Supporting Information

Role of Sediment Particle Size in Cypermethrin Toxicity to *Chironomus dilutus* and *Hyalella azteca*: Insights from Bioavailability and Exposure Pathways

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Determination of TOC in sediments

The TOC content of sediments of various particle sizes was determined using an elemental analyzer (ElementarVario EL III, Hanau, Germany). Prior to instrumental analysis, the sediment samples underwent a series of pretreatment procedures. Initially, approximately 2 g of chemically treated sediment was weighed into a 50 mL beaker, and 1 mol/L dilute hydrochloric acid was added. The sample was stirred until effervescence ceased, ensuring the removal of inorganic carbon, and then allowed to stand for 12 h. Subsequently, the sediment was rinsed with deionized water until the pH reached approximately 7, filtered, and the wet sediment was dried in an oven at 60 ^{o}C. After drying, the sample was divided into two portions; one portion was used for TOC determination. Approximately 20–30 mg of the sediment was accurately weighed, sealed in a tin capsule to prevent sample loss, and then analyzed using the elemental analyzer.

Sediment spiking

The 10 d LC50 of cypermethrin for *C. dilutus* and *H. azteca* in sediments was 1.34 and 0.38 μg/g OC, respectively [1]. The five concentration gradients for toxicity testing were 0.25 LC50, 0.5 LC50, 1 LC50, 2 LC50 and 4 LC50. Cypermethrin was spiked individually into the four sediments (Original sediment, <20 μm, 63–180 μm, and 180–500 μm) at each concentrations, using acetone as the carrier (33 μL acetone kg^{-1} sediment maximum). Spiked sediments were thoroughly mixed using a stainless paddle driven by an overhead motor for 2 h, and aged at 4 ^{o}C in dark for 30 d. All sediments were re-homogenized before testing.

Instrumental Analysis

The quantitative analysis of cypermethrin was performed by Shimazu GC-MS

(QP2010plus) with negative chemical source (NCI) ion selection model (SIM). The metabolites were analyzed on HPLC-MS/MS. An Agilent Zorbax Eclipse Plus C18 column (100 × 2.1 mm i.d., 1.8 μm) was used to separate the analytes. Acetonitrile and water containing 0.1% of formic acid was used as the mobile phase at a flow rate of 0.3 mL/min. The acetonitrile content was 40% when the gradient elution procedure was set to 0–0.5 min, and increased to 80% within 1.2–4.5 min. Then acetonitrile dropped to 40% at 4.5-5 min, and finally maintained to 6 min. The injection volume was 3 μL. The ionspray voltage (IS) is 4500V, and the ion source temperature (TEM) is 550℃. The entrance potential (EP) and the collision cell exit potential (CXP) are -10.0V and -16.0eV, respectively. The quantitative calibration range was 0.1–20 ng/mL.

Data Analysis

C_{SPME} (μg/g OC) was calculated from the freely dissolved concentration (C_{free}) of the compound in sediment porewater measured by SPME using the following equation:

$$C_{SPME} = C_{free} \times K_{OC} = \frac{C_{fiber} \times K_{OC}}{K_{fw}}$$

which K_{oc} was the partition coefficient of the compound between sediment organic carbon and porewater, C_{fiber} was the concentration of the compound on the SPME fiber, and K_{fw} was the partition coefficient of the compound between the coating material (polydimethylsiloxane) on the SPME fiber and water.

Table S1. TOC and measured concentration of sediments with different particle sizes, including the total sediment concentration (C_S), Tenax extractable concentration ($C_{S\text{-}24h}$) and SPME measured concentration (C_{SPME}), and the ratio of bionic extraction concentration to total concentration.

