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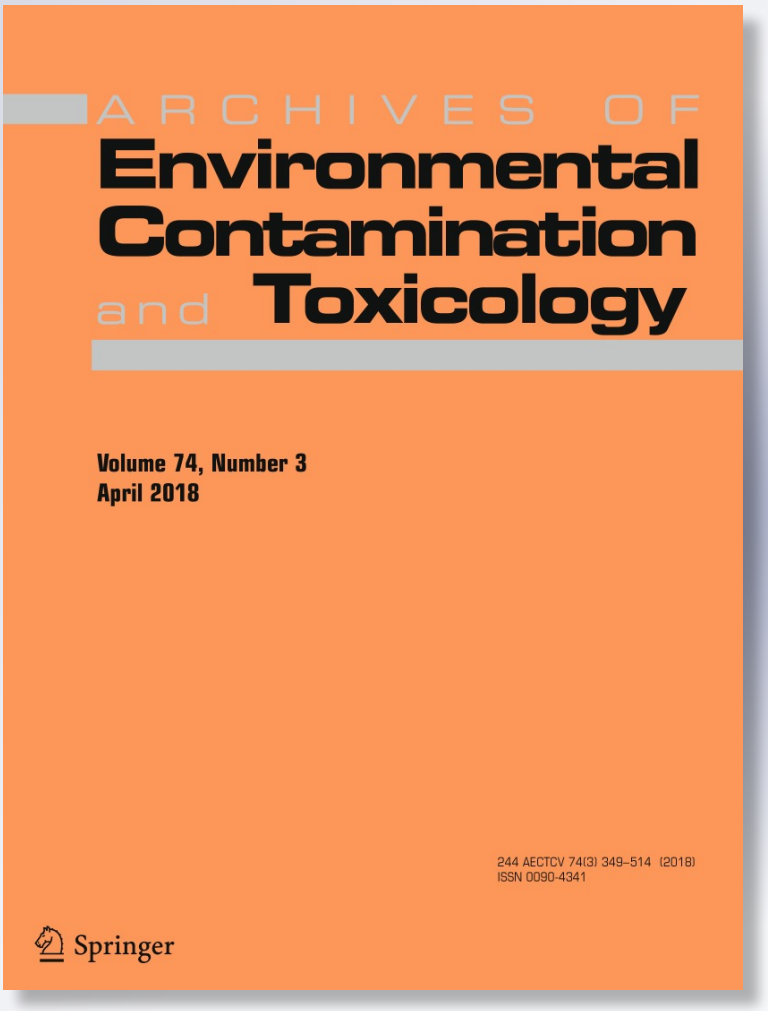

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The Value of Using Multiple Metrics to Evaluate PCB Exposure

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Abstract Current methods for evaluating exposure in ecosystems contaminated with hydrophobic organic contaminants typically focus on sediment exposure. However, a comprehensive environmental assessment requires a more holistic approach that not only estimates sediment concentrations, but also accounts for exposure by quantifying other pathways, such as bioavailability, bioaccumulation, trophic transfer potential, and transport of hydrophobic organic contaminants within and outside of the aquatic system. The current study evaluated the ability of multiple metrics to estimate exposure in an aquatic ecosystem. This study utilized a small lake contaminated with polychlorinated biphenyls (PCBs) to evaluate exposure to multiple trophic levels as well as the transport of these contaminants within and outside of the lake. The PCBs were localized to sediments in one area of the lake, yet this area served as the source of PCBs to aquatic invertebrates, emerging insects, and fish and terrestrial spiders in the riparian ecosystem. The Tenax extractable and biota PCB concentrations indicated tissue concentrations were localized to benthic invertebrates and riparian spiders in a specific cove. Fish data, however, demonstrated that fish throughout the lake had PCB tissue concentrations, leading to wider exposure risk. The inclusion of PCB exposure measures at several trophic levels provided multiple lines of evidence to the scope of exposure through the aquatic and riparian food web, which aids in assessing risk and developing potential future remediation strategies.

To evaluate fully contaminant exposure at a site, it is important to examine multiple exposure metrics, including sediment concentrations and concentrations in biota at various trophic levels within the aquatic and surrounding riparian ecosystem. These metrics provide unique information on the contaminant risk at a site. For example, traditional exhaustive sediment extractions of hydrophobic organic contaminants (HOCs) provide information on the chemicals present in the system, but they fail to evaluate fully bioavailability, even when organic carbon is used to normalize the sediment concentrations (Maruya et al. 1996; Tracey and Hansen 1996; You et al. 2006; Harwood et al. 2013). Bioavailability-based metrics, however, provide better exposure estimates by quantifying sediment bioavailability and bioaccumulation potential (USEPA 2012; Lydy et al. 2015; Harwood et al. 2015). Tenax extractable concentrations estimate bioavailability based on the contaminant fraction that desorbs from a sediment matrix (Harwood et al. 2015). Additionally, previous research demonstrated that bioavailability-based techniques, such as Tenax, accurately estimate the bioaccumulation of HOCs, such as PCBs, potentially removing the need for laboratory-based bioaccumulation assays (Cornelissen et al. 2001; ten Hulscher et al. 2003; Trimble et al.

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2008; Mackenbach et al. 2014). While accurate exposure assessments at the sediment–water interface provide essential data to estimate risk, more detailed evaluations often are needed to define completely the exposure within and around a contaminated location. Without fully evaluating the exposure in the ecosystem, the risks determined at a location can be inaccurate and lead to poorly conceived remediation decisions.

