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Joint toxicity of sediment-associated permethrin and cadmium to *Chironomus dilutus*: The role of bioavailability and enzymatic activities

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A B S T R A C T

Pyrethroid insecticides and metals commonly co-occurred in sediment and caused toxicity to benthic organisms jointly. To improve accuracy in assessing risk of the sediments contaminated by insecticides and metals, it is of great importance to understand interaction between the contaminants and reasons for the interaction. In the current study, permethrin and cadmium were chosen as representative contaminants to study joint toxicity of pyrethroids and metals to a benthic invertebrate *Chironomus dilutus*. A median effect/combination index-isobologram was applied to evaluate the interaction between sediment-bound permethrin and cadmium at three dose ratios. Antagonistic interaction was observed in the midges for all treatments. Comparatively, cadmium-dominated group (the ratio of toxicity contribution from permethrin and cadmium was 1:3) showed stronger antagonism than equitoxicity (1:1) and permethrin-dominated groups (3:1). The reasons for the observed antagonism were elucidated from two aspects, including bioavailability and enzymatic activity. The bioavailability of permethrin, expressed as the freely dissolved concentrations in sediment porewater and measured by solid phase microextraction, was not altered by the addition of cadmium, suggesting the change in permethrin bioavailability was not the reason for the antagonism. On the other hand, the activities of metabolic enzymes, glutathione S-transferase and carboxylesterase in the midges which were exposed to mixtures of permethrin and cadmium were significantly higher than those in the midges exposed to permethrin solely. Cadmium considerably enhanced the detoxifying processes of permethrin in the midges, which largely explained the observed antagonistic interaction between permethrin and cadmium.

1. Introduction

The occurrence and toxicological effects of pyrethroid insecticides in the environment have been well documented following their increasing use as alternatives of now-restricted organochlorine and organophosphate insecticides (Li et al., 2014; Siegler et al., 2015). Due to their high hydrophobicity, pyrethroids readily associated with sediment particles after entering aquatic systems and became one of the major threats to benthic invertebrates in urban waterways (Ding et al., 2010; Gan et al., 2005; Mehler et al., 2011a).

The occurrence of other contaminants in urban environment and their contribution to the toxicity should neither be overlooked. Heavy metals were frequently detected in sediment along with pyrethroids and results of whole-sediment toxicity identification evaluation testing suggested that the toxicity of sediment samples from urban waterways in China to the midge, *Chironomus dilutus*, was caused by insecticides and metals jointly (Yi et al., 2015). Interaction between pyrethroids and metals may change their individual toxicity (Liu et al., 2009; Mehler et al., 2011b), introducing uncertainty in evaluating ecological risk when only focusing on the toxicity of individual constituents. Rather, it was imperative to take mixture effects into consideration when assessing the risk, particularly the contaminants commonly co-occurred in the environment.

Concentration addition (CA) and independent action (IA) models have been proposed to assess joint toxicity of contaminants with similar and dissimilar modes of toxic action, respectively (Altenburger et al., 2003; Barata et al., 2006; Jonker et al., 2005; Mehler et al., 2011b). In some cases, however, modes of action of the toxicants were unclear, making model selection a challenge. To circumvent the requirement of determining the modes of action for

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individual chemicals, a median effect/combination index (ME/CI)-isobologram equation was introduced in pharmacology to evaluate the interactions among chemicals (Chen et al., 2015; Chou, 2006). This method allowed for modeling the degree of synergistic, additive and antagonistic effects at different concentrations and effect levels (Chou, 2006).

The observed joint toxicity may result from toxicodynamic processes on target sites, as well as alterations of bioavailability and toxicokinetic processes of the chemicals in a mixture. Svendsen et al. (2010) explained synergistic interactions between organophosphate and neonicotinamide insecticides in the nematode *Caenorhabditis elegans* from toxicodynamic and toxicokinetic aspects. Because organophosphate-oxon is an inhibitor of acetylcholinesterase and neonicotinamide is an agonist of postsynaptic acetylcholine receptors, these insecticides synergistically enhanced neuroexcitation at the target site. From toxicokinetic aspect, neonicotinoides induced the activities of P450 enzymes, which speeded up the biotransformation of organophosphates to their more toxic oxon forms, and subsequently increased their toxicity to *C. elegans*. So far, most studies have been conducted for mixture effects among pesticides (Svendsen et al., 2010). Pesticides and metals commonly co-occurred in urban waterways, but little information is known about the reasons for their joint toxicity.

The objectives of the current study were (1) to evaluate the joint toxicity between sediment-bound pyrethroids and metals to *C. dilutus*, (2) to determine the type of interaction between the contaminants at different concentrations using the ME/CI-isobologram, and (3) to elucidate the reasons for the joint toxicity by assessing chemical bioavailability and enzymatic activity. Permethrin was one of the dominant pyrethroids in sediment, for example, permethrin was detected in 96% and 45% of the sediment samples collected from the Pearl River Delta (PRD), China and Illinois, USA, respectively (Ding et al., 2010; Mehler et al., 2011a). In addition, cadmium was frequently detected in sediments in the PRD and posed high ecological risk in this area (Li et al., 2007). To more accurately perform sediment risk assessment in areas with high co-occurrence of pyrethroids and metals, like the PRD, it is of great importance to study the joint toxicity of these two risky constituents. Therefore, the frequently detected permethrin and cadmium in field sediments were selected as the representative pyrethroid and metal, respectively.

