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Optimization of Tenax extraction parameters for polychlorinated biphenyls in contaminated sediments

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ABSTRACT

Tenax extraction is a chemical technique used to provide a rapid estimate of exposure to chemicals from contaminated sediments. However, an absence of standardization has limited the implementation of Tenax extraction in regulatory venues. In the current study, the operational parameters of extraction solvent volume, Tenax sorption rate from water, and Tenax:OC (organic carbon) ratios were investigated employing polychlorinated biphenyls (PCBs) as model compounds. The highest extraction efficiency of the analytes from Tenax resulted from a 10 mL extraction volume. Recoveries of PCBs from spiked-sediment ranged from 79% to 100% with relative standard deviations between 1% and 9%. For the Tenax sorption rate from water, 0.01 g of Tenax cleared >95% of the initial solution concentration of individual PCBs from 40 mL of water in less than 30 min. This Tenax mass is capable of clearing PCBs from the 40 mL of water 413 times in 24 h. Thus, a 24 h single-point Tenax extraction would be sufficient to remove all of the desorbing PCBs from a contaminated sediment. Finally, the influence of the Tenax:OC ratio becomes more evident as the hydrophobicity of the compound and OC content (%) of the sediment increases. To obtain more reliable Tenax extractable concentrations, a minimum Tenax:OC ratio of 5:1 is suggested to conduct single-point Tenax extractions. In summary, a solvent volume of 10 mL extracted the compounds efficiently from the Tenax, and the rapid sorption from water using at least the minimum Tenax:OC ratio should lead to good measures of rapidly desorbing compound and thus represent bioaccessibility.

1. Introduction

Tenax extraction of hydrophobic organic compounds (HOCs) was introduced to the scientific community in 1990 [1] and since that time, a number of studies have used Tenax extractable concentrations to estimate exposure to the bioaccessible portion of compounds from contaminated sediments [2]. Tenax extractions have served as an applicable technique for the assessment of biodegradation processes [3–5], toxicity [6,7], and bioaccumulation of HOCs by benthic organisms [8–12].

The porous Tenax resin, a 2,6-diphenyleneoxide polymer, has been used for studying desorption of HOCs in soils and sediments and for correlating bioaccessibility with bioavailability [13]. Bioavailability is defined as the amount of chemical present in the sediment that can interact with biological systems [14]. The basis for use of Tenax to measure bioaccessibility is founded on the principle that compounds are not uniformly distributed among sediment particles, but rather are in different compartments or fractions with distinct desorption characteristics [5,12] . The contaminant fractions are operationally defined based on the measured desorption rate of compounds from the solid phase and include a rapidly desorbing fraction (F_{rap}), a slow desorbing fraction (F_s) and a very slow desorbing fraction (F_{vs}) [15]. While all fractions are known to contribute to what is bioaccessible to organisms, studies have shown that exposure correlated well with F_{rap} and that this fraction can be used for measuring bioaccessibility and toxicity of HOCs in sediments [7,15–17] . In practice, F_{rap} can be determined by consecutive Tenax extractions [18,19] or estimated from single-point Tenax extractions (e.g., 24 h) [10,17]. However, consecutive extractions are time consuming and labor intensive, whereas a 24 h single-point Tenax extraction provides reliable information on the bioaccessible pool of a contaminant in sediment in less time [10,17,20]. As a result, the 24 h single-point Tenax extractable concentrations are applied to measure the most bioaccessible fraction of HOCs from sediment more often than the consecutive extraction measures of F_{rap} [6,7,10,17], [21–24] .

A procedure to conduct single-point Tenax extractions has been reported previously [25]. In general, a small amount of sediment (1–5 g dry weight) is added to a glass vial, followed by the addition of test

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water to obtain a sediment slurry. Then, a small amount of Tenax (i.e., 0.5–2.0 g) is added to the slurry; the vial is capped, and rotated for a fixed period of time. At the end of the rotation period, the Tenax beads are separated from the sediment slurry. To extract the compound sorbed to the Tenax, a mixture of organic solvents and sonication are used and the extract is analyzed using chromatographic techniques. Although, the Tenax extraction represents a simple and rapid technique to estimate exposure by measuring desorption from sediment; currently, there is no consensus on the best operational conditions to conduct Tenax extractions, including the solvent volume used to extract the Tenax, and the Tenax mass needed relative to the amount of organic carbon in the sample [2]. Both of these aspects of the Tenax extraction method could impact exposure estimates provided by single-point Tenax extractions through error in measures of bioaccessibility.

There are three critical aspects investigated in the current study. First, what is the appropriate solvent volume to extract the contaminants from the Tenax? Second, is the Tenax mass used adequate to clear the contaminant from water such that desorption is the effective process measured? Third, is there a minimum Tenax:OC ratio needed to ensure that desorption is effectively measured? Here, the Tenax extractable concentrations from the Tenax:OC ratio(s) were compared to bioaccumulation data. These questions were investigated using 28 PCB congeners to elucidate the best operational conditions to conduct Tenax extractions as a means of estimating bioaccessibility. The PCBs were chosen as model compounds, because they are known for their chemical stability, low biodegradability, toxicity, and wide range of water solubility and hydrophobicity [26,27]. Moreover, PCBs remain as pollutants of ongoing interest to risk assessors and health practitioners, because they are still detected in elevated concentrations in soils and sediments worldwide [28,29].

2. Experimental

2.1. Reagents and chemicals

A commercial PCB mix (C-WNN, Mix #5; purity: > 99%) containing 28 congeners in *iso*-octane (10 mg L^{-1}) was purchased from AccuStandard Inc. (New Haven, CT, USA). The following PCB congeners were screened: IUPAC Nos. 8 (di-chlorobiphenyl; di-CB congener), 18, 28 (tri-CB congeners), 44, 52, 66, 77, 81 (tetra-CB congeners), 101, 105, 114, 118, 123, 126 (penta-CB congeners), 128, 138, 153, 156, 157, 167, 169 (hexa-CB congeners), 170, 180, 187, 189 (hepta-CB congeners), 195 (octa-CB congener), 206 (nona-CB congener), and 209 (deca-CB congener). Surrogates DBOFB (4,4′-dibromooctafluorobiphenyl) and PCBs (PCB-186, PCB-191 or PCB-204) were purchased from Supelco Inc. (Bellefonte, PA, USA) and AccuStandard Inc., respectively. Internal standards ^{13}C-labeled PCB congeners (^{13}C- PCB-15, ^{13}C-PCB-52, ^{13}C-PCB-144, and ^{13}C-PCB-209) were purchased from Cambridge Isotope Laboratory (Andover, MA, USA). All materials and solvents (pesticide grade), including anhydrous Na_2SO_4, hexane, acetone, and dichloromethane were purchased from Fisher Scientific (Pittsburgh, PA, USA). Nitrogen (purity: 99.9990%) and helium (99.9990%) were supplied by Airgas Inc. (Marion, IL, USA). Tenax-TA beads (60/80 mesh) were purchased from Scientific Instrument Services Inc. (Ringoes, NJ, USA).

2.2. Experimental design for Tenax operational parameters

Three experiments were conducted in the current study. The first experiment examined the volume of extraction solvent needed to optimize the extraction of PCBs from the Tenax. For these experiments, 24 h single-point Tenax extractions were conducted using PCB-spiked sediment with a range of Tenax masses and either 5 mL or 10 mL solvent washes to extract the PCBs from the Tenax. Comparisons of the PCB concentrations reported with 5 mL or 10 mL washes were made to determine the amount of solvent required to remove PCBs sorbed to the Tenax after a single-point Tenax extraction. The second experiment evaluated the Tenax sorption rate to measure how fast Tenax can remove PCBs from water over time to understand if the Tenax extractable concentrations of PCBs from sediment were indeed limited by desorption and not the uptake kinetics from water. For this experiment, single-point Tenax extractions, liquid-liquid extractions, and a mass balance approach were used. In the third experiment, Tenax:OC ratios were investigated using various Tenax masses relative to the OC content in sediments. This experiment was conducted using three field-collected sediments containing PCBs, 24 h single-point Tenax extractions, bioaccumulation, lipid analysis and OC determinations. The 24 h single-point Tenax extractable PCB concentrations obtained utilizing the varying Tenax:OC ratios were compared to tissue concentrations in *Lumbriculus variegatus* to determine how altering the Tenax:OC ratios impacted exposure estimates provided by the Tenax extraction. Details of the experimental design and methods for these experiments are described in the following sections.