The current study evaluated multiple exposure metrics to assess exposure in a PCB-contaminated ecosystem. Specifically, the major objective was to evaluate the ability of these exposure metrics to quantify the spatial extent of PCB bioavailability and exposure in the aquatic and riparian ecosystem. This study focuses on a small lake, Campus Lake, in southern Illinois and how the scale and number of relevant exposure metrics can be simplified in a small closed system. Polychlorinated biphenyls served as the representative HOCs to test this approach as they are well studied, and there is a history of PCB contamination in the Lake. The following exposure metrics were examined: exhaustive chemical extraction of the sediment, Tenax-extractable sediment concentrations, invertebrate bioaccumulation assays, and contaminant concentrations in field-collected emergent insects, riparian spiders, and three fish species. By evaluating PCB exposure at several trophic levels, multiple lines of evidence could be used which provided a more thorough evaluation of exposure potential and demonstrated the benefits and drawbacks of implementing a sampling plan that incorporates multiple exposure metrics.

Methods

Solvents and Chemicals

A total of 119 PCB congeners were quantified for the bulk of testing for the current project, whereas a 28 PCB congener mix was quantified for some of the initial source tracking and quality assurance/quality control samples. Both PCB congener solutions were purchased from Accustandard (New Haven, CT) with certified >98% purity. A surrogate, dibromooctafluorobiphenyl (DBOFB), was purchased from Supelco (Bellefonte, PA) and surrogates PCB-204 and PCB-191 were purchased from Accustandard. Internal standards included four ^{13}C-labeled PCB congeners: ^{13}C-PCB-15, ^{13}C-PCB-52, ^{13}C-PCB-141, ^{13}C-PCB-209 (Cambridge Isotope Laboratory, Andover, MA). Tenax TA, 60–80 mesh, was purchased from Scientific Instrument Services, Incorporated (Ringoes, NJ). All other materials and pesticide-grade solvents were purchased from Fisher Scientific (Pittsburgh, PA) unless otherwise noted.

Location Description and Sampling Design

Campus Lake (16.2 ha), Carbondale, Illinois, is located at 37°7′N and 89°2′W on the southwest edge of Southern Illinois University—Carbondale. To confirm the presence of PCBs and locate their general distribution, sediments were sampled from 30 locations (Fig. 1a). This screening revealed elevated PCB congener concentrations on the west side of the lake (Fig. 1a). To measure PCB bioavailability, five additional sampling locations were chosen within the contaminated area (Figs. 1a, b). At these locations, sediment was collected for subsequent exhaustive chemical and

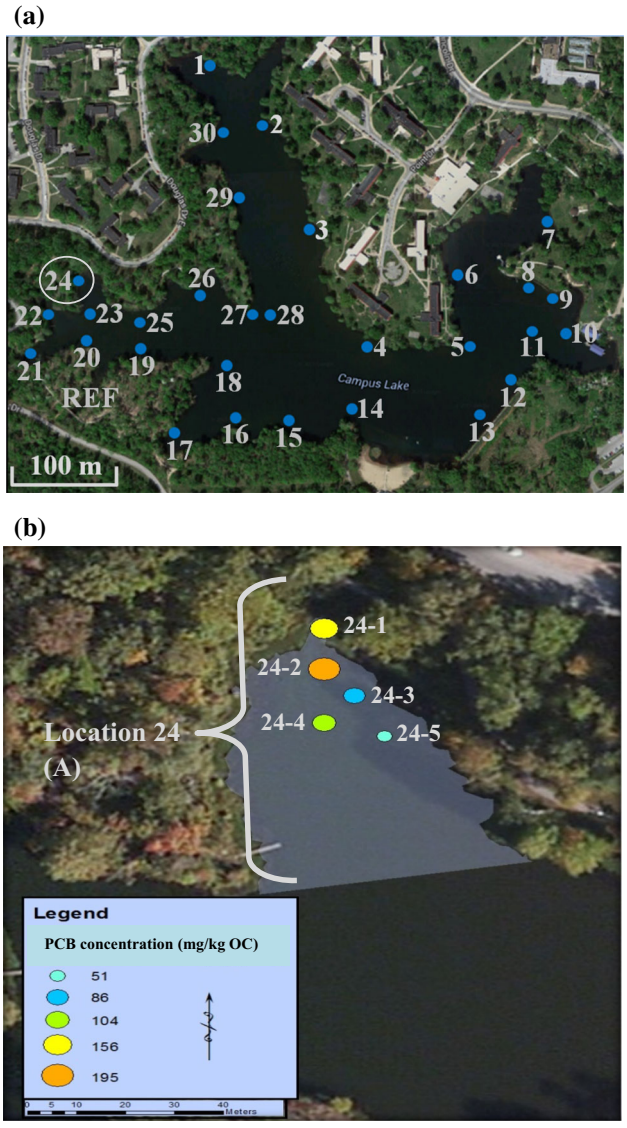

Fig. 1 Initial sediment screening sampling locations (30 total) indicated by spheres with contaminated location indicated by a *circle* (**a**). Close up image of the contaminated area in Campus Lake with sampling locations and concentrations indicated by *spheres* (**b**). Emergent insects and spiders were collected from both the contaminated (**b**) and reference (indicated by "*REF*" in **a**) locations

Tenax extraction, and laboratory-based *Chironomus dilutus* and *Lumbriculus variegatus* bioaccumulation assays also were performed. Additionally, emergent aquatic insects and riparian spiders were collected from the contaminated area. To determine whether PCBs were being transported out of the contaminated area, emergent insects and spiders were collected from a reference location on Campus Lake (indicated by an "REF" in Fig. 1a). Bluegill (*Lepomis macrochirus*), largemouth bass (*Micropterus salmoides*), and yellow bullhead catfish (*Ameiurus natalis*) also were collected from several sampling areas throughout the lake.

