

TOXICITY OF PERMETHRIN TO *CHIRONOMUS RIPARIUS* IN ARTIFICIAL AND NATURAL SEDIMENTSRACHEL J. FLEMING, DAWN HOLMES,* and SAMANTHA J. NIXON
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Abstract—Standard sediments are required for prospective risk assessments so that comparable fate and effects data can be generated within and between laboratories. One approach is to use artificial media that can be easily reproduced and characterized. However, a concern is that these media may not simulate natural sediments in terms of binding and toxicological properties. In this study, the acute and chronic effects of permethrin were compared in two artificial and one natural sediment using larvae of the midge *Chironomus riparius*. The influence of clay and carbon content and organic matter type on permethrin toxicity was also investigated. The toxic response measured in natural sediment was lower than that in the two artificial sediments, one of which contained peat and the other which contained α -cellulose as the organic carbon source. Of the two, the peat-based medium gave a more comparable response to the natural sediment. Acute and chronic permethrin toxicity was influenced by all sediment factors investigated with a consistently lower toxicity measured in the peat-based sediment compared with the α -cellulose sediment. A decrease in toxicity, coupled with an increase in bulk sediment permethrin concentration, was seen as both clay and organic carbon contents increased. Consideration should be given to improving the environmental realism of simple artificial formulations if the intention is to represent effects measured in natural sediments.

Keywords—Artificial sediment Natural sediment Toxicity Permethrin *Chironomus riparius*

INTRODUCTION

Methods for assessing the toxic effects of chemicals bound to aquatic sediments are currently being standardized for prospective risk assessment applications by international standards organizations [1,2]. In most cases this involves the exposure of single test species to a sediment amended with the chemical of concern under controlled laboratory conditions. The sediment selected will ultimately determine the measured toxic response because the physicochemical properties of the sediment matrix influence chemical bioavailability and hence toxicity [3–5]. Therefore, to generate toxicity data that are comparable between fate and effects studies, between chemicals, and within and between laboratories, standard test sediments are required.

Two approaches have been suggested for selection of a standard test sediment [6,7]. First, a natural sediment may be used that has selected physicochemical properties such as organic carbon and clay/silt content, which lie between prescribed ranges necessary for the growth and survival of test species [8]. However, one difficulty associated with the use of a natural sediment is that the understanding of factors that influence bioavailability is limited to a small number of substances, such as some nonpolar organic chemicals and divalent cations [3,4]. Little quantitative information is available on the extent to which differences in sediment character influence bioavailability even for these chemicals. Therefore, even though natural reference sediments may fall within guideline specifications, differences in observed toxicity may be attributable to small differences in sediment type. An additional difficulty with the use of natural sediment for this application is caused by spatial and temporal heterogeneity and changes

in sediment characteristics due to sampling, handling, and manipulation. Taken together, these factors mean that the probability of achieving a consistent natural test sediment is low.

The second approach is to construct artificial media from commercially available components. The advantages of artificial sediments are that they can be easily reproduced and characterized, they will be free of contaminants in toxic amounts (whereas clean natural sediments have to be carefully selected and analyzed), and they do not require the removal of indigenous predators that may influence the results of toxicity tests with natural sediments. However, one concern over the use of artificial sediments for risk assessment purposes is that they may be too simplistic to simulate the binding and toxicity of contaminants in natural sediments [6]. They do not contain the complex biological and physicochemical gradients found in natural media, and their limited biological activity and unique redox potentials may affect some sorption and desorption processes [9].

Most artificial sediments studied to date have been based on simple three-component formulations comprising sand, silt/clay, and organic matter. *Sphagnum* moss peat has been used as organic matter in the Organization for Economic Co-operation and Development (OECD) artificial soil medium for earthworm toxicity testing and has been used in several studies to formulate an aquatic sediment [10–13]. Various other organic matter sources have also been used, including manure, compost, plant material, and fish food [11,13–15]. Ribeiro et al. [16] recommended the use of synthetic α -cellulose, which has since been used successfully in effects studies [12,13].