		C. dilutus				*H. azteca*			
	Conc.	<20 μm	63–180 μm	180–500 μm	Original	<20 μm	63–180 μm	180–500 μm	Original
TOC (%)		1.78	1.04	1.66	1.04				
C_S (μg/g OC)	level 1	0.15±0.077	0.020±0.011	0.28±0.15	0.051±0.0015	0.0057±0.0010	0.048±0.038	0.15±0.018	0.018±0.00076
	level 2	0.27±0.16	0.10±0.013	0.23±0.025	0.12±0.0028	0.027±0.0017	0.028±0.0044	0.12±0.032	0.031±0.0011
	level 3	0.40±0.0091	0.24±0.028	0.64±0.058	0.23±0.0052	0.15±0.077	0.069±0.013	0.28±0.13	0.073±0.0018
	level 4	0.52±0.028	0.37±0.044	2.4±0.21	0.33±0.013	0.27±0.16	0.14±0.017	0.23±0.024	0.14±0.0031
	level 5	1.4±0.017	0.94±0.12	3.5±0.44	0.82±0.062	0.40±0.0091	0.24±0.028	0.58±0.053	0.22±0.01051
$C_{S\text{-}24h}$ (μg/g OC)	level 1	0.14±0.016	0.32±0.10	0.29±0.094	0.22±0.027	0.031±0.0090	0.049±0.0083	0.18±0.033	0.090±0.013
	level 2	0.43±0.063	0.28±0.066	0.26±0.049	0.46±0.029	0.043±0.012	0.064±0.021	0.25±0.11	0.15±0.039
	level 3	0.38±0.090	0.52±0.19	0.53±0.055	0.79±0.036	0.14±0.016	0.32±0.096	0.29±0.094	0.22±0.027
	level 4	0.63±0.085	0.79±0.11	1.3±0.32	1.1±0.13	0.43±0.063	0.28±0.066	0.26±0.049	0.46±0.029
	level 5	2.7±0.051	1.7±0.20	2.0±0.29	3.1±0.12	0.38±0.090	0.52±0.19	0.53±0.055	0.79±0.036
C_{SPME} (μg/g OC)	level 1	0.020±0.012	0.013±0.0019	0.020±0.0060	0.043±0.0051	ND	0.0012±0.00019	0.0036±0.0013	0.0098±0.0022
	level 2	0.038±0.0051	0.025±0.0022	0.026±0.0019	0.060±0.0062	ND	0.0067±0.00015	0.0023±0.00023	0.012±0.0010
	level 3	0.049±0.0064	0.091±0.019	0.036±0.0026	0.12±0.027	0.020±0.0064	0.013±0.0019	0.020±0.0060	0.043±0.0051
	level 4	0.10±0.014	0.11±0.0071	0.19±0.026	0.17±0.024	0.038±0.014	0.026±0.0022	0.026±0.0019	0.060±0.0062
	level 5	0.29±0.048	0.45±0.036	0.26±0.024	0.35±0.064	0.050±0.048	0.091±0.019	0.036±0.0026	0.12±0.027
$C_{S\text{-}24h}/C_S$	level 1	0.93	16	0.93	4.3	5.4	1.0	1.2	5.0
	level 2	1.6	2.8	1.1	3.8	1.6	2.3	2.1	4.8
	level 3	0.95	2.2	0.83	2.4	0.93	4.6	1..0	3.0
	level 4	1.2	2.1	0.54	3.3	1.6	2.0	1.1	3.3
	level 5	1.9	1.8	0.57	3.8	0.95	2.2	0.91	3.6

C_{SPME}/C_S	level 1	0.13	0.65	0.071	0.84	-	0.025	0.024	0.54
	level 2	0.14	0.25	0.11	0.50	-	0.24	0.020	0.39
	level 3	0.12	0.38	0.060	0.52	0.13	0.19	0.071	0.59
	level 4	0.19	0.30	0.080	0.52	0.14	0.19	0.11	0.43
	level 5	0.21	0.48	0.074	0.43	0.13	0.38	0.062	0.55

Table S2. The survival (%) of organisms in toxicity testing.

