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PARTITIONING, BIOAVAILABILITY, AND TOXICITY OF THE PYRETHROID INSECTICIDE CYPERMETHRIN IN SEDIMENTS

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Abstract—The partitioning, bioavailability, and toxicity of cypermethrin in water–sediment systems was investigated. Cypermethrin adsorbed extensively and rapidly, with an overall mean organic carbon (OC) adsorption partition coefficient (K_{oc}) of 350,000, and approximately 99% adsorption occurred within 24 h. Bioavailability was measured via body burdens of *Daphnia magna* and *Chironomus tentans*. Mean biota–sediment accumulation factors (BSAFs), that is, the concentration in the organism as a proportion of the concentration in the sediment, decreased with increasing OC content. The BSAF values were 0.31, 0.14, and 0.08 for *D. magna* and 0.63, 0.19, and 0.08 for *C. tentans*, in 1, 3, and 13% OC sediments, respectively. The 10-d median lethal sediment concentrations (LC50s) of cypermethrin were 3.6, 18, and 32 mg/kg for *Hyalella azteca* and 13, 67, and 62 mg/kg for *C. tentans* in 1, 3, and 13% OC sediments, respectively. Predictions of aqueous concentrations at the LC50 in sediments (based on K_{oc}) compared well to each other and to effect concentrations from studies in water alone, suggesting that equilibrium partitioning theory could be used reasonably to predict and normalize the toxicity of cypermethrin across sediments of differing OC content.

Keywords—Cypermethrin Sediment Adsorption Bioavailability Toxicity

INTRODUCTION

Synthetic pyrethroid insecticides have been used for more than 20 years to control insect pests in a variety of crops. Historically, concerns have existed regarding toxicity to aquatic organisms, particularly fish and arthropod invertebrates, because of the high degree of toxicity observed in standard laboratory studies (where exposures are maintained for periods of days to weeks). However, it has also been widely recognized that aquatic organisms are less likely to be affected under field conditions. The amelioration of effects results from a reduction in exposure because of the tendency of this group of insecticides to bind rapidly and extensively to suspended particulate matter, sediments, and aquatic plants [1,2]. Although this adsorption provides significant mitigation of possible effects for water-column organisms, it raises the question of the potential influence of those chemical residues adsorbed to sediment on benthic and infaunal organisms.

Studies on pyrethroids and other chemicals of similar lipophilicity indicate that a number of factors may affect toxicity and bioavailability in sediment. Pyrethroids readily adsorb to sediments, which greatly reduces bioavailability to water-column organisms [3]. However, once associated with sediments, the potential exists for exposure of benthic organisms via sediment particles (by ingestion or contact) or from interstitial water [4]. The extent to which lipophilic compounds such as pyrethroids are bioavailable in sediments has been the focus of recent research. To date, indications are that bioavailability for nonionic, organic chemicals is determined by a chemical equilibrium between water, sediment, and organism phases, with bioavailability best predicted from the concentration of chemical in the water phase (so-called equilibrium partitioning theory). It has been demonstrated [5] that reasonable predictions of toxicity (within a factor of two to three) can be made from predicted water-phase concentrations calculated with the sediment organic carbon (OC) partition coefficient (K_{oc}). Furthermore, for chemicals of similar lipophilicity to pyrethroids, it has been demonstrated that OC content is often the most significant sediment component in determining the partitioning [6,7]. In this paper we describe a range of studies in which we investigated the extent of adsorption, bioavailability when adsorbed, and the resulting toxicity in sediment of the synthetic pyrethroid cypermethrin (IUPAC name (*RS*)-α-cyano-3-phenoxybenzyl (1*RS*,3*RS*;1*RS*,3*SR*)-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate).

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MATERIALS AND METHODS

Test sediments

Sediments were selected for use in the studies to cover a range of OC contents. A number of natural sediments were obtained from researchers in the United States from which three sediments that contained approximately 1, 3, and 13% OC were selected for use in the studies (Table 1). The 1% OC sediment (Mississippi 2) that was used in the first study to assess bioavailability to *Daphnia* (see below) was substituted by an alternative 1% OC sediment (Florissant) in subsequent studies.