Artificial sediments have been shown to support the survival and growth of toxicity test organisms over typical laboratory exposure durations [10,17], but the fate and effects of contaminants in these media have not been widely studied. In

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the study presented here, the acute and chronic effects of permethrin were investigated in two artificial sediments using larvae of the freshwater dipteran *Chironomus riparius*. The results obtained with the artificial media were compared with effects measured in a natural freshwater sediment with similar physicochemical characteristics. Both artificial sediments were based on the OECD earthworm soil recipe using sand, clay, and calcium carbonate as a buffer. In the first sediment, finely ground peat was used as the organic matter component. In the second, α -cellulose was used. α -Cellulose is a synthetic substance that can be obtained commercially; thus, it offers a greater potential for standardization than peat, which differs in composition depending on the source. To investigate the influence of physicochemical properties on the bioavailability of permethrin in these media, a range of organic carbon and clay contents was used.

MATERIALS AND METHODS

Preparation of sediments

Artificial sediments were constructed using acid-washed sand, kaolin clay, 1% calcium carbonate, and either finely ground *Sphagnum* moss peat or α -cellulose. All ingredients were obtained commercially, apart from the peat, which was supplied by Joseph Bentley, Yorkshire, United Kingdom. This source has been used in previous studies [10]. Organic carbon content was determined using a carbon hydrogen nitrogen elemental analyzer (Perkin Elmer 2400) for each ingredient prior to formulation, and the kaolin clay was subsequently ashed at 550°C to remove the organic component. Sediments were constructed to give ranges of organic carbon and clay content on a percentage dry weight basis. Sediments were moistened with clean groundwater and mixed on a rolling machine for 24 h to ensure homogeneity. After mixing, each sediment was conditioned in clean groundwater for 7 d in semistatic conditions to allow stabilization of pH and establishment of microbial populations [14].

The natural sediment used for comparative purposes was removed from an experimental freshwater pond at WRc, Medmenham, UK, using an Ekman grab. The sediment was previously collected from a "clean site," and chemical analysis of metals and organic chemicals (such as polycyclic aromatic hydrocarbons and pesticides) indicated that no substances were present at levels of concern. This sediment was sieved to 500 μm , after which particle size distribution (measured by laser defraction granulometry) and organic carbon content were determined.

Test organisms

Chironomus riparius egg ropes were obtained from a continuous culture held at WRc and hatched in groundwater at a controlled temperature of $20 \pm 2^\circ\text{C}$ and a 16:8-h light:dark photoperiod. For chronic tests, larvae were inoculated into test systems within 48 h of hatching. For acute tests, first-instar larvae were transferred from water-only cultures to 5-L glass aquariums with a 1-cm layer of acid-washed sand. These culture vessels were continuously aerated, and the animals were fed with a suspension of ground Tetramin fish food every 48 h. After 10 d (posthatch), the larvae were transferred from culture vessels into the test systems with a glass pipette after gently agitating the substrate surface.

Acute toxicity test

Fourteen artificial sediments were constructed using either peat or α -cellulose as the organic carbon source. Five levels of organic carbon were investigated: 0.2, 0.6, 1.0, 2.0, and 3.0%, and three levels of clay: 25, 50, and 75%. At 50% clay content, all five organic carbon levels were prepared and at 1.0% organic carbon content all three clay levels were prepared (i.e., 0.2% organic carbon/50% clay, 0.6% organic carbon/50% clay, 1.0% organic carbon/50% clay, 2.0% organic carbon/50% clay, 3.0% organic carbon/50% clay, 1.0% organic carbon/25% clay, and 1.0% organic carbon/75% clay). Additional percentage weight in each sediment was made up with sand. All sediments were spiked with permethrin at the following nominal concentration range: 0, 400, 800, 1,600, and 3,200 ng/g (dry weight sediment). Spiking was achieved by rolling wet sediment with permethrin in an acetone carrier for 90 min with additional overlying water. Each treatment was left to stand for 24 h to allow suspended material to settle, after which the overlying water was decanted off and the sediment distributed into test vessels. Three replicates of each treatment were prepared by placing 40 ml of the spiked sediment into three 250-ml Pyrex® tall-form vessels and adding 160 ml of uncontaminated borehole groundwater (pH 7.7–8.0, hardness 250 mg calcium carbonate per liter) to each beaker. Test vessels were left for 1 h before sediment samples were removed for analytical confirmation. Wet sediment samples were prepared for analysis by extraction into hexane, concentration, and treatment with tetrabutyl ammonium hydrogen sulfate (to remove any sulfur present). Following removal of any humic material using solid-phase extraction, the extracts were concentrated to 1 ml and analyzed by gas chromatography with electron capture detection using a DB1701 column and programmed on-column injection. Water quality determinants (pH, dissolved oxygen, and temperature) were measured in the overlying water of one toxicity test vessel per treatment. Fifteen 10-d-old larvae were then added to each vessel at random. After a 10-d static exposure, during which the animals were unfed and the vessels were continuously aerated, the surviving larvae were sieved from the sediment and counted. Statistical analysis of mean percentage survival against the individual parameters of nominal or measured concentration, organic carbon, clay content, organic matter type, and interactions terms between parameters was carried out by logistic regression analysis using a generalized linear modeling approach implemented in Genstat, Version 3.1.

