–5). Low survival of *H azteca* in a treatment might result in more food and increased weight of surviving organisms in that treatment (for example, Orr and others, 2004). Mean biomass of *H. azteca* at day 28 and day 42 tended to be more consistent for both laboratories compared to survival or weight (figs. A5–3 and A5–6). The biomass endpoint integrates effects on survival and weight of test organisms. Hence, survival tended to be lower and weight tended to be higher in some of the *H. azteca* interlaboratory samples tested by USACE–Vicksburg. However, the biomass endpoint that integrates effects on survival or weight tended to reduce overall variability in responses observed between the two laboratories. Mean number of young/female was relatively consistent between the two laboratories (fig. A5–7) and expressing reproduction as the number of young/female normalized to percent survival within a replicate tended to reduce the overall variability between the two laboratories (fig. A5–8).

For sediment 19, there were no major differences in metal concentrations of the peeper obtained from tests performed at USACE–Vicksburg as compared to the same sediment tested at USGS–Columbia that might help explain the greater mortality of *H. azteca* observed in that sediment when tested at USACE–Vicksburg. For sediment 30, concentrations of bismuth, lead, and zinc were substantially greater in the peeper obtained from that sediment when tested at Vicksburg [2, 2, and 58 micrograms per liter (µg/L), respectively] as compared with the peeper obtained from that sediment when tested at USGS–Columbia (less than 0.1, 0.2, and 6 µg/L, respectively), or to peeper concentrations obtained from the *C. dilutus* tests for sediment 30 at either laboratory (appendix 1, table A1–5). Furthermore, those three values (2, 2, and 58 µg/L) were among the greatest concentrations measured for those three

elements in any peeper sample. Thus, it is possible that the presence of those three elements in the pore water contributed to the greater *H. azteca* mortality that was observed in sediment 30 when tested at USACE–Vicksburg; however, such concentrations typically would not be expected to be lethal to *H. azteca*. Notably, sediment 30 had the fourth greatest concentration of total Pb, and perhaps for this particular sediment there existed localized concentration differences for Pb or other metals (caused by the presence of metal-enriched granules, for example). If so, that scenario also might apply for sediment 19, which had the greatest overall concentration of Pb, despite the fact the Pb and other metals were not remarkably elevated in any of the peeper samplers obtained from that sediment.

Figure A5–21 illustrates the concentration-response relation between concentrations of total PCBs sediment to day 28 biomass of *H. azteca* in cycle 1a and in cycle 1b intralaboratory testing by USACE–Vicksburg (the same figure as fig. C5–7). The interlaboratory testing of select samples of sediment by USGS–Columbia also is plotted in with green symbol in fig. A5–21. The response *H. azteca* in the samples tested by USGS–Columbia tended to be less than the response of *H. azteca* in the samples tested by USACE–Vicksburg, with a relatively consistent overall dose response pattern between the two sets of samples.

Figure A5–22 illustrates the concentration-response relation between concentrations of total PCBs sediment to day 42 young/female normalized to survival of *H. azteca* in cycle 1a and in cycle 1b intralaboratory testing by USACE–Vicksburg (the same figure as fig. C5–1). The interlaboratory testing of select samples of sediment by USGS–Columbia also is plotted in green symbols in fig. A5–22. The response *H. azteca* in the samples tested by USGS–Columbia was similar to the response of *H. azteca* in the samples tested by USACE–Vicksburg, with a consistent overall dose-response pattern between the two sets of samples.