Methods for the analysis of the sediment physicochemical properties were as follows. The sediment pH was determined by slurrying a suspension of one part sediment to two parts water and measuring the pH with a Phillips CE13 sleeve junction combined electrode (Radiometer, Crawley, UK). Particle size analysis was performed by wet sieving for sand content (50- to 2,000-μm fraction), sequential sedimentation, and analysis of supernatant for silt (2 to 50-μm fraction) and clay (<2-μm fraction). Organic matter was determined by the method of Walkley and Black [8], which involves oxidation with

Table 1. Properties of sediments used in the studies. Mississippi 2 and 3 are from the University of Mississippi Biological Field Station (Oxford, MS, USA); Florissant is from the Midwest Science Center (Columbia, MO, USA); and Duluth is from the University of Wisconsin–Superior (Superior, WI, USA)

	Mississippi 2[a]	Florissant[b]	Mississippi 3[ab]	Duluth[ab]
% Organic carbon	1	1	3	13
Textural properties				
% Clay	10	24	25	25
% Sand	61	6	10	30
% Silt	29	70	65	45
CEC[c] (meq/100 g)	4.0	14.5	13.2	43.6
pH	4.9	6.0	5.1	7.2
Classification	Sandy loam	Silt loam	Silt loam	Loam

[a] Used for *Daphnia* bioaccumulation study.
[b] Used for adsorption–desorption studies, *Chironomus* bioaccumulation, and *Chironomus* and *Hyalella* toxicity studies).
[c] CEC = cation exchange capacity.

$K_2Cr_2O_7$ followed by titration of the excess dichromate with $FeSO_4$ with barium diphenylamine sulfonate as an indicator. Organic matter content was then divided by 1.724 to estimate OC content. Cation exchange capacity was measured by sodium saturation at pH 7 and flame photometry.

Test chemical

In all of the studies, ^{14}C-phenoxy-labeled cypermethrin (Fig. 1) with a specific activity of 2.1 G Bq/mmol and a purity of >99% was used. Cypermethrin is used on a variety of agricultural crops, but also has a number of public health and veterinary uses. The compound has also been extensively studied in laboratory and field ecotoxicological studies [2,9]. The water solubility of cypermethrin at 20°C and pH 7 is 4 μg/L [10].

Rate and extent of partitioning of cypermethrin in sediment

Measurement of adsorption dynamics and equilibria are important factors for understanding the bioavailability and toxicity of chemicals in sediments. Previous studies have shown that adsorption of pyrethroids is extensive and rapid, occurring in the most part within several hours [11]. To confirm these data for cypermethrin, adsorption and desorption properties were determined in the three sediments. Measurements were made with methodologies that broadly complied with U.S. Environmental Protection Agency test guideline 163-1 [12]. In addition, to determine the rate at which cypermethrin adsorbed, measurements of adsorption were made at various time intervals after addition of the chemical to the sediment–water system.

In all studies, an air-dried mass of 1 g of sediment (gamma irradiated with 35 kilograys, a standard dose for medical sterilization, to prevent microbial degradation of the test chemical) together with 25 ml of 0.01 M $CaCl_2$ solution was placed in a 50-ml centrifuge tube with a ground-glass stopper. The test system was mixed before the addition of test chemical by shaking on an end-over-end shaker at approximately 1,300 revolutions per hour.

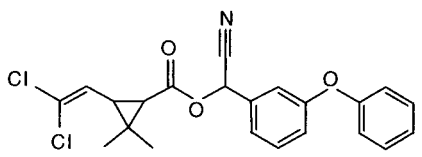

Fig. 1. Chemical structure of cypermethrin.

To estimate the time to equilibrium in the test system, cypermethrin was added to the test system in 100 μl of acetone to achieve a nominal concentration in the water phase of 0.045 μg/ml. Ten replicate test systems were prepared for each sediment. The test systems were then placed on an end-over-end shaker at approximately 1,300 revolutions per hour. Two replicate test systems were then analyzed after 2, 6, 24, 48, and 72 h. Test systems without sediment were analyzed after 72 h.

Before analysis, the water–sediment slurries were centrifuged at 1,200 *g* for 15 to 30 min until the supernatant appeared clear. A 15-ml aliquot of supernatant was then removed and extracted with 5 ml of hexane. The hexane was subsampled for liquid scintillation counting (LSC) on an LKB 1217 Rackbeta counter (Perkin-Elmer, Cambridge, UK), with Optiphase Safe (Fisher Scientific, Loughborough, UK) as the scintillation cocktail. The remainder of the supernatant aqueous phase was transferred to a glass vial and weighed. The residual sediment pellet was air-dried in the tube, and transferred as completely as possible to a plastic centrifuge tube for extraction with acetonitrile (30 ml, with end-over-end shaking for 2 h). The extract was centrifuged at 1,600 *g* for 15 min, and the supernatant was removed. This extraction process was repeated two further times for each sample. Supernatants were then combined and made up to 100 ml with acetonitrile, and extracts were analyzed by LSC to quantify the amount of radiochemical recovered. The remaining sediment pellet was then air-dried and combusted to determine the total amount of radioactivity present with a Harvey OX300 Biological Oxidizer (Lab Impex, Teddington, UK). Evolved $^{14}CO_2$ was trapped in 2-methoxyethylamine and analyzed by LSC. Pyrethroids readily adsorb to glassware, and, therefore, all centrifuge tubes were extracted twice by shaking in acetonitrile and the combined extracts were subsequently analyzed by LSC.