Analytical Quantification

Twenty-eight PCB congeners were analyzed for in the sediments from the 30 screening locations in Campus Lake, and this analysis was performed on a gas chromatograph equipped with a micro-electron capture detector (ECD) (Agilent 6890, Palo Alto, CA) using dual-column confirmation (You et al. 2007). All other extracts were analyzed for a total of 119 congeners on a gas chromatograph-mass spectrometer (6890GC- 5973 N MS, Agilent Technologies) (Mackenbach et al. 2012). Specifications of these analyses can be found in Supplementary Materials.

Sediment Collection and Storage

Surficial sediments (top 6–8 cm) were collected from Campus Lake using a stainless steel bottom sampling dredge (LaMotte, Chestertown, MD) or a core sampler (WaterMark Universal 60, Forestry Suppliers Incorporated, Jackson, MS). The global positioning system (GPS) coordinates associated with each sampling location were recorded (Garmin GPS map 625C, Olathe, KS). Three samples were collected from each sampling location and homogenized in a stainless-steel mixing bowl, while in the field. Sediments were sieved (2 mm) in the laboratory to remove large rocks and plant debris and stored in the dark at 4 ± 2 °C for no more than 2 weeks.

Sediment Extractions

Sediments were extracted using an accelerated solvent extraction system (ASE Dionex 200, Sunnyvale, CA) using aliquots of sediment freeze-dried after sieving (LabConco FreeZone Plus freeze-dryer, Kansas City, MO). Stainless-steel ASE cells were equipped with cellulose filters and loaded with 2 g of silica gel, 2 g of copper powder, 3 g dry of sediment, and scientific grade sea sand to fill the void space in the cells. Samples were then spiked with 50 ng of surrogates (DBOFB and PCB-204 or PCB-191; You et al. 2007). Extractions were performed at 1500 lb per square inch (psi), using a 1:1 (v:v) mixture of acetone: methylene chloride. The extraction process was performed at 100 °C, using two heat-static cycles of 10 min each. Following extraction, samples were solvent exchanged to hexane, evaporated to 1 mL using a nitrogen evaporator (Zymark Turbovap II, Charlotte, NC) and cleaned using the methods described in Supplementary Materials. Because sediment samples were dried before extraction, all sediment concentrations were reported on a dry weight basis. In addition to extraction, subsamples of sediment were analyzed for organic carbon (OC) using the Association of Occupational and Environmental Clinics method (AOEC, 993.13; Table S1) by Midwest Laboratories (Omaha, NE).

Bioaccumulation Assays

Fourteen- and 10-day bioaccumulation assays were performed with laboratory-cultured *L. variegatus* and *C. dilutus*, respectively, following a modified U.S. EPA protocol (U.S. EPA 2000). Although the U.S. EPA method for *L. variegatus* calls for a 28-day test, a modified 14-day test was found more effective for *L. variegatus* exposed to PCBs (Van Hoof et al. 2001; You et al. 2006; Trimble et al. 2008), because reproduction halts feeding at 14-days, thus influencing the contaminant accumulation in the organism (Leppanen and Kukkonen 2006). After their respective exposure periods, organisms were removed using a 500-µm sieve, and *L. variegatus* were placed in fresh MHW to depurate gut contents for 6 h. Depuration is recommended for this species, because the gut contains contaminated sediment (Mount et al. 1999). After depuration of *L. variegatus*, organisms were then blotted dry, weighed, and frozen at −20 ± 2 °C for subsequent extraction. The *C. dilutus* were not depurated and were weighed directly into the aluminum weighing pans. Depuration in species where gut content weight does not contribute contaminant concentration, such as *C. dilutus*, is not suggested (Hare et al. 1989). The frozen samples were then extracted according to the methods described in Trimble et al. (2008). Additionally, two *L. variegatus* or *C. dilutus* from each replicate were subsampled for lipid analysis using the methods of Folch et al. (1957) and Lu et al. (2008). The lipid mass was determined by mixing the sample with a vanillin/phosphoric acid reagent and the absorbance at 525 nm was measured (Spectronic 20 Genesys, Rochester, NY). Using a standard curve from vegetable oil, the lipid concentration was quantified in each organism. Additional details on the bioaccumulation methods are provided in Supplementary Materials.

Tenax Extractions

To assess the bioavailable fraction of PCB congeners in sediment, single-point 24-h Tenax extractions were

performed on sediment from the five locations within the contaminated area, according to the methods of Mackenbach et al. (2012). Approximately 3 g wet sediment (~2 g dry weight (dw)) was placed in a 50-mL screw top vial with 45 mL of moderately hard water (MHW). Tenax beads (0.5 g) and copper powder (0.5 g) were then added to each vial. The copper powder reduced the sulfur content in the extractions. The vial was then placed on a rotator (BBL BioQuest, Cockeysville, MD) and rotated at 20.75 revolutions per minute (rpm). After 24 h, the vials were removed and centrifuged (Eppendorf Centrifuge 5702 R, Hauppauge, NY) for 5 min at 2300 rpm. Because the Tenax beads float, they were removed from the water surface using a clean metal spatula. The Tenax was placed in clean scintillation vials and extracted using acetone (5 mL) and sonication for 5 min. The acetone was removed from the vial and placed into a clean scintillation vial. The Tenax was further extracted twice more using an acetone: hexane (1:1, v:v) solution (5 mL) for a total of three extractions. The extracts were combined and surrogate (50 ng) was added. After surrogate addition, the extracts were solvent exchanged to hexane and evaporated using a steady nitrogen gas flow to a final volume of 2 mL. To remove any remaining water, the extracts were passed through drying columns (14.6-cm Pasteur pipettes packed with ~1.75 g of anhydrous sodium sulfate). The pipette column was primed with hexane (1 mL) and eluted with hexane (1 mL). Extracts were then stored at 4 ± 2 °C overnight until they were cleaned up as described in Supplementary Materials.

