SOLID-PHASE MICROEXTRACTION FOR PREDICTING THE BIOAVAILABILITY OF 2,4,6-TRINITROTOLUENE AND ITS PRIMARY TRANSFORMATION PRODUCTS IN SEDIMENT AND WATER

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Abstract—Disposable solid-phase microextraction fibers (SPMEs) were used to measure the availability of 2,4,6-trinitrotoluene (TNT) and its two primary transformation products, 2-amino-4,6-dinitrotoluene (2ADNT) and 4-amino-2,6-dinitrotoluene (4ADNT). The SPMEs (85-μm polycarbonate) and sediment-dwelling oligochaetes (Tubifex tubifex) were exposed to TNT-spiked sediment, to TNT-spiked sediment amended with activated carbon, and to TNT-, 2ADNT-, and 4ADNT-spiked water. Sediment concentration was a poor predictor of bioavailability in unamended and carbon-amended sediments (r² = 0.14–0.73). The activated carbon amendment reduced the bioavailability of compounds in carbon-amended sediment, causing the relationships between Tubifex concentrations and sediment concentrations to differ significantly between unamended and carbon-amended sediment for all compounds. In contrast, SPME TNT concentrations predicted Tubifex TNT concentrations (r² = 0.54–0.79), and regression models did not differ significantly among the three TNT-spiked matrices. The SPME 2ADNT and 4ADNT concentrations also were predictive of Tubifex 2ADNT and 4ADNT concentrations (r² = 0.44–0.90). Relationships between Tubifex concentrations and SPME concentrations were the same between unamended and carbon-amended TNT-spiked sediments for 2ADNT and 4ADNT; however, the relationship in sediment (pooled data) differed from the relationship found in 2ADNT- and 4-ADNT-spiked water. The SPMEs provided carbon amendment-independent measures of ADNT availability in sediment and matrix-independent measures of TNT availability among the three matrices. The SPMEs show promise for predicting bioavailable organic compounds in sediment and water.

Keywords—Solid-phase microextraction fibers Explosives Nitroaromatics Biomimetic device

INTRODUCTION

Traditional chemical techniques (strong solvent extractions) for measuring organic compounds in complex environmental matrices are not well-related to the proportion of compounds that are bioavailable to organisms. Recently, a number of novel techniques have been applied to provide more accurate biomimetic chemical predictors of bioavailability [1,2], including [C¹⁸]empore disks [3–5], weak-solvent extractions [6–8], gut fluid extractions [9], Tenax beads [10,11], semipermeable membrane devices [12], and solid-phase microextraction fibers (SPMEs) [13–16]. Although their designs, applications, and weaknesses differ, biomimetic approaches generally exceed the ability of strong-solvent extractions to predict bioavailability and toxicity among different soils, sediments, or waters.

Because of their ease of use, low cost, and freedom from impurities, SPMEs have been used successfully to predict toxicity and bioavailability of organic compounds in soil, sediment, and water [2,13,14,17–19]. The SPMEs are thin silica fibers (diameter, ∼110 μm; length, 1 cm) coated with a microlayer (thickness, 5–100 μm) of organic polymer. During exposure to environmental matrices, the polymer sorbs organic compounds to concentrations several orders of magnitude higher than that in the surrounding matrix [2,20]. As long as the fiber-coating volume to sample volume ratio remains small, the SPME coating does not exhaustively extract compounds and only sorbs dissolved or weakly dissociable molecules from solution [1,2,20]. In aquatic sediments, the SPME approach may yield a more accurate surrogate measurement of the partitioning process between organisms and the sediment matrix compared to sediment concentrations normalized by organic carbon; thus, SPME measurements may be representative of bioavailability [2,16].

Recently, we have developed a disposable SPME technique to measure 2,4,6-trinitrotoluene (TNT) and its nitroaromatic (NA) transformation products in sediment during toxicity and/or bioaccumulation experiments [15]. A persistent contaminant at many military installations, TNT is the most toxic, widely used explosive compound [21–23]. It readily transforms in TNT-spiked sediments in the laboratory, complicating the assessment of toxicity and bioavailability during laboratory-controlled experiments [24]. Our SPME burial technique was simple, rapid, and less expensive than traditional chemical measures of TNT and its NA transformation products in TNT-spiked sediments [15]. In subsequent experiments, we found that estimates of median lethal toxicity based on SPME concentrations for Tubifex tubifex exposed to TNT-spiked sediment and TNT-spiked water were nearly identical, suggesting that SPMEs may provide matrix-independent chemical estimates of lethal dose [19].

