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The robustness of single-point Tenax extractions of pyrethroids: Effects of the Tenax to organic carbon mass ratio on exposure estimates

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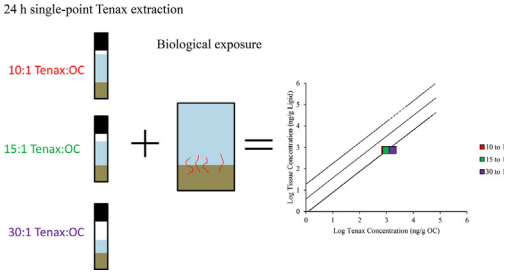
HIGHLIGHTS

- Variation in Tenax extraction methods may limit widespread use in risk assessments.
- Increasing Tenax:OC ratios had minimal impact on Tenax extractable concentrations.
- Tenax exposure estimates are as consistent as other extraction techniques.
- Using correct toxicological endpoints to model exposure is critical for success.
- Single-point Tenax extractions should use Tenax:OC ratios of at least 5:1.

GRAPHICAL ABSTRACT



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ABSTRACT

Use of Tenax extractable concentrations to estimate biological exposure to hydrophobic organic contaminants is well documented, yet method variation exists between studies, specifically in the ratio of Tenax mass to organic carbon mass in the sediment (Tenax:OC ratio) being extracted. The effects of this variation on exposure estimates are not well understood. As Tenax is theoretically in direct competition with organic carbon for freely dissolved chemical in sediment interstitial water, varying the Tenax:OC ratio could impact single-point Tenax extraction (SPTE) exposure estimates. Therefore, the effects of varying Tenax:OC ratios on SPTE pyrethroid concentrations from field-contaminated and laboratory-spiked sediments were compared to bioaccumulation by *Lumbriculus variegatus*. The Tenax:OC ratio had minimal effect on SPTE pyrethroid concentrations. The SPTE pyrethroid concentrations obtained using the highest and lowest Tenax:OC ratios ranged from 0.85- to 3.91-fold different, which is unlikely to contribute substantial error to bioaccessibility estimates. Comparisons to Tenax exposure endpoints from previous research reveal the variation in these endpoints is likely due to toxicokinetic and toxicodynamic differences; processes common to exposure estimates provided by any chemical extraction technique. As the pyrethroid concentrations in the experimental sediments caused toxicity to *L. variegatus*, thus affecting bioaccumulation, the SPTE concentrations overestimated bioaccumulation. However, SPTE concentrations strongly correlated with growth inhibition regardless of the Tenax:OC ratio, providing accurate estimates of the correct exposure endpoint. Tenax masses of 0.500–0.800 g

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should provide sufficient Tenax to achieve Tenax:OC ratios of at least 5:1, which will provide accurate exposure estimates while retaining the ease of conducting SPTEs.

1. Introduction

The success of evaluating risk of hydrophobic organic contaminants (HOCs) in aquatic sediments hinges on obtaining the most accurate estimates of the bioavailable compound present (DiToro et al., 1991; Simpson and Batley, 2016; USEPA, 1997). As such, substantial research within sediment toxicology has focused on finding accurate, reliable, and easy to use methods for evaluating HOC exposure. The most available form of chemical in sediment for uptake by biota is the chemical concentration freely dissolved in the interstitial water, which represents the chemical activity of the contaminant in sediment. While exposure can also occur through ingestion of contaminated sediment, the chemical concentration in the interstitial water can be used to estimate bioaccumulation and toxicity of HOCs in aquatic environments (DiToro et al., 1991; Lydy et al., 2014; Reichenberg and Mayer, 2006; Semple et al., 2004). Organic carbon normalization utilizes the principles of partitioning to account for the chemical fraction in sediment that will contribute to the freely dissolved interstitial water concentration by accounting for the fraction of sediment to which the contaminant is sorbed (DiToro et al., 1991). At equilibrium, the chemical activity of the contaminant on the organic carbon will equal that in the interstitial water, such that the partition coefficient between these two phases, the organic carbon water partition coefficient (K_{OC}), can be used to predict the chemical concentration in one phase or the other (DiToro et al., 1991). Use of organic carbon normalization to estimate exposure; however, does not always work well, as additional factors other than just organic carbon partitioning are responsible for the exposure concentration in sediments (Cornelissen et al., 2001; Morrison et al., 2000). Therefore, the development of other extraction techniques to more clearly represent exposure continues to be a major focus of environmental risk assessments.

The most common of these extraction techniques are passive samplers, such as solid-phase microextraction (SPME) fibers, which represent bioavailable sediment concentrations through equilibrium partitioning with the freely dissolved chemical in the environment (Lydy et al., 2014; OSWER, 2012; Parkerton and Maruya, 2014). The utility of passive samplers has been well documented and application of this technique in risk assessment is widely accepted (OSWER, 2012). Similarly, Tenax extractions, either sequential or single-point extractions, provide accurate measures of desorption of HOCs from sediment, and can be used to estimate both dermal and dietary exposure of HOCs to benthic invertebrates (Du et al., 2013, 2014; Harwood et al., 2012, Harwood et al. 2013a,b; Kraaij et al., 2001, Kraaij et al. 2002; Landrum et al., 2007; Mackenbach et al., 2012, 2014; Shor et al., 2003; ten Hulscher et al., 2003; You et al., 2006, 2008). Tenax extractions provide exposure estimates by measuring the bioaccessible chemical fraction, or the chemical fraction in the environment that may become available to cross a biological membrane in a given time frame (Du et al., 2013, 2014; Harwood et al., 2012, Harwood et al. 2013a,b; Kraaij et al., 2001, Kraaij et al. 2002; Landrum et al., 2007; Mackenbach et al., 2012, 2014; Semple et al., 2004; Shor et al., 2003; ten Hulscher et al., 2003; You et al., 2006, 2008). Since bioaccessibility is linked to the rapidly desorbing fraction (F_{rap}), which represents the labile chemical fraction available for uptake through both interstitial water and dietary release, improvements in Tenax extraction methods have been sought to provide accurate estimates of F_{rap} with the least laboratory effort and time (Cornelissen et al., 1998; Harwood et al., 2012; Kraaij et al., 2001, 2002; Kukkonen et al., 2004; Lydy et al., 2015; Sormunen et al., 2008; Trimble et al., 2008; You et al., 2007, 2009). While sequential Tenax extractions can be used to measure desorption of HOCs from sediment and offer the most comprehensive view of desorption, these experiments are time-consuming and labor intensive (Cornelissen et al., 1998, 2001). A simplification of this technique uses single-point Tenax extractions (SPTEs), generally lasting 6–30 h with 24 h extractions being most common (Cornelissen et al., 2001; Harwood et al., 2015; Lydy et al., 2015). Single-point Tenax extractable concentrations of HOCs correlate well with the chemical concentration in F_{rap} and thus, provide rapid estimates of exposure (Cornelissen et al., 1998, 2001; Kraaij et al., 2001, Kraaij et al. 2002; Kukkonen et al., 2004; Lydy et al., 2015; Sormunen et al., 2008; Trimble et al., 2008; Xu et al., 2008; Yang et al., 2008; You et al., 2007, 2009). Many studies exist demonstrating the utility of the SPTE as an estimate of bioaccessibility and exposure, but the methods used with this technique vary across studies (Lydy et al., 2015). A recent review of the Tenax literature revealed the largest variation in the application of the SPTE exists in the choice of Tenax mass used relative to the organic carbon mass in the Tenax extraction system (Lydy et al., 2015). Tenax to organic carbon mass (Tenax:OC) ratios have ranged between 0.132:1 to 109:1 to estimate exposure of different HOC classes across sediments, and only one study has briefly investigated the effects of this ratio on estimates provided by the Tenax method (White et al., 1999).

