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APPLICATION OF SPECIES SENSITIVITY DISTRIBUTION IN AQUATIC PROBABILISTIC ECOLOGICAL RISK ASSESSMENT OF CYPERMETHRIN: A CASE STUDY IN AN URBAN STREAM IN SOUTH CHINA

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Abstract: A tiered ecological risk assessment was applied to quantitatively refine the overall probabilistic risk of cypermethrin, a pyrethroid insecticide, to aquatic organisms. These results were then validated through the bioassays using field water from an urban stream, Chebei Creek in Guangzhou, South China. Seventeen water samples were collected along Chebei Creek for evaluation. In total, 71% of the field waters were acutely toxic to *Hyallela azteca* and 24% of the waters caused 100% mortality. Toxic unit evaluation suggested that cypermethrin was one of the main contributors to toxicity. The tiered ecological risk assessment approach (deterministic quotient method and probabilistic methods, including joint probability curve and Monte Carlo Simulation) suggested that cypermethrin posed significant threats to aquatic ecology in this stream. The overall probabilistic risk of cypermethrin to aquatic species in Chebei Creek reached 66% when acute-to-chronic ratios were set at 125. An exceedance probability of cypermethrin in Chebei Creek that affected *H. azteca* as modeled using the joint probability curve method was 88%, suggesting that most sites were at risk due to cypermethrin exposure. This value was similar to the results obtained from acute toxicity tests (71% of field water samples were acutely toxic to *H. azteca*), indicating the effectiveness of the tiered approach to assess risk of cypermethrin in urban waterways. To the authors' knowledge, the present study is the first to provide a focused probabilistic evaluation of ecological risk for cypermethrin in a complex urban waterway environment. Despite uncertainties existing in the ecological risk assessment procedure, this approach provides a comprehensive assessment of ecological risk of cypermethrin, and subsequently, a foundation for further risk diagnosis and management in urban waterways. *Environ Toxicol Chem* 2015;34:640–648. © 2014 SETAC

Keywords: Cypermethrin Probabilistic ecological risk assessment Urban waterways South China

INTRODUCTION

Ecological risk assessment has been developing continually over the past 30 yr to provide science-based information for risk management and decision-making processes [1,2]. The simplest and perhaps most widely used ecological risk assessment approach is the deterministic hazard quotient method, which provides a conservative screening-level risk assessment for chemicals. The advantages of this approach are in its simplicity, its transparency, and its few data requirements [2]. However, this point estimation fails to quantify the likelihood and magnitude of the risk. As a result, probabilistic risk assessment techniques have been developed to quantitatively characterize the variability and uncertainty in exposure and effect determination [1–6]. Furthermore, a tiered ecological risk assessment, which employs multiple approaches (including the deterministic method and probabilistic methods), has been recommended to produce a more comprehensive risk assessment [2,5].

Species sensitivity distributions were proposed in the late 1970s [7] to describe the variations in sensitivity of species to environmental stressors [8]. The species sensitivity distribution is a statistical distribution constructed by fitting a cumulative distribution function to a series of species toxicity data against the rank-assigned centile [9]. From the cumulative distribution, hazardous concentrations (HC) can be calculated, which provide the percentage of species expected to be affected; for instance, an HC5 value in species sensitivity distribution based on chronic toxicity data presents the probability of 5% of species being affected and has been commonly used to derive the water quality guidelines [10–13]. In addition, species sensitivity distributions are refined in the form of probabilistic risk assessment techniques, which combine the effect data with the distributions of environmental exposures of contaminants. This in turn better characterizes the probability of exceedance of exposures and more accurately predicts the probability of effects. Traditional probabilistic ecological risk assessment uses only measured or predicted exposure data and literature values on effects, whereas data on observed toxicity for the test samples are typically not applied or not available. This evaluation on effects could provide additional weight to the risk assessment results and further strengthen the validity of the analysis. To date, probabilistic ecological risk assessments have been conducted successfully for various legacy contaminants (e.g., polycyclic aromatic hydrocarbons [6], organochlorine pesticides [5], and trichlorobenzene [2]); studies on emerging contaminants, however, have been limited [14].

Pyrethroids have been used extensively in agricultural settings as well as in professional and household control of pests in urban areas. As replacements of organophosphate insecticides, pyrethroids account for approximately 17% of the current worldwide insecticide usage [15]. This shift to insecticides with less mammalian toxicity (e.g., pyrethroids) has also occurred in China, and cypermethrin has been reported to be the prevailing pyrethroid in Asia [16,17]. These reports of usage are consistent with the literature; cypermethrin has been the most frequently detected pyrethroid in various media in China, including air [18], agricultural drainage waters [19], and urban waterway

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sediments [20,21]. In addition, recent work conducted in the Pearl River Delta in South China identified cypermethrin as the primary toxicant of concern to benthic organisms in urban waterways [21–23]. To date, no studies have extended beyond evaluations of environmental residues and toxicological effects to assess the overall probabilistic risk of cypermethrin in urban waterways in China.

The objective of the present study was to apply a tiered probabilistic ecological risk assessment approach to refine the overall risk of cypermethrin in urban waterways in South China. The following 3 steps were performed to reach this objective: 1) establish a species sensitivity distribution curve for cypermethrin using the exposure–effect data gathered from US Environmental Protection Agency (USEPA) ECOTOX database; 2) perform a tiered probabilistic ecological risk assessment of cypermethrin in an urban stream of South China; and 3) validate the results of probabilistic risk of cypermethrin using bioassays with *Hyallela azteca* with field waters of this stream.