Interlaboratory Toxicity Testing with *Chironomus dilutus*

Control responses of *C. dilutus* in the interlaboratory testing (and in the intralaboratory testing; chapter 4) met U.S. Environmental Protection Agency (2010) and American Society for Testing and Materials International (2012) test acceptability requirements (appendix 3, table A3–26). Mean starting weight of *C. dilutus* was 0.05 milligrams per individual (mg/ individual) at USACE–Vicksburg and was 0.14 mg/individual at USGS–Columbia (appendix 3, table A3–26). Mean control survival of *C. dilutus* was greater than 94 percent at day 13 for USGS–Columbia and USACE–Vicksburg (appendix 3, table A3–26 and fig. A5–9). Mean ash-free-dry weight (AFDW) and biomass of *C. dilutus* at day 13 in the control sediment were higher at USACE–Vicksburg (AFDW: 1.41 mg/individual and biomass: 16.5 mg/treatment) compared to USGS–Columbia (AFDW: 0.93 mg/individual and biomass: 10.5 mg/treatment; appendix 3, table A3–26 and figs. A5–10 and A5–11). Mean percent adult emergence and median emergence time in the controls were similar between both laboratories (percent emergence ranging from 74.0 to 77.1 and median emergence time ranging from 27.2 to 31.6 days; appendix 3, table A3–26 and figs. A5–12 and A5–13). Similarly, the mean survival time of adults, number of egg cases/treatment, number of eggs/egg case, percent hatch, young/treatment, and young/replicate, and adult biomass in the controls were similar between both laboratories (figs. A5–14 to A5–20). Whereas, the USACE–Vicksburg *C. dilutus* in the control averaged about twice the weight of the USGS–Columbia *C. dilutus* at day 13, subsequent emergence, adult biomass, and reproduction of *C. dilutus* in the control sediment was similar between both laboratories.

Figures A5–9 to A5–19 illustrate relations in responses of *C. dilutus* in the interlaboratory toxicity testing with the five samples of Anniston PCB Site sediments. Mean survival, AFDW and biomass of *C. dilutus* at day 13 in the Anniston PCB Site sediments were relatively consistent between both laboratories, but tended to be higher in the interlaboratory samples tested by USGS–Columbia (figs. A5–9 to A5–11). Mean percent adult emergence and median emergence time tended to be relatively consistent between laboratories (figs. A5–12 and A5–13); whereas, adult survival time was longer for the interlaboratory samples tested by USACE–Vicksburg (fig. A5–14). The reproductive endpoints tended to be more variable between laboratories (number of egg cases, eggs/case, percent hatch, young produced, and adult biomass (figs. A5–15 to A5–20) compare to the weight, biomass or emergence endpoints (figs. A5–10 to A5–13).

Figure A5–23 illustrates the concentration-response relation between concentrations of total PCBs sediment to day 13 biomass of *C. dilutus* in cycle 1a and in cycle 1b intralaboratory testing by USGS–Columbia (the same figure as fig. C5–9). The interlaboratory testing of select samples of sediment by USACE–Vicksburg also is plotted in green symbols in fig. A5–23. The response of *C. dilutus* in the samples tested by USACE–Vicksburg tended to be lower than the response of C. *dilutus* in the samples tested by USGS–Columbia, but with a consistent overall dose-response pattern between the two sets of samples.

Figure A5–24 illustrates the concentration-response relation between concentrations of total PCBs sediment to percent emergence of *C. dilutus* in cycle 1a and in cycle 1b intralaboratory testing by USGS–Columbia (the same figure as fig. C5–3). The interlaboratory testing of select samples of sediment by USACE–Vicksburg also is plotted in green symbols in fig. A5–24. The response of *C. dilutus* in the samples tested by USACE–Vicksburg was similar to the response of *C. dilutus* in the samples tested by USGS–Columbia, with a consistent overall dose-response pattern between the two sets of samples.

Summary

Results of the interlaboratory testing of Anniston PCB Site sediments demonstrated relatively consistent responses between samples tested by USGS–Columbia and USACE–Vicksburg. Hence, there was not likely a substantial bias in the results generated by the USACE–Vicksburg or USGS–Columbia toxicity testing laboratories associated with the toxicity testing of Anniston PCB Site sediments (chapter 4). Mean biomass and number of young/female normalized to survival were the two most consistent endpoints and exhibited a broad response range in the interlaboratory toxicity testing with *H. azteca*. Mean biomass and percent emergence were the two most consistent endpoints and exhibited a broad response range in the interlaboratory toxicity testing with *C. dilutus*. The endpoint of biomass integrates effects on survival and weight and the endpoint of young/female normalized to survival integrates effects on reproduction and survival. These integrated endpoints reduced some of the interlaboratory variability observed in individual endpoints of survival, weight, or reproduction. These integrated endpoints also helped to reduce the variability observed in concentration-response models reported in chapter 5.