Chronic toxicity test

Eight artificial sediments were constructed using peat as the organic matter fraction. Four levels of organic carbon were investigated: 0.5, 1.0, 2.0, and 3.0%, at both 20 and 50% clay content. Additional percentage weight in each sediment was made up with sand. All sediments were spiked with permethrin at the following concentration range: 0, 200, 400, 800, 1,600 ng/g (dry weight sediment). For this test, an alternative method of spiking was used to avoid any loss of test substance in excess water decanted off after spiking. This was achieved by placing 300 ml of wet sediment in an industrial food mixer (Crypto Peerless KNM6) and adding test substance in an acetone carrier dropwise onto the sediment as it mixed. The sediment was left to mix for an additional hour. Three replicates of each concentration were prepared by placing 40 ml of the spiked sediment into three 250-ml Pyrex tall-form vessels and adding 160 ml of uncontaminated borehole ground-

water to each beaker. Test vessels were left for 24 h before sediment samples were removed for analytical confirmation as described above, and water quality determinants were measured. Twenty first-instar larvae were added randomly beneath the water surface of each vessel. Care was taken not to expose the animals to air. Test animals were fed with 0.5 mg ground tetramin fish food per larva per day for the first 10 d and 1 mg per larva per day for the remainder of the test. All test vessels were continuously aerated, and water lost through evaporation was replaced using deionized water. Vessels were observed for adult emergence from day 15 onward. Adult midges were counted daily, and discarded pupal cases were removed from the test systems. The test was terminated 5 d after the last emergence was seen in control vessels. At this point, water quality determinants were measured, the contents of each vessel sieved, and surviving larvae were counted. Statistical analysis of mean number of emerged adults against permethrin concentration, organic carbon content, and clay content was carried out by logistic regression analysis using a generalized linear modeling approach implemented in Genstat, Version 3.1.

Artificial versus natural sediment

Two artificial sediments were constructed with peat and α -cellulose to match the natural sediment in terms of organic carbon content (1.23%) and particle size distribution (38% >100 μm as sand, 60% <100 μm as clay). All three sediments were spiked with permethrin at the following nominal concentration range: 0, 200, 400, 800, and 1,600 ng/g (dry weight sediment) using the industrial food mixer method described above, and chronic toxicity tests were performed.

RESULTS

Sediments

On test termination, the artificial sediments were easier to sieve than the natural sediment, thus facilitating test organism recovery. Of the two artificial sediments, the α -cellulose was more homogeneous within the test system. In the peat-based sediment, small amounts of peat floated to the surface of test vessels despite all precautions, and the ingredients tended to separate when overlying water was added. Separation was more pronounced for sediments containing less than 50% clay content. Water quality determinants remained within prescribed limits (pH 6–8, dissolved oxygen >3 ppm, temperature $20 \pm 0.5^\circ\text{C}$) for the duration of the tests, and organism survival was higher than 80% for all control sediments in acute and chronic tests.

Acute toxicity tests

Permethrin concentrations measured in the highest treatment of each sediment type can be seen in Table 1. Spiking efficiency (i.e., measured/nominal concentration) ranged from 150 to 250%, depending on organic matter type, organic carbon content, and clay content. In general, bulk sediment concentrations were higher in peat sediments than in α -cellulose sediments, and an increase was seen in both sediments with increasing carbon and clay contents. As sediment concentrations of permethrin were higher than nominals, mortality was higher than expected. For those sediment types with lower levels of carbon and clay, complete mortality of larvae was observed in the overlying water of the two highest treatments (1,600 and 3,200 ng/g dry weight sediment) on test initiation.