Tenax-extractable PCB concentrations were compared to lipid-normalized bioassay concentrations for both test species, and then these concentrations were compared to the Bioaccumulation-Tenax Model (BTM) developed by Mackenbach et al. (2012, 2014). This model related the 24-h Tenax extractable concentration to laboratory-based bioaccumulation assay biota concentrations after exposure to contaminated sediment. The data from the current study was applied to this model to determine if the BTM was appropriate to assess Campus Lake sediments. If the data fit the BTM model, the model can be used to assess exposure and confirms that the bioavailability estimates (tissue and Tenax extractable concentrations) follow expected trends.

Invertebrate and Fish Collection

Emerging aquatic insects were collected using 12 emerging insect traps deployed at the contaminated and reference locations, for a total of 24 traps. From August to October 2014, aquatic insects were removed from the traps tri-weekly or before rain events using an aspirator (Forestry Suppliers Incorporated, Jackson, MS), and frozen at −20 ± 2 °C until they were identified and extracted. In addition to aquatic insects, riparian Tetragnathid spiders were collected for subsequent PCB analysis. These obligate consumers of aquatic insects were manually collected from the littoral-terrestrial region (within 1 m of the waterline) of each location (contaminated and reference) on a biweekly basis (April to October 2014) and also were stored at −20 ± 2 °C.

Three fish species were collected throughout the lake using an electrofisher (Smith Root GPP 5.0 pulsed direct current (DC) Vancouver, WA) mounted on a boat. A random distribution of males and females were collected for a total of 20 bluegill (*L. macrochirus*), 10 large-mouth bass (*M. salmoides*), and 6 yellow bullhead (*A. natalis*). Fish targeted for collection were adults between 150 and 250 mm for bluegill, 300–400 mm for large-mouth bass, and 150–350 mm for bullhead. Fish were humanely euthanized using a >250 mg/L dose of MS-222 (Argent Chemical Laboratories, Redmond, RA), according to the Institutional Animal Care and Use Committee protocols. The total length and weight of each fish were recorded and the fish were then frozen at −20 ± 2 °C for 1 week until they were homogenized using a meat grinder (Every China, Model QJHC12A, Beijing, China). The fish were then freeze-dried for subsequent extraction.

Invertebrate and Fish Extractions

The insects and spider samples were freeze-dried and ground and homogenized via mortar and pestle, extracted using ASE (as described above), and cleaned up using sulfuric acid digest (Supplementary Materials). Aliquots of homogenized, freeze-dried fish samples were extracted using the ASE methods described above. The ASE extracts were solvent exchanged to methylene chloride and filtered through 0.2-μm filters (Whatman, Fisher Scientific) primed with methylene chloride (1 mL). These filtered extracts were collected in conical volumetric vials and the volumes were adjusted to 6 mL with methylene chloride to perform gel permeation chromatography (GPC). The GPC system coupled with a fraction collector (Agilent Technologies with Foxy® Jr. Fraction Collector (ISCO, Inc. Lincoln, NE)) separated lipids from sample extracts using an Envirogel column (Waters 300 mm × 19 mm GPC clean-up column with a 5 mm × 19 mm pre-column). Extract fractions were collected during the GPC separation between 8 and 12 min in two fractions (Koch et al. 2006). The fractions were combined, solvent exchanged to hexane, and evaporated to a final volume of 2 mL for subsequent cleanup (described in Supplemental Materials). In addition, the lipid content of each fish was measured using the colorimetric method previously described.

Stable Isotope Analysis

Stable isotope analysis (SIA) was performed by the U.S. EPA National Risk Management Research Laboratory in Cincinnati, OH (Raikow et al. 2011). The SIA was performed on aliquots of sediment, adult dragonflies (Libellulidae), spiders, and fish. Briefly, samples were first freeze-dried and ground via mortar and pestle to homogeneity. Approximately 0.1 to 4 mg of each sample was then weighed in 3- × 5-mm capsules (Thermo Scientific Finnigan, Germany). The samples were then analyzed using an elemental analyzer (Carlo Erba NC1500 EA; Carlo Erba Instrumentazione, Milan, Italy) coupled to an isotope ratio mass spectrometer (Conflo III unit and Finnigan Delta-Plus Thermo Electron, Waltham, MA) to quantify $\delta^{13}C$ and $\delta^{15}N$. The oxidation furnace, reduction furnace, and column of the EA were set to 1020, 750, and 50 °C, respectively. The carrier gas used for the SIA was He at a 100 mL/min flow rate.

Quality Assurance/Quality Control

To ensure the accuracy of the analytical method, surrogates were added to the sediment, Tenax, *L. variegatus, C. dilutus,* aquatic insects, and fish (spiked at 50–100 µg/L) during the extraction to monitor extraction efficiency. Blanks, laboratory-controlled spikes, matrix spike, and matrix spike duplicates were prepared every 20 samples for sediment, Tenax, aquatic insects, and fish extractions. A calibration check standard was analyzed every seven samples, and daily calibration check standards had to be within 20% for all 119 congeners and surrogates for the run to pass. If these values were not within 20%, the data were rejected and re-run on the GC–MS. The internal standard peak areas in the samples also were checked to see if they were within 50% of the average peak areas measured in the calibration curve. The reporting limits (RL) of the current study were set to three times the product of the standard deviation (s) of seven replicate injections of a 5 µg/L standard for the 119 PCB congeners and surrogates and the Student's t value for 99% confidence interval ($n = 7$). The PCB congener concentrations that were below $s \times t$ were reported as non-detects (nd). The PCB congener concentrations that were above $s \times t$, but below the RL, were replaced with one-half the lipid- or OC-normalized RL.