2.2.1. Volume of extraction solvent experiment

For the extraction solvent experiment, 500 g of an uncontaminated reference sediment (TON: Touch of Nature, IL, USA) containing 1% OC was spiked at 10 ng g^{-1} dry weight (dw) using the 28 PCB congener mix and equilibrated for 21 d at 23 ± 1 °C. The experiment was initiated by adding PCBs to wet sediment and then mixing it thoroughly in a 1-L glass jar. Subsamples (3 g dry weight, dw) of the spiked sediment were used for the 24 h single-point Tenax extraction as described below. Seven Tenax masses were used including 0.025, 0.50, 0.75, 1.0, 1.25, 1.50 and 1.75 g. These masses were chosen to represent a range of Tenax masses that have been used in the past [2]. The PCBs sorbed to the Tenax were extracted using extraction solvent volumes of either 5 mL or 10 mL per wash, for a final extract volume of 15 mL or 30 mL, respectively. The surrogate mix (DBOFB and PCB-204) was added to the Tenax prior to the first solvent wash as described below.

2.2.2. Tenax sorption rate experiment

For the sorption rate experiments, 1 L of moderately hard reconstituted water (MHRW) [30,31] was spiked with the 28 PCB congener mix at a concentration below the water solubility limits for each congener (each spiked at 1.25 ng mL^{-1} in acetone) [27]. The water was prepared and stirred in Erlenmeyer flasks. A preliminary study investigating the sampling rate of PCBs from water showed that mixing for 15 min using a magnetic stir plate (VWR®, Chicago, IL, USA) was long enough to allow the spiked solutions to homogenize, and allow time for the water and glassware to equilibrate before adding the Tenax. Subsamples of the spiked water were then distributed into 50-mL glass tubes (water volume: 40 mL) followed by the addition of a single mass of Tenax (0.010 g) to each tube. The tubes containing the Tenax and water were capped and mixed using a wheel rotator at a rate of 25 rpm (revolutions per minute) (BioQuest, Cockeysville, MD, USA). The Tenax was removed from the tubes at 5, 15, 30, 45, 60 and 1440 min of rotation. The sorption rate of PCB congeners from water by Tenax was determined following Eqs. (1) and (2) as described in Landrum et al. [32]:

$$Q_t = \frac{(k_T \bullet A)(1 - e^{-(k_T + k_e)t})}{k_T + k_e} \quad (1)$$

where, Q_t is the quantity of PCBs (ng) absorbed on the Tenax at time t, k_T is the accumulation rate constant for PCBs onto the Tenax (h^{-1}), A is the mass of PCBs in the experimental system (ng) at t_O (initial time), k_e is the compound loss rate constant from the Tenax (h^{-1}), and t is time [32]. This equation describes the accumulation rate constant of PCBs by Tenax as a system dependent value. Data were fit using Scientist 2.01 (MicroMath Inc., St. Louis, MO, USA). The k_T was determined for each individual PCB congener (n=3) for each time point. To remove the system dependence and estimate the sorption rate of PCBs from water

by Tenax, a second equation was used:

$$k_u = k_T \left(\frac{V_w}{M_T}\right) \quad (2)$$

where, k_u is the PCB congener sorption rate from water by Tenax ($L\ g^{-1}\ h^{-1}$), V_w is the volume of water in the experimental system (0.04 L), and M_T is the mass of Tenax (0.01 g) used. To account for the initial PCB concentrations present in the aqueous phase, subsamples of spiked water were used to determine concentrations of PCBs at t_O, and incorporated into the Tenax sorption rate model.

2.2.3. Mass balance experiment

The mass balance for the Tenax system (Tenax, water and glassware) was determined as part of the Tenax sorption rate experiment. For this purpose, the PCB concentrations sorbed to the Tenax beads, remaining in the water of the extraction system after removing the Tenax, and bound to the glassware (50-mL tubes) were measured at each time point. Furthermore, the mass balance was also determined for t_O (water and glassware only) in order to obtain the initial PCB concentrations present in the spiked water. The methods used to measure PCBs on the Tenax, the remaining PCBs in the water and bound to the glassware are described below. Finally, the mass balance for the individual PCB congeners was determined by summing the PCB concentrations from each component of the Tenax system at each time point.

2.2.4. Tenax:OC ratio experiment

Three field-collected sediments historically contaminated with PCBs were used for the Tenax:OC ratio experiments and included: Manistique (MQ-1; 0.89% OC) (Manistique River, MI, USA), Campus Lake Site 4 (CLS-4; 5.65% OC) and Campus Lake Site 5 (CLS-5; 8.87% OC) (Carbondale, IL, USA). Two reference sediments uncontaminated with PCBs (Touch of Nature, TON; 1% OC and LaRue, LR; 2% OC) were also included for quality control / quality assurance purposes, and have been used in other studies [6,21,33,34] . For all sediments, large debris was removed and sediments were passed through a 500 μm sieve, mechanically homogenized, and stored at 4 °C in acetone-rinsed glass jars until use. The Tenax:OC ratios investigated were 1:1, 3:1, 5:1, 8:1, 10:1 and 15:1. The Tenax mass required for a given Tenax:OC ratio was calculated based on the % OC of each field sediment. For the reference sediments, the highest Tenax:OC ratio (15:1) was used for the matrix spike samples. The surrogate mix (DBOFB and PCB-186) was added to the Tenax extracts prior to the first solvent wash as described below.

2.3. Extraction procedures and analyses

2.3.1. Tenax extractions

Before use and to eliminate any carryover of impurities, the Tenax beads were rinsed once with acetone, and twice with a mixture of acetone/hexane 1:1 (v/v) (each solvent rinse 30 mL g^{-1} Tenax) and bath sonicated for a total of 3 h (each solvent rinse included 1 h of sonication). The Tenax was allowed to dry at room temperature (25 ± 1 °C) for a few days [15]. For the 24 h single-point Tenax extractions of the sediment slurries, 2 g dw of the spiked sediment (volume of solvent extraction experiment) or field-collected sediments (Tenax:OC ratio experiment) were weighed and placed in a 50-mL glass tube with a given Tenax mass, 0.5 g copper powder, 3 mg $HgCl_2$ to prevent microbial degradation, and 45 mL MHRW (moderately hard reconstituted water) [35]. For PCB-spiked water (sorption rate experiment), a single Tenax mass (0.01 g) was added into a 50-mL glass tube with 40 mL MHRW. Next, either the slurry or water sample was then mixed on a wheel rotator at a rate of 25 rpm. After a 24 h rotation period, the tubes were removed and centrifuged for 5 min at 2000*g* (Centrifuge 5702 R, Eppendorf Inc.). Tenax beads float and were collected using a metal spatula. The Tenax beads were then transferred into a vial containing acetone, the volume of which was determined based on the results of the extraction solvent experiment (5 mL or 10 mL). Next, the surrogate mix was added and the extract was placed into a bath sonicator for 10 min. The acetone extract was removed and transferred into a Turbovap vial. The Tenax beads were solvent washed and sonicated two additional times using 5 mL or 10 mL of a hexane-acetone mix (1:1, v/v) each time. The extracts were combined (15 mL or 30 mL) in the vial, concentrated to 2 mL under a steady nitrogen stream, exchanged into hexane (10 mL), cleaned up with sulfuric acid, concentrated to 1 mL and transferred with hexane rinses to GC vials for quantitative analysis.