Logistic regression analysis of survival against permethrin

Table 1. Permethrin concentration (ng/g)^a

	α -Cellulose	<i>Sphagnum</i> moss peat
Sediment 1 (clay 25%, organic carbon 1.0%)	4,920	2,150 ^b
Sediment 2 (clay 50%, organic carbon 0.2%)	5,670	6,700
Sediment 3 (clay 50%, organic carbon 0.6%)	5,100	6,357
Sediment 4 (clay 50%, organic carbon 1.0%)	5,890	7,310
Sediment 5 (clay 50%, organic carbon 2.0%)	6,370	7,895
Sediment 6 (clay 50%, organic carbon 3.0%)	6,780	3,270 ^b
Sediment 7 (clay 75%, organic carbon 1.0%)	5,260	4,840

^a Nominal concentration = 3,200 ng/g.

^b Difficulty in achieving a representative analytical sample.

concentration was performed initially using both measured and nominal values. As would be expected, both these factors were significant descriptors of the survival data, but a number of large standardized residuals showed that permethrin concentration alone did not completely explain the trends in the survival data. Carbon and clay content and organic matter type were then incorporated into the model that led to a significantly improved fit of the data, indicating that all three factors exerted an influence on survival. The following results are based on nominal concentrations of permethrin. The two highest treatments (1,600 and 3,200 ng/g dry weight sediment) were excluded from the data set due to the high level of mortality observed. α -Cellulose sediment with an organic carbon content of 1.0% and a clay content of 50% was used as a standard against which other sediment types were compared using *t* values derived from estimates of regression coefficients and standard error terms in the regression analysis. The variation between replicates of a treatment was generally less than 25% with only 10 of the 70 treatment groups showing greater variability.

Organic matter type. There were no significant differences between control survival in the peat or α -cellulose sediments. However, at nominal concentrations of 400 and 800 ng/g, survival in peat sediments was significantly higher than in α -cellulose sediments (*t* values = 2.32 and 4.95, respectively; $p < 0.05$).

Organic carbon content. For the α -cellulose sediment, survival increased as organic carbon content increased. This could be seen even in the controls, although the effect was more pronounced in the permethrin treatments. There was significantly decreased survival in an organic content of 0.20% when compared with 1.0% (*t* value = 2.96, $p < 0.05$) and significantly increased survival in 3.0% when compared with 1.0% (*t* value = 4.95, $p < 0.05$). Survival in organic carbon content sediments of 0.60 and 2.0% was not significantly different from that in 1.0% sediments. Similarly, survival in peat sediment increased as organic carbon content increased in both controls and permethrin treatments. Survival in 0.2% peat sediment was significantly reduced compared with that in 1.0% α -cellulose (*t* value = 2.57, $p < 0.05$). Mean larval survival (%) at a nominal permethrin concentration of 800 ng/g can be seen in Figure 1 for the different levels of organic carbon at a clay content of 50%.

Clay content. For α -cellulose sediment, survival increased with an increase in clay content. Again, this was seen in controls, although the trend was more pronounced in permethrin treatments. Survival in a sediment with a clay content of 25% was not significantly different from survival in 50% clay, although survival in 75% clay was higher (*t* value = 4.81, $p <$

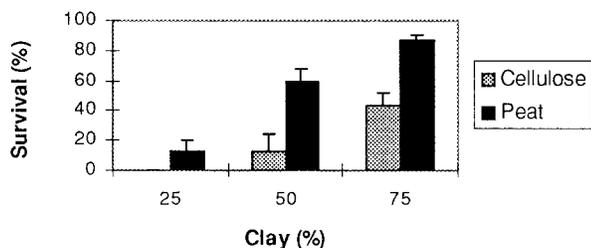


Fig. 1. Mean larval survival (%) at 800 ng/g permethrin (dry weight) in 50% clay sediments with differing organic content and type (error bars represent standard deviation from the mean).

0.05). Survival in the peat-based sediments also showed an increase in survival with increasing clay content. As in the α -cellulose sediment, survival was not significantly different for 25 and 50% but was higher at 75% (t value = 2.30, $p < 0.05$). Mean larval survival (%) at a nominal permethrin concentration of 800 ng/g can be seen in Figure 2 for the different levels of clay at an organic carbon content of 1%.

Chronic toxicity tests

Chemical confirmation of permethrin concentrations in bulk sediment samples could not be performed for this test due to poor recovery during sample extraction. Therefore, all statistical analyses were based on nominal concentrations. As seen with the acute tests, larval mortality was seen immediately in the overlying water of the two highest permethrin treatments for those sediments with lower organic carbon and clay contents. These data were excluded from statistical analyses. In general, adult emergence reflected survival at each treatment with few surviving larvae found on test termination.