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Figures

Appendix 5 figures are in a PDF file. The PDF file is available at *http://pubs.usgs.gov/sir/2013/5125*.

Figure A5–1. Graph showing *Hyalella azteca* day 28 survival in cycle 1a samples evaluated in interlaboratory toxicity testing done at U.S. Geological Survey (USGS)–Columbia and at U.S. Army Corps of Engineers (USACE)–Vicksburg (normalized to percent of control response).

Figure A5–2. Graph showing *Hyalella azteca* day 28 weight in cycle 1a samples evaluated in interlaboratory toxicity testing done at U.S. Geological Survey (USGS)–Columbia and at U.S. Army Corps of Engineers (USACE)–Vicksburg (normalized to percent of control response).

Figure A5–3. Graph showing *Hyalella azteca* day 28 biomass in cycle 1a samples evaluated in interlaboratory toxicity testing done at U.S. Geological Survey (USGS)–Columbia and at U.S. Army Corps of Engineers (USACE)–Vicksburg (normalized to percent of control response).

Figure A5–4. Graph showing *Hyalella azteca* day 42 survival in cycle 1a samples evaluated in interlaboratory toxicity testing done at U.S. Geological Survey (USGS)–Columbia and at U.S. Army Corps of Engineers (USACE)–Vicksburg (normalized to percent of control response).

Figure A5–5. Graph showing *Hyalella azteca* day 42 weight in cycle 1a samples evaluated in interlaboratory toxicity testing done at U.S. Geological Survey (USGS)–Columbia and at U.S. Army Corps of Engineers (USACE)–Vicksburg (normalized to percent of control response).

Figure A5–6. Graph showing *Hyalella azteca* day 42 biomass in cycle 1a samples evaluated in interlaboratory toxicity testing done at U.S. Geological Survey (USGS)–Columbia and at U.S. Army Corps of Engineers (USACE)–Vicksburg (normalized to percent of control response).

Figure A5–7. Graph showing *Hyalella azteca* day 42 young per female in cycle 1a samples evaluated in interlaboratory toxicity testing done at U.S. Geological Survey (USGS)–Columbia and at U.S. Army Corps of Engineers (USACE)–Vicksburg (normalized to percent of control response).

Figure A5–8. Graph showing *Hyalella azteca* day 42 young per female (normalized to survival) in cycle 1a samples evaluated in interlaboratory toxicity testing done at U.S. Geological Survey (USGS)–Columbia and at U.S. Army Corps of Engineers (USACE)–Vicksburg (normalized to percent of control response).

Figure A5–9. Graph showing *Chironomus dilutus* day 13 survival in cycle 1a samples evaluated in interlaboratory toxicity testing done at U.S. Geological Survey (USGS)–Columbia and at U.S. Army Corps of Engineers (USACE)–Vicksburg (normalized to percent of control response).

Figure A5–10. Graph showing *Chironomus dilutus* day 13 ash-free dry weight in cycle 1a samples evaluated in interlaboratory

toxicity testing done at U.S. Geological Survey (USGS)–Columbia and at U.S. Army Corps of Engineers (USACE)–Vicksburg (normalized to percent of control response).

Figure A5–11. Graph showing *Chironomus dilutus* day 13 biomass in cycle 1a samples evaluated in interlaboratory toxicity testing done at U.S. Geological Survey (USGS)–Columbia and at U.S. Army Corps of Engineers (USACE)–Vicksburg (normalized to percent of control response).