MATERIALS AND METHODS

Water sampling, toxicity, and chemical analysis

Chebei Creek was chosen as a representative urban stream in Guangzhou, South China, to assess the probabilistic risk of cypermethrin to aquatic ecosystems for several reasons. First, Chebei Creek is a 25.4-km watershed with multiple inputs, receiving agricultural, residential, and industrial discharges. The heavily-influenced characteristics of this stream are expected to be similar to the other 231 urban streams of Guangzhou. Second, the profile of sediment-associated pyrethroids and the corresponding toxicity of pyrethroids to aquatic organisms were similar for Chebei Creek sediments and other urban waterway sediments in the Pearl River Delta in South China [20,21,23]. Last, Chebei Creek is one of the many streams that discharge into the Pearl River (the largest river in South China); therefore, evaluation of risk in Chebei Creek could provide the foundation for future risk assessment work in the Pearl River itself.

Seventeen surface water samples were collected from sampling sites along Chebei Creek in March of 2011 (Figure 1). Water was collected in 2.5-L brown glass jars, transported to the laboratory, and stored at 4 °C until use. The coordinates and brief descriptions of the sampling sites are included in a previous study by Li et al. [21].

Acute 48-h toxicity testing was performed with *H. azteca* immediately after water samples were brought into the laboratory. Testing was conducted with 5 replicates per sample water. In each 60-mL jar, 1 g of sand, 20 mL of field water, and 5 organisms were added. Reconstituted water prepared according to USEPA protocol [24] was used as control water. Toxicity testing was conducted under a 16:8-h light:dark cycle at 23 ± 1 °C. No water change or feeding was performed during the testing. Water quality parameters—including dissolved oxygen, conductivity, temperature, and pH—were monitored on a daily basis. At the termination of the bioassay, survival was enumerated. Meanwhile, the immobility of the surviving amphipods was compared with that of control organisms to diagnose the impairment of mobility.

Contaminants in dissolved and suspended particle phases were assessed in field waters. To separate dissolved and particle phases, 1 L of water was filtered through a 0.45-μm glass fiber filter (Whatman), which was baked at 450 °C for 4 h prior to use. We attempted to extract contaminants in the dissolved phase using liquid-liquid extraction with dichloromethane, but the extraction failed due to severe emulsification in the solution. Even with the addition of NaCl and extended settling time, phase separation in extraction was not possible. Because of the short time frame required to complete water analysis (within 14 d [25]), an additional assay was not conducted to obtain the aqueous concentrations. As such, the total concentrations of contaminants in water samples were calculated based on the concentrations of cypermethrin in suspended particles

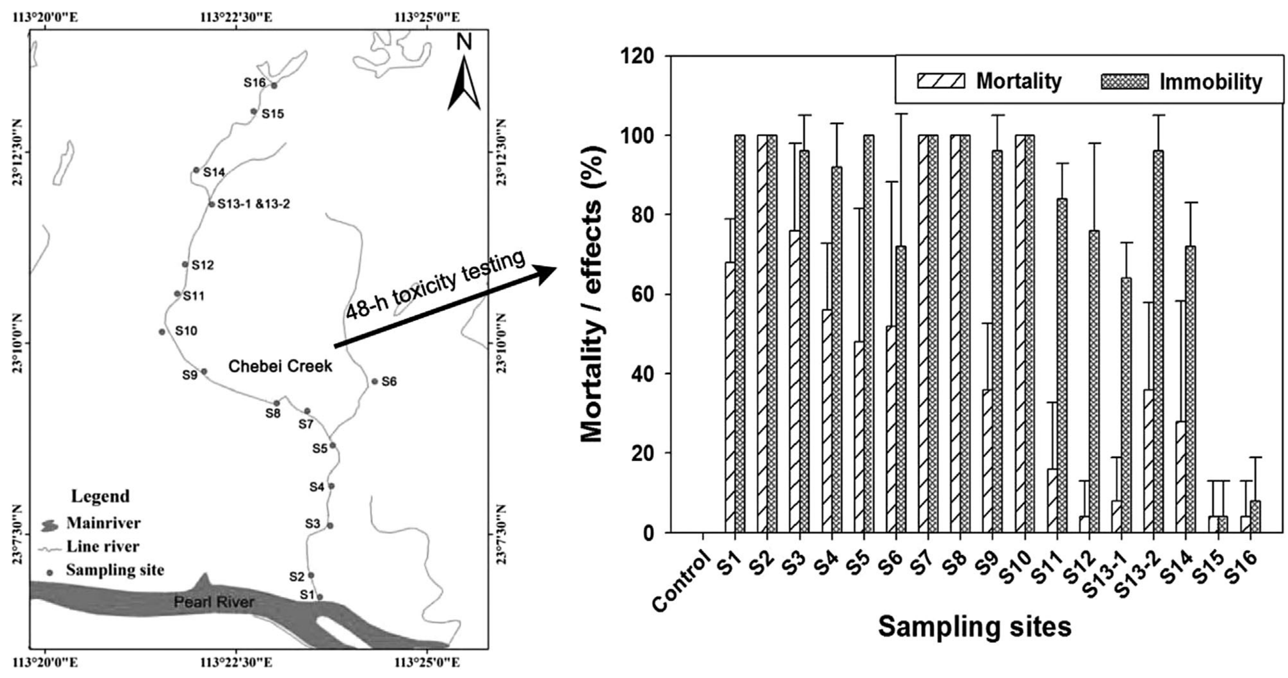

Figure 1. Map of sampling sites and 48-h acute toxicity to *Hyallela azteca* (mortality and sublethal effect of immobility) of water samples from Chebei Creek in Guangzhou, China. The error bars represent the standard deviations of the percentages ($n = 5$). The toxicity in most field water samples was significantly different from the controls ($p < 0.05$), except for 5 sites (S11, S12, S13–1, S15, and S16) for mortality and 2 sites (S15 and S16) for immobility.

(additional descriptions in *Data analysis*). Toxicity tests and chemical analysis were completed within 1 wk after sampling.