The success of Tenax extractions to estimate bioaccessibility of different compound classes across a wide range of environments depends on the ability of the Tenax to absorb all of the compound desorbed to the interstitial water from the sediment over the length of the extraction, without affecting the desorption kinetics of the compound (Cornelissen et al., 1998; Harwood et al., 2012; Kraaij et al., 2001, 2002; Kukkonen et al., 2004; Lydy et al., 2015; Sormunen et al., 2008; Trimble et al., 2008; You et al., 2007, 2009). The Tenax:OC ratio is important, because organic carbon is the sorbent in most sediments controlling sorption and desorption of HOCs and thus is in direct competition with the Tenax beads for freely dissolved chemical in the extraction system (DiToro et al., 1991; Pignatello and Xing, 1996). Thus in a Tenax extraction, both organic carbon and Tenax compete for the compound of interest through sorption of freely dissolved chemical in the interstitial water and the extent of sorption depends on the law of mass action and the relative affinities and capacities of organic carbon and Tenax. If this is true, then the relationship between organic carbon and Tenax should be governed by competitive sorption, and the Tenax:OC ratio would need to be large enough to prevent significant re-adsorption to the organic carbon after desorption. Too little Tenax relative to the organic carbon mass could underestimate bioaccessibility, while too much Tenax could result in an overestimate of bioaccessibility due to alterations of desorption kinetics of the residual fractions by creating an extremely large chemical activity gradient between the chemical in the organic carbon and on the Tenax during the extraction. Using different Tenax:OC ratios

during SPTEs of pyrethroids from sediment was hypothesized to cause the most variation in Tenax exposure estimates of bioaccumulation or toxicity (Lydy et al., 2015). Understanding how variation of the Tenax mass affects the estimates of bioaccessibility for SPTEs is crucial to standardizing the method and implementing this technique on a wider scale.

The current study is designed to better understand how the Tenax:OC ratio affects estimates of exposure provided by SPTEs by 1) evaluating the variation in pyrethroid SPTE concentrations obtained with varying Tenax:OC ratios, and 2) understanding how this variation impacts exposure estimates in the form of pyrethroid bioaccumulation by *Lumbriculus variegatus*. Pyrethroid-contaminated sediments collected from the field and laboratory-spiked sediments aged for 7, 60, and 120 d were used to represent different conditions under which Tenax extractions may be used to evaluate pyrethroid exposure. Single-point Tenax extractions were conducted with varying Tenax:OC ratios and compared in two ways. First, the difference between the SPTE pyrethroid concentration obtained with the highest and lowest Tenax:OC ratios used during 24 h Tenax extractions of each sediment were determined to understand the variability introduced into the SPTE by altering the methodology of the extraction. Second, whether these differences in the SPTE concentrations would result in significant error in estimates of bioaccumulation and toxicity of pyrethroids was determined by comparing the variation found in the first analysis to the variability observed in Tenax exposure estimates from previous research, as well as pyrethroid bioaccumulation and toxicity caused to *L. variegatus* during bioaccumulation assays with field-contaminated and laboratory-spiked sediments in the current study. Pyrethroids were chosen as a model compound class for this study due to their acute toxicity, the widespread use and presence of pyrethroids in aquatic environments worldwide, and to provide information on the utility of using this technique to estimate exposure and bioaccessibility of current-use chemicals (Amweg et al., 2005; Cui et al., 2009; Ding et al., 2010; Harwood et al., 2013b; Maul et al., 2008; Mokry and Hoagland, 1990).

2. Material and methods

2.1. Chemicals

Tefluthrin, bifenthrin, fenpropathrin, λ-cyhalothrin, permethrin, cypermethrin, and esfenvalerate were purchased as individual compounds from Chem Service, Inc. (West Chester, PA, USA) (purity $\geq$ 98%). A custom pyrethroid mixture containing all of the target pyrethroids was purchased from AccuStandard, Inc. (New Haven, CT, USA) and was used for calibration curves during analytical analysis and added to matrix spike samples. Octachlorobiphenyl (polychlorinated biphenyl (PCB)-204), flucythrinate, and deuterated (d6) versions of the target pyrethroids were used as internal standards and were purchased from Chem Service, Inc., Cambridge Isotope Laboratories, Inc., (Tewksbury, MA, USA), AccuStandard, Inc., and donated by Kalexsyn, Inc. (Kalamazoo, MI, USA), respectively. Surrogates, 4,4'-dibromooctafluorobiphenyl (DBOFB) and decachlorobiphenyl (PCB-209) were purchased from Sigma-Aldrich (St Louis, MO, USA). Tenax (60–80 mesh) was purchased from Scientific Instrument Services, Inc. (Ringoes, NJ, USA). Anhydrous sodium sulfate and Supelco dual layer ENVI™-Carb II/PSA 300/600 mg solid-phase extraction (SPE) cartridges were purchased from Sigma Aldrich. Pesticide grade solvents, including hexane, acetone, and methylene chloride, as well as acetic acid and mercuric chloride were purchased from Thermo-Fisher Scientific (Waltham, MA, USA).