Figure A5–12. Graph showing *Chironomus dilutus* percent emergence in cycle 1a samples evaluated in interlaboratory toxicity testing done at U.S. Geological Survey (USGS)–Columbia and at U.S. Army Corps of Engineers (USACE)–Vicksburg (normalized to percent of control response).

Figure A5–13. Graph showing *Chironomus dilutus* median emergence time in cycle 1a samples evaluated in interlaboratory toxicity testing done at U.S. Geological Survey (USGS)–Columbia and at U.S. Army Corps of Engineers (USACE)–Vicksburg (normalized to percent of control response).

Figure A5–14. Graph showing *Chironomus dilutus* adult time-to-death in cycle 1a samples evaluated in interlaboratory toxicity testing done at U.S. Geological Survey (USGS)–Columbia and at U.S. Army Corps of Engineers (USACE)–Vicksburg (normalized to percent of control response).

Figure A5–15. Graph showing *Chironomus dilutus* number of egg cases in cycle 1a samples evaluated in interlaboratory toxicity testing done at U.S. Geological Survey (USGS)–Columbia and at U.S. Army Corps of Engineers (USACE)–Vicksburg (normalized to percent of control response).

Figure A5–16. Graph showing *Chironomus dilutus* eggs per case in cycle 1a samples evaluated in interlaboratory toxicity testing done at U.S. Geological Survey (USGS)–Columbia and at U.S. Army Corps of Engineers (USACE)–Vicksburg (normalized to percent of control response).

Figure A5–17. Graph showing *Chironomus dilutus* percent hatched in cycle 1a samples evaluated in interlaboratory toxicity testing done at U.S. Geological Survey (USGS)–Columbia and at U.S. Army Corps of Engineers (USACE)–Vicksburg (normalized to percent of control response).

Figure A5–18. Graph showing *Chironomus dilutus* total number of young in cycle 1a samples evaluated in interlaboratory toxicity testing done at U.S. Geological Survey (USGS)–Columbia and at U.S. Army Corps of Engineers (USACE)–Vicksburg (normalized to percent of control response).

Figure A5–19. Graph showing *Chironomus dilutus* average number of young per replicate in cycle 1a samples evaluated in interlaboratory toxicity testing done at U.S. Geological Survey (USGS)–Columbia and at U.S. Army Corps of Engineers (USACE)–Vicksburg (normalized to percent of control response).

Figure A5–20. Graph showing *Chironomus dilutus* adult biomass in cycle 1a samples evaluated in interlaboratory toxicity testing done at U.S. Geological Survey (USGS)–Columbia and at U.S. Army Corps of Engineers (USACE)–Vicksburg (normalized to percent of control response).

Figure A5–21. Graph showing concentration-response model for total polychlorinated biphenyls (PCBs; µg/kg DW) in sediment and *Hyalella azteca* day 28 biomass tested in cycle 1a and cycle 1b by U.S. Army Corps of Engineers (USACE)–Vicksburg. Interlaboratory testing of *Hyalella azteca* with select cycle 1a samples of sediment by U.S. Geological Survey (USGS)–Columbia are plotted with green symbols.

Figure A5–22. Graph showing concentration-response model for total polychlorinated biphenyls (PCBs; µg/kg DW) in sediment and *Hyalella azteca* day 42 young per female (normalized to survival) tested in cycle 1a and in cycle 1b by U.S. Army Corps of Engineers (USACE)–Vicksburg. Interlaboratory testing of *Hyalella azteca* with select cycle 1a samples of sediment by U.S. Geological Survey (USGS)–Columbia are plotted with green symbols.

Figure A5–23. Graph showing concentration-response model for total polychlorinated biphenyls (PCBs; µg/kg DW) in sediment and *Chironomus dilutus* day 13 biomass tested in cycle 1a and in cycle 1b by U.S. Geological Survey (USGS)–Columbia. Interlaboratory testing of *Chironomus dilutus* with select cycle 1a samples of sediment by U.S. Army Corps of Engineers (USACE)–Vicksburg are plotted with green symbols.