The objective of the present study was to investigate the ability of SPMEs to predict the bioavailability of TNT and its two primary NA transformation products, 2-amino-4,6-dinitrotoluene (2ADNT) and 4-amino-2,6-dinitrotoluene (4ADNT). The log Kow values for these compounds are approximately two [25]. Because the main route of uptake by aquatic organisms for compounds with log Kow values of less than five is predicted to be mainly via absorption of dissolved

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compounds from water [26], we hypothesized that the relationship between SPME concentrations and organism concentrations would be constant regardless of matrix (e.g., carbon-rich sediment, carbon-poor sediment, and water). This hypothesis was evaluated in three NA-spiked matrices with different relative bioavailabilities (bioavailable molecules relative to total mass balance): Carbon-amended sediment (low relative bioavailability), sediment (medium relative bioavailability), and water (high relative bioavailability). Spiked sediments were used because site-contaminated sediments were not readily available. However, this approach would be valid for experiments using site-contaminated sediments in the laboratory or in situ. To our knowledge, this is the first evaluation regarding the ability of SPMEs to predict bioavailability among aquatic matrices of different relative bioavailabilities.

MATERIALS AND METHODS

Experimental matrices and exposure conditions

Sediment used in the present experiment was obtained from the top 15 cm of uncontaminated ponds at the University of North Texas Water Research Field Station (Denton, TX, USA). Sediment was prepared as described by Conder et al. [15]. Briefly, sediment was dried for 24 h (105°C), ground, and sieved (particle size, <250 µm). Crystalline TNT (purity, 98%; Chem Service, West Chester, PA, USA) was dissolved in pure high-performance liquid chromatography (HPLC)–grade acetonitrile and added in 4-ml aliquots to each sediment replicate of 100 g of dry sediment. Unamended sediments were spiked at initial nominal concentrations of 661 to 1321 nmol TNT/g sediment dry weight. Carbon-amended sediments were spiked at a slightly higher range of initial nominal concentrations (969–1540 nmol TNT/g sediment dry wt). The carbon amendment (see below) was expected to sorb NAs; thus, a higher range of initial TNT concentrations was necessary to yield levels of bioavailable NAs similar to those in unamended sediments spiked at a lower range of initial TNT concentrations. Three replicates were spiked at each concentration. After mixing sediment and acetonitrile thoroughly by hand (30 s), solvent was allowed to evaporate in the dark for 24 h. Unamended sediment was then wet with 500 ml of ultrapure water; carbon-amended sediment was wet with 490 ml of ultrapure water and 10 ml of carbon slurry. The carbon slurry was composed of 0.0200 g of powdered activated carbon (Darco G-60; Fisher Scientific, Fair Lawn, NJ, USA) and 10 ml of ultrapure water. To hydrate the carbon, which aided incorporation into the sediment, the slurry was mixed continuously with an end-over-end rotating mixer (60 rpm) for 18 h before addition to the sediment. Activated carbon was used as a bioavailability-reducing amendment (200 µg carbon/g sediment dry wt), because it is a homogenous material capable of efficiently sequestering TNT and its transformation products [6, 27] at amendment levels nontoxic to Tubifex. After hydration, sediments were mixed vigorously with a magnetic stirrer for 1 min and placed in an environmental chamber (23 ± 1°C, 16:8-h light:dark photoperiod). To maintain dissolved oxygen during the aging and exposure periods, the overlying water of each beaker was aerated gently and continuously using a pipette immersed below the water’s surface. Sediments were aged for 14 d before SPME, and organism exposures were initiated according to recommendations and procedures detailed by Conder et al. [24]. During the 14-d aging period, sediment NA concentrations decrease by an order of magnitude as TNT and its primary transformation products (i.e., ADNTs) become unextractable [24]. This phenomenon, as studied in TNT-contaminated soil, has been hypothesized to occur via irreversible covalent binding of NA transformation products to organic matter [28–30].