Contaminants in suspended particles were extracted using a CW-2000 ultrasound-assisted microwave extractor (Xintuo Company). Freeze-dried glass fiber filter membrane which contained the suspended particles, 100 mL of hexane:acetone (1:1, v/v), 2 g of copper powder, and 50 ng of analytical surrogates (4,4'-dibromooctafluorobiphenyl and decachlorobiphenyl) were added to a 250-mL flask and extracted for 6 min. The microwave and ultrasound power were set at 100 W and 50 W, respectively. The extraction was repeated once with 50 mL of fresh extraction solution. The extracts were combined, filtered, concentrated, and solvent exchanged to hexane. Solid phase extraction cartridges packed with primary-secondary amine and granular black carbon (Supelco) were used to purify the extracts. After the cartridge was conditioned with hexane and the extract was loaded, 7 mL of a mixture of hexane and dichloromethane (7:3, v/v) was used as the elution solvent. The cleaned sample was concentrated, solvent exchanged to hexane, and then analyzed on a gas chromatograph–mass spectrometer (GC/MS) for target compounds after adding the internal standard (d6-*trans*-cypermethrin). Quality control samples, including control blanks and matrix-spiked water and glass fiber filter membrane samples, were analyzed simultaneously with field waters.

Previous studies suggested that pyrethroids, especially cypermethrin, were the main toxicity contributors to benthic organisms, such as *Chironomus dilutus* and *H. azteca* in Pearl River Delta waterways [21–23]. Therefore, chemical analysis in the present study focused solely on pyrethroids. Nine pyrethroids, including: bifenthrin, cyfluthrin, *lambda*-cyhalothrin, cypermethrin, deltamethrin, esfenvalerate, fenpropathrin, permethrin, and tefluthrin, were analyzed using a Shimadzu QP-2010-plus series GC/MS in negative chemical ionization (NCI) mode. A DB-5HT column (15 m × 0.25 mm; 0.1 μm film thickness) was used to separate the analytes. Helium at a flow rate of 1.5 mL/min and methane were used as carrier gas and NCI reaction gas, respectively. The temperatures of ion source and transfer line were set at 250 °C and 280 °C, respectively. The initial oven temperature was set at 60 °C, held for 1 min, heated to 200 °C at 10 °C /min, heated to 220 °C at 3 °C /min, held for 8 min, then ramped to 300 °C at 50 °C /min, and held for 15 min. A programmable temperature vaporizing injection was used, and the initial injector temperature was set at 60 °C, held for 0.1 min, then quickly heated to 280 °C at 300 °C /min, and held for 17 min.

Collection of toxicity data

Published data of toxicity for cypermethrin to aquatic species were gathered from the ECOTOX database [26]. Use of chronic toxicity data in developing species sensitivity distributions has been recommended [27]; however, few chronic toxicity data are available for current-use pesticides, such as cypermethrin. Therefore, the available acute median lethal concentrations (LC50s) and median effect concentrations (EC50s) were collected and acute-to-chronic ratios (ACRs) [3] were applied as an alternative method to derive the species sensitivity distribution values in the present study. Freshwater species of algae, crustaceans, insects, and fish were chosen as representative taxonomic groups for establishing species sensitivity distributions. The endpoints were limited to LC50s and EC50s focusing on immobility with test duration of less than 10 d for all species. Furthermore, values that were 2 orders of magnitude different from other studies for the same species were considered outliers and removed from the list. In total, 257 toxicity values were obtained for 88 species. If a species had more than one toxicity data point available, the geometric mean of available data was applied for species sensitivity distribution simulation [5]. A summary of the selected toxicity data for the establishment of species sensitivity distribution is presented in the Supplemental Data, Tables S1 and S2.

Tiered ecological risk assessment of cypermethrin in the urban stream

The USEPA Ecological Committee on FIFRA Risk Assessment (ECOFRAM) proposed a draft report [4] on terrestrial risk assessment and divided the risk assessment methods into 3 categories in the order of increasing complexity and realism, namely deterministic quotients, methods involved in comparing the distribution of exposure with a fixed value or a distribution of effect, and methods incorporating functions to integrate distributions of exposure and effect. Based on this protocol, a 3-tiered approach was proposed to generate a probabilistic ecological risk assessment of pesticides for non-target species [5]. This tiered approach was adopted in the present study to perform an ecological risk assessment for cypermethrin in an urban stream. The various levels, including deterministic methods and probabilistic methods that were employed in the ecological risk assessment, are discussed below.

Level 1. In this level, hazard quotients were calculated by dividing point values of exposure by effect. Contaminant concentrations in waters of Chebei Creek represented point values of exposure, and effect values were determined by using HC5 values at various ACRs derived from the species sensitivity distribution. Significant variability in ACRs has been observed among species and chemicals with different modes of action [28], and wide range of ACRs from 1 to more than 18 000 has been reported for organic chemicals. For 93% of the chemicals, however, the mean ACR was equal to or less than 25 [29]. Thus, Kenaga [29] suggested using an ACR of 25 or less to predict the chronic toxicity from acute toxicity data. For cypermethrin, specifically, Shen et al. [30] reported that its ACRs for *Ceriodaphnia dubia* varied for different toxicity endpoints (e.g., 14.3, 66.6, and 33.3 for survival, growth, and reproduction, respectively). Therefore, an ACR of 25 along with lower and upper limits (10 and 125, respectively) were employed in the present study to better elucidate the uncertainties associated with varying ACRs. Also, an ACR of 1, which directly linked the observed acute effect values (LC50 or EC50) to the exposure data, was included to assess the acute toxicity of cypermethrin to aquatic species.