Results and Discussion

Quality Assurance/Quality Control

For the bioaccumulation assays, controls (4 replicates) were prepared using clean reference sediment in each test. Water quality parameters including dissolved oxygen, pH, conductivity, ammonia, and temperature all remained within acceptable ranges during the assays (U.S. EPA 2000). Control survival of *L. variegatus* and *C. dilutus* in the bioaccumulation assays was ≥95%, and there was no observed avoidance of the sediment. Surrogate recoveries for sediment, Tenax, and bioassay organisms were 69–142% for DBOFB, 51–115% for PCB-204, and 51–128% for PCB-191 across all samples. Quality assurance sample recovery (laboratory-controlled spikes, matrix spikes, and matrix spike duplicates) for sediment, Tenax, laboratory-cultured organisms, aquatic insects, spiders, and fish fell within 56–83%. No target analytes were detected in the blank samples.

PCB Exposure Assessments and Transfer in Campus Lake

The initial sediment screening revealed elevated PCB levels in the sediment from a limited area on the western side of Campus Lake (Fig. 1b). The total PCB concentration in sediment from this location was 3.97 mg/kg (dry weight, dw; Table 1). Sediment from the remaining 29 sampling locations in Campus Lake revealed concentrations that were on average 50 times less than at the contaminated location (Table 1). Additionally, a reference location adjacent to the contaminated location (Fig. 1a) was selected for comparison of contaminant concentrations in aquatic insects and spiders. The reference location had sediment concentrations 90 times lower than those from the contaminated location (0.05 mg/kg compared with 3.97 mg/kg total PCBs; Table 1).

After identifying the source area of PCB contamination in Campus Lake, additional sediments were collected in this contaminated area. These sediments were examined using traditional exhaustive chemical extraction methods using a 119 congener analysis (Fig. 1b) with OC normalization. The total PCB concentrations at the five contaminated locations ranged from 51 to 195 mg/kg OC, with sediment OC levels ranging from 5.65–8.87% (Table S1). Location 2 (Fig. 1b) had the highest total PCB concentration (195 mg/kg OC).

While exhaustive chemical extractions were highest for sediment samples in the contaminated area of the lake, these data did not indicate if PCBs were bioavailable to organisms (Lydy et al. 2015). The U.S. Environmental Protection Agency has recently recommended the use of bioavailability-based techniques to monitor HOCs to better represent the contaminant fraction responsible for exposure (USEPA 2012). Additionally, desorption-based techniques, such as Tenax extractions, are well recognized to provide accurate exposure estimates (Cornelissen et al. 2001; You et al. 2011). The 24-h, single-point Tenax extractions

Table 1 Polychlorinated biphenyl (C-WNN 28 congener list) concentrations detected in the 30 sediment sampling locations (Fig. 1a) used in the pilot study

Site	Total PCBs (mg/kg, dry weight)
1	0.035
2	0.028
3	0.24
4	0.015
5	0.012
6	0.027
7	0.052
8	0.096
9	0.046
10	0.044
11	0.060
12	0.032
13	0.072
14	0.044
15	0.038
16	0.036
17	0.026
18	0.048
19	0.061
20*	0.045
21	0.12
22	0.15
23	0.030
24	*3.97*
25	0.059
26	0.17
27	0.084
28	0.034
29	0.044
30	0.042

Compared with the contaminated location (#24), all other concentrations were on average 50 times less and therefore considered background contamination. The contaminated cove examined in this study is indicated by italics; the asterisk indicates the sampling site closest to the reference location (approximately 100 times less than contaminated cove)

performed on the sediment from the five locations from the contaminated area of Campus Lake had total PCB concentrations from 13.0 to 122 mg/kg OC (Fig. 2). The Tenax bioavailability-based exposure metric provided the advantage of estimating bioaccumulation potential in organisms as well as providing information on PCB distribution in sediments. Because PCBs were present in the Tenax extracts, the PCBs were bioavailable to benthic organisms that inhabit the contaminated sediment (Landrum et al. 2007; Trimble et al. 2008; Mackenbach et al. 2012, 2014).

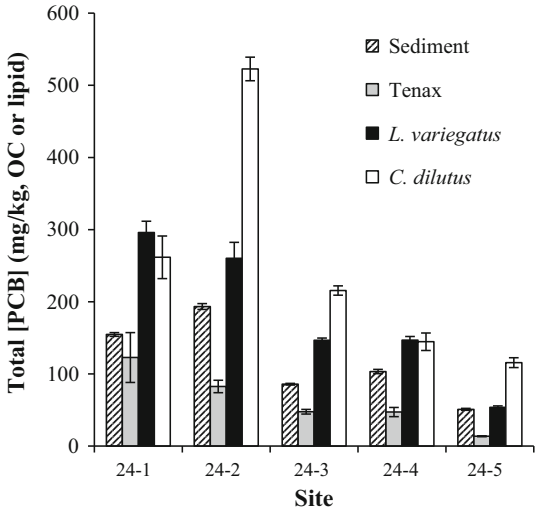

Fig. 2 Comparison of average total polychlorinated biphenyl concentrations found in exhaustive chemical extraction, Tenax extractable fraction, and *Lumbriculus variegatus* and *Chironomus dilutus* tissues at each of the five locations within the contaminated area of Campus Lake. *Error bars* indicate standard deviation between replicates ($n = 4$)