Species	Particle-sizes	Conc.	Parallel 1	Parallel 2	Parallel 3	Parallel 4	Parallel 5
C.dilutus	<20 μm	level 1	100	90	90	90	100
		level 2	100	90	80	90	100
		level 3	70	60	70	60	60
		level 4	50	30	40	30	50
		level 5	30	10	20	10	30
	63–180 μm	level 1	100	80	100	100	80
		level 2	90	80	90	100	100
		level 3	70	90	70	70	80
		level 4	70	90	80	80	80
		level 5	40	60	50	70	60
	180–500 μm	level 1	100	80	90	100	100
		level 2	90	80	90	80	100
		level 3	70	70	50	70	100
		level 4	80	85	90	80	70
		level 5	50	70	60	50	70
	Original	level 1	100	100	90	90	100
		level 2	70	90	80	80	80
		level 3	70	80	80	80	90
		level 4	80	70	80	70	70
		level 5	40	40	40	20	30
H.azteca	<20 μm	level 1	70	90	80	90	70
		level 2	80	60	60	80	60
		level 3	50	60	40	40	50
		level 4	20	20	20	20	10
		level 5	0	0	0	0	0
	63–180 μm	level 1	90	90	100	90	70
		level 2	70	80	80	80	80
		level 3	70	70	80	70	80
		level 4	70	50	50	30	40
		level 5	20	0	0	0	10
	180–500 μm	level 1	80	80	90	100	100
		level 2	80	70	90	80	90
		level 3	60	80	80	80	80
		level 4	50	60	60	100	100
		level 5	50	40	40	100	100
	Original	level 1	80	100	70	80	80
		level 2	40	50	60	60	50

	level 3	20	20	20	20	10
	level 4	10	10	0	0	0
	level 5	0	0	0	0	0

Table S3. The average wet weight (mg/individual) of surviving organisms in each group at the end of the toxicity testing.

Particle-sizes	*C.dilutus*	*H.azteca*
<20 μm	12.0 ± 1.2	2.4 ± 0.5
63–180 μm	11.9 ± 2.4	2.0 ± 0.3
180–500 μm	11.7 ± 2.2	2.2 ± 0.4
Original	11.9 ± 4.2	2.0 ± 0.3

Table S4. The slope of the dose-response curves for *C.dilutus* and *H.azteca* based on the total sediment concentration (C_S), Tenax extractable concentration ($C_{S\text{-}24h}$) and SPME measured concentration (C_{SPME}) of cypermethrin with different sediment particle-scale.

Species	Particle-sizes	Slope of C_S	Slope of $C_{S\text{-}24h}$	Slope of C_{SPME}
C.dilutus	<20 μm	1.724 ± 0.1673	1.654 ± 0.1423	2.446 ± 0.1488
	63–180 μm	1.595 ± 0.1692	1.368 ± 0.1893	1.929 ± 0.1769
	180–500 μm	0.980 ± 0.0735	1.035 ± 0.0782	1.476 ± 0.1254
	Original	2.154 ± 0.1521	1.742 ± 0.1476	2.450 ± 0.1811
H.azteca	<20 μm	1.581 ± 0.1468	1.572 ± 0.1632	2.426 ± 0.1647
	63–180 μm	0.521 ± 0.1356	1.140 ± 0.1539	1.440 ± 0.2160
	180–500 μm	1.115 ± 0.1202	1.087 ± 0.1246	1.581 ± 0.2539
	Original	0.945 ± 0.1914	1.195 ± 0.1418	1.712 ± 0.1826

Table S5. LC50 values of *C. dilutus* and LC25 values of *H. azteca* were compared between different particle sizes based on the total sediment concentration (C_S), Tenax extractable concentration ($C_{S\text{-}24h}$) and SPME measured concentration (C_{SPME}) of cypermethrin. Different superscript letters indicate significant differences (p<0.05) of the ratio among the different concentrations, while the same letter indicates no significant differences.