Because LSC only determines the total amount of radioactivity present, further analyses were conducted on all aqueous and sediment extracts to determine what proportion of the extracted radioactivity was parent cypermethrin (as opposed to other breakdown products). Aliquots from each extract were adjusted in volume to approximately 100 μl, which was applied in a 1.5-cm band to a thin-layer chromatography (TLC) plate (CAM-LAB precoated, Fisher Scientific). The plate was immediately placed in a 60:20:4:1 (v/v/v/v) toluene:hexane:chloroform:acetonitrile solvent system. Extracts were cochromatographed with an analytical standard of cypermethrin. Radioactivity on the plates was quantified with either a Rita 68000 or 3200 Automatic TLC Analyzer (Raytex, Sheffield, UK). Autoradiograms were made with a Fuji BAS Phosphorimager (Raytex).

As a result of the time to equilibrium studies (see below), an equilibration time of 24 h was selected for subsequent adsorption and desorption studies. The same test systems as described above were treated with radiolabeled cypermethrin to obtain nominal concentrations in the aqueous phase of 0.015, 0.045, 0.135, and 0.405 μg/ml. Four replicates of each of the three test sediments were prepared for each concentration. After 24 h, two replicate tubes were analyzed to determine the concentration in the water and sediment phase, with the same methods as described above. Two further replicate tubes were used for the desorption step. The tubes were centrifuged, and an aliquot was removed and extracted into hexane for LSC.

The aqueous phase was removed and replaced with an equivalent volume of 0.01 M $CaCl_2$ to permit desorption from the sediment. The test system was then shaken for 24 h at 1,300 revolutions per hour, after which the water and sediment phases were analyzed as described above.

Adsorption and desorption were expressed as the sediment adsorption/desorption coefficient (K_d), normalized to the OC content of the sediment (K_{oc}), where

$$K_d = \frac{\mu g \text{ chemical per g sediment dry weight}}{\mu g \text{ chemical per } cm^3 \text{ of the aqueous phase}}$$

$$K_{oc} = \frac{K_d}{\% \text{ OC}} \times 100$$

Bioavailability of cypermethrin to Daphnia magna *and* Chironomus tentans

Estimating bioavailability of pyrethroids simply from measurements of the chemical in the aqueous phase of water–sediment systems can be difficult because small amounts of particulates, dissolved organic carbon (DOC), or colloids in the water can adsorb the chemical, reducing bioavailability [13,14]. Therefore, ascertaining what is truly in the water phase in such small test systems can be difficult. Determination of the relative influence of the various routes of uptake in a multiphase system without affecting the chemical equilibria also is difficult. Therefore, the approach to estimating bioavailability that was considered most likely to succeed was by measuring organism body burdens. Bioaccumulation studies were performed with radiolabeled cypermethrin in three water–sediment systems. One study utilized *D. magna* to estimate bioavailability to organisms exposed principally through the overlying water phase. The second used *C. tentans* to measure bioavailability to a benthic organism additionally exposed through interstitial water and solid phases (by contact or ingestion).

Late (third to fourth)-instar *C. tentans* and adult female *D. magna* were obtained from laboratory cultures. Organisms of this size were required to allow sufficient tissue to be present for subsequent analyses. Test systems consisted of 500-ml glass jars containing 10 g dry weight of sediment and 250 ml of water. In the test systems containing *C. tentans,* 8 mg of ground TetraMin® (Tetra Sales, Blackburg, VA, USA) also was added as a food source. *Daphnia magna* were fed with algal and yeast suspensions 24 h after their addition to the test systems.

For the *C. tentans* studies, nominal application rates were 40, 100, and 150 μg/kg sediment dry weight for the Florissant, Mississippi 3, and Duluth sediments, respectively. For the *D. magna* studies, nominal application rates were 423, 1,260, and 5,320 μg/kg sediment dry weight for the Mississippi 2, Mississippi 3, and Duluth sediments, respectively. Application rates were selected to provide concentrations that were high enough to permit test organisms to accumulate sufficient chemical to quantify, while being low enough to avoid acute toxic effects, based on the water-only toxicity data and the expected partitioning of the compound [2]. After application of the test chemical in a 100-μl aliquot of acetone, the jars were placed on a rolling mill overnight to ensure even mixing of the chemical in the test system. The test systems were then allowed to settle for 2 d before the introduction of 10 organisms and were then incubated in a water bath at 20°C and 23°C for the *D. magna* and *C. tentans,* respectively. After 24, 48, 72, and 96 h, test systems were analyzed for concentrations of the test chemical in the sediment, sediment pore water, overlying water, and test organisms. For the *D. magna* studies, two replicates were analyzed at each time point. For the *C. tentans* study, a further replicate was analyzed at each time point because an additional test system was required for analysis of the sediment pore water (this was not possible after removal of the test organisms because of disruption of the test system).