2.2. Field-contaminated sediments

Three field-contaminated sediments were collected from urban landscapes, and included Mosher Slough (MSH, 1.70% organic carbon (OC); Stockton, CA, USA), Curry Creek (CRY, 3.64% OC; Roseville, CA, USA), and Springfield (SPFD, 4.24% OC; Springfield, IL, USA) sediments. Field-contaminated sediments were passed through a 2 mm sieve upon collection to remove any large debris, homogenized, and then held at 4 ± 2 °C until use. All three field-contaminated sediments contained detectable pyrethroid concentrations as determined by preliminary SPTEs. Organic carbon content of all sediments was determined by Midwest Laboratories, Inc. (Omaha, NE, USA) using the American Society for Testing and Materials method (d) 5373.

2.3. Laboratory-spiked sediments

Two sediments were collected from Southern Illinois for use in the laboratory-spiking experiment and included Bay Creek (BC, 0.56% OC; Pope County, IL, USA) and LaRue-Pine Hills (LPH, 2.04% OC; Shawnee National Forest, IL, USA) sediments. These sediments were passed through a 0.500 mm sieve to remove large debris, homogenized, and then stored at 4 ± 2 °C until use. Both reference sediments were found to contain no detectable concentrations of any of the target pyrethroids as determined by preliminary SPTEs.

The two reference sediments were spiked with pyrethroids solubilized in acetone carrier. Each sediment was spiked with appropriate volumes of individual stock solutions of each of the target pyrethroids to achieve nominal concentrations of 300 ng/g (dry weight (dw)) of each compound. This sediment concentration was chosen to provide detectable concentrations after the respective aging times of the study. Sediments were spiked in bulk and rolled for 4 h after a homogenization step via 5 min of hand mixing. After spiking, laboratory-spiked sediments were stored in darkness at 4 ± 2 °C for 7, 60, and 120 d prior to experimental use, with rolling occurring every two weeks for 1 h at 23 ± 2 °C during aging to ensure that the sediments were well homogenized. The bulk sediments were hand mixed prior to use in experiments at each aging time.

2.4. Single-point Tenax extractions

Twenty-four hour SPTEs were performed using varying amounts of Tenax with the field-contaminated and laboratory-spiked sediments. These experiments were conducted to examine the effects of the Tenax:OC ratio on the Tenax extractable pyrethroid concentration and the effect on estimates of exposure.

Twenty-four hour SPTEs were performed by adding approximately 3 g (dw) of sediment, as well as 4.5 mg of mercury (II) chloride to prevent microbial degradation of the compounds during the Tenax extraction, to a 50 mL glass vial. Three grams of sediment represented 0.051 g OC, 0.109 g OC, 0.127 g OC, 0.017 g OC, or 0.061 g OC for MSH, CRY, SPFD, BC, or LPH sediments, respectively. Forty milliliters of moderately hard water (MHW) (Smith et al., 1997) as well as a predetermined Tenax mass representing the range of Tenax masses most commonly found in the literature (e.g. Tenax:OC ratios between 5:1 and 60:1; Table A.1 (Lydy et al., 2015)) were then added to the vial. After addition of the Tenax, the vials were briefly shaken, and then rotated at 20.8 revolutions per minute (rpm) via a BBL BioQuest tube rotator (Cockeysville, MD, USA) for 24 h. Four replicates were used with each Tenax mass and sediment, as well as two blanks and three matrix spikes containing uncontaminated LPH reference sediment. The Tenax mass used with the blank and matrix spike samples represent the largest Tenax mass used in each experiment (1.64 g of Tenax for the MSH

and CRY sediment experiments, 1.65 g of Tenax for the SPFD sediment experiment, and 1.53 g of Tenax for the BC and LPH sediment experiments) to ensure no target analytes were present on the Tenax and the solvent volumes used to wash these Tenax masses were adequate to fully recover pyrethroids bound to the Tenax beads. After the 24 h extraction period, the Tenax was extracted by sonication with the wash procedure, determined from a preliminary solvent wash experiment, described below.

2.5. Tenax cleanup

After 24 h, the Tenax extraction vials were centrifuged at 2000 rpm for 5 min to separate the Tenax beads, which float, from the sediment. The Tenax beads were then removed from the vial and placed in a clean 20 mL vial containing either 5 or 10 mL of acetone. Tenax masses ≤0.800 g were washed with two 5 mL washes of acetone followed by two subsequent washes with 5 mL of an acetone:hexane (1:1, v/v) mixture. Tenax masses >0.800 g were washed with two 10 mL washes of acetone followed by two subsequent washes with 10 mL of an acetone:hexane (1:1, v/v) mixture. Each wash consisted of sonicating the vial containing the Tenax and solvent for 10 min, after which the solvent was removed, placed in a clean 20 mL vial for 5 mL washes or 300 mL Turbovap vial for 10 mL washes, and the solvent removed from the Tenax vial was replaced with the solvent required for the next wash. Matrix spike samples were processed by adding 50 ng of the pyrethroid mix to the Tenax matrix with the first solvent wash. The two surrogates, DBOFB and PCB-209 (50 ng), were added to all samples with the addition of Tenax prior to the first sonication. After the four washes were complete, the wash solvent, totaling 20 or 40 mL, was concentrated to 5 mL, solvent exchanged with 10 mL of hexane for 5 mL wash samples or 20 mL of hexane for 10 mL wash samples, and further concentrated to 5 mL, allowing for clear differentiation of the aqueous and organic layers in the extract. Next, the aqueous layer was washed by vortexing the samples at 2000 rpm for 5 min followed by 5 min of centrifugation at 3000 rpm. The hexane was removed from the aqueous layer of each extract, collected in a 20 mL vial, and the aqueous layer was then washed two more times with 3 mL of hexane. The initial hexane layer and two washes were collected in clean 20 mL vials. The Tenax extracts were concentrated to 1 mL and passed through ~1.75 g of anhydrous sodium sulfate with hexane to remove any residual water. The final extracts were then concentrated to 1 mL, transferred to a GC vial, and acidified with 1 μL of acetic acid for analysis via gas chromatograph-mass spectrometer (GC-MS) operated in negative chemical ionization (NCI) mode. The extract was acidified to prevent isomerization of the pyrethroids (You and Lydy, 2007). All pyrethroid concentrations reported using Tenax extractions were normalized for the organic carbon mass in the sediment of the Tenax extraction system and reported as ng/g OC.

