Environmental Toxicology and Chemistry—Volume 40, Number 4—pp. 1188–1193, 2021
Received: 13 July 2020 | Revised: 26 August 2020 | Accepted: 17 December 2020

Environmental Toxicology

Acute Polychlorinated Biphenyl Benthic Invertebrate Toxicity Testing to Support the 2017 Chronic Dose–Response Sediment Injury Model

Kenneth Finkelstein,[a,]* Edward F. Wirth,[b] K.W. Chung,[c] B.S. Shaddrix,[c] E.C. Pisarski,[c] and C. Rios[d]

[a]Office of Response and Restoration, National Oceanographic and Atmospheric Administration, Boston, Massachusetts, USA
[b]National Centers for Coastal Ocean Science, National Oceanic and Atmospheric Administration, Charleston, South Carolina, USA
[c]CSS, Contracted to National Oceanic and Atmospheric Administration, Charleston, South Carolina, USA
[d]New York University, New York, New York, USA

Abstract: As managers and decision makers evaluate pollutant risk, it is critical that we are able to measure an assessment of the injury. Often, these estimates are difficult to determine for benthic organisms, so in 2017 a chronic polychlorinated biphenyl (PCB) sediment dose–response model to predict benthic invertebrate injury was proposed. Given both natural resource trustee and consultant questions following publication concerning that the aqueous chronic toxicity testing data used in the 2017 model development were primarily from the 1970s and 1980s, this follow-up short communication is meant to provide the user some additional data that are more recent. With the advances in analytical and quantitative environmental chemistry (i.e., better detection limits and congener separation), we chose to complete acute aquatic toxicity testing using 3 estuarine invertebrates and lethal endpoints (20 and 50% lethal concentrations). This acute testing was selected because chronic aquatic testing for PCBs outside of the data used in the 2017 study was not available to us. The aquatic results used in the present study were changed to sediment using equilibrium partitioning, as done in the 2017 chronic model, after using the same organic–carbon partition coefficient and total organic carbon for our equilibrium partitioning (EqP)–measured calculations. Based on these acute aquatic toxicity results and a general acute-to-chronic injury concentration ratio of approximately 10, we found that the 2017 model was valid and, hence, that a 1.0 μg/g chronic PCB sediment criterion is a reasonable estimation of potential benthic invertebrate injury. This was followed by spiked sediment tests where percent acute sediment injury was compared to the EqP-derived chronic value and the results from 2017; modest agreement is shown. *Environ Toxicol Chem* 2021;40:1188–1193. Published 2020. This article is a U.S. Government work and is in the public domain in the USA.

Keywords: PCBs; Aqueous acute and chronic toxicity; Equilibrium partitioning; PCB sediment dose response; Sediment injury

INTRODUCTION

In 2017 Finkelstein et al. published a polychlorinated biphenyl (PCB) sediment dose–response "look-up table" used to estimate chronic benthic injury for natural resource damage assessments and ecological risk assessments when provided a site-specific sediment concentration (Table 1). However, we, and others, noted that the available aquatic PCB toxicity test data were mostly from the 1970s and that more recent data were not found that fit the requirement of avoiding acute ≤96-h tests. Given that more recent chronic aqueous PCB toxicity data still are not available, we decided to complete a study focusing on the acute aqueous toxicity of PCBs to benthic invertebrates and similarly predict sediment concentrations using equilibrium partitioning (EqP) theory and total organic carbon (TOC), to assess any agreement of the resulting acute toxicity data and the predictive 2017 chronic model. Subsequently, we measured PCB-spiked sediment toxicity to learn how well the 2017 dose–response model corresponds to these new laboratory data.

Acute aqueous exposure toxicity tests using Aroclor 1254 (A1254) were completed using larval, juvenile, and adult life stages of 3 crustacean species. Mortality across multiple treatments was determined every 24 h, and estimates of the

* Address correspondence to Ken.Finkelstein@NOAA.gov
Published online 28 December 2020 in Wiley Online Library (wileyonlinelibrary.com).
DOI: 10.1002/etc.4977

TABLE 1: Partial reprint of the sediment polychlorinated biphenyl benthic predicted injury look-up table that used chronic exposures (cf Finkelstein et al. 2017, Table 5)[a]