In addition to the Tenax extractions, laboratory bioaccumulation assays were performed using *L. variegatus* and *C. dilutus* exposed to sediments collected from the contaminated locations. Ideally, in situ organisms would have been collected from these areas of the lake to provide estimates of benthic organism exposure, but significant tissue masses could not be obtained during sampling. In laboratory exposures, the body residues for *L. variegatus* and *C. dilutus* were 53.8–296 and 116–523 mg/kg lipid, respectively (Fig. 2). In laboratory assays, the mean lipid values for *L. variegatus* and *C. dilutus* were 3.31 ± 0.70 and $2.10 \pm 0.32\%$, respectively. *Chironomus dilutus* demonstrated greater bioaccumulation than *L. variegatus* when exposed to the same sediments, but both species bioaccumulated PCBs. Both exposure metrics, Tenax extractions and laboratory bioaccumulation assays, showed PCBs in the contaminated area of Campus Lake were bioavailable and capable of moving from the sediment to benthic invertebrates. Importantly, Tenax extractions are less expensive (\$400 vs. ~\$4000) and faster (24 h vs. 14–28 days) than traditional bioaccumulation assays, yet provide comparable information. Furthermore, because the BTM has been demonstrated to correlate single-point Tenax extractable concentrations to bioaccumulation data (Harwood et al. 2015; Mackenbach et al. 2012, 2014), single-point Tenax extractions provide an alternative to laboratory bioaccumulation assays or in situ sampling when cost, time, or lack of sufficient field-collected tissue masses becomes a concern. This is demonstrated by the ability of the BTM to predict bioaccumulation for both species (Fig. 5).

Although the benthic life stages of aquatic emergent insects residing in Campus Lake could not be collected, adult life stages were collected and analyzed for PCBs. Total PCB concentrations detected in the emerged adult aquatic insects Aeshnidae/Libellulidae (dragonflies), Coenagrionidae (damselflies), and Chaoboridae/Ceratopogonidae/Chironomidae (midges) from the contaminated locations were 8.41, 46.6, and 99.4 mg/kg lipid (Table 2; Fig S1), respectively. A comparison of PCB congener patterns in sediment to emerged aquatic insects confirmed that the adult insects collected from the contaminated location were exposed to PCBs in these sediments. This further confirmed the bioavailability estimated from the Tenax extractions and laboratory bioaccumulation assays (Fig. S1). The presence of PCBs in aquatic insects, however, appeared to be localized to those emerging from the contaminated area. Midges collected from the reference location only accumulated one PCB (153) congener at an average concentration of 1.98 mg/kg lipid (Table 2), whereas dragonflies and damselflies did not contain detectable PCBs.

To assess potential transport from the lake to the riparian zone, terrestrial spiders collected from the riparian areas surrounding the contaminated and reference areas were analyzed for PCBs. Tetragnathid spiders collected from the contaminated locations had an average total PCB concentration of 70.9 mg/kg lipid, while spiders collected from the reference area only accumulated PCB 153 at 1.69 mg/kg lipid (Table 2; Fig. 3). Tetragnathid spiders, which are emerging aquatic insect obligates, feed at the littoral-terrestrial interface of water bodies (within 1–3 m). The PCB contamination in spiders creates the potential for further terrestrial transport via arachnivorous birds (Maul et al. 2006; Walters et al. 2010). Because spiders near the contaminated locations accumulated PCBs and similar congener patterns were found for emergent aquatic insects and spiders (Fig. 3), it was concluded that the spiders surrounding the contaminated area were exposed to PCBs through the consumption of emergent insects from this contaminated sediment. The comparison of emergent insect and spider congener distribution data reveals the pathway for the PCBs transport was into the riparian area immediately surrounding the contaminated locations, but the lack of PCBs in insects and spiders collected from the reference area suggests this exposure pathway was limited spatially (Fig. 1a). Therefore, even with the use of multiple exposure metrics (insect, spider, and sediment data) to evaluate the risk of PCB exposure within Campus Lake, one may

Table 2 Total polychlorinated biphenyl concentrations present in invertebrates and fish collected from Campus Lake

	PCB (mg/kg lipid)	
	Contaminated	Reference
Invertebrates		
Midges	99.4	1.98
Damselflies	46.6	<RL
Dragonflies	8.41	<RL
Spiders	70.9	1.69
Fish		
Bluegill	49.8	
Largemouth bass	232	
Bullhead	312	

RL reporting limit

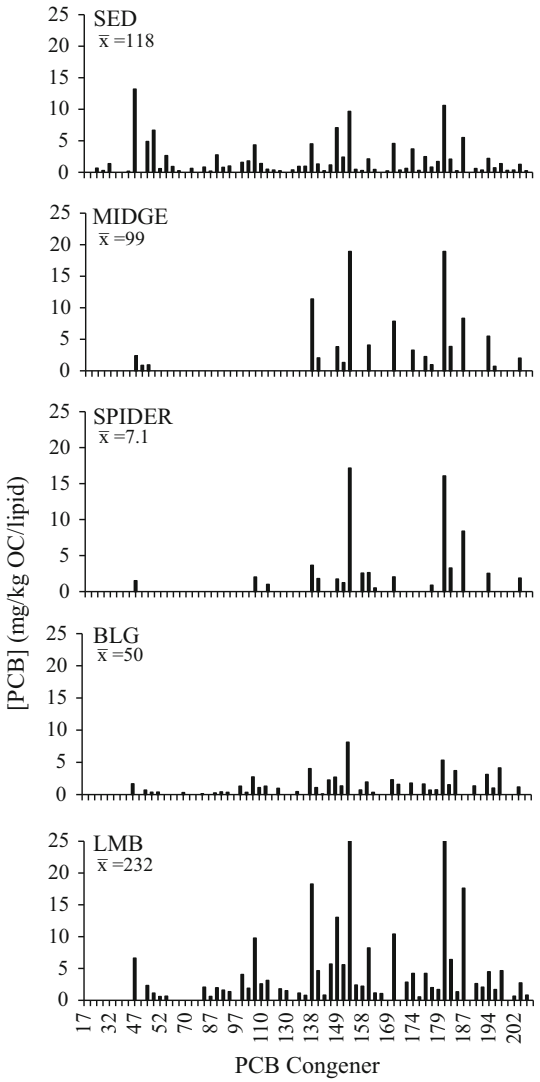

Fig. 3 Average polychlorinated biphenyl congener concentrations present in sediment (SED) midges, spiders (sub-sample averages, $n = 3$) bluegill (BLG; $n = 20$) and largemouth bass (LMB; $n = 10$) collected from Campus Lake. All PCB concentrations are normalized to either organic carbon or lipid content; $\bar{x}$ represents the mean total PCB concentration