Figure A5–24. Graph showing concentration-response model for total polychlorinated biphenyls (PCBs; µg/kg DW) in sediment and *Chironomus dilutus* percent emergence tested in cycle 1a and in cycle 1b by U.S. Geological Survey (USGS)–Columbia. Interlaboratory testing of *Chironomus dilutus* with select cycle 1a samples of sediment by U.S. Army Corps of Engineers (USACE)–Vicksburg are plotted with green symbols.

Appendix 6. Age Comparison of Midge, *Chironomus dilutus*, Sensitivity with Exposure to PCB-contaminated Sediments from Anniston, Alabama

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Introduction

A study was done to evaluate the relative sensitivity of the midge *Chironomus dilutus* in exposure to Anniston Polychlorinated Biphenyl Site (PCB Site) sediment when exposures were started with about 7-day (d)-old larvae compared to exposures started with less than 24-hour (h)-old larvae. The long-term reproduction sediment toxicity tests with the amphipod, *Hyalella azteca*, and *C. dilutus*, were done with samples collected from the Anniston PCB Site in accordance with guidance provided in U.S. Environmental Protection Agency (2000) and American Society for Testing and Materials International (2012). Results of these toxicity tests are described in chapter 4. The decision was made to perform the *C. dilutus* toxicity tests starting with 7-d-old larvae (appendix 3, table A3–2) rather than starting tests with less than 24-h-old larvae (as is recommended in U.S. Environmental Protection Agency, 2000 and in American Society for Testing and Materials International, 2012). This decision was based on problems observed with control acceptability with tests started with less than 24-h-old larvae at USGS–Columbia, at USACE–Vicksburg and at other laboratories (for example, Ingersoll and others, 2005; Norberg King and others, 2006). Specifically, poor survival of larvae has been observed at day 20 of the sediment exposures before *C. dilutus* pupate and emerge as adults. For example, in interlaboratory testing of the methods described in U.S. Environmental Protection Agency (2010) and American Society for Testing and Materials International (2012), only 63 percent of laboratories successfully completed the 20-d sediment exposures starting with less than 24-h-old *C. dilutus* (Norberg-King and others, 2006).The low survival of sediment toxicity tests started with less than 24-h-old *C. dilutus* in control sediment is likely because of difficulties in handling these young larvae at the start of the exposures.

In August 2008, USGS–Columbia and USACE–Vicksburg performed a preliminary study to evaluate emergence of *C. dilutus* in three control sediments (included West Bearskin Lake control sediment used in the current study). Methods used to perform this preliminary study were the same as the methods outlined in appendix 3, table A3–1 (USGS–Columbia Study Outline 08–20–25). Both laboratories observed good performance of *C. dilutus* in exposures started with 7-d-old larvae (for example, 13-d survival of *C. dilutus* at USGS–Columbia ranged from 90 to 98 percent). However, survival of *C. dilutus* in exposures started with less than 24-h-old larvae was poor (for example, 20-d survival at USGS–Columbia ranged from 7 to 21 percent; Chris Ingersoll, USGS–Columbia, unpub. data, 2008).

Based on the results of the preliminary 2008 study and based on inconsistent results by USGS–Columbia and by other laboratory performing long-term sediment exposures started with less than 24-h-old larvae of *C. dilutus*, the decision was made in the current study to start sediment exposures with about 7-d-old larvae (chapter 4). This modification to the method is consistent with the guidance provided in U.S. Environmental Protection Agency (2000) and American Society for Testing and Materials International (2012). Specifically, section A7.1.2 in American Society for Testing and Materials International (2012) states that the standard "describes general guidance for performing a long-term sediment toxicity test with *C. dilutus* that can be used to evaluate sublethal effects of contaminants associated with sediment. More definitive methods may be described in future versions of this standard after additional laboratories have successfully used the method."