2.6. Lumbriculus variegatus bioaccumulation assays

Bioaccumulation assays were conducted with all field-contaminated and laboratory-spiked sediments using *L. variegatus* to determine the ability of different Tenax:OC ratios to estimate pyrethroid bioaccumulation and toxicity. *Lumbriculus variegatus* were cultured at Southern Illinois University and bioaccumulation assays followed a modified protocol from the U.S. Environmental Protection Agency (USEPA, 2000). The U.S. EPA protocol specifies a 28-d exposure for bioaccumulation assays with *L. variegatus*, but previous research has demonstrated that *L. variegatus* begin reproduction at 14-d, which causes feeding to halt, affecting bioaccumulation (Leppänen and Kukkonen, 1998). Therefore, a 14-d test, which has been demonstrated to be long enough for the organisms to reach steady-state with PCBs, was deemed most appropriate for the current study (Mackenbach et al., 2012; Trimble et al., 2008). Approximately 100 g (wet weight (ww)) of MSH, CRY, SPFD, BC, or LPH sediment was weighed into four replicate 600 mL beakers and covered with 500 mL overlying MHW. After allowing the sediment to settle for 24 h, 50 *L. variegatus* were added to each beaker and placed in a flow through system, which conducted 100 mL automated water changes three times daily. The *L. variegatus* were allowed to reside in the sediment for 14 d at 23 ± 1 °C with a light cycle of 16:8 h light:dark. *Lumbriculus variegatus* added to six beakers with uncontaminated LPH sediment were also included with each experiment to serve as negative controls and matrix spike samples.

After 14 d, the *L. variegatus* were removed from the beakers, rinsed with MHW, placed in new beakers containing MHW, and allowed to depurate their gut contents for 6 h. Following depuration, the *L. variegatus* were removed from the beakers, patted dry, and approximately 15 mg of tissue was removed from each replicate for lipid analysis following spectrophotometric methods of van Handel (1985). Briefly, the lipids were extracted from the *L. variegatus* using a mixture of chloroform and methanol, as well as sulfuric acid digestion. Next, the lipid content was determined using a phosphoric acid vanillin reagent, the transparency of which was altered by the lipid content of the organisms. A spectrophotometer was used to measure the change in transparency of the samples and compared to calibration standards to determine the lipid mass present in the organisms from each bioaccumulation assay sample. The remaining organisms were placed in a clean 20 mL vial, and weighed to the nearest 0.1 mg using a Mettler Toledo analytical balance (Columbus, OH, USA). The organisms were then held at −20 °C until analysis.

2.7. Analysis of pyrethroids in Lumbriculus variegatus

At the conclusion of each bioaccumulation test, *L. variegatus* were collected from each sediment and sonicated in 10 mL of an acetone:hexane (1:1, v/v) solution with 20 s pulses until complete homogenization was achieved using a Sonics and Materials sonic disruptor (Newtown, CT, USA). Surrogates (DBOFB and PCB-209) were added to each sample prior to sonication and 50 ng of a standard pyrethroid mix was also added to matrix spike samples prior to sonication. The extracts were then concentrated to 2 mL and solvent exchanged to hexane. After further concentrating samples to approximately 1 mL, each sample was passed through a dual layer ENVI™-Carb II/PSA 300/600 mg SPE cartridge to remove interferences. The SPE cartridges were first primed with 3 mL of hexane, then the sample was loaded into the cartridge, and eluted with 7 mL of a hexane:methylene chloride (70:30 v/v) solution (de Perre et al., 2015a). The eluent from the SPE cartridges was solvent exchanged to hexane, transferred to a GC vial and acidified with 1 μL of acetic acid. The final extract was then quantified using GC-MS (NCI). All pyrethroid concentrations reported in the *L. variegatus* tissue samples were normalized for the lipid mass in the total tissue mass as determined from the lipid content of the individuals taken from each replicate treatment and reported as ng/g lipid.

2.8. Gas chromatography-mass spectrometry

Pyrethroid concentrations in Tenax and *L. variegatus* samples were analyzed using an Agilent 5975C GC-MS operated in NCI mode (Agilent Technologies, Palo Alto, CA, USA) with a method adapted from previous research (de Perre et al., 2015b; Li et al., 2013; Weston and Lydy, 2014). A HP-5MS (30 m × 250 μm × 0.25 μm; Agilent Technologies) column was used to separate

pyrethroids for analysis. Helium at a flow rate of 1 mL/min was used as the carrier gas, and methane was used as the NCI reaction gas. Two microliters of each sample was injected in splitless mode with an inlet temperature of 260 °C. Pyrethroids were separated by using the following GC oven parameters: initial oven temperature 50 °C, held for 1 min, then ramping to 200 °C at 20 °C/min, and then the temperature was increased to 295 °C at 10 °C/min, which was then held for 5 min. The temperature of the transfer line, ion source, and quadrupole were 300 °C, 150 °C, and 150 °C, respectively. Internal standards, PCB-204, chlorpyrifos-d10, and flucythrinate were used for standardization of response in calibration standards and samples for field-contaminated samples. Deuterated (d-6) versions of the seven pyrethroids included in the current study, as well as PCB-204 and flucythrinate were used as internal standards for standardization of response of the pyrethroids and surrogates, respectively, in calibration standards and samples for laboratory-spiked samples. The response of the target pyrethroids was calibrated using the deuterated version of the respective pyrethroid as an internal standard. Pyrethroids and surrogates were identified using known retention times, the respective quantitation ion for each compound, specific ion ratios, and at least one qualifier ion for each compound.

2.9. Quality assurance and quality control

To ensure extraction and cleanup methods were adequate for all pyrethroids being analyzed, surrogates were added to all samples, as described above. Three matrix spike and at least two blank samples were completed with uncontaminated LPH reference sediment with every extraction procedure (Tenax extractions, *L. variegatus* tissue analysis) and with each set of sediments being tested. Calibration check standards were run every eight samples during analysis with the GC-MS and the reported concentration of all pyrethroids and surrogates had to fall within 20% for the run to pass, otherwise the samples were reanalyzed.