Species	Ratio	Based on C_S (μg/g OC)	Based on $C_{S\text{-}24h}$ (μg/g OC)	Based on C_{SPME} (μg/g OC)
C. dilutus	$LC50_{<20\ \mu m}$ / $LC50_{original}$	1.3±1.8^{ab}	1.2±0.2^{a}	0.8±0.1^{b}
	$LC50_{63–180\ \mu m}$/ $LC50_{original}$	3.1±0.7^{a}	2.6±0.4^{ab}	2.2±0.2^{b}
	$LC50_{180–500\ \mu m}$/ $LC50_{original}$	6.7±1.0^{a}	4.2±0.5^{b}	3.4±0.3^{c}
	$LC50_{63–180\ \mu m}$/ $LC50_{<20\ \mu m}$	2.3±3.1^{ab}	2.2±0.4^{a}	2.8±0.3^{b}
	$LC50_{180–500\ \mu m}$/ $LC50_{<20\ \mu m}$	5.0±6.6^{ab}	3.6±0.5^{a}	4.3±0.4^{b}
	$LC50_{180–500\ \mu m}$/ $LC50_{63–180\ \mu m}$	2.2±0.4^{a}	1.6±0.1^{b}	1.5±0.1^{b}
H. azteca	$LC25_{<20\ \mu m}$ / $LC25_{original}$	0.4±0.3^{ab}	0.3±0.2^{a}	0.6±0.1^{b}
	$LC25_{63–180\ \mu m}$/ $LC25_{original}$	1.0±0.4^{ab}	1.3±0.3^{a}	0.7±0.1^{b}
	$LC25_{180–500\ \mu m}$/ $LC25_{original}$	1.1±0.4^{a}	1.0±0.2^{ab}	0.5±0.1^{c}
	$LC25_{63–180\ \mu m}$/ $LC25_{<20\ \mu m}$	2.6±1.8^{ab}	3.7±2.5^{a}	1.1±0.1^{b}
	$LC25_{180–500\ \mu m}$/ $LC25_{<20\ \mu m}$	2.8±1.8^{a}	2.9±1.9^{ab}	0.9±0.1^{c}
	$LC25_{180–500\ \mu m}$/ $LC25_{63–180\ \mu m}$	1.1±0.4^{a}	0.8±0.2^{a}	0.8±0.1^{a}

Table S6. Differences in toxicity between metabolites and parents of pyrethroid.

Species		Chemicals	Description of toxicity	Reference
Eisenia fetida	Parents	*α*-cypermethrin	α-CYP had significant effects on EC10, EC50, NOEC values (the end point of effect was reproduction) and LC50 values of *E. fetida* *E. fetida* tends to accumulate metabolites, which are difficult to excrete and may reach concentrations that cause sublethal toxic effects	2
	Metabolites	Hydroxy-cypemethrin; 3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylic acid		
Rana catesbeiana	Parents	*α*-cypermethrin	Parents and metabolites were detected in bullfrog organs by oral and water exposure. 3-PBA is an endocrine disrupting compound that may lead to reduced adult fitness or mortality	3
	Metabolites	Cis-3-(2',2-dichlorovinyl)−2,2-dimethylcyclopropane carboxylic acid (cis-DCCA); 3-phenoxybenzoic acid (3-PBA)		
Danio rerio	Parents	Deltamethrin	Both parents and metabolites lead to increased embryo/larval mortality, delayed hatching time, reduced hatching rate, increased heart rate, and deformed tail. But deltamethrin is more toxic than 3-PBA.	4
	Metabolites	3-PBA		
Danio rerio	Parents	Permethrin and *β*-cypermethrin	Both parents and metabolites showed significant developmental and neurotoxicity, which caused abnormal vascular development and altered the motor activity of the larvae. β-cypermethrin was more toxic than permethrin, and PBCHO was the most toxic.	5
	Metabolites	(3-phenoxybenzoic alcohol (PBCOH); (4-3-phenoxybenzaldehyde (PBCHO); 3-PBA		