Level 2. In level 2, the distribution of water concentrations was used to calculate the probability that exposure levels exceeded the preselected effect levels as determined in *Level 1* (e.g., HC5 values at various ACRs). Potential affected fraction, which shows the proportion of affected species at a certain exposure concentration, was also estimated based on this information

Level 3. Two methods were proposed to evaluate the probabilistic risk of cypermethrin in Chebei Creek, namely level 3–1 (joint probability curve) and level 3–2 (distribution-based hazard quotient). In level 3–1, exposure and effect distributions were integrated to establish a joint probability curve, and it was derived from the exceedance probability function (the reverse cumulative distribution of exposure) and species sensitivity distribution [1–3,5,6]. The joint probability curve was applied to assess the probability of exceeding the concentration for a particular degree of effect and vice versa (i.e., the proportion of affected species for a particular exceedance of the concentration. The overall ecological risk was quantitatively characterized using the overall risk probability as suggested in Wang et al. [5]. The overall risk probability was calculated as the area under the joint probability curve using the following equation [5].

$$\mathrm{ORP} = \int_0^1 \mathrm{EPr}(x)dx \qquad (1)$$

where ORP is the overall risk probability, EPr (x) is the exceedance probability for x percent of species affected ($0 \leq x \leq 1$). As discussed, various ACRs were introduced to take different protection levels into account; therefore, a series of joint probability curves at the preselected 4 ACRs was attained herein.

In level 3–2, a distribution-based hazard quotient was calculated. This hazard quotient characterized the risk by calculating the probability of exceeding the preselected hazard quotients that corresponded to the aforementioned series of ACRs. A Monte Carlo analysis (Cristal Ball, Ver 11.1; Oracle) was employed to estimate the probability of exceedance by simulating the distributions of exposure and effect after 20 000 simulations.

Data analysis

Water toxicity was determined by comparing the toxicity (mortality and immobility) of the amphipods in field waters with control organisms using a student's *t*-test. A significant difference of $p < 0.05$ indicated that test waters were acutely toxic to *H. azteca*. Toxic units (TU) were calculated for individual pyrethroids to evaluate their toxicity contribution. The toxic unit was calculated by dividing the total concentration of a contaminant in water (C_t) by its LC50 to *H. azteca* as found in the literature [31]

$$\mathrm{TU} = \frac{C_t}{\mathrm{LC50}} \qquad (2)$$

The total concentration was determined as the sum of concentrations in the dissolved phase (C_d) and in the suspended particles (C_p; Equation 3). As mentioned previously, C_d was not able to be analytically determined due to experimental failure, thus it was computed from C_p using Equation 4 with the assumption that the partitioning of the chemical between the dissolved and suspended particle phases reached equilibrium

$$C_t = C_d + C_p \qquad (3)$$

$$C_d = \frac{C_p}{K_d[\mathrm{SS}]} \qquad (4)$$

where [SS] is the amount of suspended particles in a sample and K_d is the partition coefficient between the suspended particle and water. The K_d values for pyrethroids were derived from the literature [32].

The species sensitivity distribution curves of cypermethrin were simulated using BurrliOZ program (Ver I.O. 14; CSIRO) [33]. A 3-parameter Burr type III distribution was applied in the BurrliOZ program, and these parameters (b, c, and k) were used to calculate HC5 values with 95% confidence intervals and potential affected fraction values. As the 3 parameters approach their limiting values, the Burr type III distribution moves toward other limiting distributions. For example, if k becomes exceedingly large (>100), the Burr type III distribution tends toward the Reciprocal Weibull distribution, whereas if c becomes exceedingly large (>80), it tends toward a Reciprocal Pareto distribution [10]. Consequently, the most appropriate model can be selected using Equations 5 through 7

$$\text{Burr type III}: F(x) = \frac{1}{[1 + (b/x)^c]^k} \qquad (5)$$

$$\text{Reciprocal Weibull}: F(x) = \exp(-\alpha/x^\beta) \qquad (6)$$

$$\text{Reciprocal Pareto}: F(x) = \exp(x/x_0)^\theta \qquad (7)$$

where, x is the exposure concentration (μg/L) and b, c, k, α, β and θ are parameters for the distributions.

RESULTS AND DISCUSSION

Pyrethroid insecticides in water

No target pyrethroids were detected in laboratory control waters, and the recoveries of the target pyrethroids in matrix spikes were 117 ± 33%. The recoveries of the 2 surrogates, 4,4'-dibromooctafluorobiphenyl and decachlorobiphenyl, which were added to the suspended particle samples before extraction, were 69 ± 17% and 97 ± 25%, respectively. Four pyrethroids, including bifenthrin, *lambda*-cyhalothrin, cypermethrin, and permethrin, were detected above the reporting limit in at least 1 field water sample. The C_t of target pyrethroids (which were computed from C_p using Equations 3 and 4) and C_p are presented in Table 1 and Supplemental Data, Table S3, respectively.

Excluding the headwaters of Chebei Creek (S16), in which no pyrethroids were detected, the other 16 samples had at least 1 of the 4 pyrethroids detected at concentrations greater than the reporting limit; the following discussion is based on these 16 sites. Sum concentrations of pyrethroids (Σpyrethroid) in Chebei Creek ranged from 2.01 ng/L to 109 ng/L. The profile of pyrethroid residues in the field waters was similar to that in bottom sediments in this area [20,21], as cypermethrin was the predominant pyrethroid detected in water. Cypermethrin accounted for 64% of the Σpyrethroid, with cypermethrin concentrations ranging from 2.01 ng/L to 71.5 ng/L. Cypermethrin residues in Chebei Creek waters were similar to those in drainage channel water in South China (<reporting limit to 220 ng/L) [19]. Compared with concentrations found in other studies, concentrations in Chebei Creek were slightly greater than those in urban runoff in California (<reporting limit to 12.3 ng/L) [34]; however they were much lower than that in floodwater (106–710 ng/L) and runoff (<50–490 ng/L) in agricultural streams of Argentina [35].