Use of large Tenax masses (> 0.5 g) tends to result in a significant amount of water being included in the Tenax extracts after the washing procedure. To address this issue, the solvent layer (≈2 mL) must be separated from the water after solvent exchanging the samples into hexane and concentrating the samples. After removing and collecting the initial hexane layer in a clean 20 mL vial, the remaining water was washed again by adding hexane (3 mL), followed by vortexing for 5 min at 2000 rpm and centrifugation for 5 min at 3000 rpm. The overlying solvent was then removed and filtered through anhydrous Na_2SO_4 packed-columns (10 g) to remove any remaining water prior to combining the extracts in the vial. The procedure was repeated one additional time. The combined extract (≈8 mL) was concentrated to 1 mL under a steady nitrogen stream and transferred with hexane rinses to GC vials for quantitative analysis.

2.3.2. Liquid-liquid extraction

Liquid-liquid extraction (LLE) was used to extract PCBs from the spiked aqueous solutions, which were employed to determine the mass balance as part of the Tenax sorption rate experiment [36]. Briefly, each water sample from the 40-mL tubes was transferred to a 100-mL separatory funnel. At this point, the surrogate mix (DBOFB and PCB-204) was added to the water in the funnel. The PCBs were extracted by shaking the aqueous solution with 10 mL of dichloromethane for 5 min. This step was repeated two additional times. The extracts (30 mL) were combined in a Turbovap vial (Zymark® ZW640-3), concentrated to 2 mL under a gentle nitrogen stream, exchanged to hexane (10 mL), cleaned with sulfuric acid, filtered through anhydrous Na_2SO_4 packed-columns (10 g) to remove any remaining water, concentrated and transferred with hexane rinses to GC vials for quantitative analysis.

2.3.3. Glassware extraction

Sonication and solvent washes of the glassware were conducted to extract the PCBs bound to the glassware used in the sorption rate experiment. Briefly, once the Tenax beads and water (MHRW) were removed from the 50-mL tube(s), 10 mL of acetone was added to each tube followed by the addition of the surrogate mix (DBOFB and PCB-204). Next, the tubes were placed into a bath sonicator for 10 min. The acetone extract was removed and transferred into a Turbovap vial. The tubes were solvent washed and sonicated two additional times using 10 mL of a hexane-acetone mix (1:1, v/v) each time. The extracts were combined (30 mL) in the vial, concentrated to 2 mL under a steady nitrogen stream, exchanged into hexane (10 mL), cleaned up with sulfuric acid, concentrated to 1 mL and transferred with hexane rinses to GC vials for quantitative analysis.

2.3.4. Bioaccumulation tests and tissue extraction

Standard bioaccumulation tests using the oligochaete *L. variegatus* were conducted following modified U.S. Environmental Protection Agency (EPA) protocols [37]. The modification includes the use of a 14-d time frame rather than the 28-d bioaccumulation outlined in the original protocol. The alternative time frame was established due to reproduction of *L. variegatus* that begins at 14 d, which could influence bioaccumulation [18]. For all bioaccumulation tests, *L. variegatus* were obtained from populations cultured at Southern

Illinois University, Carbondale, IL, USA. Tests were conducted in 600 mL beakers containing 100 g wet sediment, 500 mL overlying MHRW, and adult *L. variegatus* (50 individuals, ≈0.25 g wet weight (ww). All beakers were placed in a water bath with an automated water delivery system, in which the overlying water was renewed daily with three water changes (80–100 mL) to ensure dissolved oxygen (DO) levels remained above 5 mg L^{-1}, and a constant photoperiod of 16:8 h light:dark. The water-quality parameters were measured daily to assure the test conditions remained within acceptable ranges (Euteach Instruments, Singapore; YSI Company, Yellow Springs, OH, USA). The 14 d sediment bioaccumulation assays were conducted using three replicates for the spiked sediments, plus controls conducted with sediments containing no PCBs. After 14 d, the worms were sieved from the sediment (500 μm mesh sieve), and transferred to fresh MHRW for 6 h to depurate gut contents. After depuration, the worms were blotted dry, weighed, and frozen at −20 °C prior to tissue extraction as described below. Two individuals of each experimental replicate were randomly chosen and frozen at −20 °C in a culture tube for lipid analysis.

For the tissue extraction procedure, *L. variegatus* from the bioaccumulation tests were removed from the freezer and spiked with the surrogate mix (DBOFB and PCB-186). The tissue was extracted using a high-intensity sonicator (Newtown, CT, USA) for 20 s with acetone (10 mL). The sonication was repeated two additional times. Finally, hexane (10 mL) was added to the vial and the samples were subjected to bath-sonication for 10 min. The extracts were filtered through anhydrous Na_2SO_4 packed-columns (10 g) to remove tissue debris and any remaining water. The tissue extracts were concentrated under a gentle nitrogen stream to 2 mL, exchanged into hexane (10 mL), cleaned with sulfuric acid, concentrated, and transferred with hexane rinses to GC vials for quantitative analysis.

2.3.5. Sample cleanup

The acid cleanup of PCB extracts was conducted following a published procedure [35]. Briefly, concentrated sulfuric acid (1 mL) was added to each 2-mL extract, vortexed for 5 min at 2000 rpm and centrifuged for 5 min at 2000*g*. The hexane layer was removed and passed through anhydrous Na_2SO_4 packed-columns (10 g) to remove any remaining water. Hexane (2 mL) was added to the remaining sulfuric acid layer, vortexed, centrifuged, and filtered as described above two additional times. The combined filtered extract was concentrated to 1 mL under a steady nitrogen stream and transferred with hexane rinses to GC vials. The final volume of the extracts was 1 mL for Tenax, sediment and tissue samples. Some sediment extracts required additional cleanup, because of sulfur and other types of interferences and were treated with 1 g of activated copper granules (Restek Corp., Bellefonte, PA, USA), and shaken for 24 h. The resulting extract was transferred to a clean GC vial prior to analytical quantification.

2.3.6. Lipid analysis

Lipid content in *L. variegatus* was analyzed using individuals from each bioaccumulation test. Two individuals from each experimental replicate were randomly chosen, blotted dry, weighed (≈0.025 g), placed in a glass culture tube, and extracted with chloroform and methanol (1:1, v/v) as previously described [38]. A vanillin/phosphoric acid reagent was added and transmittance was read at 525 nm using a spectrophotometer (Spectronic 20 Genesys™; Spectronic Instruments). A five-point calibration curve was constructed using three-fold dilutions of vegetable oil, and treated the same as tissue samples. The transmittance readings were conducted in triplicate to obtain a mean and standard deviation for each sample.

2.3.7. Organic carbon analysis

The OC content of each spiked and field-collected sediments were determined by Midwest Laboratories (Omaha, NE, USA) using hydrochloric acid digestion, followed by combustion using standard methods (ASTM D 5373). The OC analysis was conducted in triplicate, and data was reported as percent of OC (%) per gram of sediment (dw).

2.4. Analysis and quantification

Quantification of the 28 PCB congeners was completed on a gas chromatograph-mass spectrometer (Agilent 6850 GC 5975 XL MS, Agilent Technologies). Analytes were separated on a DB-XLB column (30 m×0.18 mm×0.18 μm film thickness; Agilent Technologies) initially set at 100 °C and heated to 265 °C at 1.2 °C min^{-1}. Inlet, ion source, and quadrupole temperatures were 260, 230, and 150 °C, respectively. The mass spectrometry detector and mode was set as an electronic ionization/selected-ion monitoring (EI/SIM) mode. A 2.0 μL sample was injected in pulsed split-less mode at 50 psi. Helium was the carrier gas, and column flow was 1.0 mL min^{-1}. Quantification was performed using internal standard calibration in electron impact mode. The selected ion for the GC/EI/MS/SIM experiments have been previously reported in the literature [39,40]. Ten calibration standards were prepared at levels of 1, 2, 5, 10, 25, 50, 75, 150, 200 and 300 μg L^{-1} of each PCB congener and surrogate in hexane, and concentrations of the four internal standards were kept constant at 20 μg L^{-1} for each standard and added to each sample and calibration standard in GC vials (1 mL).