Methods

After completion of the cycle 1b sediment toxicity testing described in chapter 4, an additional study was done comparing the relative sensitivity of *C. dilutus* in sediment toxicity tests started with 7-d-old larvae with tests started with less than 24-h-old larvae (cycle 1c in appendix 3, table A3–26). The toxicity tests started with less than 24-h-old larvae were done for 20 days; the toxicity tests started with 7-d-old larvae were done for 13 days. Hence the developmental stage at the end of these exposures would be about at the fourth instar. Endpoints measured in the toxicity tests included survival, ash-free-dry weight (AFDW), and biomass of surviving larvae. See chapter 4 and appendix 3, tables A3–1 to A3–7 for a description of methods used to perform the whole-sediment

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toxicity tests with *C. dilutus*. SPME fibers were placed in additional replicate chemistry exposure chambers containing sediments and test organisms, but there were no additional replicate chemistry exposure chambers with peeper for sampling metals in pore water (chapter 2). The sediments evaluated in this study were Anniston PCB Site sediments 02, 15, 17, 20, 27, and the West Bearskin Lake control sediment (sediment 33; table C2–1). These five Anniston PCB Site sediments were initially tested in cycle 1b starting in January 2011. These Anniston PCB Site sediments contained moderate to high concentrations of total PCBs (990 to 120,000 µg/kg dry weight; appendix 1, table A1–3a) and exhibited moderate toxicity to *C. dilutus* at day 13 of the cycle 1b exposures. For example day 13 biomass of *C. dilutus* with exposure to these five Anniston PCB Site sediments tested in cycle 1b were below reference responses, with biomass ranging as low as 72.6 percent of the control response in sediment 27 (chapter 4 and appendix 3, table A3–26).

Results and Discussion

An initial study that attempted to evaluate relative life stage sensitivity of *C. dilutus* with exposure to Anniston PCB Site sediments was started May 3, 2011. In the initial study, mean control survival of *C. dilutus* in the 13-d exposures started with about 7-d-old larvae was 87.5 percent. However, mean control survival of *C. dilutus* in the 20-d exposures started with less than 24-h-old larvae was only 16.7 percent, not meeting control test acceptability survival criterion of 70 percent (U.S. Environmental Protection Agency, 2000; American Society for Testing and Materials International, 2012).

A second study was started July 5, 2011, in an attempt to improve control survival of *C. dilutus* in exposures started with less than 24-h-old larvae (designated as cycle 1c in appendix 3, table A3–26). In this second study, mean control survival of *C. dilutus* in the 13-d exposures started with about 7-d-old larvae was 95.8 percent with an AFDW of 0.98 mg/individual, meeting control test acceptability requirements for survival of 70 percent and AFDW of 0.48 mg/individual (U.S. Environmental Protection Agency, 2010; American Society for Testing and Materials International, 2012). The AFDW of 7-d-old *C. dilutus* at the start of the cycle 1c exposures and at the start of the cycle 1b exposures was 0.14 mg/individual (appendix 3, table A3–26). Hence, 13-d control survival, AFDW, and biomass in exposures started with 7-d-old larvae were consistent between cycle 1b testing started in January 2011 and cycle 1c testing started in July 2011 (figs. A6–1 to A6–3).

Figures A6–1 to A6–3 illustrate relations in responses of *C. dilutus* in exposures to Anniston PCB Site sediments started with about 7-d-old larvae in cycle 1b testing compared to cycle 1c testing. As expected, only moderate effects were observed on survival, AFDW, or biomass of *C. dilutus* in the cycle 1c testing started in July 2011. Moreover, the responses in cycle 1c testing were relatively consistent with responses observed in the cycle 1b testing (chapter 4). However, survival and biomass of *C. dilutus* in exposures started with 7-d-old larvae tended to be lower in the cycle 1c testing started in July 2011 compared to these responses in the initial cycle 1b testing started in January 2011 (figs. A6–1 and A6–3).