Water-only exposures were conducted with TNT-, 2ADNT-, or 4ADNT-spiked reconstituted hard water [19]. Both 2ADNT and 4ADNT were obtained from R. Spanggord (SRI International, Menlo Park, CA, USA). To spike test water, aliquots of 50:50 (v/v) acetonitrile:water solution containing dissolved NA were added to a volumetric flask. Aliquot volumes varied according to final concentration desired (as much as 3.6 ml). Acetonitrile was evaporated from the aliquot under a gentle stream of He, leaving crystalline NA in water. This was redissolved in, and diluted to volume with, reconstituted hard water to obtain the highest exposure concentration (TNT, 36 nmol/ml; 2ADNT and 4ADNT, 20 nmol/ml) before serial dilution to generate remaining concentrations (n = 3 replicates/concentration). Each replicate contained 100 ml of water and 10 cm³ glass beads (diameter, 1 mm). Glass beads served as an inert substrate in which Tubifex could burrow, because Tubifex placed in an environment devoid of substrate will form a tight cluster, a behavior that may influence the absorption of contaminants and/or toxic response. Each beaker was covered with perforated aluminum foil to reduce evaporation. The SPME and organism exposures were initiated immediately after water was prepared. Exposure solutions were not renewed during the experiments.

SPME preparation

The SPME (85-µm polyacrylate) was purchased in bulk (without syringe applicator) from Supelco (Bellefonte, PA, USA). Fiber was cut into 1.00-cm pieces using a double-bladed, stainless-steel razor blade apparatus. Each 1-cm fiber contained 0.521 µl of polyacrylate. We present SPME data as concentrations (number of molecules absorbed by polyacrylate divided by volume of polyacrylate), as presented in previous studies [13, 14, 19, 31]. The SPMEs were inserted halfway through pierced, stapled, Teflon®-coated silicone disks (septa for glass vials; diameter, 10 mm; thickness, 1 mm). The SPMEs (in disk holders) were then rinsed with 50:50 HPLC-grade acetonitrile:ultrapure water, rinsed with ultrapure water, and allowed to dry at room temperature. The Teflon-disk holder allowed SPMEs to be handled and buried in sediment (using forceps) without damage to or loss of the SPME. The staple in each holder allowed both holder and fiber to be recovered easily with a strong magnet. The Teflon-disk holder method was found to be much simpler than the stainless-steel mesh envelope method [15] but not as rugged and, probably, inappropriate for in situ deployment. Fiber uptake of NAs is independent of holder (stainless-steel mesh envelope or Teflon disk); this was verified by measurement.

Experimental procedure

After sediments were aged 14 d, each sediment replicate received 10 adult Tubifex (age, eight to nine weeks) and one SPME, which was buried 1 cm below the surface of the sediment. This depth was chosen because most organism activity occurs at depths of 0 to 2 cm, as observed by the presence of burrowing activity (galleries) during previous sediment experiments with Tubifex. Each replicate in NA-spiked water-only exposures received 10 adult Tubifex (age, eight to nine weeks) and one SPME. Exposure time for most experiments was 48 h, which is sufficient for NAs to reach steady-state concentrations in SPMEs [15] and Tubifex [32]. Exposures for...
several experimental levels in the water-only TNT experiment were extended to 96 h. No time-based differences were found between data obtained at 48 h and data obtained at 96 h; NA concentrations in organisms and SPMEs were at steady state at both durations.