2.5. Quality assurance/quality control

For extraction purposes, two surrogate mixes DBOFB and PCB-204, and DBOFB and PCB-186 were used unless otherwise specified. The surrogate mix (50 ng mL^{-1}) was added to individual extracts (Tenax, water and tissue) prior to extraction in order to verify performance of the extraction method. Method blanks, laboratory control blanks, matrix spikes and matrix spike duplicates were included every 20 samples. To maintain the quality of the extraction and cleanup methods for all matrices, absolute percent (%) recoveries that lie in the range of 70–120% were considered acceptable.

For quality assurance purposes, a calibration standard at 50 μg L^{-1} was analyzed every eight samples on the GC-MS to determine Relative Standard Deviation (%RSD) within runs. The relative differences between the calibration curve and the daily calibrations must be within 20% for all congeners in order for the results to be deemed acceptable. Reporting limits for the PCB congeners were (the lowest concentration that could be accurately quantified) 1 ng mL^{-1} for water and Tenax, and 2 ng g^{-1} (ww) for tissue.

2.6. Statistical analyses

A Student's *t*-test was used to compare whether the means of PCB congener concentrations recovered with 5-mL and 10-mL extractions were statistically different. A one-way ANOVA was used to determine whether there were any significant differences among the mean of PCB congener concentrations among the time points chosen for the sorption rate of Tenax of PCBs from water. A linear regression model was used to fit the data of the PCB congener concentrations of Tenax:OC ratios versus tissue concentrations. A one-way ANCOVA was used to further test any significant differences among the slopes of regression lines of Tenax extractable concentrations of different Tenax:OC ratios and tissue concentrations from bioaccumulation data. Finally, a significance level of α equal to 0.05 was chosen to determine whether statistically significant differences existed among the treatments ($p < 0.05$). Each experimental treatment and control was conducted in triplicate (n=3) unless otherwise specified. For statistical calculations and regressions, the software SAS, version 9.0 for windows (SAS Institute Inc. Cary, NC, USA) was used.

3. Results and discussion

3.1. Effect of the extraction solvent volume

The literature consistently reports acetone and hexane as the appropriate solvents to extract non-polar analytes from Tenax; however, there is no consistency on the appropriate volume of solvent to use among studies [15,35,41]. Extraction depends on the relative solubility of the compound and the law of mass action, such that if too small a solvent volume is used to remove compounds sorbed to the Tenax the efficiency will be low. Thus, at the end of a single-point extraction, the reported Tenax extractable concentration may underestimate bioaccessibility, and thus exposure.

To determine the influence of the extraction solvent volume on Tenax extractable concentrations, two extraction solvent volumes were tested (5 mL versus 10 mL), and were chosen based on the volumes commonly reported within the Tenax literature [15,35,41]. A surrogate mix (DBOFB and PCB-204) was used to determine the recoveries (measurement of accuracy) and relative standard deviation (RSDs, measurement of precision) of the 28 PCB congeners from both low and high Tenax masses, and determine if any loss of analyte(s) may have occurred during the cleanup process. The DBOFB was used to measure extraction recovery for the lower-chlorinated PCBs, and PCB-204 was used for the higher-chlorinated PCBs. The comparison of surrogate recoveries from 5 mL and 10 mL of extraction solvent are shown in **Table S1** of the Supporting material. For both low and high Tenax masses, the 10 mL extraction volume provided better relative recoveries for the surrogates DBOFB (nominal 50 ng g^{-1}) and PCB-204 (nominal 50 ng g^{-1}). The only exception was that similar recoveries were found for both 5 mL and 10 mL volumes for DBOFB extracted from low Tenax mass (0.025 g). Furthermore, the RSD values for the low and high Tenax masses using 5 mL ranged from 1.9% to 2.0% and from 6.5% to 14.0%, respectively, while the overall RSD values were lower for the 10 mL extractions for both Tenax masses, ranging from 1.2% to 2.9% for the low Tenax masses, and from 2.5% to 9.3% for the high Tenax masses (Table S1).

To investigate the influence of the extraction solvent volume on PCB recoveries, seven Tenax masses (0.025–1.75g) and two extraction volumes (5 mL and 10 mL) were used to obtain 24 h single-point Tenax extractable concentrations for individual PCB congeners from the same sediment. The same spiked sediment was used to conduct the Tenax extractions using each solvent volume. Thus, the extractions for each Tenax mass were expected to quantify the same PCB concentration, if both extraction solvent volumes were capable of fully extracting the PCBs from the Tenax. At this point, the appropriate amount of Tenax to fully extract the bioaccessible concentration has not been established, because the purpose of this experiment was to determine the influence of the extraction solvent volume. Therefore, the Tenax masses in Fig. 1 should not be considered equivalent to the amount of Tenax required for sediment extraction (the optimum amount of Tenax needed is discussed in the effect of Tenax:OC ratio results section). Tenax extractable concentrations of representative PCB congeners, based on their homolog groups, using the two extraction solvent volumes are displayed in Fig. 1. For comparison purposes, the quantification is reported for the lowest Tenax mass (0.025 g) and the highest Tenax mass (1.75 g) used. As shown in Fig. 1, significant statistical differences ($p < 0.05$) were found for the Tenax extractable concentrations of the higher-chlorinated PCBs (hexa-, hepta-, octa- and nona-CB) extracted using 10 mL versus the smaller extraction volume (Fig. 1a). In contrast, the Tenax extractable concentrations of the lower-chlorinated PCBs (di-, tri-, tetra-, and penta-CB) for the lowest Tenax mass were not affected by the volume of extraction solvent used (Fig. 1b).

The results of the Tenax extractable concentrations for the other two lower Tenax masses (0.5 g and 0.75 g) followed the same trend as noted for the lower-chlorinated PCBs as shown in Fig. 1. Whereas, Tenax extractable concentrations of the higher-chlorinated PCBs for the other three higher Tenax masses (1.0, 1.25 and 1.5 g) using 10 mL were always significantly greater than concentrations using 5 mL ($p < 0.05$), except for the di-CB congeners (Table S2). These results indicate that a solvent volume of 10 mL would be appropriate to remove all of the PCBs present on the Tenax. This is also supported by the higher surrogate recoveries (Table S1) and smaller RSD values found for the 10 mL extraction. Thus, for PCBs, 10 mL of solvent is recommended to remove all of the congeners from Tenax, including the higher-chlorinated congeners.

3.2. Effect of the Tenax sorption rate

3.2.1. Mass balance

To calculate the sorption rate of Tenax requires that accurate estimates be made of the initial PCB concentration present in the aqueous phase (t_O) [32]. To determine this concentration, the mass balance of the PCB concentrations in the Tenax systems (Tenax, water and glassware) was measured at t_O, and at each time point to understand how the PCBs were distributed among the Tenax, water and glassware. The overall mass balance of the Tenax system ranged from 91% to 112% across all time points (Table 1). The quality assurance for the mass balance showed that the mean surrogate recoveries from the Tenax systems were generally acceptable for all matrices and ranged from 80-95% (DBOFB) and 83–97% (PCB-204) for Tenax, 76–96% (DBOFB) and 82–102% (PCB-204) for water, and 85–95% (DBOFB) and 80–98% (PCB-204) for glassware. No target analytes were detected in the blank samples. The percent recoveries of the matrix spikes from Tenax, water and glassware ranged from 83-90%, and the RPD between the matrix spikes fell within an acceptable range (< 25%).