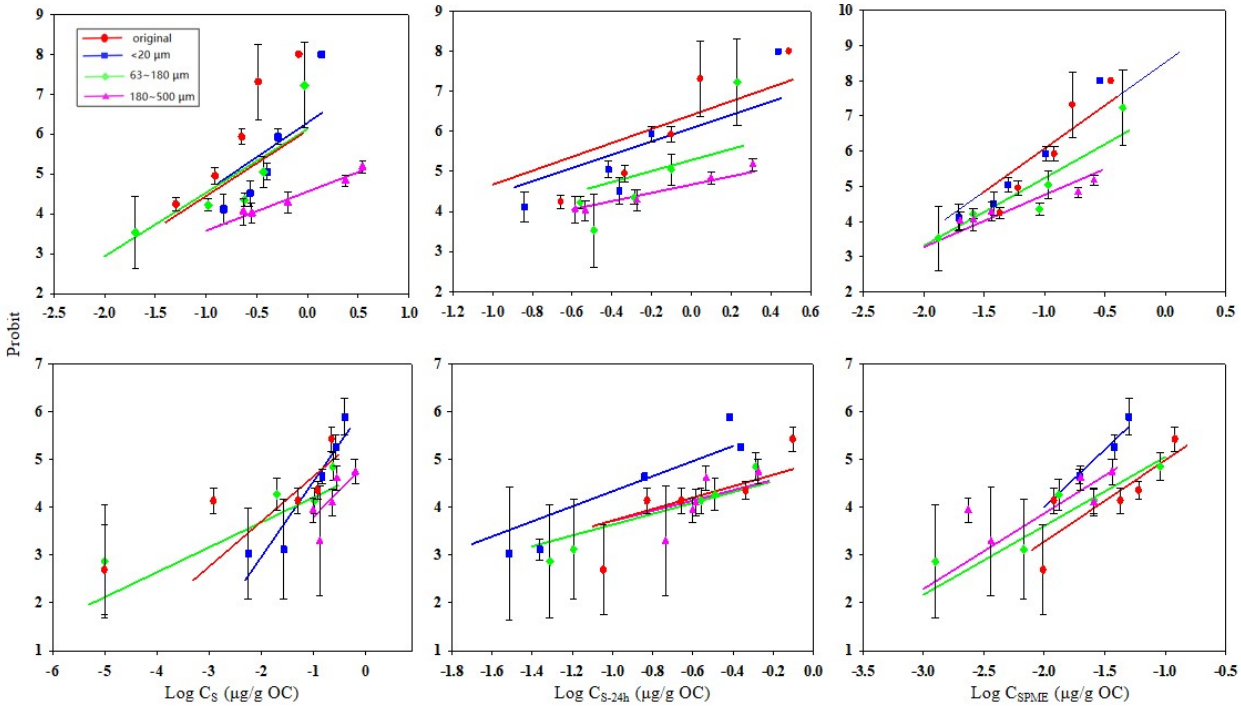

Fig. S1. The dose-response curves for *C.dilutus* (in the above) and *H.azteca* (in the bottom) based onthe total sediment concentration (C_S), Tenax extractable concentration ($C_{S\text{-}24h}$) and SPME measured concentration (C_{SPME}) of cypermethrin with different sediment particle-scale.

REFERENCES

1. D. P. Weston, J. You and M. J. Lydy, Distribution and toxicity of sediment-associated pesticides in agriculture-dominated water bodies of California's Central Valley, *Environ. Sci. Technol.*, 2004, **38**, 2752-2759.
2. T. Hartnik and B. Styrishave, Impact of biotransformation and bioavailability on the toxicity of the insecticides α-cypermethrin and chlorfenvinphos in earthworm, *Journal of Agricultural and Food Chemistry*, 2008, **56**, 11057-11064.
3. G. Yao, X. Jing, C. Liu, P. Wang, X. Liu, Y. Hou, Z. Zhou and D. Liu, Enantioselective degradation of alpha-cypermethrin and detection of its metabolites in bullfrog (*rana catesbeiana*), *Ecotoxicol. Environ. Saf.*, 2017, **141**, 93-97.
4. R. S. Kuder and H. P. Gundala, Developmental toxicity of deltamethrin and 3-phenoxybenzoic acid in embryo–larval stages of zebrafish (*Danio rerio*), *Toxicol. Mech. Methods*, 2018, **28**, 415-422.
5. C. Xu, X. Li, M. Jin, X. Sun, L. Niu, C. Lin and W. Liu, Early life exposure of zebrafish (*Danio rerio*) to synthetic pyrethroids and their metabolites: A comparison of phenotypic and behavioral indicators and gene expression involved in the HPT axis and innate immune system, *Environ. Sci. Pollut. Res.*, 2018, **25**, 12992-13003.