3. Statistical analysis

A one-way analysis of variance (ANOVA) was used to determine statistical differences between the Tenax extractable pyrethroid concentration (ng/g OC) for each different Tenax:OC ratio ($\alpha = 0.05$) using SAS statistical software version 9.2 (SAS Institute, Inc., Cary, NC). In cases when the results of the ANOVA were significant, a post-hoc Tukey's test was used to determine differences between Tenax:OC ratios that resulted in similar or dissimilar Tenax extractable pyrethroid concentrations. A linear regression was used to evaluate the relationship between Tenax extractable concentrations and growth inhibition of *L. variegatus*, described below.

4. Results and discussion

4.1. Quality assurance and quality control

No target analytes were detected in any blank samples from the 24 h SPTEs or bioaccumulation assays. The percent recoveries of pyrethroids from matrix spike samples from the 24 h SPTEs and bioaccumulation assays ranged from 70.5 to 106% and 78.6 to 98.7%, respectively. The percent recoveries for the surrogates from the 24 h SPTEs and bioaccumulation assays ranged from 32.6 to 122% and 52.9 to 97.8%, respectively, for DBOFB, and 70.3 to 132% and 62.3 to 91.5%, respectively, for PCB-209. The low recoveries of DBOFB were found in SPTE samples from BC and LPH sediments aged for 7 d. The pyrethroid concentrations reported in these samples were not corrected for this low recovery, as similar recoveries have been observed for DBOFB from previous research using SPTEs to measure both PCB and pyrethroid concentrations in sediments (Harwood et al., 2013a; Landrum et al., 2007; Mackenbach et al., 2012, 2014). Furthermore, the recovery of the target pyrethroids from the matrix spike samples included with the SPTEs of these sediments demonstrated much higher recoveries, suggesting that the DBOFB was a poor representation of the pyrethroid recovery in these samples. Therefore, no correction for the low DBOFB recovery was conducted for these samples.

4.2. Effect of Tenax:OC ratio on Tenax extractable pyrethroid concentrations

The range of Tenax:OC ratios used was between 5:1 and 60:1 with a range of 2.5- to 4- fold difference for each sediment, depending on the sediment used (Table A.1). Increasing the Tenax:OC ratio used during a 24 h SPTE did not have as large an effect on the Tenax extractable pyrethroid concentration as was initially hypothesized. While statistical differences did exist in the SPTE concentration measured using the various Tenax:OC ratios, the overall variation in the SPTE pyrethroid concentration between the Tenax:OC ratios was not exceedingly large (Table 1). For example, bifenthrin extracted from MSH sediment demonstrated the largest variability in the Tenax extractable concentration when the highest (30:1) and lowest (10:1) Tenax:OC ratios were used (Table 1). This also represented the largest difference in Tenax mass used in a single-point experiment (1.02 g). However, the difference between the bifenthrin extractable concentration by these two ratios only varied by a factor of 3.91 ± 1.64 (mean ± standard

Table 1
The differences between the 24 h single-point Tenax extractable pyrethroid concentration obtained when using the highest and lowest Tenax to organic carbon (Tenax:OC) ratio during experiments with Mosher Slough (MSH, 1.70% organic carbon (OC)), Curry Creek (CRY, 3.64%OC), Springfield (SPFD, 4.24% OC), Bay Creek (BC, 0.56% OC), and LaRue-Pine Hills (LPH, 2.04% OC) sediments. The values represent the 24 h single-point Tenax extractable pyrethroid concentration obtained using the highest Tenax:OC ratio divided by the Tenax extractable pyrethroid concentration for the lowest Tenax:OC ratio, and thus represent the difference between these two ratios for the particular sediment and compound being evaluated. Values in parentheses represent ± one standard deviation.

Sediment	Tefluthrin	Bifenthrin	Fenpropathrin	λ-cyhalothrin	Permethrin	Cypermethrin	Esfenvalerate
MSH	ND[a]	3.91 (1.64)	ND	ND	ND	ND	ND
CRY	ND	1.86 (0.76)	ND	ND	ND	ND	ND
SPFD	ND	1.40 (0.66)	ND	ND	ND	ND	ND
BC 7d	0.90 (0.06)	1.52 (0.15)	1.05 (0.08)	1.21 (0.11)	1.19 (0.16)	1.15 (0.08)	1.42 (0.12)
BC 60d	0.85 (0.05)	1.36 (0.10)	0.90 (0.09)	1.09 (0.06)	1.06 (0.10)	1.10 (0.07)	1.20 (0.11)
BC 120d	0.89 (0.03)	1.47 (0.18)	0.96 (0.02)	1.19 (0.14)	1.12 (0.14)	1.18 (0.02)	1.49 (0.10)
LPH 7d	0.93 (0.05)	1.42 (0.08)	0.97 (0.08)	1.22 (0.09)	1.11 (0.05)	1.18 (0.06)	1.34 (0.10)
LPH 60d	0.88 (0.05)	1.28 (0.16)	1.28 (0.08)	0.99 (0.16)	0.99 (0.09)	1.12 (0.04)	1.15 (0.13)
LPH 120d	0.91 (0.05)	1.33 (0.09)	0.98 (0.09)	1.28 (0.13)	1.11 (0.15)	1.18 (0.11)	1.27 (0.09)

[a] ND = Not detected, pyrethroid was not found in sediments according to mass spectrum, specific ion ratios, and retention times of these compounds.

deviation) (Table 1). Similar comparisons made for the remaining eight sediments, constituting 48 experimental units, revealed between 0.85- to 1.86-fold difference when the Tenax concentrations extracted by the largest and smallest Tenax:OC ratios were compared (Table 1). The average variation in this comparison was 1.23 ± 0.46, thus demonstrating that this method yields fairly consistent estimates of the bioaccessible concentration regardless of the Tenax:OC ratio used. Bay Creek sediment demonstrated the largest difference in Tenax:OC ratios of any of the sediments used in the current study representing a four-fold difference, with the highest ratio used set at 60:1 and the lowest set at 15:1. This difference represented a factor of four between the Tenax:OC ratios. Yet, despite this variation in the Tenax:OC ratios, the largest fold difference in the SPTE concentrations was only a factor of 1.52 ± 0.15 for bifenthrin extracted from this sediment after 7 d of aging (Table 1). Therefore, even with a four-fold difference in the Tenax:OC ratio, the total Tenax extractable concentrations varied by less than a factor of two.