Log10 A1254 sediment concentration (μg/g OC)	A1254 sediment concentration (μg/g OC) at 1% OC	A1254 sediment concentration (μg/g)	Benthic injury (%)	Lower 95% CI	Upper 95% CI
1.604	40.18	0.40	7.44	−1.8	16.7
1.629	42.53	0.43	8.05	−1.6	17.7
1.653	45.01	0.45	8.69	−1.4	18.8
1.678	47.65	0.48	9.38	−1.1	19.8
1.703	50.43	0.50	10.13	−0.7	21.0
1.727	53.38	0.53	10.92	−0.3	22.1
1.752	56.50	0.56	11.77	0.2	23.3
1.777	59.80	0.60	12.67	0.7	24.6
1.801	63.30	0.63	13.64	1.4	25.9
1.826	67.00	0.67	14.66	2.1	27.2
1.851	70.91	0.71	15.75	2.9	28.6
1.875	75.06	0.75	16.90	3.8	30.0
1.900	79.44	0.79	18.11	4.9	31.4
1.925	84.09	0.84	19.40	6.0	32.8
1.949	89.00	0.89	20.75	7.2	34.3
1.974	94.20	0.94	22.17	8.6	35.8
1.999	99.71	1.00	23.66	10.0	37.3
2.023	105.54	1.06	25.22	11.6	38.9
2.048	111.71	1.12	26.84	13.3	40.4
2.073	118.24	1.18	28.53	15.1	42.0
2.097	125.15	1.25	30.28	17.0	43.6
2.122	132.46	1.32	32.09	19.0	45.2
2.147	140.20	1.40	33.95	21.0	46.9

[a]A sediment concentration of 1.0 μg/g estimates benthic injury of approximately 23%.
A1254 = Aroclor 1254; OC = organic carbon; CI = confidence interval.

50 and 20% lethal concentrations (LC50 and LC20, respectively) were determined for the 3 benthic species to compare to the chronic model as detailed in Finkelstein et al. (2017). The objective of the present study was to learn if these acute results would, with some adjustment, confirm the original chronic model and therefore provide support in using the relationship detailed in the 2017 look-up table in assessing damages to natural resources from exposure to PCBs.

METHODS

Aquatic

A series of acute 96-h aqueous toxicity tests were completed for 3 species (common name; life stage): *Palaemonetes pugio* (grass shrimp; adults), *Leptocheirus plumulosus* (benthic amphipod; juveniles), and *Americamysis bahia* (mysid shrimp; larvae) following ASTM standard toxicity testing protocols (ASTM International 2014a). We exposed each species to A1254 (item N-11091; ChemService) dissolved in acetone in standard 96-h aqueous toxicity tests. In summarizing the test methods, we exposed these species to A1254 using standard 96-h static renewal protocols (6 or 7 treatments plus control). The A1254 concentrations in water were analytically determined by isolating PCBs from water samples via Strata-X solid-phase extraction (SPE) cartridges (Phenomenex, Torrance, CA), followed by gas chromatographic–mass spectrometric (GC/MS) analysis (Agilent 6890/5973) in each treatment over the duration of the test at each dose and 24-h postdose (Wirth et al. 2014).

TABLE 2: Average survival results for each aquatic treatment[a]

TWA A1254 concentration (μg/L)	Average survival (%)	%CNR	%Injury	Predicted %Injury
Grass shrimp (adult)				
0	95	100.0	0.0	—
1.02	90	94.7	5.3	1.4
2.09	95	100.0	0.0	3.9
6.4	95	100.0	0.0	16.6
17.0	97.5	102.6	−2.6	44.5
21.4	90	94.7	5.3	52.8
59.7	62	65.8	34.2	82.9
560	0	0.0	100.0	99.2
Mysid (larval)				
0.002	100	100	0	0.0
3.84	95	95	5	20.3
11.7	97.5	97.5	2.5	31.7
31.6	77.5	77.5	22.5	57.9
69.4	5	5	95	78.5
144	0	0	100	94.5
570	0	0	100	95.5
Amphipod (juvenile)				
0.0	95	95	5	0.0
1.50	97.5	102.6	2.5	19.8
2.27	87.5	92.1	12.5	54.8
4.86	35	36.8	65	83.4
9.63	0	0	100	93.9
28.3	0	0	100	97.8
32.8	0	0	100	99.7

[a]Survival data were normalized as a percent (%CNR) and then percentage injury was calculated (100 – %CNR) to compare the difference between the injury results from the measured acute tests (%Injury) of the present study and the predicted injury based on chronic exposure (Predicted %Injury) from Finkelstein et al. (2017).
A1254 = Aroclor 1254; CNR = control normalized response; TWA = time-weighted average.

