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Do pyrethroid-resistant *Hyalella azteca* have greater bioaccumulation potential compared to non-resistant populations? Implications for bioaccumulation in fish☆

Leslie L. Muggelberg [a, 1], Kara E. Huff Hartz [a], Samuel A. Nutile [a], Amanda D. Harwood [b], Jennifer R. Heim [a], Andrew P. Derby [a], Donald P. Weston [c], Michael J. Lydy [a, *]

[a] Center for Fisheries, Aquaculture and Aquatic Sciences and Department of Zoology, Southern Illinois University, Carbondale, IL 62901, USA
[b] Biology and Environmental Studies, Alma College, Alma, MI 48801, USA
[c] Department of Integrative Biology, University of California, Berkeley, CA 94720, USA

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ABSTRACT

The recent discovery of pyrethroid-resistant *Hyalella azteca* populations in California, USA suggests there has been significant exposure of aquatic organisms to these terrestrially-applied insecticides. Since resistant organisms are able to survive in relatively contaminated habitats they may experience greater pyrethroid bioaccumulation, subsequently increasing the risk of those compounds transferring to predators. These issues were evaluated in the current study following toxicity tests in water with permethrin which showed the 96-h LC50 of resistant *H. azteca* (1670 ng L^{-1}) was 53 times higher than that of non-resistant *H. azteca* (31.2 ng L^{-1}). Bioaccumulation was compared between resistant and non-resistant *H. azteca* by exposing both populations to permethrin in water and then measuring the tissue concentrations attained. Our results indicate that resistant and non-resistant *H. azteca* have similar potential to bioaccumulate pyrethroids at the same exposure concentration. However, significantly greater bioaccumulation occurs in resistant *H. azteca* at exposure concentrations non-resistant organisms cannot survive. To assess the risk of pyrethroid trophic transfer, permethrin-dosed resistant *H. azteca* were fed to fathead minnows (*Pimephales promelas*) for four days, after which bioaccumulation of permethrin and its biotransformation products in fish tissues were measured. There were detectable concentrations of permethrin in fish tissues after they consumed dosed resistant *H. azteca*. These results show that bioaccumulation potential is greater in organisms with pyrethroid resistance and this increases the risk of trophic transfer when consumed by a predator. The implications of this study extend to individual fitness, populations and food webs.

1. Introduction

Pyrethroids are currently the dominant insecticide in residential environments, and have major agricultural use as well (Kemble et al., 2013; Hunt et al., 2016). Pyrethroids were detected in 80% and 75% of sediment samples taken from urban and agricultural water bodies in California (USA), respectively (Amweg et al., 2006; Weston et al., 2004). Many of these sediments were found to cause mortality in test organisms during laboratory exposures and toxic unit analysis identified pyrethroid contamination as the main cause. There have been similar findings with sediments from other waterways across the United States (Ding et al., 2010; Hintzen et al., 2009; Kemble et al., 2013; Weston et al., 2005). Although pyrethroids are hydrophobic and tend to adsorb to organic matter in sediments, these compounds also have been detected in water samples at concentrations toxic to biota (Feo et al., 2010; Weston and Lydy, 2010; Weston et al., 2009). While little is known about how elevated pyrethroid concentrations will impact aquatic systems, some significant changes among populations have emerged.

Pyrethroid resistance has recently been documented in several wild populations of *Hyalella azteca*, an epibenthic amphipod commonly used in toxicity testing (Weston et al., 2013). Found in

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* Corresponding author.
E-mail address: mlydy@siu.edu (M.J. Lydy).
[1] Present address: Department of Biological Sciences, Sage College of Albany, Albany, NY 12208, USA.

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pyrethroid-contaminated waters in central California, these *H. azteca* were up to 550 times less sensitive to two common pyrethroids (cyfluthrin and bifenthrin) than non-resistant lab populations. Gene sequencing of the resistant animals revealed mutations in the voltage-gated sodium channels of the nervous system, the target site of pyrethroid insecticides (Weston et al., 2013). Typically, pyrethroids bind to the sodium channels, preventing or delaying their closing (Davies et al., 2007; Soderlund and Bloomquist, 1989). This results in repetitive firing of the neurons, which manifests as convulsions, tremors and loss of coordinated movements. After some time, the organism becomes paralyzed and dies (Davies et al., 2007; Soderlund and Bloomquist, 1989). However, the mutation of the sodium channels in the pyrethroid-resistant *H. azteca* prevents pyrethroid binding. Research suggests that the alternative mode of toxic action in the resistant organisms is oxidative stress, though occurring at much higher concentrations (Weston et al., 2013).