Logistic regression analysis of emergence against nominal permethrin concentration and organic carbon and clay content once again showed that all factors, and the interaction between them, exerted an influence on survival. Significant differences in emergence between treatments, as determined by t values, were more difficult to observe in the chronic tests due to between-replicate variability. This is likely to reflect the difficulty in inoculating test vessels with first-instar larvae as opposed to with the 10-d-old larvae used in the acute tests. The only significant difference was observed at a clay content of 20%, where organic carbon contents of 2.0 and 3.0% led to an increase in emergence compared with 0.5 and 1.0% (t values = 3.97 and 3.80, respectively; $p < 0.05$). In general, increases in clay content and organic content both led to increased survival, as was also observed in the acute tests.

Comparison between the acute and chronic test results is hindered by the lack of bulk sediment chemistry data for the latter. For sediment types containing 50% clay and 2.0% peat,

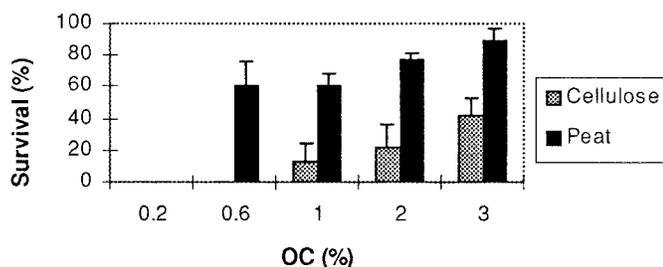


Fig. 2. Mean larval survival (%) at 800 ng/g permethrin (dry weight) in 1.0% organic carbon sediments with differing clay content (error bars represent standard deviation from the mean).

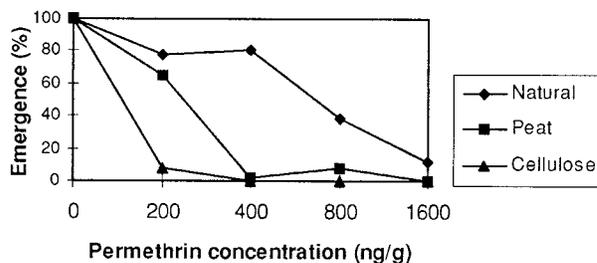


Fig. 3. Mean number of adults emerged (%) in natural and artificial sediments at differing permethrin concentrations.

which were used in both tests, comparison of effects based on nominal concentrations suggest that the chronic test was more sensitive. In the chronic test, mean percentage number of emerged adults at a nominal concentration of 200 ng/g was reduced to 27% compared to 100% emergence in control vessels. In the acute test, no reduction in larval survival was seen at a nominal concentration of 400 ng/g.

Artificial versus natural sediment

Logistic regression analysis showed that the nominal permethrin concentration strongly affected the total number of adults emerging, although this response was also influenced by sediment type. In the natural sediment, a significant reduction in mean emergence of 63% at a nominal permethrin concentration of 800 ng/g compared to the controls. In the peat sediment, a significant reduction in emergence of 62% was observed at 200 ng/g. In the α -cellulose sediment, no emergence was seen at the lowest permethrin concentration of 200 ng/g compared with 100% emergence in the controls. Therefore, the toxicity of permethrin in the three sediment test systems was α -cellulose > peat > natural sediment. Mean number of adults emerged (%) can be seen in Figure 3 for the three sediment types at the nominal permethrin concentrations tested.

DISCUSSION

Higher than expected bulk sediment concentrations of permethrin seen in this study have been observed in previous unpublished studies with artificial and natural sediments performed at WRC. This phenomenon has also been observed for spiking of phenanthrene in natural sediments [18]. The reason for this is unclear, although it may be due to preparation errors during sediment spiking or to analytical interferences. Concentrations of permethrin were below the analytical limit of detection in all control treatments.

In the artificial sediments tested in this study, the acute and chronic toxicity of permethrin decreased as the organic carbon content and bulk sediment concentration increased. This observation is consistent with the equilibrium partitioning theory of DiToro et al. [4], which postulates that the bioavailability of some hydrophobic compounds is determined by sorption to organic carbon, which in turn influences dissolved chemical concentrations in the overlying and pore water. The importance of the overlying water in mediating the acute toxic response in this study was clear. In sediments with low levels of organic carbon, larval mortality in the water column of the highest permethrin treatments was seen immediately on inoculation of the test organisms. If additional toxicity over a longer test duration was seen in these tests, as suggested by comparison of the acute and chronic data based on nominal concentrations,

direct contact or ingestion of sediment particles may also have influenced toxic response. However, this increased sensitivity could also have been due to the extended exposure duration, additional sensitivity of earlier larval instars, or enhanced sensitivity during emergence.