The results of the mass balance of PCB congeners across the time points are summarized as homolog group concentrations in Table 1. There was limited loss of compound due to glassware binding (< 2 ng mL^{-1} for each congener) and this loss did not change over time. This suggested there was little to no desorption of PCBs from the Tenax into the water with subsequent attachment to the glassware. Moreover, the mass balance results indicate that almost all of the PCBs present in the aqueous phase were adsorbed onto the Tenax within 30 min as observed in Table 1. Therefore, the plateau observed in Fig. 2 was due to the Tenax sorbing the entire available PCB mass in the Tenax system, and not a reflection of the capacity of the Tenax being exceeded by the PCB mass in the system.

3.2.2. Tenax sorption rate

The Tenax mass used in a single-point Tenax extraction is directly linked to the sorption rate of chemicals from water by the Tenax. The Tenax sorption rate is important, because the dissolved fraction of the chemical in the aqueous phase must be maintained near zero to promote desorption. However, the variability in the Tenax mass used, specifically in reference to the organic carbon mass in the extraction system range widely among studies [2]. Thus, establishing better estimates of the ability of Tenax to clear non-polar contaminants from water should allow better estimates of the mass of Tenax required to maintain desorption for measures of bioaccessibility. The current experiment was designed to measure the sorption rate of PCBs from water by Tenax to demonstrate that removal of dissolved compounds is limited by desorption, and not the clearance of the water surrounding the sediment particles.

The sorption rate (k_u) of PCB congeners by Tenax from water was determined from the mass of congener absorbed onto Tenax at the following sampling times (t) 5, 15, 30, 45, 60 and 1440 min. The sorption rates from the current study ranged from 68.98 to 91.4 L g $Tenax^{-1}$ h^{-1} for the individual PCB congeners (Table 2). The sorption curves exhibited rapid absorption and reached a plateau after about 30 min, indicating that the Tenax had removed all of the PCB mass present in the water (Fig. 2 and S1). The proportion of congeners adsorbed on the Tenax represented on the y-axis was determined by

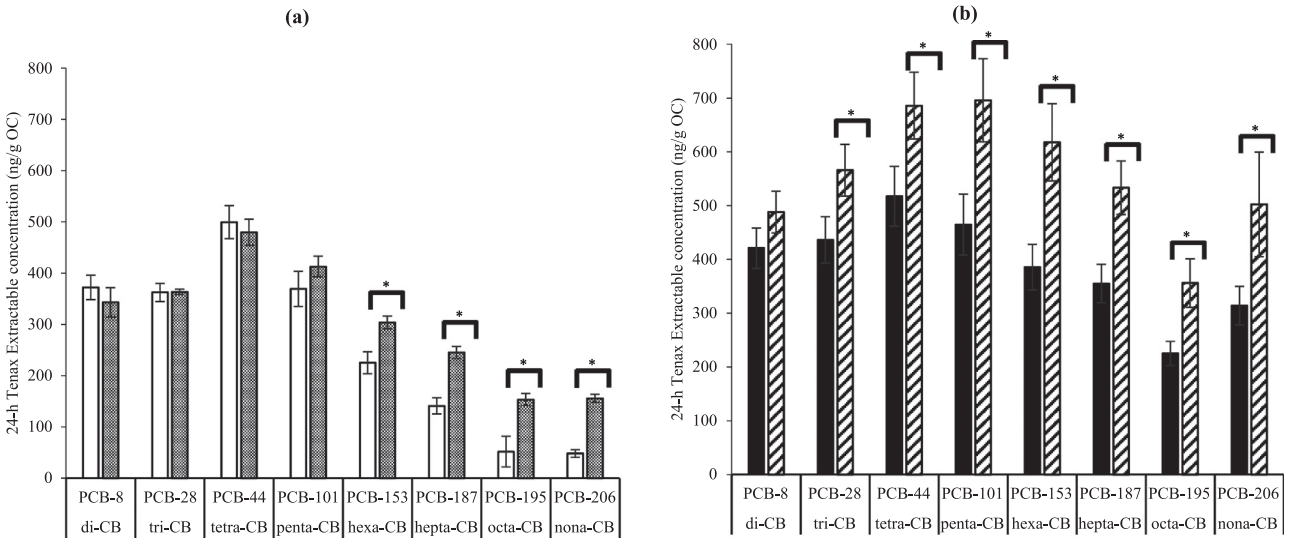

Fig. 1. The influence of different extraction solvent volumes on 24 h single-point Tenax extractable concentrations of representative PCB congeners. The congeners are grouped by homolog groups. (a) Bioaccessible PCBs extracted using either 5 mL (white square) or 10 mL (fine diagonal square) (0.025 g Tenax mass). (b) Bioaccessible PCBs extracted using either 5 mL (black square) or 10 mL (thick diagonal square) (1.75 g Tenax mass). Each bar represents the mean ± SD (standard deviation) (n=3). All significant comparisons are $p < 0.05$ and denoted by the bars and (*). The PCB congener concentrations are shown on an organic carbon (OC) normalized basis.

Table 1
Summary of the mass balance data for PCB congeners organized by homolog groups (0–1440 min). For clarity purposes, standard deviations of measurements (n=3) are omitted. The % mass balance was calculated relative to homolog group concentration measured at t_O. nq implies below limit of quantification. Mass balance showed only from t_O to t_{1440} min.

Time (min)	Matrix	di-CB	tri-CB	tetra-CB	penta-CB	hexa-CB	hepta-CB	octa-CB	nona-CB	deca-CB
Concentrations of homolog groups (ng/mL)										
0	Tenax	nq	nq	nq	nq	nq	nq	nq	nq	nq
	Water	40.9	87.2	239	283	334	190	46.9	49.7	50.1
	Glassware	nq	nq	nq	nq	nq	nq	nq	nq	nq
	Σ	40.9	87.2	239	283	334	190	46.9	49.7	50.1
5	Tenax	32.5	70.6	194	230	270	151	37.0	40.6	40.4
	Water	9.31	12.18	39.3	46.3	57.1	28.6	6.07	6.77	3.94
	Glassware	nq	nq	1.99	7.92	8.96	3.84	1.54	3.59	1.38
	Σ	41.8	82.7	235	284	336	183	44.6	50.9	45.7
	%	102%	95%	98%	100%	101%	97%	95%	103%	91%
15	Tenax	39.5	82.8	234	279	328	184	44.2	48.5	48.7
	Water	3.28	2.14	14.5	20.6	25.9	13.3	2.93	4.23	1.51
	Glassware	nq	nq	1.37	7.43	7.46	2.67	nq	3.00	nq
	Σ	42.7	84.9	249	307	361	199	47.1	55.7	50.2
	%	105%	98%	105%	109%	108%	105%	101%	112%	100%
30	Tenax	39.3	83.8	240	286	338	188	45.1	49.2	48.4
	Water	nq	nq	1.63	5.75	7.78	4.02	1.29	3.12	nq
	Glassware	nq	Nq	nq	4.90	7.59	4.48	2.00	4.34	3.03
	Σ	39.3	83.8	241	296	353	196	48.3	56.6	51.4
	%	96%	96%	102%	105%	106%	103%	103%	114%	103%
Concentrations of homolog groups (ng/mL)										
45	Tenax	39.8	83.9	241	285	339	190	46.9	49.4	48.8
	Water	nq	nq	1.54	4.94	6.04	2.06	nq	2.33	nq
	Glassware	nq	nq	nq	4.24	3.99	nq	nq	3.41	1.37
	Σ	39.8	83.9	242.5	294	349	192	46.9	55.1	50.1
	%	97%	96%	101%	104%	104%	101%	99%	110%	100%
60	Tenax	39.23	83.3	242	286	340	189	46.5	50.2	48.7
	Water	nq	nq	5.35	10.06	14.44	7.94	1.92	2.43	nq
	Glassware	nq	nq	nq	3.74	4.93	1.17	nq	1.87	nq
	Σ	39.23	83.3	247	300	359	198	48.4	54.5	48.7
	%	96%	95%	104%	106%	107%	104%	103%	110%	97%
1440	Tenax	39.8	83.9	242	286	340	190	46.6	50.2	48.4
	Water	nq	nq	nq	3.64	7.09	nq	nq	nq	nq
	Glassware	nq	nq	nq	3.61	5.69	nq	nq	nq	nq
	Σ	39.8	83.9	244	293	353	190	46.6	50.2	48.4
	%	97%	96%	102%	104%	106%	100%	99%	101%	97%

dividing the amount of the congener quantified at a given time period by the amount of the same congener quantified in the water at t_0. These results demonstrate a high sorption capacity and a rapid clearance of PCBs by the Tenax for compounds across a wide range of hydrophobicities (e.g., Log K_{ow} values of 4.48, 6.11 and 7.98 for CB-8, CB-101 and CB-208, respectively [27]).