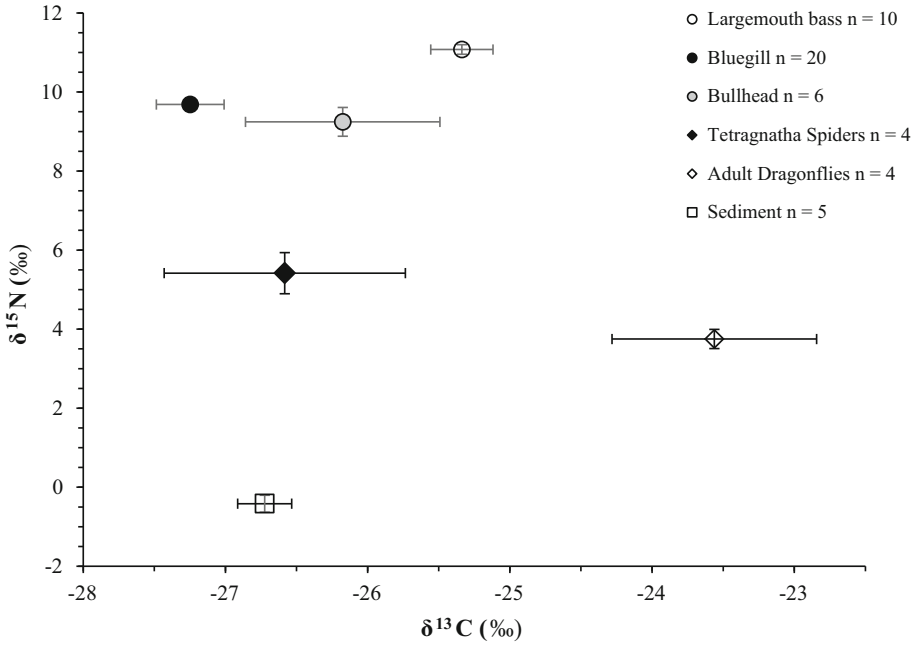

Fig. 4 Stable isotope abundances from samples collected from both the contaminated and reference locations in Campus Lake. Stable isotope abundances were expressed as $\delta^{15}N$ and $\delta^{13}C$, where $\delta X = [(R_{sample}/R_{standard} - 1) \times 1000‰]$, where X was ^{15}N or ^{13}C and R was the corresponding ratio of $^{15}N/^{14}N$ or $^{13}C/^{12}C$. *Error bars* show standard error of replicates. Dragonflies and spiders were pooled from clean and dirty locations. Spiders were pooled from specimen collected in 2013 and 2014

conclude a localized spatial risk through transport within and outside of the lake. However, the spatial scale of the metrics (aquatic insects, spiders, and sediment) must be considered along with other exposure pathways (e.g., fish) to fully characterize the risk to the receptors.

The exposure pathway that includes fish provides a much different estimate of exposure. Fish species often are used as a metric to evaluate HOCs exposure, particularly for PCBs, because of the extent these compounds biomagnify and the potential for exposure of higher trophic levels (Watanabe et al. 2003). Therefore, contaminant concentrations in fish were quantified to assess transport to other parts of the Campus Lake. Bluegill, largemouth bass, and bullhead had average total PCB concentrations of 49.8, 232, and 312 mg/kg lipid, respectively (Table 2). The PCB concentrations found in fish also were averaged within species and plotted by congener (Fig. 3; Fig. S1). A comparison of PCB congener patterns in fish to other organisms collected from Campus Lake revealed similar congener patterns among fish, aquatic insects, and spiders, with the heavier chlorinated congeners being most prevalent (Fig. 3; Fig. S1). These pattern similarities supported a common source of contamination and are logical, because the more hydrophobic congeners have a higher tendency to bioaccumulate compared with the lower chlorinated congeners. Additionally, stable isotope ratios of N and C were used to confirm the respective trophic level of organisms collected from Campus Lake. Largemouth bass had the highest $\delta^{15}N$ enrichment (11.08‰ ± 0.12%, $n = 10$), followed by bluegill (9.69‰ ± 0.22%, $n = 20$) and bullhead (9.24‰ ± 0.38%, $n = 6$; Fig. 4). The $\delta^{15}N$ was enriched between 2‰ and 5‰ per trophic level (Jardine et al. 2006), thus the largemouth bass occupied the highest trophic level, followed by the yellow bullhead, and finally, bluegill (Fig. 4). The differences in trophic level and life histories of the three fish species help to explain the overall differences in PCB bioaccumulation within the fishes. For a more detailed discussion of this topic, see the following references as this discussion was outside the scope of this research project (Tanabe et al. 1984; Kidwell et al. 1994; Leblanc 1995; Meier et al. 2015). Regardless of the differences in bioaccumulation among the three fish species, the fish exposure metric revealed that all of the fish collected from Campus Lake accumulated elevated levels of PCBs compared with the lower tropic levels. Alone, the fish data suggest that PCB exposure in Campus Lake may be coming from a ubiquitous source or multiple locations, because the fish freely move throughout the lake. However, the aquatic insect data indicated localized contamination. Therefore, neither the fish data nor the aquatic insect data alone indicated a complete characterization of the PCB exposure. This difference constitutes one of the major flaws in using a single metric, as discussed below. Only through considering the sediment, invertebrate, and fish data can one determine that the PCBs were localized to one area of Campus Lake at bioavailable concentrations, leading to transport to the surrounding riparian areas through emerging insects and to the wider aquatic environment through bioaccumulation by fish and export to the riparian system as indicated by spiders.