The toxicity of Chebei Creek waters to *H. azteca* is presented in Figure 1. The water parameters, including dissolved oxygen, conductivity, temperature and pH in water were 4.9 ± 1.0 mg/L, 334 ± 21 μS/cm, 23 ± 1 °C, and 7.2 ± 0.3, respectively. No adverse effects (for either lethality or immobility) were observed for *H. azteca* in control replicates. On the contrary, 71% of field waters from Chebei Creek showed significant mortality, and 24% of field waters caused 100% mortality to the amphipods. In addition, 88% of field waters showed significant

Table 1. The total water concentrations (C_t, ng/L) of detected pyrethroids in Chebei Creek in Guangzhou, China

Sample site	Bifenthrin	*lambda*- Cyhalothrin	Cypermethrin	Permethrin	Σpyrethroid	%Cypermethrin[b]
S1	<RL[a]	5.70	27.1	14.0	46.8	58
S2	2.76	9.29	41.6	22.8	76.4	54
S3	2.26	8.66	34.8	21.7	67.4	52
S4	1.64	7.14	38.9	20.6	68.3	57
S5	1.44	5.00	25.3	9.98	41.7	61
S6	<RL	<RL	18.2	13.0	31.3	58
S7	2.54	16.2	71.5	18.8	109	66
S8	2.13	8.73	44.3	13.3	68.5	65
S9	<RL	<RL	4.22	<RL	4.22	100
S10	<RL	13.3	26.7	3.67	43.6	61
S11	<RL	1.18	15.9	11.3	28.4	56
S12	<RL	1.78	20.8	38.8	61.4	34
S13–1	<RL	1.38	16.4	9.30	27.1	61
S13–2	<RL	1.32	19.0	15.4	35.7	53
S14	<RL	1.14	18.8	1.81	21.8	86
S15	<RL	<RL	2.01	<RL	2.01	100
S16	ND	ND	ND	ND	ND	

[a]Below the reporting limit (RL). The reporting limit of the total concentration in water was based on the reporting limit in the suspended particles, which was calculated by multiplying the lowest concentration of the calibration standards by the volume of the cleaned extract, and then dividing it by the volume of the water extracted. The reporting limit in suspended particles was 1 ng/L.
[b]Percent based on cypermethrin concentration over the sum concentration of detected pyrethroids.
ND = not detected.

impairment to the behavior of *H. azteca*, whereas 35% of the field waters caused swimming impairment to all of the test organisms for a given site (Figure 1). Spatially, highly toxic waters were generally located near residential areas in the middle and downstream areas of Chebei Creek, which was consistent with previous results of sediment toxicity in this stream [21,22]. The estimates of toxic unit approach suggested that cypermethrin was the main toxicity of concern to *H. azteca* in Chebei Creek waters because of the high detection frequency and concentration of cypermethrin in field waters as well as its status as the most toxic pyrethroid of those monitored. As shown in Figure 2, a significant relationship between exposure (toxic unit of cypermethrin) and effect (mortality presented as probit) was observed, suggesting that cypermethrin plays a large role in the observed toxicity to *H. azteca* in Chebei Creek waters.

Ecological risk of cypermethrin

The species sensitivity distribution curves of cypermethrin for algae, crustaceans, insects, and fish are shown in Figure 3.

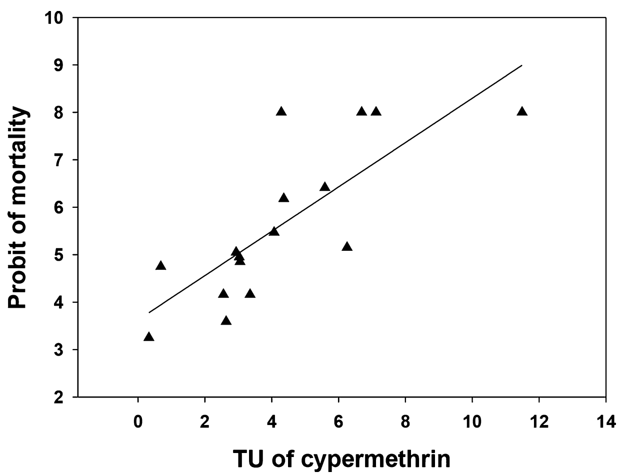

Figure 2. The relationship between cypermethrin-based toxic unit (TU) and mortality to *Hyallela azteca* as presented using probit for the waters in Chebei Creek in Guangzhou, China. The equation of the regression line is Probit = 0.46 TU + 3.62, $r^2 = 0.61$, $p < 0.001$.

The simulated parameters and calculated HC5 values at 4 ACRs are presented in Table 2. The species sensitivity distribution curve of crustaceans suggested that they, as a taxonomic group, were generally the most sensitive to cypermethrin (the curve was the closest to the *y*-axis), followed by insects, fish, and algae. The sensitivity ranking was also observed by examining their corresponding HC5 values at an ACR of 1 (0.005 μg/L, 0.019 μg/L, 0.70 μg/L, and 280 μg/L, respectively). The HC5 values of algae were much higher than the other 3 taxa, as expected, because the mode of action for cypermethrin is not applicable in plants. Overall, the predicted HC5 at an ACR of 1 was 0.013 μg/L by considering the 4 taxonomic groups. Maltby et al. [8] reported similar HC5 values of cypermethrin of 0.003 μg/L and 0.17 μg/L to arthropods and vertebrate communities, respectively.

Level 1. The calculated geometric mean ($HQ_{geomean}$) and maximum (HQ_{max}) hazard quotients for each taxonomic group

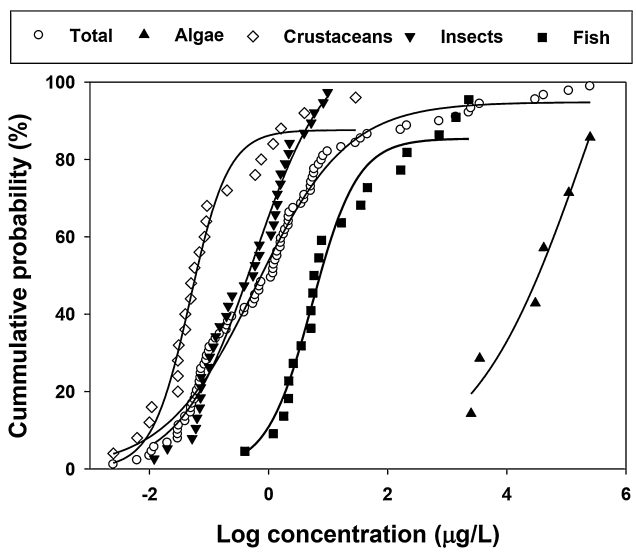

Figure 3. The species sensitivity distribution curves of cypermethrin for all examined species (total), algae, crustaceans, insects, and fish based on the acute toxicity data.