To analyze the overlying water, a 100-ml aliquot was removed and extracted into 5 ml of hexane and subequently analyzed by LSC. The remaining overlying water was then removed, and in the case of the *D. magna* studies, the organisms were also removed. Sediment pore water was then extracted from the sediment of one replicate. The sediment was placed in a centrifuge tube and centrifuged at 2,100 *g* for 15 min to separate the pore water from the sediment. The pore water was then removed and extracted with hexane for subsequent analysis by LSC (as described above). For the *C. tentans* studies, the organisms were removed from the sediments in the remaining two replicates. The sediments from all replicates were extracted with acetonitrile in an end-over-end shaker for 1 h, and were centrifuged at 2,100 *g* for 15 min. The extracts were then quantified by LSC. Any remaining sediment was dried and combusted (as described above) to determine the amount of radioactivity remaining in the sediment. The organisms were counted, rinsed with water, blotted dry, then wet-weighed in a combustion cone before analysis by LSC.

Aliquots of water and sediment extracts were also analyzed by TLC (as above) to determine what proportion of the extracted radioactivity was cypermethrin. Extraction and characterization of the radioactivity in the organisms was not possible because too little radioactivity was present. However, because the breakdown products of cypermethrin are polar and readily excreted, the majority of radioactivity measured in the organisms was assumed to be parent cypermethrin. Some ^{14}C may have been incorporated into tissues, and assuming that this was cypermethrin would have overestimated the amount bioconcentrated, and hence the bioavailability.

Toxicity of cypermethrin in sediment to Hyalella azteca *and* C. tentans

Sediment toxicity tests were conducted with *H. azteca* and *C. tentans,* to evaluate effects on mortality and growth over 10 d. The test method was based on the methods of the U.S. Environmental Protection Agency [15].

Test systems were prepared in the same way as those described in the bioavailability studies above. For *C. tentans,* nominal sediment test concentrations ranged from 2.2 to 180, 3.4 to 300, and 5.6 to 450 μg/kg, for the Florissant, Mississippi 3, and Duluth sediments, respectively. For *H. azteca,* nominal sediment test concentrations ranged from 2.5 to 40, 0.74 to 60, and 1.9 to 150 μg/kg for the Florissant, Mississippi 3, and Duluth sediments, respectively. Six replicates were prepared for each test concentration. In addition, dilution water and solvent controls were prepared for each sediment and organism. Test organisms were obtained from laboratory cultures and third-instar *C. tentans* (confirmed by head capsule measurements) and 7- to 14-d-old *H. azteca* (selected by sieving through a 500-μm mesh and retaining on a 250-μm mesh) were used to initiate the tests. Ten organisms were added to each test system, after the test system had equilibrated. Test systems were covered to reduce evaporation and were incu-

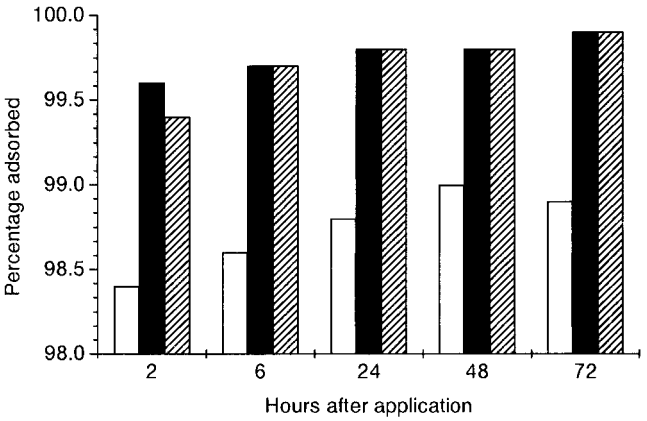

Fig. 2. Rate of adsorption of cypermethrin to three aquatic sediments (column legend: white = Florissant; black = Mississippi 3; cross-hatched = Duluth).

bated in a water bath at 23°C on a 16:8 h light:dark cycle at approximately 800 lux (lumen per square meter). *Chironomus tentans* were fed at discretion (i.e., if no food was visible at the sediment surface) with ground TetraMin. In practice, larvae were provided with 20 mg of TetraMin on two to three occasions during the study. After 10 d, a 100-ml aliquot of water was removed from the overlying water of all of the test systems. This was extracted into 5 ml of hexane and analyzed by LSC (as above).