At termination of the exposures, each SPME was retrieved and removed from its holder with Teflon-coated forceps and placed into an HPLC autosampler vial containing 400 μl of 50:50 HPLC-grade acetonitrile:ultrapure water for 10 min to desorb compounds from the fiber [15]. After Tubifex were removed from water-only experiments using forceps or recovered from sediment using a 500-μm sieve, they were blotted on absorbent paper and stored frozen (−10°C) until tissue analysis. Each experimental replicate of 10 Tubifex was pooled for tissue analysis. Tubifex tissue samples (0.0200–0.0500 g) were weighed and homogenized for 100 s in 750 μl of HPLC-grade acetonitrile using 1.0-mm glass beads in a Mini-Beadbeater (Biospec Products, Bartlesville, OK, USA; http://www.biospec.com). The homogenate then received 750 μl of 1% CaCl2 before sonication for 1 h in a cool water bath (16 ± 2°C). After sonication, the homogenate was centrifuged for 10 min at 10,000 g. The supernatant was then filtered through a 0.45-μm glass microfiber filter. For Tubifex exposed to sediment, the pellet (extracted tissue and ingested sediment) was transferred to preweighed crucibles, ashed at 450°C for 12 to 18 h, and reweighed to estimate organismal sediment content (see Data analysis). Sediment samples (1–5 g wet wt) obtained at the termination of sediment exposures were weighed and extracted with 10 ml of HPLC-grade acetonitrile, mixed for 16 h using an end-over-end rotating mixer (60 rpm), and centrifuged for 2 min at 2,000g [15,133]. Supernatants (extracts) were filtered with 0.45-μm glass microfiber filters and diluted with an equal volume of ultrapure water. To minimize possible compound transformation, samples were not air-dried before extraction. Sediment sample dry weights were determined via gravimetric moisture determination (dried at 105°C for 24 h) of the postextraction sediment. The SPME, Tubifex, and sediment extracts were analyzed via HPLC using a Waters Nova Pak C-18 column (Milford, MA, USA) with an 82:18 isopropanol:water isotropic mobile phase (fixed ultraviolet detector, 254 nm).

Quality-assurance/quality-control measures included spike and method blank analyses for SPME, Tubifex, and sediment extractions. For Tubifex tissue samples, average percentage recoveries ranged from 92 to 96%, with approximate method detection limits of 1.5 to 6.9 nmol NA/g wet weight. The SPME spike recoveries were determined by spiking a known aliquot of NAs directly on the fiber before extraction with solvent. The SPME recoveries ranged from 104 to 110%, with method detection limits of 0.06 to 0.23 μmol NA/ml. For sediment, average percentage recoveries ranged from 109 to 121%, with approximate detection limits of 0.5 to 2.5 nmol NA/g dry weight.

Data analysis

For sediment-exposed Tubifex, body weights and NA burdens were corrected for mass and NAs originating from sediment in Tubifex digestive systems at the time of collection. The following formulas were used [34,35]:

\[
S_{\text{dry wt}} = \frac{A}{(S_{\text{wet wt}}/100)}
\]

(1)

\[
S_{\text{wet wt}} = \frac{S_{\text{dry wt}}}{(S_{\text{wet wt}}/S_{\text{dry wt}})}
\]

(2)

\[
O_{\text{NA wet wt}} = \frac{E_{\text{NA}} - [(S_{\text{dry wt}}) - S_{\text{dry wt}}]}{O_{\text{wet wt}} - S_{\text{wet wt}}}
\]

(3)

where \(S_{\text{dry wt}}\) = ingested sediment mass (g dry wt), \(A =\) measured organism pellet ash mass (g dry wt), \(S_{\text{wet wt}}\) = percentage ash content of the sediment (constant, 94.1% dry wt), \(S_{\text{dry wt}}\) = ingested sediment mass (g wet wt), \(S_{\text{wet wt}}\) = measured dry sediment sample mass (g dry wt), \(S_{\text{dry wt}}\) = measured wet sediment sample mass (g wet wt), \(O_{\text{NA wet wt}}\) = organism NA concentration (nmol/g wet wt), \(E_{\text{NA}}\) = measured organism extract NA (nmol), \([S_{\text{dry wt}}]\) = measured sediment sample concentration (nmol NA/g dry wt), and \(O_{\text{wet wt}}\) = measured organism mass (g wet wt). Aside from assumptions concerning homogeneity of sediment NAs and ash content of sediment, the above calculations are based on several key requirements. The first is that the ash content of Tubifex is 0% (i.e., 100% of pellet ash is from sediment-derived ash). This was verified by ash content measurement of sediment-free Tubifex. The second requirement is that Tubifex consume sediment indiscriminately. Previous research suggests that Tubifex selectively consumes sediment particles [36]. To investigate this, we analyzed the ash content of fecal pellets collected from unspiked sediment inhabited by Tubifex. The mean ash content (standard deviation) of fecal pellets was 94.3% (0.62); this was not significantly different from the ash content of sediment (p = 0.6805). This suggests that Tubifex did not selectively consume portions of this homogenized sediment with relatively low organic matter content (0.96%). The third main requirement of this approach is that sediment-associated NAs in the Tubifex digestive tract have not been absorbed by tissue at the time of sampling; assessment of this assumption was beyond the scope of our experiment.