Table 2. The results for the 3 simulation types used as well as values for the parameters used for the species sensitivity distribution curves for assessing cypermethrin risk in Chebei Creek in Guangzhou, China[a]

			Parameter								HQ (Level 1)		PAF (Level 2)		
Species	ACR	Simulation type	*b*	*c*	*k*	x_0	θ	α	β	HC5 (µg/L)	Geomean	Max	Geomean	Max	Probability (Level 2)
Total															
	1	BurrIII	0.007	0.47	5.4					0.013	1.6	5.6	8%	21%	88%
	10	BurrIII	0.0004	0.46	6.8					0.001	15.3	53.5	37%	56%	100%
	25	BurrIII	0.0002	0.46	6.8					0.0005	38.5	134	52%	68%	100%
	125	BurrIII	0.00003	0.46	6.8					0.0001	195	681	73%	83%	100%
Algae															
	1	RePareto				251 000	0.44			280	0.00	0.00	0%	0%	0%
	10	RePareto				25 100	0.59			157	0.00	0.00	0%	0%	0%
	25	RePareto				10 040	0.59			62.9	0.00	0.00	0%	0%	0%
	125	RePareto				2008	0.59			12.6	0.00	0.01	0%	0%	0%
Crustaceans															
	1	BurrIII	0.004	0.65	5.1					0.005	4.1	14.1	24%	50%	88%
	10	BurrIII	0.00014	0.63	8.5					0.0005	37.4	130	70%	85%	100%
	25	BurrIII	0.00011	0.63	8.5					0.0004	472	1647	93%	97%	100%
	125	BurrIII	0.00006	0.63	8.5					0.0002	92.8	323	100%	100%	100%
Insects															
	1	BurrIII	0.086	0.77	2.5					0.019	1.1	3.7	3%	15%	88%
	10	BurrIII	0.009	0.77	2.4					0.003	7.4	25.8	36%	65%	94%
	25	BurrIII	0.003	0.77	2.4					0.001	18.5	64.6	58%	80%	100%
	125	BurrIII	0.0007	0.77	2.4					0.0002	92.7	323	84%	93%	100%
Fish															
	1	ReWeibull						2.5	0.56	0.70	0.03	0.10	0%	0%	0%
	10	ReWeibull						0.68	0.56	0.070	0.29	1.01	0%	5%	6%
	25	ReWeibull						0.41	0.56	0.028	0.73	2.5	3%	17%	31%
	125	ReWeibull						0.17	0.56	0.006	3.64	12.7	23%	48%	88%

[a]Level 1 data shown consists of the hazardous concentration for 5% of the species (HC5) and the calculated hazardous quotient (HQ) based on both geometric mean (Geomean) and maximum value (Max). The potential affected fraction (PAF) based on geometric mean (Geomean) and maximum concentrations (Max), and the probability of the environmental concentrations exceeding the corresponding HQ at various acute-to-chronic ratios (ACRs) is also presented. Toxicity data at various ACRs was derived from median lethal (LC50) and median effect (EC50) concentrations in the literature.

at different ACRs are presented in Table 2. In general, hazard quotient > 1 suggests potential ecological effects [5]. Therefore, cypermethrin posed potential risk to the aquatic community in Chebei Creek with $HQ_{geomean}$ and HQ_{max} being 1.6 and 5.6, respectively (ACR = 1). Individually, crustaceans showed the highest risk with hazard quotients >1 even at the low ACR of 1, implying acute toxicity. Although the insects did not show as at risk as the crustaceans, the hazard quotients of insects were greater than 1 when the ACR was set at 10 or greater, suggesting potential risk to these taxa as well. Cypermethrin only posed chronic risk to fish communities as the HQ_{max} was 2.5 at an ACR of 25. Conversely, hazard quotients were all < 0.01 for algae even at an ACR of 125, suggesting cypermethrin would not cause any direct risks to algae communities in Chebei Creek. However, Friberg-Jensen et al. [36] and Wendt-Rasch et al. [37] demonstrated that indirect adverse effects of cypermethrin on abundance and community structure may be evident in algae species due to the co-dependent relationship between planktonic and periphytic alga and those of crustacean and other invertebrate communities.

The hazard quotient method is commonly used as a screening stage of the ecological risk assessment process. This screening provides a quick measure of potential risk based on limited information (e.g., geometric mean and maximum concentrations); however, these point estimates offer neither information on a spatial scale nor quantitative information on probability and magnitudes of effects. Therefore, additional tiers of assessment were conducted.

Level 2. The probabilities of environmental exposure levels exceeding the HC5 values at the 4 selected ACRs were calculated (Table 2). As suggested by Wang et al. [5], a probability greater than 10% indicated potential risk. Using the 3 trophic levels (algae, invertebrates, and fish) examined, 88 % of the water samples (14 of 16 sites) collected in Chebei Creek were projected to cause acute effects (ACR = 1) to more than 5% of aquatic species at the site. In addition, using those same 3 trophic levels, all 16 water samples from Chebei Creek were projected to cause chronic effects (ACR ≥ 10). For crustaceans and insects, 88% of Chebei Creek field waters would pose acute risk (ACR = 1) and more than 94% would pose chronic risk (ACR ≥ 10) based on the ecological risk assessment calculations. The results for algae and fish were similar to the estimates attained in level 1. Chebei Creek waters exhibited no direct risk to algae, and chronic risk to fish only in certain areas (i.e., 6% of the collected field waters were projected to cause chronic effects to fish when the ACR was set at 10).