The variation noted between the 24 h SPTE pyrethroid concentrations obtained with the different Tenax:OC ratios within each sediment was smaller than the variation observed in estimates of bioaccumulation or toxicity benchmarks established using the SPTE with similar or wider ranges of Tenax:OC ratios (Harwood et al., 2013b; Mackenbach et al., 2012). This suggests that variation in exposure estimates provided by SPTEs is not due as much to methodological variation, but instead to toxicokinetic and toxicodynamic processes that result in differences in biological exposure across study sites (Harwood et al., 2013b; Mackenbach et al., 2012). The most comprehensive collection of Tenax data in one model was the development of the Bioaccumulation Tenax Model (BTM), which utilized data from three studies and Tenax extractions involving Tenax:OC ratios ranging between 3.1:1 to 109.1:1 (Mackenbach et al., 2012). The variation in this model, represented by the 95% confidence intervals, spans two orders of magnitude, which is much larger than the variation observed among the SPTE concentrations of the current study (Mackenbach et al., 2012). Although the range of Tenax:OC ratios used to generate the BTM was larger than the ranges used in the current study, the low variation introduced into the Tenax method by altering the Tenax:OC ratio used during a SPTE would suggest the variation in the BTM is due to aspects other than the range of Tenax:OC ratios used (Mackenbach et al., 2012). Indeed, the range of confidence limits in the BTM has been attributed to toxicokinetic differences that exist in the bioaccumulation of different compound classes by benthic invertebrates, and not due to error in the estimates of bioaccessibility provided by Tenax (Mackenbach et al., 2012). Similar conclusions have been drawn when utilizing SPTEs to develop toxicity benchmarks for bifenthrin in sediment (Harwood et al., 2013b). Toxicity benchmarks for *Hyalella azteca* and *Chironomus dilutus* exposed to bifenthrin in three sediments were developed using SPTEs with Tenax:OC ratios between 7.3:1 and 20.8:1 by Harwood et al. (2013b). The bifenthrin toxicity benchmarks determined for the three sediments varied by as much as 4.39 times across all the experimental conditions (Harwood et al., 2013b). The variation in these toxicity benchmarks was approximately two times larger than the variation between the Tenax:OC ratios observed from the data of the current study when using a wider range of ratios to extract bifenthrin compared to the Tenax:OC ratios used by Harwood et al. (2013b). While the variation in these estimates may have been due in part to the variation of the SPTE, toxicity benchmarks developed for the same sediments using a passive sampler and exhaustive sediment extractable concentrations normalized for organic carbon also demonstrated similar variability (2.91 and 3.33 times across the toxicity benchmarks of all experimental conditions for organic carbon normalized sediment concentrations and passive sampler concentrations, respectively), suggesting the variation observed when using SPTEs was due to toxicokinetic and toxicodynamic differences between the sediments, a variable common to any extraction technique (Harwood et al., 2013b).

While minimizing the Tenax:OC ratios used across different Tenax studies may help to develop a standardized Tenax method for use during estimates of exposure, standardizing the method may not greatly alter most of the variation observed between studies involving SPTE estimates of biological exposure. The variation observed in estimates of bioaccumulation or toxicity after adjusting for exposure concentration is due largely to biological differences that alter uptake and elimination of chemicals across sediments; a complicating factor that would introduce variation based on any exposure metric (Harwood et al., 2013b; Mackenbach et al., 2012). Alteration of the toxicokinetics of exposure may result in a failure of the exposed organisms to reach steady-state during the bioaccumulation assay or toxicity test, thus violating the assumption of the test. If the assumption of steady-state is violated during the exposure phase, then concentrations provided by any exposure metric will fail to properly assess exposure. The low variability in the estimates of exposure provided by SPTEs, however, helps to reduce any extraneous variability (i.e. changes in bioaccessibility between study sites) that may be introduced into these estimates, and as such, stands as a valuable, robust tool for evaluating exposure of HOCs in sediment. As most of the variation in exposure estimates provided by Tenax extractions is likely attributed to differences in how organisms respond to different HOCs, standardizing the use of the SPTE should focus on proper application of Tenax extractable concentrations to exposure endpoints.

4.3. Estimating pyrethroid exposure using single-point Tenax extractions

The most common use of SPTE concentrations in previous research has been comparisons to bioaccumulation representing the exposure to sediment-associated contaminants (Harwood et al., 2012; Kraaij et al., 2001, 2002; Landrum et al., 2007; Mackenbach et al., 2012, 2014; ten Hulscher et al., 2003; You et al., 2006). Strong relationships exist between the Tenax extractable concentration extracted from sediments and tissue concentrations of organisms at steady-state, regardless of environmental variables (i.e. organic carbon content/composition, aging time, chemical concentration) (Harwood et al., 2012; Kraaij et al., 2001, 2002; Landrum et al., 2007; Mackenbach et al., 2012, 2014; ten Hulscher et al., 2003; You et al., 2006). However, there are examples in the literature where these correlations do not exist (Lydy et al., 2015). While it is not always clear why Tenax extractable concentrations do not correlate to bioaccumulation, the poor fit could be caused by sampling tissue concentrations of organisms that have failed to reach steady-state or due to biotransformation of chemicals by exposed organisms (Mackenbach et al., 2014). However, SPTE concentrations of biotransformed compounds, such as pyrethroids, have been demonstrated to strongly correlate with bioaccumulation of the parent form of the compound (Harwood et al., 2012). As only parent pyrethroid was measured in both the SPTEs and bioassays, it is unlikely that biotransformation of the pyrethroids caused the reduced fit of the data to the BTM observed in the current study (see below). The failure to reach steady state was likely due to the toxic responses in the organisms, as shown below, or when feeding behavior is altered, such as was suggested for *L. variegatus* residing in post-remediation superfund site sediments (Mackenbach et al., 2014). A similar observation showing bioaccumulation that was less than expected based on Tenax

extractable concentrations was seen with the *L. variegatus* in the current study (Figs. 1 and 2, A.1).