2. Materials and methods

2.1. Sediment and chemicals

Control sediment was collected from a drinking water reservoir in Conghua, China and this sediment was also used to prepare the spiked sediments for toxicity testing. More information on chemicals and reagents used in the current study and the procedures for sediment collection and spiking and the measurements of organic carbon (OC) content in sediment are presented in the Supplementary Material.

2.2. Joint toxicity test

The midge, *C. dilutus* was chosen as the test organism because it was recommended as model organism for sediment toxicity testing by the U.S. Environmental Protection Agency (USEPA) (2000) and has been previously applied to evaluate toxicity of a variety of sediment-bound contaminants, including pyrethroids and metals (Maul et al., 2008; Mehler et al., 2011a; Yi et al., 2015). The midges were cultured at Guangzhou Institute of Geochemistry, Chinese Academy of Sciences (GIGCAS) according to the standard protocols by the USEPA (2000).

Joint toxicity tests of permethrin and cadmium were conducted at fixed ratios of toxicity contribution (1:1, 3:1 and 1:3) and nominal sediment concentrations were selected according to toxicity data of individual constitutes. The 10-d median lethal concentration (LC50) of cadmium to *C. dilutus* was 170 μg/g dry weight (dw) and was obtained in a pilot experiment, and a value of 24.5 μg/g OC from the literature (Maul et al., 2008) was used for permethrin. Toxicity testing with permethrin only was also conducted along with joint toxicity tests. As shown in Table 1 and Fig. S1 ("S" represents figures and tables in the Supplementary Material thereafter), the tests included four groups, namely permethrin-only (G0), equitoxicity (G1, the ratio of permethrin and cadmium was 1:1), permethrin-dominated (G2, 3:1), and cadmium-dominated (G3, 1:3) groups. Toxicity tests in each group were conducted using five concentrations. For groups G0 and G1, the highest concentration was set at 4 times of LC50 for each component and then was divided by a dilution factor of 1.83 to generate a series of concentrations at 0.35, 0.65, 1.19, 2.18 and 4 times of LC50, respectively. Similarly, the highest concentration was set at 6 times of LC50 for permethrin and 2 times of LC50 for cadmium in group G2, whereas the highest concentration was set at 2 times of LC50 for permethrin and 6 times of LC50 for cadmium in G3. A dilution factor of 1.83 was also used in groups G2 and G3 to generate a series of five concentrations (Table 1 and Fig. S1).

Appropriate amounts of permethrin and $CdCl_2$ were spiked into control sediment with acetone (100 μL/600 g wet sediment) and MiliQ water as carriers, respectively. A solvent control was also prepared by adding the same amount of acetone into control sediment. After spiking, the sediments were thoroughly mixed for 2 h using a stainless steel paddle which was driven by an overhead motor, and then aged at 4 °C in the darkness for 28 days.

After 28-d aging, the sediments were homogenized again and 10-d sediment toxicity tests were conducted in six replicates with *C. dilutus* following the standard methods (USEPA, 2000) with some modifications. Solvent and negative controls were included in the testing. In brief, 70 g of wet sediment was put into a 400-mL beaker and then 250 mL of reconstituted water prepared according to the USEPA protocol (2000) was added as overlying water. After the sediment was settled overnight, ten 3rd instar midge larvae were randomly introduced into each beaker. The tests were carried out at 23 ± 1 °C with a light: dark photoperiod of 16:8 h, and pH, conductivity, temperature and dissolved oxygen of overlying water were monitored every day. Ammonia was measured at the beginning and end of the testing. Approximately 150 mL of overlying water was renewed twice daily using an automatic water exchange system. The midges were fed with 6 mg of grinded fish food daily. At the termination of 10-d exposure, *C. dilutus* were removed from the sediment using a 500-μm sieve. Surviving organisms were counted, rinsed, blotted dry, and stored at −20 °C for measuring the activities of metabolic enzymes.

2.3. Sediment analysis

Du et al. (2013) reported that the degradation of permethrin in sediment was negligible during 10-d toxicity tests. We also found no significant difference between cadmium concentrations at the beginning and end of 10-d testing in our pilot experiments. Thus, concentrations of permethrin and cadmium in sediment which were analyzed at the beginning of toxicity tests (after 28-d aging) were used for all calculations.

Permethrin was extracted from the sediments by sonication (Li et al., 2010), purified with solid phase extraction cartridges packed with primary secondary amine/graphitized carbon black, and finally quantified by Shimadzu QP-2010 plus series gas chromatography/mass spectrometry (GC/MS). Cadmium in sediment was