Organisms were carefully removed from four of the six replicate test systems. The number surviving was recorded and remaining organisms then were preserved for length (*H. azteca* only) and dry weight determinations. The overlying water from the two remaining test systems was then removed and the sediment pore water was extracted as described in the bioavailability studies above. The remaining sediment from all replicates was analyzed for radioactivity as described above. Some of the sediment extracts were also analyzed by TLC (as described above) to characterize the radioactivity.

Survival data were analyzed by the technique of iteratively reweighted linear regression of logit response on log 10 concentration. Length and weight data were analyzed by analysis of variance with the Statistical Analysis System (SAS®, Cary, NC, USA).

RESULTS AND DISCUSSION

Adsorption dynamics

The average recovery of radioactivity applied was 109%, indicating that the extraction procedures had been effective. The TLC analysis of sediment extracts confirmed that >97% of the radioactivity in the extracts was cypermethrin. The TLC analyses of aqueous extracts were very difficult to quantify because of the low amounts of radioactivity present. Cypermethrin typically constituted >60% of the radioactivity, but small amounts of more polar compounds were sometimes apparent, suggesting that some degradation had occurred. However, for all calculations of partition coefficients, all of the radioactivity in the water phase was assumed to be parent cypermethrin. Because this would increase the estimate of water concentration, this approach would underestimate the degree of partitioning.

As expected, cypermethrin adsorbed rapidly to sediments, reaching equilibrium in the sediment in all cases in less than 24 h (Fig. 2). Indeed, the vast majority (>98%) of total adsorption had already occurred within 2 h of application of the chemical. For all three sediments, approximately 99% or more of the total amount of chemical applied to the test system was adsorbed to the sediment at equilibrium.

A direct relationship was found between adsorption and increasing OC content, with mean sediment partition coefficients (K_ds) of 2,360, 15,700, and 23,600 for 1, 3, and 13% OC sediments, respectively. In theory, if partitioning was only determined by OC content, a direct relationship would exist between OC and K_d, which would result in an expected ratio of 1:3:13 between these K_d values. Here, the average ratio was 1:6:10, which seems to support the hypothesis that adsorption is closely related to OC content, within a factor of two [5].

Normalizing adsorption to sediment OC content, mean adsorption K_{oc} values were 238,000 (standard deviation [SD] = 38,000; coefficient of variation [CV] = 16%), 502,000 (SD = 27,000; CV = 5%), and 177,000 (SD = 40,000; CV = 23%) for the 1, 3, and 13% OC sediments, respectively. The overall mean adsorption K_{oc} of cypermethrin was 350,000. Theoretically, K_{oc} should be a constant for a particular chemical, that is, an increase in OC content should lead to a direct increase in adsorption. However, adsorption probably also is affected by the physical nature of the OC present in the sediment and the surface area available for adsorption (the latter being a function of particle size distribution within the soils). Organic carbon that is present in small, often cominuted particles or coating the surface of mineral particles is likely to present a greater potential for adsorption than larger intact particles of OC, because of increased surface area. However, the method of Walkley and Black of analyzing for OC content (as used here) does not take surface area into account, because OC is digested from the sediment in its entirety [8]. Therefore, two sediments that have the same measured OC content, but different surface areas of OC, possibly could adsorb the chemical slightly differently. This factor probably accounts for the variation in K_{oc} values observed in this study. Furthermore, aggregated OC would be expected to cause more variability in adsorption measurements because of the more heterogeneous distribution of larger particles. This was observed for the 13% OC sediment in this study, whose adsorption characteristics were more variable than the lower OC sediments (see coefficients of variation for adsorption K_{oc} values described above and desorption K_{oc} values described below).

Measured K_{oc} values after the desorption step were similar to, although somewhat higher, than those measured after the adsorption step, averaging 281,000 (SD = 28,000; CV =10%), 582,000 (SD = 75,000; CV = 13%), and 182,000 (SD = 73,000; CV = 40%) for the 1, 3, and 13% OC sediments, respectively. This suggests that the adsorption of cypermethrin may not be entirely reversible, indicating that adsorption K_d values might be somewhat conservative in estimating potential bioavailability.