The pyrethroid concentrations in both the field-contaminated and laboratory-spiked sediments were high enough to cause a reduction in the size of the *L. variegatus* and alterations of the feeding behavior as suggested by the sediment avoidance in laboratory-spiked sediments compared to controls. This behavior modification resulted in an overestimation of bioaccumulation by a majority of the SPTE concentrations, regardless of the sediment (Fig. 1), compound (Fig. 2), or Tenax:OC ratio (Fig. A.1) being evaluated. Separating the bioaccumulation and SPTE data by these different factors did not reveal any trends in the data set; thus, choosing to relate the SPTE pyrethroid concentrations to bioaccumulation by the *L. variegatus* was not appropriate (Figs. 1 and 2, A.1). The toxicokinetic processes involved in bioaccumulation by the *L. variegatus* were affected due to the toxicity of the pyrethroids and avoidance of the sediments, such that the worms were not exposed to the total exposure potential of the sediment, thus bioaccumulation was underestimated compared to estimates predicted by the BTM based on the SPTE concentrations (Figs. 1 and 2, A.1).

Despite the poor fit of the bioaccumulation data to the BTM, the Tenax extractions provided a good estimate of toxicity through evaluations of growth inhibition due to pyrethroid exposure. The percent growth inhibition was calculated using the following equation and compared to log transformed sum pyrethroid SPTE concentrations using a linear regression:

$$\%\ \text{Growth Inhibition} = \left(\frac{\text{g Tissue Control} - \text{g Tissue Treatment}}{\text{g Tissue Control}}\right)*100 \quad (1)$$

where, $g_{\text{Tissue Control}}$ was the average tissue mass in grams of the *L. variegatus* recovered from the blank and matrix spike controls

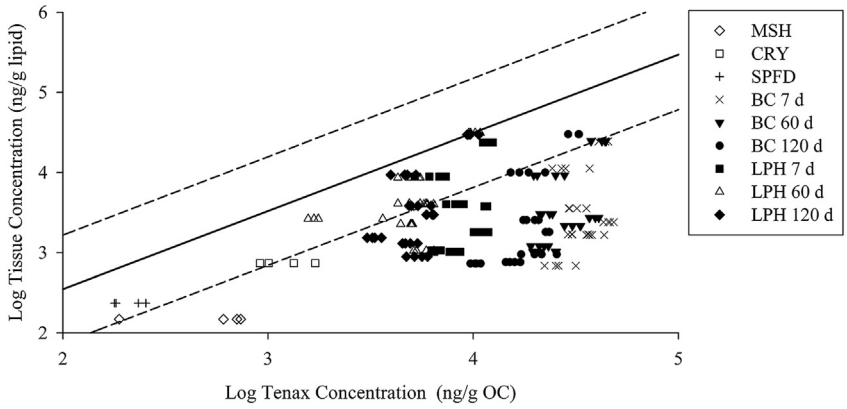

Fig. 1. The 24 h single-point Tenax extractable pyrethroid concentration obtained using various Tenax to organic carbon ratios versus the pyrethroid tissue concentration in *Lumbriculus variegatus* exposed to Mosher Slough (MSH, 1.70% organic carbon (OC)), Curry Creek (CRY, 3.64% OC), Springfield (SPFD, 4.24% OC), Bay Creek (BC, 0.56% OC), and LaRue-Pine Hills (LPH, 2.04% OC) sediments aged for 7, 60, and 120 d overlaid on the Bioaccumulation Tenax Model (BTM) (Mackenbach et al., 2012). The solid line represents the model line and the dashed lines represent the 95% confidence limits of the model. The different points represent the log Tenax concentrations obtained with the four Tenax:OC ratios used with each sediment demonstrating the effects of sediment and aging time on the utility of the Tenax extraction as an estimate of pyrethroid bioaccumulation.

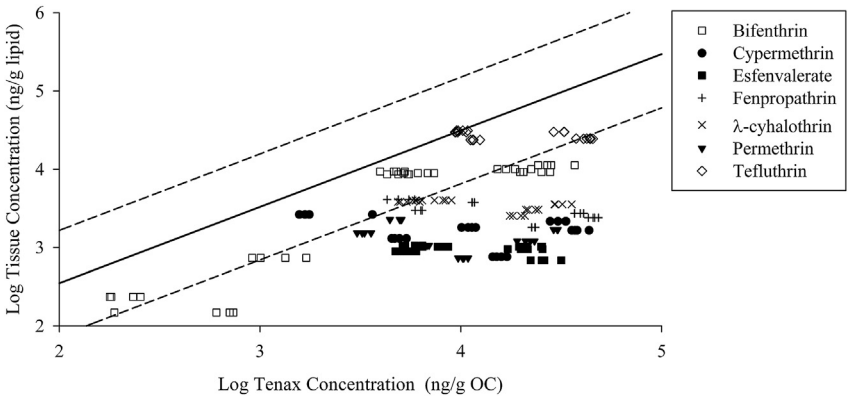

Fig. 2. The 24 h single-point Tenax extractable pyrethroid concentration obtained using various Tenax to organic carbon ratios versus the pyrethroid tissue concentration in *Lumbriculus variegatus* exposed to Mosher Slough (MSH, 1.70% organic carbon (OC)), Curry Creek (CRY, 3.64% OC), Springfield (SPFD, 4.24% OC), Bay Creek (BC, 0.56% OC), and LaRue-Pine Hills (LPH, 2.04% OC) sediments aged for 7, 60, and 120 d overlaid on the Bioaccumulation Tenax Model (BTM) (Mackenbach et al., 2012) as individual pyrethroids. The solid line represents the model line and the dashed lines represent the 95% confidence limits of the model. This comparison demonstrates the effects of individual pyrethroids on the utility of the Tenax extraction as an estimate of bioaccumulation.