Linear regression was used to evaluate relationships between bioavailability (Tubifex NA concentrations) and chemical measures of availability (sediment concentrations and SPME concentrations). The SAS/LAB® (Ver 8.02; SAS Institute, Cary, NC, USA) was used to test parametric assumptions and to identify outliers. For significant (\(\alpha = 0.05\)) regression models, model slopes and -intercepts were compared using Student’s t tests [37]. Tubifex NA concentrations were transformed using \(\log_{10}\) operand, because heteroscedasticity led to a positive correlation of regression residuals with dependent variables [38,39], Transformation enabled the use of parametric regression to develop models with which to examine the ability of SPMEs to predict bioavailability; however, the need for transformation may suggest that the relationship in the untransformed data may not be linear. Addressing the theoretical nonlinearity of this relationship is beyond the scope of this paper and is not necessary for assessing the ability of SPMEs to predict empirically the NA concentrations in Tubifex. In Results and Discussion, regression models are presented with the standard error (SE) of parameters in parentheses: \(\log_{10} y = \text{slope (slope SE)}x + \text{y-intercept (y-intercept SE)}\). Model coefficients of determination (\(r^2\)) and probabilities of type I error (p) also are presented.
RESULTS AND DISCUSSION

Sediment concentrations as predictors of bioavailability in sediment

Sediment concentrations of TNT, 2ADNT, and 4ADNT were not predictive of Tubifex concentrations between unamended and carbon-amended sediments (Fig. 1 and Table 1). Tubifex TNT concentrations were linearly related to sediment TNT concentrations in the unamended sediment but not in the carbon-amended sediment (Table 1). For both 2ADNT and 4ADNT, Tubifex ADNT concentrations were linearly related to sediment ADNT concentrations in unamended and in carbon-amended sediment (Table 1). For 2ADNT, slopes of the unamended and carbon-amended sediment models were not significantly different; however, a significant difference was found in $y$-intercepts. Both slopes and $y$-intercepts for 4ADNT models were significantly different between unamended and carbon-amended sediment.

Differences in slopes and $y$-intercepts of the regression models for unamended and carbon-amended sediments can be explained by the lower bioavailability (but similar acetonitrile extractability) of compounds in carbon-amended sediment. Whereas Tubifex NA concentrations are similar for both sediments, total sediment concentrations are two- to ninefold higher in carbon-amended sediments. The difference in relationships between Tubifex NA concentrations and sediment concentrations suggests that sediment concentrations are poor predictors of NA bioavailability in sediment.

SPME concentrations as predictors of bioavailability in sediment and water

Concentrations of TNT, 2ADNT, and 4ADNT in SPMEs were predictive of Tubifex NA concentrations in unamended and carbon-amended sediments (Fig. 2), since significant relationships were found between Tubifex NA concentrations and SPME NA concentrations (Table 1). For all three compounds, slopes did not differ significantly between unamended and carbon-amended sediment models. Similarly, $y$-intercepts did not differ significantly between unamended and carbon-amended sediment models. The lack of differences between model parameters suggests that the compound-specific relationships between Tubifex NA concentrations and SPME NA concentrations were similar regardless of the presence of the bioavailability-reducing carbon amendment. The stability of the SPME to Tubifex relationships in two very different levels of relative bioavailability suggests that SPMEs are predictive of bioavailable NAs.

The $r^2$ values for relationships between Tubifex concentrations and SPME concentrations in carbon-amended sediments were consistently lower than relationships in unamended sediment. This may indicate that the activated carbon amendment may not have been as homogeneously distributed as the natural sources of carbon in the sediment. Regardless, it should be noted that during the 48-h SPME and Tubifex exposure period, the NA concentration in the sediment–water matrix changed slightly (by $\sim 10\%$) because of the transformation of TNT to ADNTs and overall decrease in extractability [24]. Thus, TNT, 2ADNT, and 4ADNT sediment concentrations are not at a true steady state. Similarly, NA concentrations in Tubifex are not at steady state because of metabolism of TNT to 2ADNT and 4ADNT [32]. Variation in sampling time (on the order of minutes) from sample to sample could lead to slight experimental error, but the largest source of error pertains to ADNT concentrations in SPME and Tubifex. The Tubifex ADNT concentrations represent the sum of absorption of ADNT from sediment plus metabolism of absorbed TNT to ADNT within Tubifex. Regarding sediments in which the bioavailable ADNT to TNT ratio is different than that observed in the unamended and carbon-amended sediments of the present study, the relationship between Tubifex and SPME concentrations may differ from the relationships we have reported (Fig. 2b and c). Thus, the relationship between bioavailable sediment-associated ADNT and tissue concentrations of ADNT probably will never be constant in the presence of TNT; any attempt to
Table 1. Regression models predicting \( \log_{10} \) 2,4,6-trinitrotoluene (TNT), 2-amino-4,6-dinitrotoluene (2ADNT), and 4-amino-2,6-dinitrotoluene (4ADNT) concentrations in *Tubifex tubifex* exposed to unamended and carbon-amended TNT-spiked sediments a