In the second study, mean control survival of *C. dilutus* in the 20-d exposures started with less than 24-h-old larvae was 68.8 percent with an AFDW of 1.29 mg/individual (appendix 3, table A3–26). Hence, in the second exposure started with less than 24-h-old larvae, AFDW met the test acceptability criterion of 0.48 mg/individual; however, survival was slightly below the control test acceptability survival criterion of 70 percent (U.S. Environmental Protection Agency, 2000; American Society for Testing and Materials International, 2012). The 13-d control survival was higher and 13-d control AFDW was lower in cycle 1c testing started with less than 7-d-old larvae compared to cycle 1c testing started with less than 24-h-old larvae (figs. A6–4 and A6–5). However, the control biomass in the 13-d exposures and in the 20-d exposures was consistent in cycle 1c testing.

Figures A6–4 to A6–6 illustrate relations in responses of *C. dilutus* in exposures to Anniston PCB Site sediments started with about 7-d-old larvae in cycle 1c testing compared to exposures started with 24-h-old larvae in cycle 1c testing. Survival of *C. dilutus* was relatively variable in cycle 1c exposures started with about 7-d-old larvae compared to the responses in cycle 1c exposures started with less than 24-h-old larvae. Weight and biomass of *C. dilutus* was relatively consistent in cycle 1c exposures started with about 7-d-old larvae compared to the responses in cycle 1c exposures started with less than 24-h-old larvae. Only sediment 02 exhibited a somewhat lower response in cycle 1c testing started with less than 24-h-old larvae (that is, 32.0 percent biomass relative to the control) compared to testing started with about 7-d-old larvae (that is, 52.8 percent biomass relative to control; fig. A6–6).

Figures A6–7 to A6–9 illustrate the concentration-response relation between concentrations of total PCBs sediment to day-13 survival, weight, or biomass of *C. dilutus* in cycle 1a and in cycle 1b interlaboratory testing performed by USGS–Columbia (chapter 4). The cycle 1c data for *C. dilutus* exposures started with 7-d-old larvae are plotted with green symbols and the data for *C. dilutus* exposures started with less than 24-h-old larvae are plotted with red symbols in figs. A6–7 to A6–9. Survival and biomass of *C. dilutus* in cycle 1c were similar in exposures started with 7-d-old larvae in exposures started with less than 24-h-old larvae, but survival and biomass tended to be lower in cycle 1c testing compared to cycle 1a or cycle 1b testing (figs. A6–7 and A6–9). The overall dose response for survival and biomass of *C. dilutus* tended to be lower in the cycle 1c testing compared to the cycle 1a and 1b testing (figs. A6–7 and A6–9). Weight of *C. dilutus* in cycle 1c was similar in exposures started with 7-d-old larvae and in exposures started with less than

24-h-old larvae, and weight was similar in cycle 1c testing compared to cycle 1a and cycle 1b testing (fig. A6–8). The overall dose response for weight of *C. dilutus* in cycle 1c testing was similar to the dose response for weight in cycle 1a and 1b testing (fig. A6–8).

Summary

Survival, weight, and biomass of *C. dilutus* were relatively consistent in cycle 1c exposures started with about 7-d-old larvae compared to the responses in cycle 1c exposures started with less than 24-h-old larvae. These results indicate that long-term exposures in the definitive cycle 1a and cycle 1b tests started with about 7-d-old larvae (chapter 4) would not likely underestimate the toxicity of the Anniston PCB Site sediments to *C. dilutus* compared to starting the exposures with less than 24-h-old larvae.