Using the concentrations measured, we then calculated a time-weighted average (TWA) dose and determined the LC50 for each species test using a Probit analysis (SAS; Table 2). Then, for each treatment, we calculated the percentage control normalized response and the percentage injury (%Injury) as described in Finkelstein et al. (2017) and then compared the measured %Injury to the predicted %Injury proposed (Equation 1).

$$\text{Predicted \%Injury} = 100/(1 + 10^{(\log \text{LC50} - \log\,[\text{A1254}] \times \text{slope})}) \quad (1)$$

The published 2017 model lists assumptions related to EqP theory. For each measured TWA A1254 treatment, EqP was used to estimate A1254 sediment concentrations applying the suggested log organic–carbon partition coefficient (K_{OC}) of 4.82 as reported by Finkelstein et al. (2017) and a TOC content of 1%. This log K_{OC} was described in the Estimation Program Interface Suite, Ver 4.11 (US Environmental Protection Agency 2016). The predicted sediment A1254 concentration (as micrograms per gram organic carbon) was then used to predict %Injury from the look-up tables in Finkelstein et al. (2017). This predicted injury based on chronic exposure (Predicted %Injury) was then compared to the measured injury (%Injury) from the acute tests provided in the present study (Table 2 and Figure 1). Figure 1 is a representation of the relationship between calculated and predicted injury. The dashed line is the linear trendline, and the dotted line is the polynomial trendline; R^2 is 0.79 and 0.85, respectively. These results indicate that the model proposed by Finkelstein et al. (2017) is generally a good estimator of predicted benthic injury.

The 2017 chronic model was generated from literature values describing aqueous toxicity results and included a range of crustacean species and endpoints based on survival, growth, and reproduction; the resulting toxicity value versus sediment concentration may be found in figures 1 and 2 and tables 4 and 5 of Finkelstein et al. (2017). To compare the toxicity response for the 2017 study and this update, both were normalized to the respective control response and plotted against the measured A1254 concentrations (Figure 1).

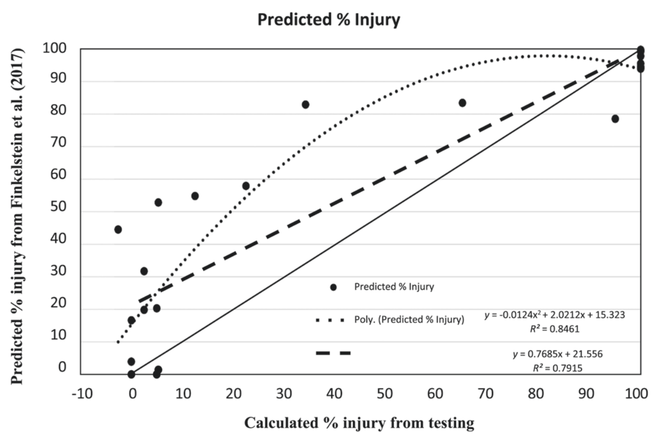

FIGURE 1: The relative fit of calculated percentage injury from this research was plotted against the predicted percentage injury as described in Finkelstein et al. (2017). Kendall's coefficient of concordance ($W = 0.85$) was calculated to determine the relative agreement of the calculated and predicted injury using concordance analysis. The modeled relationship between these parameters resulted in a linear trendline where predicted percentage injury results in an R^2 of 0.79 and a polynomial trendline where predicted percentage injury results in an R^2 of 0.85.

Sediment

Then, we performed spiked sediment toxicity tests to compare the results with the EqP model. We exposed juvenile *L. plumulosus* to A1254 spiked sediment for 10 d following ASTM standard methods for sediment toxicity testing (ASTM International 2014b). A total of 3 sediments were tested with variable moisture and organic carbon content. Two sediment types (Folly River and Leadenwah Creek) were collected from known, long-term reference sites near Charleston, South Carolina, USA (Fulton et al. 2007); and the third is the culture sediment, a commercially available sediment used by Aquatic Biosystems. Field sediment was collected in January 2020, sieved through a 1-mm mesh, and stored at 4 °C for 14 to 20 d. The culture sediment was received 48 h prior to test setup. The moisture content was determined by mass difference after drying for 48 h at 105 °C. Total organic carbon was determined for each sediment type by combustion (Heiri et al. 2001). The dry fraction for each sediment was 0.2272, 0.5795, and 0.6914 for the culture, Folly River, and Leadenwah Creek sediments, respectively. Total organic carbon percentages were determined to be 15, 3, and 4 for the culture, Folly River, and Leadenwah Creek sediments, respectively.