Since the resistant *H. azteca* survive in environments with relatively high concentrations of pyrethroids, these organisms must differ from non-resistant *H. azteca* with respect to toxicokinetic processes (e.g., uptake, biotransformation and elimination) and/or bioaccumulation potential. The resistant organisms either have higher biotransformation or excretion rates, or alternatively the compounds accumulate in the organisms' tissues to higher concentrations than in non-resistant *H. azteca*, who would succumb to toxicity before attaining high body burdens. This may have implications on both environmental assessments and ecosystem functioning. For instance, significant energy allocation to biotransformation and/or high body residues of pyrethroids may reduce fitness by making individuals more sensitive to other stressors and by reducing fecundity (Chandler, 1990; Werner et al., 2002). Furthermore, a high degree of pyrethroid bioaccumulation in lower trophic level organisms increases the probability of trophic transfer of these compounds in the food web. For that reason, we investigated how resistance to the pyrethroid insecticide, permethrin affected bioaccumulation in *H. azteca* and the potential for subsequent bioaccumulation in a predator, the fathead minnow *(Pimephales promelas)*. Specifically, our objectives were to 1) determine the lethal permethrin concentration to 50% of the individuals (LC50) in non-resistant and resistant *H. azteca* through water-only exposures, 2) compare the bioaccumulation of permethrin in non-resistant and resistant populations, and 3) examine bioaccumulation potential of permethrin in fathead minnows fed resistant *H. azteca* dosed with ^{14}C-labeled permethrin.

2. Methods

2.1. Chemicals

Permethrin (40% *cis*, 60% *trans*) and two surrogates, dibromooctofluorobiphenyl (DBOFB) and decachlorobiphenyl (DCBP), were purchased from ChemService (West Chester, PA, USA) and Sigma-Aldrich Co. (St. Louis, MO, USA), respectively. Radiolabeled permethrin (^{14}C—ring-labeled, specific activity 260 mCi mmol^{-1}, purity ≥95%) was purchased from Moravek Biochemicals (Brea, CA, USA). The purity of the radiolabeled permethrin was evaluated by separating the parent compound from degradation products using an Agilent 1100 high-pressure liquid chromatograph (HPLC) equipped with a fraction collector (Agilent Technologies, Palo Alto, CA, USA) using methods similar to those in You et al. (2009) and measuring the radioactivity of resulting fractions on a Packard TriCarb 2900TR liquid scintillation counter (LSC) (Packard Instrument Company, Meriden, CT, USA). Scintillation cocktail (ScintSafe Plus 50%), all solvents (pesticide-grade acetone, hexane, methylene chloride and acetonitrile) and MS-222 were purchased from Fisher Scientific (Pittsburgh, PA, USA).

2.2. Organisms

Non-resistant and resistant *H. azteca* were cultured according to standard protocols (USEPA, 2000) at Southern Illinois University (Carbondale, IL, USA). The non-resistant culture was initiated in 2001 with organisms acquired from a culture at the U.S. Environmental Protection Agency, Duluth laboratory. Resistant *H. azteca* were collected in 2014 from Mosher Slough, Stockton, CA, USA, where resistance to cyfluthrin and bifenthrin had previously been documented (Weston et al., 2013). The Mosher Slough animals in the current study had been cultured in the laboratory for at least 16 months (approximately 16 generations) prior to testing. For all tests, juvenile *H. azteca* were collected from cultures by isolating individuals that passed through a 1 mm mesh sieve, but were retained by a 500 μm mesh sieve.

Bioaccumulation and toxicity of hydrophobic compounds can be affected by tissue lipid content; therefore, this metric was measured prior to testing to confirm lipid levels for the two different *Hyalella* populations and the fish (Klosterhaus et al., 2003). Lipid levels in all of the test organisms were determined using a phosphor-vanillin spectrometric method (Van Handel, 1985).

2.3. Water toxicity tests

Toxicity tests using both non-resistant and resistant *H. azteca* followed standard procedures for 96-h static water tests outlined by the U.S. EPA (2000). Briefly, moderately hard water (MHW) (Smith et al., 1997) was spiked using permethrin dissolved in an acetone carrier. Treatments consisted of seven permethrin concentrations determined from preliminary testing, as well as solvent and negative controls. The volume of acetone added as the carrier in permethrin treatments and added in the solvent control accounted for a minimal amount (<100 μL L^{-1}) of the total spiked water volume. Dosed water (500 ml) was distributed into five replicate 600-ml beakers for each treatment, four of which were used for toxicity testing and one was used for determination of initial permethrin concentration using methods described below in Section 2.4. The beakers were then stored at 23 °C for approximately 24 h prior to test initiation to allow for equilibration of the permethrin with the glassware.

Upon test initiation, 10 *H. azteca* were added to four replicate beakers for each permethrin concentration and control beakers after which they were stored in an incubator at 23 °C with a 16:8 h light:dark photoperiod for the duration of the tests. No feeding was provided during the tests and termination of the tests consisted of enumeration of living organisms. Final permethrin concentrations were determined by pooling 125 ml aliquots of water from each of the four replicate beakers and extracting permethrin using methods described in Section 2.4. Water quality parameters were measured at the beginning and end of each test and those details are included in Supplemental Information.

2.4. Toxicity test water extractions and analyses

Non-radiolabeled permethrin was extracted from water using a liquid-liquid extraction (LLE) and concentrations were analyzed via gas chromatography (GC). Water samples (500 ml) from the beginning and end of each test were extracted with 50 ml of methylene chloride three times by hand shaking for 3 min. Surrogate compounds (DBOFB and DCBP) were added to each sample prior to extraction. Quality assurance included a matrix spike and matrix spike duplicate that were spiked with permethrin and the