In addition to probability distribution of exposure, the probability of effect expressed as potential affected fraction was also calculated (Table 2). The geometric mean concentration of cypermethrin in the site waters would be expected to pose potential risk to 8% of the species evaluated when the ACR equaled 1, and the risk reached a much higher level (73%) when the ACR increased to 125. Perhaps more alarming was that potential affected fraction reached 100% and 84% for crustaceans and insects, respectively, when the ACR increased to 125. Similar to the distribution of exposure results noted above, potential affected fractions were zero for algae, indicating no direct adverse effects to algae. For fish, at an ACR = 125, geometric mean and maximum potential affected fractions were 23% and 48%, respectively, indicating only potential chronic effects to fish, which were similar to the results discussed above.

The 2 approaches used in the level 2 assessment present 2 dimensions of calculating the magnitudes of risk in Chebei Creek and are considered to be relatively complementary to one another. The probability distribution for exposure analysis demonstrates the relative extent of risk in the studied area by considering the spatial variability of exposures, whereas the probability for effect analysis reveals the intensity of toxic effects regardless of spatial scale [2]. However, potential affected fractions were calculated only on certain exposure levels (e.g., geometric and maximum concentrations), and the overall variability and uncertainty of ecological effects on the whole watershed were not taken into consideration in this level of assessment.

Level 3. To gain a better understanding of the overall risk, a series of joint probability curves using all species examined at different ACRs was constructed by using a combination of the probability distributions for exposures and effects analyses (level 3–1; Figure 4). The *x*-axis of the joint probability curve is the percentage of species affected, and the *y*-axis shows the probability of the effect.

The overall risk probability, which equals the area under the joint probability curve, was applied to quantitatively assess the overall ecological risk of cypermethrin in this urban stream. A larger overall risk probability value indicates higher risk at an area for this contaminant. An overall risk probability greater than 0.1% indicates potential risk, and an overall risk probability greater than 1% indicates clear risk [5]. In the present ecological risk assessment, overall risk probabilities of cypermethrin in Chebei Creek were 9%, 36%, 43%, and 67% when ACRs were set at 1, 5, 25, and 125, respectively. These overall risk probability values indicated that cypermethrin in Chebei Creek posed both acute and chronic threats to the stream ecosystem. The overall risk probabilities derived from the joint probability curve analysis were similar to potential affected fractions based on geometric mean concentrations of cypermethrin (8%, 37%, 52%, and 73% when ACRs were set at 1, 5, 25, and 125, respectively).

On the other hand, the probability of exceeding the preselected hazard quotient (hazard quotient at ACR = 1 for all examined species) was estimated from a distribution-based hazard quotient, which was derived from the distribution of exposures and acute-based species sensitivity distribution using

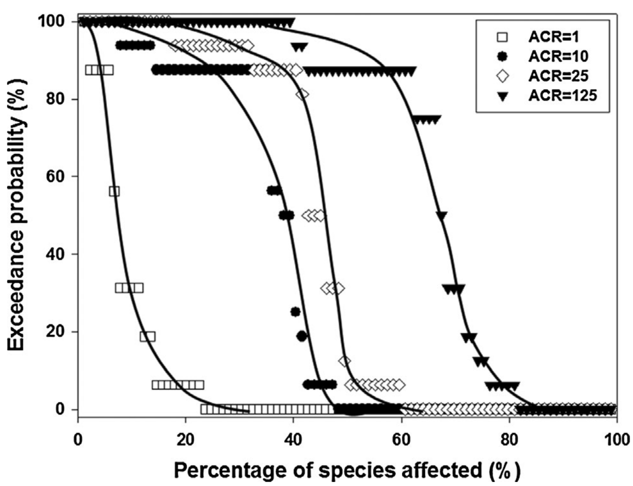

Figure 4. The joint probability curves (level 3–1) for all examined species at various acute-to-chronic ratios (ACRs). Toxicity data used to construct the curves were derived from acute median lethal (LC50) and median effect (EC50) concentrations in the literature.

Monte Carlo simulations (level 3–2; Supplemental Data, Figure S1). The exceedance probability using this method was 36%, approximately 5 times higher than the overall risk probability (9%) at an ACR of 1. A critical step in the Monte Carlo simulation is to appropriately define the distribution of exposures. The distribution analysis showed that a log-normal model was applicable for fitting the distribution of exposures; sample size in the present study was small, however, and might introduce relatively high errors in the Monte Carlo simulation. The differences in the joint probability curve method and Monte Carlo simulation are not surprising; although both methods are probability predictors, they represent different risk characterization techniques, as suggested by Wang et al. [5]. The joint probability curve method offers visual indication of risk, including probabilities of exposures and magnitude of effects, and provides evaluations of a wide range of possible protection levels (e.g., as conducted using various ACRs). Alternatively, the Monte Carlo simulation provides distribution of hazard quotients and then calculates the probability of exceeding the preselected hazard quotients. The distribution of hazard quotients does not vary with ACRs as in the joint probability curve. As such, the Monte Carlo simulation does not provide a range of protection levels, but instead provides a cumulative conservative assessment of risk.

In summary, the tiered ecological risk assessment approach provides quantitative information on both the distribution of exposure and the intensity of effects in iterative fashion. By using multiple analysis techniques, the limitations of a single analysis could be refined and the probability and magnitude of risk of cypermethrin to aquatic organisms in Chebei Creek were achieved. The tiered ecological risk assessment showed that cypermethrin posed significantly acute and chronic risk to the aquatic species in this stream. Whereas the fish might be chronically affected, the invertebrates in Chebei Creek might encounter acute risk by exposure to cypermethrin in water. Even with the multiple-level predictions of risk, it should be noted that some limitations exist that may influence the analysis, such as avoidance behavior of organisms and bioavailability of contaminants, which are typically not evaluated in an ecological risk assessment. Zoolezzi et al. [2] suggested that conducting toxicity testing would aid in alleviating these biases and provide additional weight to elucidate the risk of a contaminant.