<table>
<thead>
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<th>Independent variable/compound</th>
<th>Models</th>
<th>t Tests</th>
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<tbody>
<tr>
<td></td>
<td>Unamended sediment</td>
<td>Carbon-amended sediment</td>
</tr>
<tr>
<td>Sediment concentration</td>
<td>Slope</td>
<td>( y )-Intercept</td>
</tr>
<tr>
<td>TNT</td>
<td>1a 0.187 (0.0626)</td>
<td>0.519 (0.163)</td>
</tr>
<tr>
<td>2ADNT</td>
<td>1b 0.025 (0.0038)</td>
<td>0.764 (0.0654)</td>
</tr>
<tr>
<td>4ADNT</td>
<td>1c 0.017 (0.0024)</td>
<td>1.135 (0.0647)</td>
</tr>
<tr>
<td>SPME concentration</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNT</td>
<td>2a 0.148 (0.0209)</td>
<td>0.622 (0.0582)</td>
</tr>
<tr>
<td>2ADNT</td>
<td>2b 0.186 (0.0207)</td>
<td>0.649 (0.0605)</td>
</tr>
<tr>
<td>4ADNT</td>
<td>2c 0.056 (0.0070)</td>
<td>1.109 (0.0596)</td>
</tr>
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a Dependent variables for the models were sediment and solid-phase microextraction fiber (SPME) TNT, 2ADNT, and 4ADNT concentrations. Statistical results of \( t \) tests comparing slopes (standard error) and \( y \)-intercepts (standard error) between models for unamended and carbon-amended sediments also are shown.

b NA = not available. The regression model using sediment TNT concentration in carbon-amended sediment was not significant \( (p > 0.05) \). Model parameters are not reported and not compared with those of the unamended sediment model.

Table 2. Regression models predicting \( \log_{10} \) 2,4,6-trinitrotoluene (TNT), 2-amino-4,6-dinitrotoluene (2ADNT), and 4-amino-2,6-dinitrotoluene (4ADNT) concentrations in *Tubifex tubifex* exposed to TNT-spiked sediment (pooled unamended and carbon-amended sediment data) and TNT-spiked water using solid-phase microextraction fiber TNT, 2ADNT, and 4ADNT concentrations as dependent variables a

<table>
<thead>
<tr>
<th>Compound</th>
<th>Models</th>
<th>t Tests</th>
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<tbody>
<tr>
<td></td>
<td>Sediment</td>
<td>Carbon-amended sediment</td>
</tr>
<tr>
<td></td>
<td>Slope</td>
<td>( y )-Intercept</td>
</tr>
<tr>
<td>TNT</td>
<td>3a 0.101 (0.0139)</td>
<td>0.719 (0.0139)</td>
</tr>
<tr>
<td>2ADNT</td>
<td>3b 0.205 (0.0232)</td>
<td>0.548 (0.0689)</td>
</tr>
<tr>
<td>4ADNT</td>
<td>3c 0.050 (0.0060)</td>
<td>1.127 (0.0518)</td>
</tr>
</tbody>
</table>

a Statistical results of \( t \) tests comparing slopes (standard error) and \( y \)-intercepts (standard error) between models for sediment and water also are shown.
estimate this relationship, such as with SPMEs, is theoretically flawed. For example, if sediment was not aged before *Tubifex* and SPMEs were exposed (i.e., aging < 14 d), then less ADNT, but more TNT, would be present in sediment [24]. Much of the TNT absorbed by *Tubifex* in this situation would be metabolized to ADNTs, and the majority of the ADNT body burden in this case would be derived from metabolism, not from absorption. Because sediment-associated ADNTs would be lower, SPME ADNT uptake would be less than that observed in our experiments with 14-d aged sediment. The SPME models in Figure 2b and c likely would underpredict *Tubifex* ADNT concentrations in situations with more sediment-associated TNT and less sediment-associated ADNT.