These sorption rate results are comparable to those reported in the Tenax literature [5,41,42]. In one of the few studies that examined this

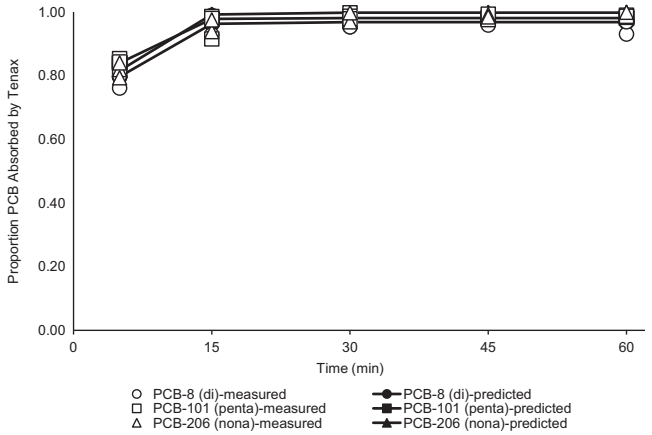

Fig. 2. Measured proportion of representative PCB congeners absorbed by Tenax and the model predicted proportion (solid lines) are illustrated (t_O to t_{60} min). For clarity purposes, only three congeners are drawn: (○) represents PCB 8 (di-CB), (□) represents PCB 101 (penta-CB) and (Δ) represents 206 (nona-CB). Each point represents the mean (n=3) ± SD (standard deviations are obscured by symbols).

issue, water (100 mL) spiked with various organic compounds was extracted for 5 min using 0.2 g of Tenax [5]. These conditions provided a sorption rate of 6000 mL g^{-1} h^{-1}. This suggested that a 100 mL volume of water would be cleared of the organic compounds 60 times per hour with 0.2 g of Tenax. Similarly, in a study determining the sorption rates and capacity of Tenax for DDT and its metabolites from an intestinal solution, 0.01 g of Tenax was sufficient to adsorb > 99% of the compounds with an incubation time of 6.3–19 min [41]. Finally, Zhao and Pignatello [42] reported that 1 g of Tenax was sufficient to clear > 95% of benzo(a)pyrene from a 50 mL volume of water in less than seven minutes. This would give a crude sorption rate of 428 mL g^{-1} h^{-1}, which suggests that a 50 mL volume would be cleared of benzo(a)pyrene 8.5 times per hour with 1 g of Tenax.

From the literature studies and our results, it is evident that Tenax is capable of removing all desorbing PCBs released from contaminated sediments to the slurry water. For example, desorption rate constants of PCBs from contaminated sediments ranged from 0.251 to 0.094 h^{-1} for the rapid desorbing fraction (readily bioaccessible), and from 0.004 to 0.001 h^{-1} for the slow desorbing fraction (slowly bioaccessible) [43]. Because one can only compare rate constants from equivalent systems, the calculated rate constant for the lowest sorption rate of 68.98 L g^{-1} Tenax h^{-1} (Table 2) in a system with 0.5 g and a volume of water of 0.04 L would be 862 h^{-1}, which translates to 4310 times faster absorption than the desorption rate constant of an average rapid desorbing fraction of PCBs (e.g., 0.251 h^{-1}). The difference would be even greater for the slow and very slow phases. Moreover, 0.5 g of Tenax would be able to clear PCBs from 40 mL of water 20,694 times in a 24 h period. The rate at which Tenax clears PCBs from the water, and the number of times that this volume is cleared by the Tenax supports the assumption that desorption of the compounds from the sediment is the limiting step in measuring bioaccessibility during Tenax extraction.

3.3. Effect of the Tenax:OC ratios on estimates of bioaccumulation

The third operational parameter that may be responsible for variation in Tenax extraction is the mass required for complete uptake of the desorbed compound relative to organic carbon mass in sediment in an extraction system. The Tenax:OC ratio varies greatly within the literature ranging from 0.132 to 109:1 without any clear information

Table 2
Tenax sorption rates of PCB congeners. The k_T represents the sampling rate constant of Tenax for PCBs, k_d represents the conditional desorption rate constant of PCBs from the Tenax, V_w represents the constant volume of water used (0.04 L), M_T represents the constant mass of Tenax used (0.01 g), and k_u represents the conditional sampling sorption rate of Tenax in volume of water per gram of Tenax per hour. Values within the parentheses represent ± SD (standard deviation). The COD is the coefficient of determination and represents how well the experimental data fit the model (calculated by Scientist 2.01).

Homolog group	Congener	k_d (h^{-1})	SD	k_T (h^{-1})	SD	V_w (L)	M_T (g)	k_u (L g Tenax^{-1} h^{-1})	COD
Di-CB	8 (2,4′)	0.67	(0.13)	20.13	(0.91)	0.04	0.01	80.51	0.89
Tri-CB	18 (2,2′,5)	1.53	(0.17)	21.54	(1.04)	0.04	0.01	86.17	0.86
	28 (2,4,4′)	0.34	(0.12)	21.21	(0.93)	0.04	0.01	84.85	0.94
Tetra-CB	44 (2,2′,3,5′)	0.18	(0.10)	20.29	(0.77)	0.04	0.01	81.15	0.99
	52 (2,2′,5,5′)	0.16	(0.12)	20.71	(0.89)	0.04	0.01	82.82	0.90
	66 (2,3′,4,4′)	0.22	(0.09)	17.95	(0.64)	0.04	0.01	71.79	0.94
	77 (3,3′,4,4′)	0.10	(0.10)	19.66	(0.70)	0.04	0.01	78.65	0.93
	81 (3,4,4′,5)	0.32	(0.14)	19.93	(1.09)	0.04	0.01	79.72	0.86
Penta-CB	101 (2,2′,4,5,5′)	0.44	(0.11)	22.85	(0.89)	0.04	0.01	91.39	0.90
	105 (2,3,3′,4,4′)	0.53	(0.06)	17.54	(0.42)	0.04	0.01	70.16	0.97
	114 (2,3,4,4′,5)	0.32	(0.15)	20.58	(1.06)	0.04	0.01	82.30	0.87
	118 (2,3′,4,4′,5)	0.04	(0.14)	20.89	(1.04)	0.04	0.01	83.56	0.87
	123 (2,3′,4,4′,5′)	0.46	(0.13)	21.04	(1.04)	0.04	0.01	84.14	0.88
	126 (3,3′,4,4′,5)	0.45	(0.07)	17.24	(0.53)	0.04	0.01	68.98	0.96
Hexa-CB	128 (2,2′,3,3′,4,4′)	0.10	(0.11)	19.15	(0.77)	0.04	0.01	76.60	0.92
	138 (2,2′,3,4,4′,5′)	0.34	(0.13)	18.34	(0.91)	0.04	0.01	73.35	0.90
	153 (2,2′,4,4′,5,5′)	0.07	(0.13)	19.87	(0.95)	0.04	0.01	79.50	0.89
	156 (2,3,3′,4,4′,5)	0.24	(0.08)	17.41	(0.59)	0.04	0.01	69.62	0.95
	157 (2,3,3′,4,4′,5′)	0.67	(0.11)	19.64	(0.83)	0.04	0.01	78.58	0.92
	167 (2,3′,4,4′,5,5′)	0.17	(0.11)	20.47	(0.80)	0.04	0.01	81.89	0.92
	169 (3,3′,4,4′,5,5′)	0.19	(0.10)	21.29	(0.82)	0.04	0.01	85.15	0.92
Hepta-CB	170 (2,2′,3,3′,4,4′,5)	0.19	(0.08)	19.54	(0.60)	0.04	0.01	78.15	0.95
	180 (2,2′,3,4,4′,5,5′)	0.44	(0.12)	19.98	(0.87)	0.04	0.01	79.94	0.90
	189 (2,3,3′,4,4′,5,5′)	0.12	(0.11)	17.57	(0.77)	0.04	0.01	70.27	0.92
	187 (2,2′,3,4′,5,5′,6)	0.49	(0.10)	19.31	(0.66)	0.04	0.01	77.24	0.94
Octa-CB	195 (2,2′,3,3′,4,4′,5,6)	0.38	(0.15)	19.05	(1.03)	0.04	0.01	76.18	0.87
Nona-CB	206 (2,2′,3,3′,4,4′,5,5′,6)	0.04	(0.12)	20.39	(0.88)	0.04	0.01	81.56	0.90
Deca-CB	209 (2,2′,3,3′,4,4′,5,5′,6,6′)	0.61	(0.11)	20.77	(0.80)	0.04	0.01	83.07	0.92