The other factor influencing the toxicity seen in the artificial sediment test systems was clay content. Increased percentage of clay also led to an increase in bulk sediment permethrin concentration and a reduction in toxicity. Zhou et al. [19] demonstrated that in the absence of organic coatings permethrin and other synthetic pyrethroids adsorb onto the surface of clay particles. The influence of clay and sand on adsorption and desorption parameters of selected pesticides has also been demonstrated by Zheng and Cooper [20]. In most natural sediments, organic coatings would effectively block mineral adsorption sites [19]. However, in the artificial sediments used in our study, any organic coatings that may have been associated with the clay particles were removed by ashing prior to formulation. The organic matter components of peat and α -cellulose that were present in these sediments were not closely associated with the clay fraction, thus leaving the mineral sites free for adsorption. The influence of the clay fraction in determining permethrin toxicity may, therefore, be more pronounced in the artificial sediments used here than in natural sediments.

The influence of clay and carbon content was also seen in artificial sediment control treatments where survival tended to increase with an increase in both factors. This suggests that test substrate requirements of *Chironomus* larvae could also be contributing to the observed toxicant effects in permethrin treatments. However, that >80% survival was seen in all control sediments in both acute and chronic tests demonstrates the wide tolerance of this organism to differences in sediment type. This finding is consistent with work on the survival of *C. tentans* in formulated and natural freshwater sediments, which showed that this species was tolerant of a wide range of sediment types, providing the organic carbon content exceeded 0.91% [14,17].

Differences in permethrin toxicity were also observed between the two artificial sediments used in this study, which differed only in organic matter type. Toxicity was lower in the peat-based medium than in the α -cellulose sediment, and this was generally reflected by higher concentrations of permethrin in the former. This could be due to the higher sorptive capacity of peat, leading to a reduction in permethrin bioavailability in the test systems. A difference in binding capacity of different organic matter types in aquatic sediments has been demonstrated for selected synthetic pyrethroids by Zhou et al. [19], who found that nonpolar humic acid bound more pyrethroid than the more polar fulvic and macromolecular acids. Rutherford et al. [21] demonstrated in soil studies that peat had a higher sorption capacity for benzene and carbon tetrachloride than did cellulose. Garbarini and Lion [22] also showed that the sorptive capacity of cellulose was lower for toluene and trichloroethylene when compared to other organic carbon sources such as soil humic and fulvic acids. Because of the different binding capacities of different types of organic matter, it is likely that the bioavailability and toxicity of substances such as permethrin will be influenced by the organic matter composition of the artificial sediment used.

Differences in organic matter type may also have contributed to the differences in toxicity observed between the artificial and natural sediments investigated in this study. Al-

though all sediments contained the same percentage of organic carbon, organic matter in the natural sediment would have been more diverse and, therefore, more likely to have contained a range of nonpolar components into which permethrin preferentially sorbed, thus reducing bioavailability and toxicity. Differences in toxicity of hydrophobic contaminants have also been reported for different natural sediments containing the same percentage of organic carbon [23,24]. Variation in organic matter type was also suggested as an explanation in these cases. The toxic response measured in the peat-based artificial sediment was closer to that of the natural sediment than was the α -cellulose sediment response. This may have been expected, given that the latter is a purely synthetic product, whereas peat is produced in a wet environment by degradation of a diversity of plant and animal materials.

CONCLUSIONS

The results of this study have implications for the use of artificial sediments if these media are intended to simulate the binding and toxicological properties of natural sediments. It is likely that the closer the artificial medium is to natural sediment in terms of organic matter type and diversity, the more representative it will be in terms of binding and toxicity. One way to improve the environmental realism of simple artificial formulations would be to include a wider variety of organic matter types. Some researchers have included humic acid in artificial formulations [16,25]. This may increase the sorptive capacity of the sediments used and provide a closer approximation to natural sediment. However, just as one natural sediment cannot mimic all other natural sediments, it would be unreasonable to assume that one artificial sediment could be used to generate data that would be applicable over a wide range of sediment types. Further comparisons are required for different classes of compound in different natural and artificial sediments before the environmental realism of these media can be assessed. Particular attention should be paid to the spiking protocols to ensure that observed differences in toxicity between artificial and natural sediments are not artifacts of the preparation techniques used.

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