Bioavailability

Measured concentrations (based on total radioactivity) in the sediment ranged from 80 to 85% of nominal in the studies with *D. magna*, and from 74 to 97% in studies with *C. tentans* (Table 2). Extractable radioactivity (which was determined to be only ^{14}C-cypermethrin by TLC) accounted for the vast majority (88–98%) of the total radioactivity, and the vast majority of total radioactivity was cypermethrin, with no other major products present. Relatively little change occurred in the concentration of cypermethrin in the water, sediment, or organism

Table 2. Measured concentrations in the water, sediment, and organism phases in bioavailability studies with *Daphnia magna* and *Chironomus tentans*

Sediment	Time (h)	Body burden (μg/kg)	Overlying water (ng/L)	Sediment (μg/kg)	Pore water (ng/L)
Daphnia magna[a]					
Mississippi 2	24	90	34	307	170
	48	110	41	377	220
	72	123	27	378	185
	96	107	21	383	145
Mississippi 3	24	109	54	1,061	480
	48	130	66	989	535
	72	130	44	1,093	285
	96	171	28	1,111	270
Duluth	24	424	535	3,959	1,850
	48	282	645	4,287	980
	72	292	390	4,410	2,000
	96	133	260	4,345	1,350
Chironomus tentans[b]					
Florissant	24	13	8	31	70
	48	21	9	30	60
	72	15	9	29	40
	96	18	9	29	40
Mississippi 3	24	16	18	95	40
	48	18	16	98	40
	72	19	13	100	50
	96	21	11	96	70
Duluth	24	11	25	133	20
	48	12	35	129	40
	72	10	22	137	40
	96	9	4	140	20

[a] Number of replicate samples for each phase was two.
[b] Number of samples for each phase was: organism, $n = 2$; overlying water, $n = 3$; sediment, $n = 3$; pore water, $n = 1$.

phases through time, indicating that the organisms rapidly reached equilibrium with the test system. Similar rapid equilibration has been found in previous studies with pyrethroids [11]. In addition to organisms and sediment, concentrations of cypermethrin in the interstitial and overlying water were analyzed (Table 2). Cypermethrin was extracted from whole water samples (no centrifugation) and hence the amount of cypermethrin truly in solution and that associated with DOC and suspended and colloidal material would not be distinguished. The analytical methods used will not readily allow the concentration truly in solution and that associated with DOC and suspended material to be distinguished at such low concentrations.

In the majority of cases, concentrations of cypermethrin in the interstitial water were up to an order of magnitude higher than those in the overlying water. This may have resulted from the centrifugation extraction process for pore water, causing additional amounts of DOC and colloids with their associated cypermethrin to be released from the sediment, thereby increasing the apparent concentration of cypermethrin in the interstitial water. Similar results have been observed with another lipophilic pesticide, chlorpyrifos [14]. Measurements of cypermethrin in the overlying water may also have reflected a similar influence of increasing DOC and colloids with increasing OC content in the sediment, Because average K_{oc} values decreased with increasing OC content in both studies (Table 3).

Table 4. Mean biota–sediment accumulation factors (BSAFs) for cypermethrin in sediment of varying organic carbon content. Values in parentheses are 95% confidence limits

Sediment organic carbon content (%)	BSAF, *Daphnia magna*	BSAF, *Chironomus tentans*	Mean BSAF
1[a]	0.31 (0.28–0.34)	0.63 (0.50–0.76)	0.47
3	0.14 (0.12–0.16)	0.19 (0.17–0.21)	0.17
13	0.08 (0.06–0.10)	0.08 (0.06–010)	0.08

[a] Studies were performed using two different 1% organic carbon sediments—Mississippi 2 and Florissant for *Daphnia* and *Chironomus*, respectively (see Table 1 for sediment characteristics).

To compare the relative bioavailability of cypermethrin to the organisms in the various studies, bioavailability was expressed as biota–sediment accumulation factor (BSAF), that is, the concentration in the organism divided by the concentration in the sediment. This ratio indicates the proportion of the chemical adsorbed to the sediment that has accumulated in the organism. For example, if the concentration in the sediment is 100 mg/kg and the concentration in the organism is 50 mg/kg, the BSAF would be 0.5; if the sediment concentration is 100 mg/kg and that in the organism is 10 mg/kg, the BSAF would be 0.1. Hence, BSAF decreases as bioavailability decreases.