Based on the moisture content of each sediment, approximately 2500 g of wet sediment in precleaned glass 4-L jars were spiked with A1254 (or the equivalent volume of acetone for control sediments). These sediment slurries were rolled on a jar-roller for at least 8 h. Sediment A1254 concentrations were determined in nondosed and spiked sediments at the beginning of the 10-d experiment. Sediment exposures without organisms were run concurrently with the organism exposure, and the A1254 concentrations were determined in the overlying water and sediment (Table 3). Sediment A1254 concentrations were determined by accelerated solvent extraction (Thermo Fisher ASE 200) with 1:1 dichloromethane:acetone, followed by cleanup with copper and alumina SPE. Sediment extracts were analyzed for PCBs through GC/MS analysis, in the same manner as the water samples. For each sediment type, there were 5 replicates for the control and spiked sediments. Survivorship of the 20 individual amphipods in each replicate was determined at the end of 10 d (Table 4).

RESULTS

Aquatic

The results show that the predicted %Injury based on chronic exposures (y-axis) is generally higher compared to the observed acute %Injury (x-axis) from these 96-h toxicity tests (Figure 1). These results were expected, yet when using a polynomial regression analysis, the result showed a fit of 0.85.

TABLE 3: Water and sediment Aroclor 1254 chemistry for spiked sediment toxicity testing

	Time (d)	Culture sediment	Control Folly River	Leadenwah Creek	Culture sediment	Spiked Folly River	Leadenwah Creek
Water (ng/L)	10	0.432	0.863	0.275	49.1	354	492
Sediment (ng/g dry)	0	0.463	0.206	0.038	7596	12 838	13 259
	10	0.286	0.207	0.104	7241	12 714	12 436
Average sediment concentration (ng/g dry)		0.374	0.207	0.071	7419	12 776	12 848

More specifically, we calculated the LC20 for the 4 acute tests shown in the present study (Table 5) to provide a range from 4.93 µg/L (larval) to 41.1 µg/L (adult), resulting in an EqP predicted acute sediment concentration range of 3.30 to 27.50 A1254 µg/g. Of additional interest is the matching of the acute LC20 with the EqP calculated sediment concentration at 1% organic carbon and the resulting predicted chronic injury from Finkelstein et al. (2017). The 4 acute aqueous concentrations showing an acute lethal toxicity of 20% results in a sediment concentration that would show an approximately 3.5 to 5 times greater injury for those species measured with chronic toxicity tests. We note that the chronic test used both lethality and reproduction as endpoints, whereas the acute tests used only the latter.

The results also show that the predicted %Injury based on chronic exposures (*y*-axis) is generally higher compared to the observed acute %Injury (*x*-axis) from these 96-h toxicity tests (Figure 1). These results were expected, yet when using a polynomial regression analysis, the result showed a fit of 0.85.

Sediment

The modeled sediment LC20 for *L. plumulosus* was calculated using data from the present study, and we targeted an A1254 sediment concentration of 11.6 µg/g dry mass (Table 3). Survivorship data were used to compare observed results to the predicted injury from Finkelstein et al. (2017). Using the toxicity test results provided in Table 5 along with the sediment concentrations from Table 4, one can compare the spiked sediment results with the predicted chronic model. Thus, 38% average injury at approximately 13 µg/g total spiked PCBs for Leadenwah Creek sediment can be found using Tables 3 and 4. Similarly for the Folly River sediment and the culture sediment we, respectively, find approximately 13 and 7.4 µg/g total spiked sediments, resulting in 19 and 38% injury.

TABLE 4: Survivorship data from 10-d amphipod sediment toxicity test[a]

Control			Spiked		
Culture	Folly River	Leadenwah Creek	Culture	Folly River	Leadenwah Creek
19	20	19	14	15	13
20	20	20	14	15	13
20	20	18	11	17	12
20	20	20	11	18	12
18	20	20	12	16	12

[a]The test started with 20 individuals in each replicate, and reported mortality is the difference between the initial number of amphipods and those observed survivors.