on the effect of this variation on Tenax estimates of exposure [2]. Because desorption of HOCs from the sediment into the interstitial water is largely controlled by the composition and amount of OC in the sediment [44], the effect of Tenax:OC ratios may add variability or even underestimate bioaccessibility of contaminants. Furthermore, the organic carbon acts as a direct competitor for freely dissolved compound in the extraction system, such that sorption to the Tenax or organic carbon depends on the amount and capacity of each material.

It is clear from the Tenax sorption rate experiment that Tenax extraction is largely limited by desorption of the PCBs from the sediment provided the Tenax has a greater capacity for the PCBs than the organic carbon. This supports the observation of strong relationships between Tenax extractable concentrations and bioaccumulation or toxicity [6,8,9] . However, if the amount of Tenax is too small to be the dominant competitor for the dissolved contaminant, compared to OC, then even the high sorption rate reported above may not be sufficient to completely absorb the desorbing compound. To test the rapid removal of desorbing PCBs by Tenax from sediments, and understand how this may impact the utility of the Tenax method as an estimate of bioaccumulation, experiments varying the Tenax:OC ratio evaluated the observed Tenax concentration obtained from 24 h single-point Tenax extractions relative to PCB bioaccumulation by *L. variegatus*.

Recoveries of the surrogates for the Tenax and *L. variegatus* ranged from 75-110% for DBOFB and 80–115% for PCB 191 across all samples. No target analytes were detected in the blank samples. The recoveries for the matrix spikes from sediments ranged from 82-118%, and the RSD between the matrix spikes fell within the acceptable range (< 25%). Ranges of the water quality parameters in overlying water were temperature 23 ± 1°C, DO 5.0–6.09 mg L^{-1}, pH 7.5 ± 1, conductivity at 400 ± 1 μS cm^{-1} and ammonia < 0.05 mg L^{-1}. Mean *L. variegatus* lipid values were 1.5 ± 0.2% for MQ-1, 2.8 ± 1.1% for CL-S4 and 1.9 ± 0.3% for CL-S5.

Tenax masses between 0.05 g and 2.0 g were chosen to represent the most common range of Tenax masses used in previous Tenax research [2]. The chosen Tenax masses spanning this range resulted in Tenax:OC ratios from 1:1 to 15:1 relative to the OC content of the three sediments evaluated in the current study, and the specific Tenax masses and Tenax:OC ratios are shown in **Table S3**a–c of the Supporting material. To evaluate the impact of varying the Tenax:OC ratios on estimates of exposure represented by bioaccumulation of PCBs by *L. variegatus*, an ANCOVA analysis was used to test for differences in the linear regressions comparing the 24 h single-point Tenax extractable concentrations (Log C24, ng g^{-1} OC) from the varying Tenax:OC ratios with the tissue concentrations (Log Ca, ng g^{-1} lipid) for each field-collected sediment (Fig. 3 and Fig. S2a–c).

The resulting regressions for the MQ-1, CL-S4 and CL-S5 sediments are shown in Fig. S2a–c of the Supporting material. The ANCOVA results from each individual sediment demonstrate there were no significant differences in the relationship of 24 h Tenax extractable PCB concentrations and bioaccumulation between the different Tenax:OC ratios used within each sediment (**Fig. S2**a–c). This suggests that the mechanisms controlling the linear relationship between the Tenax extractable concentrations and bioaccumulation are the same regardless of the Tenax:OC ratio used. However, the variability in this relationship was impacted by the Tenax: OC ratio, as evidenced by the regression coefficients (r^2) of the individual regression lines. In the three field-contaminated sediments, the variability in the linear regressions was significantly reduced once a 5:1 Tenax:OC ratio was achieved or exceeded, suggesting Tenax:OC ratios ≥5:1 provide better predictability of bioaccumulation of PCBs from sediment (Fig. 3, **S2**a–c). Fig. 3 shows a summary of the regressions between 24 h single-point Tenax extractable concentrations and tissue concentrations using a minimum Tenax:OC ratio that provided the best linear fit (i.e., r^2 values closer to 1).

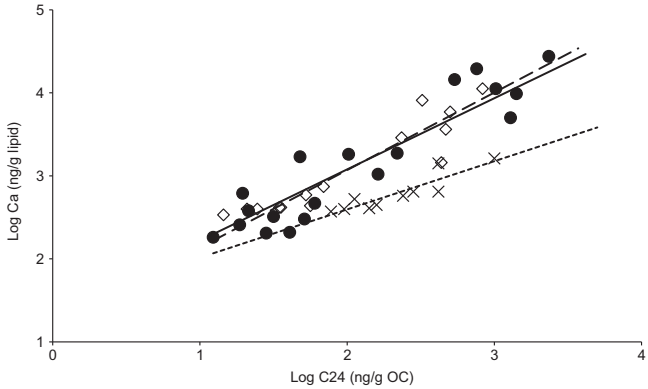

Fig. 3. Linear regression models with log transformed tissue concentrations (Ca) and 24 h single-point Tenax extractable concentrations (C24) of PCB congeners. The lines represent the minimum Tenax:OC ratios depicting a good linear fit for sediments with varying %OC content (MQ-1, 0.89% OC; CL-S4, 5.65% OC, and CL-S5, 8.87% OC). The MQ-1 sediment (x) with a 5:1 ratio (r^2=0.80); CL-S4 sediment (•) with a 5:1 ratio (r^2=0.86), and CL-S5 sediment (◇) with a 5:1 ratio (r^2=0.86).