Because regression models for *Tubifex* concentrations versus SPME concentrations were similar between sediments, we combined data from unamended and carbon-amended sediments and compared the pooled sediment data to the data obtained from water-only exposures, in which *Tubifex* and...
SPMEs were exposed to water spiked with TNT, 2ADNT, or 4ADNT (Fig. 3 and Table 2). The regression model for Tubifex TNT concentration versus SPME TNT concentration in sediment was not significantly different from the model in TNT-spiked water (Fig. 3a and Table 2), because neither slopes nor y-intercepts differed significantly. In contrast, relationships between Tubifex and SPME ADNT concentrations differed between sediment and water (Fig. 3b and c). The regression model for Tubifex 2ADNT versus SPME 2ADNT concentrations in pooled sediment data were much different than that in 2ADNT-spiked water (Fig. 3b and Table 2). Significant, sixfold differences were found in slopes, as were significant differences in model y-intercepts (Table 2). As with 2ADNT, the model for Tubifex 4ADNT versus SPME 4ADNT concentrations in pooled sediment data was very different than in 4ADNT-spiked water (Fig. 3c and Table 2). Significant, two-fold differences were found in slopes, and significant differences were found in y-intercepts (Table 2). In general, at the average SPME ADNT concentrations observed in sediment exposures, ADNT concentrations were three- to fivefold higher in Tubifex exposed to water. We expected the converse (greater ADNT concentrations in Tubifex exposed to sediment), because ADNT concentrations in Tubifex exposed to TNT-spiked sediment are composed of ADNTs originating from absorption of external ADNTs and metabolism of absorbed TNT to ADNTs, the latter of which cannot be predicted by SPMEs. Differences in relationships for ADNTs in water and sediment may result from differences in toxicokinetics between absorbed and metabolically generated ADNTs [32]. A more definitive experiment to compare the Tubifex–SPME ADNT concentration relationships between water and sediment could not be attempted during the study because of the unavailability of large amounts of ADNTs needed to spike sediments.

Although the transformation of NAs in sediment and organisms during the exposure periods in our experiments is not the perfect experimental design with which to evaluate a measurement technique that assumes equilibrium conditions, our approach is a pragmatic response to an exposure-assessment shortcoming in which a biomimetic approach is sorely needed. Organic compounds that are capable of transformation may never reach steady state in the environment, but they still represent significant risks to ecosystems. The quantification of some measure of exposure is needed to assess ecological risk. Despite the violation of the assumptions concerning true equilibrium conditions in sediment, our sediment data (Fig. 2) suggest that SPMEs represent a marked improvement over traditional chemical techniques in assessing the bioavailability of these compounds. Our results concur with a growing number of studies that suggest SPMEs may be effective predictors of bioavailability and toxicity of organic compounds in water, soil, and sediment [2,13,14,17–19].

CONCLUSIONS

The relationship between SPME and Tubifex TNT concentrations was relatively constant among TNT-spiked sediment, TNT-spiked carbon-amended sediment, and TNT-spiked water, suggesting that SPMEs may be able to provide matrix-independent measures of TNT availability. Relationships between SPME and Tubifex 2ADNT and 4ADNT concentrations differed between TNT-spiked sediment and ADNT-spiked water; however, SPMEs were predictive of ADNT bioavailability in both TNT-spiked sediments. These results concur with those of previous studies regarding SPMEs and organic compound bioavailability [2,13,14,16–18], suggesting that the SPME technique is a powerful tool for assessing the availability of organic compounds. However, because both metabolism and bioavailability determine the concentrations of the three main NAs measured in the animals, the relationships between bioavailability and SPME concentrations reported here are purely empirical and may be specific to this organism. The ability of SPMEs to predict NA bioavailability in different matrices in the present study is promising, and more research is warranted to explore the SPME approach in site-contaminated sediments, with other organisms, and with other compounds.

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body residues and toxicity of chemicals that act by narcosis.  