Research is ongoing at the U.S. Environmental Protection Agency laboratory in Duluth, Minnesota (Minn.) to improve performance of *C. dilutus* in sediment long-term sediment exposures started with less than 24-h-old larvae. Improved performance of *C. dilutus* is observed in exposures started with less than 1-h-old larvae compared to exposures started with less than 24-h-old larvae (Ted Valenti and Dave Mount, U.S. Environmental Protection Agency, Duluth, Minn., unpub. data, 2012). Similarly, USGS–Columbia has recently observed good performance of *C. dilutus* in 20-d sediment exposures started with less than 1-h-old larvae (that is control survival greater than 90 percent in sand or in the control sediment of West Bearskin Lake sediment). It may be that larvae that are away from the egg mass for more than a couple hours might be food limited, reducing their performance in subsequent exposures started with less than 24-h-old larvae (Ted Valenti, U.S. Environmental Protection Agency, Duluth, Minn., unpub. data, 2012). Moreover, the 20 percent effect concentrations for copper based on AFDW are about a factor of 2 lower in 10-d water-only exposures started with less than 1-h-old larvae compared to exposures started with 8-d-old larvae (Ted Valenti, U.S. Environmental Protection Agency, Duluth, Minn., unpub. data, 2012). Results of ongoing studies at USEPA Duluth may result in improving the guidance provided in American Society for Testing and Materials International (2012) and in U.S. Environmental Protection Agency (2000) for performing long-term sediment exposures with *C. dilutus* (that is, starting with less than 1-h-old rather than greater than 24-h-old larvae or with about 7-d-old larvae that were tested in the current study). Alternatively, if laboratories continue to have difficulty starting sediment exposures with younger larvae, it may be than either 7-d-old larvae or perhaps about 4-d-old larvae might be suggested as a life stage for starting long-term sediment exposures with *C. dilutus*.

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Figures

Appendix 6 figures are in a PDF file. The PDF file is available at *http://pubs.usgs.gov/sir/2013/5125*.

Figure A6–1. Graph showing survival of *Chironomus dilutus* in cycle 1b compared to cycle 1c tests started with about 7-day-old larvae.

Figure A6–2. Graph showing ash-free dry weight of *Chironomus dilutus* in cycle 1b compared to cycle 1c tests started with about 7-day-old larvae.

Figure A6–3. Graph showing biomass of *Chironomus dilutus* in cycle 1b compared to cycle 1c tests started with about 7-day-old larvae.

Figure A6–4. Graph showing survival of *Chironomus dilutus* in cycle 1c tests started with about 7-day-old larvae compared to cycle 1c tests started with less than 24-hour-old larvae.

Figure A6–5. Graph showing ash-free dry weight of *Chironomus dilutus* in cycle 1c tests started with about 7-day-old larvae compared to cycle 1c tests started with less than 24-hour-old larvae.

Figure A6–6. Graph showing biomass of *Chironomus dilutus* in cycle 1c tests started with about 7-day-old larvae compared to cycle 1c tests start with less than 24-hour-old larvae.

Figure A6–7. Graph showing concentration-response model for total polychlorinated biphenyls (PCBs; µg/kg DW) in sediment and *Chironomus dilutus* day 13 survival tested in cycle 1b. Data for cycle 1c retesting of sediments starting with about 7-day-old larvae are plotted with green symbols and data for testing of sediments starting with less than 24-hour-old larvae are plotted with red symbols.

Figure A6–8. Graph showing concentration-response model for total polychlorinated biphenyls (PCBs; µg/kg DW) in sediment and *Chironomus dilutus* day 13 ash-free dry weight of tested in cycle 1b. Data for cycle 1c retesting of sediments starting with about 7-day-old larvae are plotted with green symbols and data for testing of sediments starting with less than 24-hour-old larvae are plotted with red symbols.

Figure A6–9. Graph showing concentration-response model for total polychlorinated biphenyls (PCBs; µg/kg DW) in sediment and *Chironomus dilutus* day 13 biomass tested in cycle 1b. Data for cycle 1c retesting of sediments starting with about 7-day-old larvae are plotted with green symbols and data for testing of sediments starting with less than 24-hour-old larvae are plotted with red symbols.

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Back cover. Don MacDonald sampling sediment. Photograph by Chris Ingersoll, U.S. Geological Survey.