Ecological risk assessment confirmation using toxicity testing

As shown in Table S2 in the Supplemental Data, the amphipod, *H. azteca* was in the 2nd centile of the affected species in the species sensitivity distribution, suggesting that this species is highly sensitive to cypermethrin. Therefore, toxicity testing of field waters with *H. azteca* was applied to validate the present ecological risk assessment. Based on the species sensitivity distribution simulation parameters (Table 1), the hazardous concentration affecting 2% of species (HC2) was computed to be 0.006 μg/L. The probability of environmental concentrations exceeding HC2 was calculated to be 88%, implying that 88% of waters in Chebei Creek would cause acute toxicity to *H. azteca* (ACR = 1). The 48-h toxicity testing of field waters in the laboratory were consistent with the predictions, because field waters in Chebei Creek exhibited high levels of lethal and sublethal effects to *H. azteca* (71% and 88% of field waters caused mortality and immobility, respectively). The similar probability gained from the tiered ecological risk assessment analysis and the laboratory toxicity testing demonstrated that the application of the probabilistic ecological risk assessment approach in the urban stream was successful.

Uncertainty and limitations

The probabilistic ecological risk assessment was favorable in assessing risk of cypermethrin in Chebei Creek relative to the deterministic quotient method, because it accounted for probability and distribution of both exposure and effects. Uncertainties in probabilistic risk assessment are inevitable, however, as with every type of assessment. The uncertainties introduced from the ecological risk assessment methods generally include data limitations (not all species being possibly accounted for; e.g., crustaceans and insects were chosen as the representatives for invertebrates), use of non-native species as part of the species sensitivity distribution [10,38,39], variability of timeframe and toxicity endpoints in the effect distribution, issues in laboratory-to-field extrapolation, and interlaboratory differences [5]. For these reasons, the use of acute toxicity data instead of chronic toxicity data is employed, because more consistent data is available for acute exposures. Using the acute data, however, provides another level of uncertainty because ACR values must be used and these ratios cannot be confirmed. Further work in understanding ACRs and additional species-chemical related studies at both acute and chronic levels would improve the analysis and provide more accurate assessments of risk in watersheds.

Additional monitoring data for cypermethrin in aquatic systems in the study area was not available, thus the concentrations of cypermethrin reported herein are the only values that were attainable for the ecological risk assessment. Although the spatial distribution of cypermethrin residues in Chebei Creek was included in the probabilistic ecological risk assessment (levels 2 and 3), the limited numbers of sites may still introduce bias into the exposure distribution assessment. Perhaps more importantly, water samples in Chebei Creek were collected only once, because this is the first study in the area, so temporal scale evaluations of exposure distribution were not considered in the present risk assessment. Because insecticide exposures are generally event-based and/or seasonal, sampling designs are needed in insecticide ecological risk assessment evaluations that account for the fluctuations in exposure [40]. Further work in this area will provide more information to understand how spatial and temporal factors may influence risk of cypermethrin exposure in the area.

One challenge in present ecological risk assessments is the extrapolation from laboratory-based species sensitivity distributions to natural environments (e.g., from individual species to populations, communities, and ecosystems). Although Posthuma and de Zwart [41] showed successful application of species sensitivity distribution model outputs in environmental risk management, many studies have demonstrated that HC5 values derived from laboratory-based species sensitivity distributions were protective of populations in aquatic ecosystems (Maltby et al [8], Hose and van den Brink [10], and Mebane [42]). Giddings et al. [14] suggested that the HC5 may actually over-estimate risk in some cases, because activities such as reproductive rates, resistance, immigration, recovery, and refugia are not accounted for. In addition, Wendt-Rasch et al. [37] observed top-down effects to planktonic community assemblage after exposure to cypermethrin (e.g., direct toxicity on crustaceans caused indirect effects on the abundance and community structure of planktonic species). Interestingly, indirect effects on fish, however, were not observed in a mesocosm study when direct effects were observed in invertebrates after exposure to pyrethroids, suggesting that a diversity of food sources might alleviate indirect effects on fish [14].

Inherent uncertainties exist in the present ecological risk assessment approach, which ultimately may confound the decision-making process. However, the goal of ecological risk assessments is to identify priority stressors responsible for the risk and effectively reduce risk in a timely and cost-effective manner. The results of the present risk assessment do just that, providing evidence that cypermethrin poses a significant risk to many aquatic species in urban waterways in Guangzhou.

CONCLUSIONS

Using a tiered ecological risk assessment, ecological risk of cypermethrin in Chebei Creek, an urban waterway of Guangzhou, South China, was assessed. The water toxicity tests showed that 71% of the field waters collected from Chebei Creek were acutely toxic to *H. azteca*, with 24% of those field waters causing complete mortality. The toxic unit approach suggested that cypermethrin was one of the major contributors to the toxicity. This was consistent with the results of the tiered ecological risk assessment, which suggested that cypermethrin posed significant threats to the aquatic organisms in this urban stream. The exceedance probability of affecting *H. azteca* was comparable to the percentage of toxicity obtained in the bioassays, validating the effectiveness of the tiered ecological risk assessment approach. Although inevitable uncertainties exist in the present ecological risk assessment procedures, this comprehensive approach used in Chebei Creek presents a screening evaluation of ecological risk of cypermethrin and provides foundation for future risk management of pyrethroid insecticides in urban waterways of South China.

SUPPLEMENTAL DATA

Tables S1–S4.
Figures S1. (599 KB DOC).

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