For the 3 and 13% OC sediments, the mean sediment BSAFs for *Daphnia* and *Chironomus* were very similar (Table 4). For the 1% OC sediments, small differences of a factor of two occurred in bioavailability. This may have been due to the use of different sediments in the two studies (Mississippi

Table 3. Mean sediment organic carbon partition coefficients for 1, 3, and 13% organic carbon sediments measured in bioavailability studies with *Daphnia magna* ($n = 2$) and *Chironomus tentans* ($n = 3$). Standard deviations of means for each time interval are shown in parentheses below the mean values

	1% organic carbon		3% organic carbon		13% organic carbon	
Time (h)	*D. magna*	*C. tentans*	*D. magna*	*C. tentans*	*D. magna*	*C. tentans*
24	850,000	363,000	650,000	193,000	50,000	38,000
	(99,000)	(25,000)	(190,000)	(60,000)	(4,200)	(7,700)
48	920,000	382,000	470,000	228,000	47,000	34,000
	(99,000)	(128,000)	(64,000)	(68,000)	(3,500)	(12,000)
72	1,390,000	307,000	780,000	292,000	84,000	58,000
	(190,000)	(61,000)	(42,000)	(98,000)	(6,400)	(22,000)
96	1,820,000	370,000	1,250,000	438,000	118,000	192,000
	(280,000)	(136,000)	(78,000)	(249,000)	(7,100)	(98,000)
Average	1,245,000	356,000	788,000	288,000	75,000	81,000

2 for *Daphnia* and Florissant for *Chironomus*; see Table 1). For these 1% OC sediments, cypermethrin seemed to be more bioavailable in the Mississippi 2 sediment than in the Florissant sediment, as perhaps might be expected with the higher sand content of Mississippi 2. Where the same sediments were used, analysis of the data clearly demonstrated that no difference occurred in the relative bioavailability to water column or benthic organisms of cypermethrin adsorbed to sediments in the test systems used.

Comparison of the BSAFs for the different sediment types (Table 4) demonstrated decreases in bioavailability with increasing OC content. If bioavailability is inversely related to OC content, a linear decrease in bioavailability would be expected as OC content increases. Relative differences between the sediments broadly followed this pattern. The average ratio between bioavailabilities for 1 and 3% OC sediments was 1:3, exactly as expected for this difference in OC content. However, the ratio between the 3 and 13% OC sediments was 1:2, compared to an expected ratio of 1:4. Once again, this difference may be due to smaller surface area of OC in the 13% OC sediment (see above), providing slightly less adsorption as a function of OC than expected. However, a discrepancy of a factor of approximately two to three is consistent with the expected precision of bioavailability estimates based on equilibrium partitioning [5]. The bioavailability results observed for cypermethrin were consistent with those observed for another pyrethroid, λ-cyhalothrin [16].

The observed differences in the amount of chemical in the water phase (overlying and interstitial) were clearly not reflected in the bioavailability data, where bioaccumulation decreased with increasing sediment OC (Table 4). However, the relative bioavailability would have been adequately described by differences in K_d values from the adsorption–desorption study described above. The difference between the studies can be explained by differences in methodologies between the two study types. Adsorption–desorption studies overcome the problem of chemical associated with DOC and colloids in the water phase by removing these materials by addition of a flocculating agent, calcium chloride. This coagulates organic material, promoting settling and thereby reducing apparent water-phase concentrations, but giving a more realistic measurement of actual dissolved concentrations. Such additions are impractical when organisms are present because of the potentially toxic effects of concentrated calcium chloride. However, indications of a similar process occurring through settling of DOC and colloids with time were also apparent in the bioavailability studies, where K_{oc} values tended to increase throughout the study (Table 3).

In combination, the potential errors that may result from water-phase measurements (overlying or interstitial) due to the presence of DOC and colloids suggest that whole water-phase concentrations may not be a very good predictor of bioavailability and hence toxicity for pyrethroids or other highly lipophilic chemicals because of the difficulty of establishing what is truly in the aqueous phase. Concentrations of the chemical in the sediment together with sediment OC content seem to provide a more robust predictor of the bioavailability.

Sediment toxicity studies

Measured concentrations in the sediment on day 0 were generally similar to, albeit slightly lower than, the nominal concentrations. For *H. azteca* studies, extractable sediment residues on day 10 were approximately 65, 75, and 80% of day 0 values for the Florissant, Mississippi 3, and Duluth sediments, respectively. For *C. tentans* studies, extractable sediment residues on day 10 were approximately 70, 90, and 90% of day 0 values for the Florissant, Mississippi 3, and Duluth sediments, respectively. Effects on survival and growth of *H. azteca* and *C. tentans* after 10-d exposures in sediment are shown in Table 5. Effect concentrations were calculated on the basis of initial (day 0) measured concentrations in the sediment.

Table 5. Effects of cypermethrin in three sediments on survival and growth of *Hyalella azteca* and *Chironomus tentans*. Confidence limits for survival measurements are given in parentheses[a]

Sediment organic carbon content (%)	*Hyalella azteca*		*Chironomus tentans*	
	10-d LC50 (μg/kg)	NOEC growth (μg/kg)	10-d LC50 (μg/kg)	NOEC growth (μg/kg)
1	3.6 (3.1–4.2)	<1.8	13 (4.5–42)	3.8
3	18 (15–23)	2.3	67 (24–215)	25
13	23 (19–28)	1.8	62 (23–176)	14

[a] LC50 = median lethal concentration; NOEC = no-observed-effect concentration.