DISCUSSION

The acute LC20 measures shown in Table 5 for the 4 tests provide a range from 4.93 µg/L (*A. bahia* [mysid] larval) to 41.1 µg/L (*P. pugio* [grass shrimp] adults), resulting in an EqP predicted sediment concentration range of 3.30 to 27.48 A1254 µg/g. Note that the LC20 for these acute aquatic tests provides sediment concentrations greater than the approximate 1.0 µg/g chronic sediment toxicity found by Finkelstein et al. (2017), who used a similar value of approximately 20% toxicity of benthic organisms. Despite different species, one also notes the nearly order of magnitude concentration difference for a toxic response between larval and adult crustaceans (Table 5). Although these age-related results are expected, we recommend future further study using the same larvae, juvenile, and adult species.

Because the earlier model used chronic laboratory toxicity testing compared to the acute work of the present study, we reviewed the literature to find acute-to-chronic ratios used by others, to compare our acute to the 2017 chronic toxicity concentrations. For example, Raimondo et al. (2007) report using an acute-to-chronic ratio of 8.3. In addition, site-specific comparisons of acute to chronic toxicity are commonly found in Comprehensive Environmental Response, Compensation, and Liability Act baseline ecological risk assessments. For example, at the GE Housatonic River Superfund Site Rest of River Study, the US Environmental Protection Agency (2003) reports sediment total PCB concentrations >3 µg/g, which indicates significant adverse effects for sensitive (chronic) endpoints, and total PCB concentrations in the 10 to 30 µg/g range elicit acute mortality to multiple organisms.

Noting an approximate order of magnitude difference in resulting acute sediment concentration (3.3 to 27.48) for the 4 species-specific tests when examining a 20% toxicity, as shown in Table 5, and then using a 10:1 acute-to-chronic ratio, the LC20 toxicity level provides a new 0.33 to 2.75 chronic value range, close to the 1.0 µg/g (1% organic carbon) at approximately 20% sediment toxicity found in Finkelstein et al. (2017; Table 1). Therefore, by using an acute-to-chronic ratio of 10 for the 20% acute injury concentrations shown in the present study, we suggest that the chronic values found in the original model look-up table provide a close approximation to

TABLE 5: Estimating the potential acute %Injury at a 20% lethal concentration (from the PROBI T analysis) for each species based on modeled sediment concentrations calculated using aquatic toxicity and equilibrium partitioning theory

	Calculated LC50 (μg/L)	Calculated LC20 (μg/L)	Estimated A1254 sediment concentration (TWA, μg/g OC)	Estimated A1254 sediment concentration (μg/g; assumed 1% OC)	Estimated A1254 sediment concentration (μg/g; assumed 1% OC) after 10:1 ACR reduction	Modeled percentage injury from Finkelstein et al. (2017)
Grass shrimp, *Palaemonetes pugio*	71.4	41.1	2748	27.5	2.75	97.6
Amphipod, *Leptocheirus plumulosus*	27.8	17.3	1157	11.6	1.16	92.1
Mysid, *Americamysis bahia* (static)	13.02	7.89	528	5.28	0.53	78.2
Mysid, *A. bahia* (renewal)	6.97	4.93	330	3.30	0.33	70.1

A1254 = Aroclor 1254; ACR = acute-to-chronic ratio; LC20/50 = 20 and 50% lethal concentrations, respectively; TWA = time-weighted average; OC = organic carbon.

sediment PCB toxicity. Hence, we move forward in the present study with an approximate 10:1 acute-to-chronic ratio when examining the concentrations causing a specific toxicity level, in this case 20%.

The sediment toxicity data used in the present comparison study provided actual toxicity, although only acute given the 10- rather than 28-d test. When examining Table 6, both the Folly River and Leadenwah Creek sediments show a PCB concentration of 12.8 μg/g. The former shows 19% injury with 3% TOC, the latter 38% with 4% TOC. The culture sediment is different given the considerably lower 7.4 μg/g concentration and especially because of an elevated 15% TOC resulting in a much reduced toxicity of 10%. For both the Folly River and Leadenwah Creek sediments, the difference between the toxicity found in these spiked sediment samples and that from Finkelstein et al. (2017) after normalizing to TOC is approximately a factor of 1.7 to 3.8, with the chronic toxicity being the higher injury value. Only the culture sediment showing a high TOC does not follow the chronic injury > acute injury, likely because of the elevated TOC. As is generally the issue with the measure of sediment chemistry concentrations, simple relationships are hard to find. The acute-to-chronic ratio of 10 used for the aquatic and chronic concentration values is not practical

TABLE 6: Summary of acute sediment data from the present study compared to the injury found in the chronic sediment study of Finkelstein et al. (2017)

	Culture	Folly's Creek	Leadenwah Creek
Concentration (μg/g OC)	7.4	12.8	12.8
Injury (%)	38	19	38
TOC (%)	15	3	4
Concentration normalized using sample-specific OC (μg PCBs/g OC)	49	426	320
Injury found in Finkelstein et al.'s (2017) chronic sediment study when using these 3 acute study normalized concentrations (%)	10	72	63

OC = organic carbon; TOC = total organic carbon; PCB = polychlorinated biphenyl.

for the whole-sediment injury measures. However, it appears that the chronic injury values are generally greater than those acutely addressed when using the same concentration. Both the Folly River and Leadenwah Creek sediments show this, with only the high TOC found in the culture sediment resulting in the opposite.