These results are comparable to that of other studies using Tenax extractable concentrations as surrogates of exposure to PCBs from sediments with varying OC content [16,45]. For example, Tenax:OC ratios ranging from 1.3:1 to 21.2:1 provided good relationships describing bioaccessibility of polycyclic aromatic hydrocarbons and PCBs in spiked sediments (0.41–4.11% OC) [16]. Similarly, lower Tenax:OC ratios ranging from 3.0:1 to 5.1:1 were used to predict bioaccessibility of pesticides and PCBs for field-collected sediments (0.70–4.90% OC) [45]. No significant effect of Tenax:OC ratios was reported in either study with respect to the bioaccessible fraction represented by the Tenax extractable concentrations [16,45] . The current study is the first to directly address the effect of Tenax:OC ratios on Tenax extractable concentrations for a specific class of compounds. Moreover, the results of the current study demonstrated that the bioaccessibility of PCBs in contaminated sediments with low % OC (e.g., < 1.0% OC) and high %OC (e.g., > 5.0% OC) is better represented by Tenax extractable concentrations from Tenax:OC ratios ≥5:1 (Fig. 3). Since we do not accurately know where the OC cutoff would occur, we suggest that a minimum ratio of 5:1 be used to yield consistent relationships between the 24 h single-point Tenax extractable concentrations and tissue concentrations.

3.4. Validation of Tenax parameters

To further test the influence of Tenax:OC ratios on the bioaccessibility of PCBs, and therefore estimates of exposure as represented by bioaccumulation, the linear regression data was assessed using the BTM (Bioaccumulation Tenax Model) [34]. The BTM is a linear regression model (r^2=0.94) developed using data from single-point Tenax extractions and bioaccumulation from several benthic organisms (e.g., *L. variegatus*) exposed to a wide range of sediments contaminated with PCBs [34]. It represents a valid approach to compare the data presented here, because the model was developed from varying experimental conditions. Because the authors of the BTM reported a good fit of the data in the BTM, it is assumed that PCBs were efficiently extracted by Tenax, and that the Tenax masses and length of the single-point Tenax extractions (e.g., 24 h) used were sufficient to measure the bioaccessible compounds. In the current study, the 24 h single-point Tenax extractions were conducted using an extraction solvent volume of 10 mL across all the field-collected sediments. The only parameter that varied in this comparison using the BTM was the Tenax:OC ratio. Therefore, it is hypothesized that any regression data that fell outside the model is likely due to less than optimal Tenax masses being used in the extraction system.

As shown in Fig. 4, data for the field-collected sediments (MQ-1,

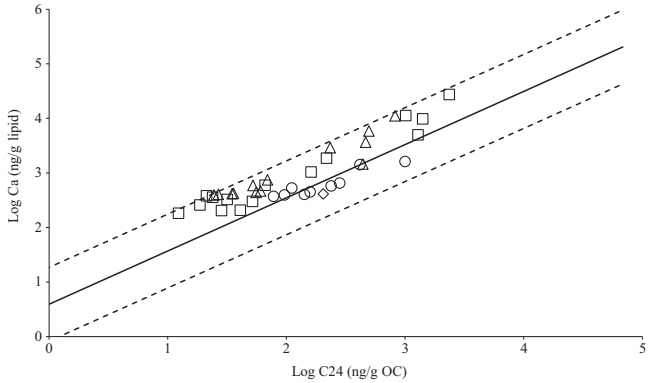

Fig. 4. Relationship between 24 h single-point Tenax extractable concentrations (C24) and tissue concentrations (Ca) of PCB congeners fit to the BTM (Biota Tenax Model [34]). The data points represent the C24 concentrations from a Tenax:OC ratio of 5:1. The symbols represent individual PCB congener data. The (○) represents data from MQ-1 (0.89% OC); (□) represents data from CL-S4 (5.65% OC) and (Δ) represents data from CL-S5 (8.87% OC). The 95% confidence intervals of the BTM are represented by the dotted lines. OC=organic carbon.

CL-S4 and CL-S5) all fell within the 95% confidence intervals (C.I.) of the BTM, when a minimum Tenax:OC ratio of 5:1 was used. The fact that these data fit the BTM further confirms that an extraction solvent volume of 10 mL and a 24 h rotation period applied to the Tenax extractions yields reliable data to predict bioaccessibility of PCBs in biota. Moreover, these results also suggest that the method variability used in Tenax extractions in the past did not seem to significantly impact overall results. However, it is best to practice standardized methods to reduce any extraneous variability that could be introduced.

To investigate the data that fell outside the 95% C.I. of the BTM, the Tenax:OC ratio data were fit to the BTM using PCB homolog groups and tissue concentrations as shown in Figs. S2, S3 and S4 of the Supporting material. Overall, 55% of these data points fell outside the 95% C.I. of the BTM for sediments varying in their OC content (0.89–8.87% OC), when single-point Tenax extractable concentrations were determined using Tenax:OC ratios of 1:1 or 3:1 (Fig. S3).

The tri-CB, tetra-CB and penta-CB groups fit the BTM independent of the OC content of the sediments, while the higher-chlorinated congener group data (hexa-, hepta- and octa-CB) fell outside of the 95% C.I. of the BTM across sediments. These results showed that the amount of compound detected by Tenax is lower at small ratios leading to a shift in the data. Similar results were reported in a study assessing the exposure to PCB-contaminated sediments in aquatic and riparian species [46]. The author also examined how well the Tenax and aquatic species data from field-collected sediments (6.1–8.0% OC) fit the BTM. In this case, the data that fell outside the 95% C.I. of the BTM corresponded to higher-chlorinated congener groups (hepta-, octa- and nona-CB) that were obtained using Tenax:OC ratios that ranged from 3:1 to 4:1 [46].

In contrast, when single-point Tenax extractable concentrations were determined using Tenax:OC ratios that ranged from 5:1 to 15:1, all the data for the various homolog groups fell within the 95% C.I. across the sediments as shown in Fig. S4. Furthermore, these results are in agreement with the good fit (r^2) of the linear regressions when ratios ≥5:1 was used as shown in Fig. S2a–c. Therefore, it is recommended that at least a 5:1 Tenax:OC ratio be used for sediments with varying OC content (0.89–8.87% OC) to prevent under-prediction of concentrations of PCBs in the biota tissue (Fig. S3).

4. Conclusions

The objective of the current study was to determine the best operational conditions for Tenax extractions by investigating the parameters of solvent extraction volume, Tenax sorption rate and Tenax:OC ratios using PCBs. For the first parameter, an extraction solvent volume of 3×10 mL provided both good recoveries (up to ≤100%) and precision (< 10% RSD) of the Tenax extractable concentrations and provided good estimates of the bioaccessible PCB concentrations in sediments. For the second parameter, 30 min was a sufficient amount of time for 0.01 g of Tenax to clear all of the available PCBs from 40 mL of water. The implication of this finding is that 0.5 g of Tenax, a mass commonly reported in Tenax studies, would clear the same volume of water of PCBs 20,694 times in a 24 h period. This supports the idea that 24 h single-point Tenax extraction would be capable of sorbing the PCBs which desorb from sediment, and thus become bioaccessible through the interstitial water surrounding the sediment particles. We hope that this finding could also be applied to other classes of organic compounds with similar water solubility and hydrophobicity as PCBs, thus allowing a better selection of Tenax masses and time points to reflect the concentration of the desorbed compound as represented by the Tenax extractable concentrations. Finally, the results from the current study indicate that the Tenax:OC ratio plays an important role in reducing uncertainties in the measurements of the Tenax extractable concentrations of contaminated sediments. This especially holds true for mixtures of compounds, such as PCBs, because they have a wide range of hydrophobicities which contributes to sorption in sediments. Overall, it is suggested that a minimum 5:1 Tenax:OC ratio be used to yield optimum relationships between the 24 h single-point extractable concentrations and tissue concentrations. Additional work using a more in-depth OC characterization will help expand the influence of OC content and type on the Tenax extractable concentrations. In summary, the optimized operational conditions for Tenax extraction should provide Tenax users/researchers and regulatory agencies with a more thorough experimental setting for assessing bioaccessibility of PCBs and other organic compounds found in contaminated sediments.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.talanta.2016.11.061.

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