Data from the toxicity studies (Table 5) were not as precise as those developed in bioavailability studies (note the confidence limits for mortality data). Two factors are likely to have influenced this. First, a number of experimental variables other than the toxicant can affect the expression of whole-organism toxic responses (e.g., energetic, growth, or reproductive status of test organism; interaction with test sediment; and so on). Second, designing tests that contain concentrations that are wholly suitable for measuring endpoints for both mortality and growth is difficult. Some compromise in the test concentrations is necessary. Nevertheless, for the 1 and 3% OC sediments, toxicity followed the same pattern that was seen in the adsorption and bioavailability studies, with decreases in toxicity observed with increasing OC content, as would be expected. Reductions in toxicity were similar to what would have been expected in the 3% OC sediment, with median lethal concentration (LC50) values approximately five times greater than the 1% OC sediment. However, toxicity of cypermethrin in the 13% OC sediment was similar to that observed in the 3% OC sediment.

As with the bioavailability studies described above, although differences in OC content (as measured by soil characterization methods) would be predicted to cause decreases in bioavailability and hence toxicity, the possibility exists that not all of the OC measured in the 13% OC sediment is available to adsorb the chemical. This would lead to differences in the apparent toxicity because of sediment sorption. Differences in adsorption are also reflected in the relatively low K_{oc} for the 13% OC sediment measured in this study (average K_{oc} was 78,000), which suggests that a smaller proportion of cypermethrin was adsorbing than would be predicted from the OC content (average K_{oc} from adsorption–desorption studies was ~350,000).

Predictions of the water-phase concentrations at the LC50 and no-observed-effect concentration in the three sediments can be made by using the K_{oc} measured for each sediment in the adsorption and bioavailability studies (see formulae in

Table 6. Effect concentrations (mortality and growth) normalized according to predicted concentrations in the water phase (based on organic carbon partition coefficients [K_{oc}s] measurements from adsorption–desorption and bioavailability studies) from sediment toxicity studies on *Hyalella azteca* and *Chironomus tentans* with cypermethrin[a]

Sediment organic carbon content (%)	K_{oc}	Predicted water-phase concentration (ng/L)			
		Hyalella azteca		*Chironomus tentans*	
		LC50	NOEC	LC50	NOEC
1	239,000[b]	1.5	<0.76	5.5	1.60
	350,000[c]	1.0	<0.52	3.8	1.10
3	503,000[b]	1.1	0.15	4.3	1.58
	350,000[c]	1.6	0.22	6.2	2.27
13	178,000[b]	1.0	0.08	2.6	0.59
	350,000[c]	0.5	0.05	1.3	0.30

[a] LC50 = median lethal concentration; NOEC = no-observed-effect concentration.
[b] Mean measured K_{oc} for sediment in adsorption–desorption studies.
[c] Overall mean adsorption K_{oc}.

methods). Hence, when the K_{oc} is 350,000, the sediment has an OC content of 3%, and the concentration on the sediment in the test system (10 g) is 10 μg/kg, then the concentration in the water phase (250 ml) would be predicted to be 0.001 μg/L. Table 6 shows predicted water-phase concentrations derived from the sediment toxicity values based on the K_{oc} measured for each sediment and from the overall mean K_{oc} (350,000).

Effect concentrations for both *Hyalella* and *Chironomus* were highly consistent when the data were normalized according to the concentration that was predicted to be in the water phase (Table 6). This demonstrates that K_{oc} (both measured for a specific sediment or as an overall constant for a particular chemical) is a reasonable approach for obtaining a preliminary indication of potential exposure concentrations for sediment organisms.

Previous studies with *H. azteca* (7–14 d old) and *Chironomus riparius* (first instar) generated 48-h LC50s in water alone of 5.3 and 6.9 ng/L, respectively (Zeneca Agrochemicals, unpublished data). When normalized to concentrations predicted in the water phase, the effect concentrations in the sediment studies were of the same order of the toxicity measured in water alone, albeit slightly lower probably because of the longer duration of the study (10 d as opposed to 2 d). The predicted water-phase values also were similar to other acute toxicity data for cypermethrin with sensitive insects and amphipods [2]. This suggests that reasonable predictions of cypermethrin toxicity in sediment could be made by estimating the concentration of cypermethrin in the aqueous phase and comparing that to toxicity data from water-only studies.

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