SUMMARY

The present study along with the chronic Finkelstein et al. (2017) study can be used together to provide the user a chronic and acute value to estimate PCB sediment toxicity. Our objective was to learn if the 1.0 μg/g 20% toxicity value found by Finkelstein et al. (2017) could be supported using the available acute aquatic toxicity analysis for PCB A1254. Ratios measuring acute and chronic concentration and injury will differ depending on the question asked. For example, in the present study, we found a literature-supported 10:1 acute-to-chronic ratio when measuring the acute to chronic PCB sediment concentration resulting in 20% toxicity. As is always the case, the user must review the primary literature before making decisions on site-specific toxicity. Hence, we encourage the user to follow the methods of both studies before addressing their estimates of sediment toxicity. The spiked sediment data we show should allow the user a picture of what they might expect when evaluating higher concentrations and the difficulty using sediment with elevated TOC concentrations.

Acknowledgment—We thank those current and former National Oceanic and Atmospheric Administration employees from the Assessment and Restoration Division of the Office of Response and Restoration and the Ecotoxicology Branch of the National Centers for Coastal Ocean Science. In particular, we thank N. Beckvar, who provided many thoughtful recommendations.

Disclaimer—C. Rios completed this work based on support through the Research Experiences for Undergraduates program of the US National Science Foundation under award DBI-1757899 to the Marine Biology Program, College of

Charleston. The scientific results and conclusions, as well as any opinions expressed herein, are those of the author(s) and do not necessarily reflect the views of the National Oceanic and Atmospheric Administration or the Department of Commerce. The mention of any commercial products is not meant as an endorsement by the agency or department.

REFERENCES

ASTM International. 2014a. Standard guide for conducting acute toxicity tests on test materials with fishes, macroinvertebrates, and amphipods. E729-96. In *Annual Book of ASTM Standards*, Vol. 11.06. Philadelphia, PA, USA.

ASTM International. 2014b. Standard test method for measuring the toxicity of sediment-associated contaminants with estuarine and marine invertebrates. E1367-03. In *Annual Book of ASTM Standards*, Vol. 11.06. Philadelphia, PA, USA.

Finkelstein K, Beckvar N, Dillon TM. 2017. Benthic injury dose–response models for polychlorinated biphenyl contaminated sediment using equilibrium partitioning. *Environ Toxicol Chem* 36:1311–1329.

Fulton MH, Hyland JL, Key PB, Wirth EF, Balthis WL, Cooksey C, Chung K, Leight AK. 2007. Characterization of toxic impacts on living marine resources in tidal rivers of the Chesapeake Bay. NOAA Technical Memorandum NOS NCCOS 64. National Oceanic and Atmospheric Administration, Charleston, SC, USA.

Heiri O, Lotter AF, Lemke G. 2001. Loss on ignition as a method for estimating organic and carbonate content in sediments: Reproducibility and comparability of results. *J Paleolimnol* 25:101–110.

Raimondo S, Montague BJ, Barron MG. 2007. Determinants of variability in acute to chronic toxicity ratios for aquatic invertebrates and fish. *Environ Toxicol Chem* 26:551–558.

US Environmental Protection Agency. 2003. Ecological risk assessment for GE/Housatonic River site, rest of river. Appendix D: Benthic invertebrates. Boston, MA.

US Environmental Protection Agency. 2016. Estimation Program Interface (EPI) Suite, Ver 4.11. In *KOCWIN User's Guide*, Section 6.1.3—Methodology. [cited 2016 November 22]. Available from: http://www.epa.gov/opptintr/exposure/pubs/episuite.htm

Wirth EF, Pennington PL, Cooksey C, Schwacke L, Balthis L, Hyland J, Fulton MH. 2014. Distribution and sources of PCBs (Aroclor 1268) in the Sapelo Island National Estuarine Research Reserve. *Environ Monit Assess* 168:8717–8726.