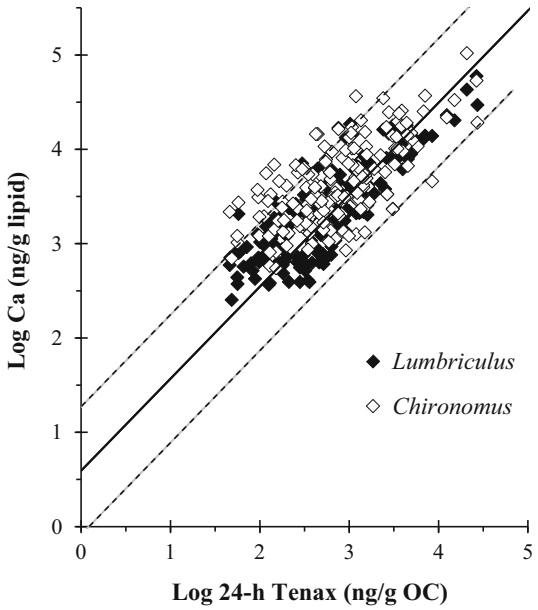

Fig. 5 Relationship between 24-h Tenax-extractable concentrations and polychlorinated biphenyl concentrations present in bioaccumulation assay test species, *L. variegatus* and *C. dilutus,* after exposure to sediment collected from the contaminated cove. Concentrations were regressed onto the Bioaccumulation-Tenax Model (BTM); the *solid line* represents BTM and the *dashed lines* represent 95% confidence intervals of the model

Implications of Exposure Metrics

While it is ideal to have all exposure metrics available when conducting a risk assessment, due to cost, time, and limitations on sample availability (e.g., limited biomass), this may not be possible. Therefore, it is crucial to understand the attributes and limitations of each exposure metric. The contaminated area of the lake was identified using sediment concentrations measured by exhaustive chemical extractions. The exposure assessments were focused to the area where the PCB sediment concentrations were highest and thus had highest risk of PCB exposure. Identifying the location of contamination was valuable for reducing the scope of the assessment and helped focus prospective mitigation work. Exhaustive chemical extractions of the sediment; however, did not indicate if PCBs were available to organisms residing in this area of the lake (Lydy et al. 2015).

The Tenax bioavailability-based exposure metric provided the advantage of estimating bioaccumulation potential in organisms, as well as a measure of the distribution of bioavailable PCBs in the sediments. The PCBs were present in the Tenax extractable fraction, which indicated that they were bioavailable to organisms (Landrum et al. 2007; Trimble et al. 2008; Mackenbach et al. 2012, 2014, Fig. 5). The bioaccumulation of PCBs in *L. variegatus* and *C. dilutus* was highest after exposure to sediment collected from locations 24–1 and 24–2 within the contaminated area of the lake, suggesting that PCBs were most available for uptake near the littoral-terrestrial interface (Fig. 2). These results were not unexpected as a known linear relationship exists between 24-h Tenax extractions and oligochaete tissue concentrations (ten Hulscher et al. 2003; Landrum et al. 2007; Mackenbach et al. 2012, Fig. 5). Furthermore, the 24-h Tenax extraction provided equivalent information as the combination of in-lab bioassays. Using Tenax extractions, however, may help to save time, money, and efforts compared with exhaustive chemical extractions and better estimate the biological exposure.

Examining PCB concentrations in riparian spiders and fish provided different information on exposure risk, local versus lake-wide, respectively, for the species selected in this study. Collecting emergent aquatic insects and riparian spiders from the contaminated area demonstrated that the PCBs were capable of being exported from the sediment to the surrounding terrestrial environment. The extent of this transport, however, was limited to the areas directly adjacent to the contaminated locations, as data from the reference location demonstrated no significant PCB contamination. Alone, these data suggest a localized area of exposure, which may easily be remediated by dredging or capping the contaminated sediments. Conversely, the fish collected throughout Campus Lake suggest a wide spread distribution of PCBs throughout the lake. However, the PCB contamination in fish actually stems from a localized source of exposure and the PCBs bioaccumulating through the food web via consumption of contaminated prey from the localized contaminated area of the lake. The life history of the fish species and the organisms that may feed on them could suggest a wide range of exposure into the surrounding terrestrial environment and even the human population that may use the fish for sustenance. The potential for human exposure; therefore, may lead to a more aggressive remediation plan to manage human or piscivorous wildlife compared with managing the risk based on an assessment solely relying on the invertebrate data.

To more efficiently conduct a weight of evidence assessment project of this type, specific metrics to address specific research objectives or assessment needs should be selected to focus data collection to meet these data quality objectives. For example, simple 24-h single-point Tenax extractions may be sufficient to assess the bioavailability of PCBs to organisms in initial screenings by estimating PCB concentrations in aquatic invertebrates using the BTM model (Harwood et al. 2015; Mackenbach et al. 2012, 2014). Additionally, if the primary concern was assessing fish for potential consumption advisories, the focus of the evaluation could be PCB concentrations in edible species. Although the time, cost, and effort

necessary to collect data using a weight of evidence approach from multiple metrics may exceed the scope of many assessments, the strategic selection of multiple metrics to address the project specific needs will provide a better assessment of exposure risk.

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