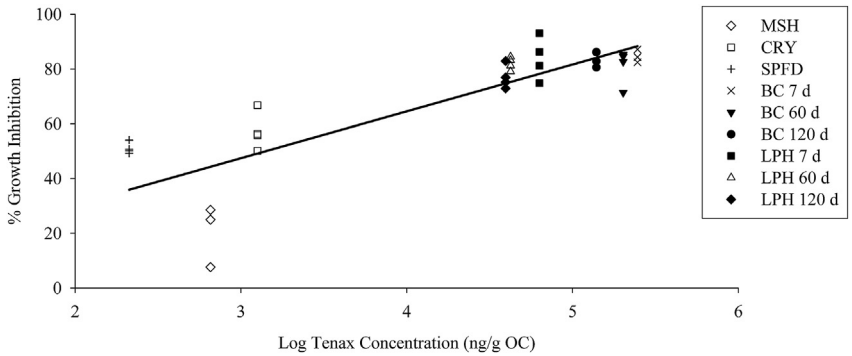

Fig. 3. Percent growth inhibition of *Lumbriculus variegatus* exposed to pyrethroids in Mosher Slough (MSH, 1.70% organic carbon (OC)), Curry Creek (CRY, 3.64% OC), Springfield (SPFD, 4.24% OC), Bay Creek (BC, 0.56% OC), and LaRue-Pine Hills (LPH, 2.04% OC) sediments aged for 7, 60, and 120 d. The equation of the line fit to the experimental data was equal to Growth Inhibition (%) = 17.1 (2.11) [Log Tenax (ng/g OC)] − 3.89 (9.17), $F = 65.6$, $p < 0.0001$, $r^2 = 0.67$. Values in parentheses in the equation represent ± standard error.

included with each bioaccumulation assay, and $g_{\text{Tissue Treatment}}$ was the tissue mass in grams of the *L. variegatus* recovered from the four experimental units of the bioaccumulation assays conducted with each contaminated sediment (MSH, CRY, SPFD, BC and LPH 7, 60, and 120 d). There was a significant linear relationship ($F = 65.6$, $p < 0.0001$, $r^2 = 0.67$) between the log of the Tenax extractable concentrations and growth inhibition (Fig. 3). For the Tenax:OC ratios for which there was sufficient data, separating these regressions by Tenax:OC ratio did not significantly impact the relationship between toxicity and Tenax extractable concentrations (Figs. A.2–A.3). This further demonstrates the importance of choosing an appropriate toxicological endpoint when evaluating exposure of chemical contaminants in sediments, as this will likely introduce the most variation in biological exposure estimates when evaluating sediment contamination.

The inability of the Tenax extraction to depict observed bioaccumulation in the presence of a toxic response points to a lesson to consider when utilizing Tenax extractions to estimate exposure of acutely toxic compounds. For example, sub-acute toxic effects of compounds may alter toxicokinetics process, which affects the potential for bioaccumulation and result in overestimations of exposure when using SPTEs. Development of toxicity benchmarks based on exposure estimates provided by Tenax extractions are likely to be a more appropriate use of this technique (Du et al., 2013, 2014; Harwood et al., 2013b, Harwood et al. 2015). Therefore, selection of appropriate endpoints is essential to optimally use the Tenax method.

4.4. Choosing an "optimum" Tenax:OC ratio

The data collected in the current study did not point to an "optimum" Tenax:OC ratio due to the low variation in the total extractable pyrethroid concentrations by different ratios and the ability of each ratio to provide similar estimates of exposure. Therefore, the authors suggest use of a Tenax:OC ratio of at least 5:1 with Tenax masses between 0.500 g and 0.800 g with 3 g (dw) of sediment to accurately reflect bioaccessibility and exposure of pyrethroids across a wide range of sediments with varying OC content. For example, a Tenax mass of 0.500 g will provide enough Tenax to create a Tenax:OC ratio of at least 5:1 for Tenax extractions with sediment containing up to 6.5% OC. This ratio should provide accurate or conservative estimates of pyrethroid exposure and bioaccumulation by benthic invertebrates residing in the sediment assuming the toxicity of the chemicals does not impact bioaccumulation (Harwood et al., 2013b, 2015). If the sediment being studied has higher than 6.5% OC, then increasing the Tenax mass used up to 0.800 g, to achieve a Tenax:OC ratio of at least 5:1 is recommended. Although increasing the Tenax mass, and thus the Tenax:OC ratio, used during a Tenax extraction should not impact estimates of bioaccessibility or exposure provided by the method, there are methodological difficulties involved with using higher Tenax masses. Increasing the Tenax mass used during a SPTE increases the solvent required to fully extract the pyrethroids sorbed to the Tenax beads. This increases the material costs of the extraction as more solvent is needed per replicate. Weighing larger Tenax masses for use during Tenax extractions can limit the number of replicates that can be performed, as more Tenax is needed per replicate. Similarly, increasing the Tenax mass increases the volume of water carried over from the Tenax extraction system to the extraction vials, as well as the volume of solvent required to wash the Tenax beads after the extraction. As one of the major attractions of the Tenax method is the ease of use and the rapidity with which extractions can be performed, modifying the Tenax method to reduce the processing time in the laboratory, while still obtaining accurate, reproducible results offers the best approach for using SPTEs. Therefore, decreasing the sediment mass used during the Tenax extraction is recommended to meet the suggested 5:1 ratio. Operating within the confines described in the current study should provide accurate estimates of bioaccessibility, exposure, and a 5:1 Tenax:OC ratio in most sediment that will be of concern to risk assessors.

5. Conclusions

The Tenax:OC ratio is the most variable methodological component of the SPTE for a fixed extraction period and this variation was hypothesized to limit widespread use of SPTEs. However, the current study demonstrated that the Tenax:OC ratio had little effect on the SPTE pyrethroid concentration obtained from sediment. Compared to the variation in exposure estimates due to toxicokinetic and toxicodynamic differences that exist between bioaccumulation and toxicity of different classes of HOCs and sediments, the variation in exposure estimates with use of different Tenax:OC ratios should be considered minimal. A more important variable in estimating bioaccumulation using Tenax extractions may be alterations of normal feeding behavior due to toxicity, resulting in variation in estimates of bioaccumulation provided by the Tenax method. Therefore, a better use of this tool for acutely toxic compounds, such as pyrethroids, may be in development of Tenax-based toxicity benchmarks (Harwood et al., 2013b). While no Tenax:OC ratio appears to be "optimal" for use in estimations of pyrethroid exposure, Tenax:OC ratios of at least 5:1 should provide accurate exposure estimates, assuming the proper endpoints are used to reflect bioaccessibility to the study organisms. To further

improve the utility of the SPTE method, future studies utilizing this technique should include information representing the total OC present in the sediments, justification that the biological exposure endpoints used to compare to the SPTE concentrations are relevant, and the Tenax:OC ratio used in the study. Furthermore, as SPTEs provide operationally defined exposure estimates, the authors feel it is relevant to clearly specify the length of the SPTEs being conducted, as well as inclusion of appropriate quality control and assurance (i.e. matrix spike and blank samples). Inclusion of this data in future manuscripts will help to identify the data requirements necessary to further the use of SPTEs, as well as allow for analysis of the limitations of this technique.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.12.045.

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