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Comparative toxicities of organophosphate and pyrethroid insecticides to aquatic macroarthropods

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HIGHLIGHTS

- We exposed crayfish and water bugs to pyrethroid and organophosphate insecticides.
- Insecticide class was a significant predictor of risk of mortality during the study.
- Pyrethroid insecticides were consistently more toxic than organophosphates.
- Malathion was the only insecticide identified as posing low risk to macroarthropods.
- Identifying low-risk insecticides is critical to minimize adverse ecosystem effects.

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ABSTRACT

As agricultural expansion and intensification increase to meet the growing global food demand, so too will insecticide use and thus the risk of non-target effects. Insecticide pollution poses a particular threat to aquatic macroarthropods, which play important functional roles in freshwater ecosystems. Thus, understanding the relative toxicities of insecticides to non-target functional groups is critical for predicting effects on ecosystem functions. We exposed two common macroarthropod predators, the crayfish *Procambarus alleni* and the water bug *Belostoma flumineum*, to three insecticides in each of two insecticide classes (three organophosphates: chlorpyrifos, malathion, and terbufos; and three pyrethroids: esfenvalerate, λ-cyhalothrin, and permethrin) to assess their toxicities. We generated 150 simulated environmental exposures using the US EPA Surface Water Contamination Calculator to determine the proportion of estimated peak environmental concentrations (EECs) that exceeded the US EPA level of concern ($0.5 \times LC_{50}$) for non-endangered aquatic invertebrates. Organophosphate insecticides generated consistently low-risk exposure scenarios (EECs $< 0.5 \times LC_{50}$) for both *P. alleni* and *B. flumineum*. Pyrethroid exposure scenarios presented consistently high risk (EECs $> 0.5 \times LC_{50}$) to *P. alleni*, but not to *B. flumineum*, where only λ-cyhalothrin produced consistently high-risk exposures. Survival analyses demonstrated that insecticide class accounted for 55.7% and 91.1% of explained variance in *P. alleni* and *B. flumineum* survival, respectively. Thus, risk to non-target organisms is well predicted by pesticide class. Identifying insecticides that pose low risk to aquatic macroarthropods might help meet increased demands for food while mitigating against potential negative effects on ecosystem functions.

1. Introduction

Global sales of insecticides have increased over the past several decades (Grube et al., 2011). Insecticide use is positively correlated with cropland (Meehan et al., 2011) and is almost certain to increase with the agricultural expansion necessary to feed the increasing global human population (Tilman et al., 2011; Tilman et al., 2001). Pyrethroid use in particular has increased worldwide, especially to control vector-borne diseases (van den Berg et al., 2012). Additionally, the organophosphate insecticides chlorpyrifos and malathion remain among the most-frequently detected insecticides in surface waters of the United States (Gilliom, 2007), even as agricultural use of organophosphates in the United States has stagnated (Grube et al., 2011; Thelin and Stone, 2013).

Agrochemical pollution from insecticide run-off can have important negative consequences for non-target taxa (Brock et al., 2000; McMahon et al., 2012; Rohr et al., 2013; Rohr et al., 2008b). Insecticides can adversely impact aquatic macroarthropods (Brock et al., 2000; Van Wijngaarden et al., 2006), which play

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many important functional roles in wetland ecosystems (Wallace and Webster, 1996), including as predators of aquatic herbivores (Kesler and Munns, Jr., 1989; Weber and Lodge, 1990) and as prey for vertebrate predators (Jordan et al., 1996). Because they occupy intermediate trophic levels, macroinvertebrates can mediate the effects of both top-down and bottom-up pressures on ecosystems (Wallace and Webster, 1996). Thus, changes in the abundances of macroarthropod predators can indirectly affect aquatic community composition and ecosystem properties (Halstead et al., 2014; Rohr and Crumrine, 2005).

As new pesticides are developed and approved for use, it is important that risk assessors can predict the risk these chemicals pose to non-target wildlife. Pesticides may vary both in their toxicity to organisms and in their estimated environmental exposures, the latter of which is based on recommended application rates and the physicochemical properties of the pesticide. Insecticides with similar modes of action often have similar safe threshold values in terms of toxic units (concentrations of different pesticides that are standardized by dividing by the geometric mean of reported EC_{50} values of the most sensitive standard test species (typically *Daphnia magna*); Brock et al., 2000). Therefore, pesticides of the same class (i.e., organophosphate vs. pyrethroid insecticides) might be expected to pose similar risk to focal species even though individual pesticides within a class might vary in their relative estimated environmental exposures and toxicities.

The United States Environmental Protection Agency (US EPA) has developed standardized methods for assessing risk to non-target organisms. Environmental exposure scenarios can be generated using the US EPA's Surface Water Contamination Calculator software (SWCC v1.106), which incorporates recommended pesticide application rates for a given crop, local weather and soil characteristics, and the physicochemical properties of the pesticide to generate a 30-year series of peak estimated environmental concentrations (EECs) for a standardized wetland (US EPA, Washington, DC, USA). The ratio of the EEC for a given pesticide relative to its median lethal concentration (LC_{50}) for an organism of concern is then used to determine a risk quotient for that organism ($RQ = EEC/LC_{50}$; US EPA, 2014). The US EPA considers RQ values of 0.5 or greater as representing acute high risk to aquatic organisms (US EPA, 2014a).

Here we compare the relative toxicities of three insecticides in each of two classes of compounds (three organophosphates: chlorpyrifos, malathion, and terbufos; and three pyrethroids: esfenvalerate, λ-cyhalothrin, and permethrin) for two important macroarthropod predators of snails: the crayfish *Procambarus alleni* and the water bug *Belostoma flumineum*. Additionally, as an exploration of relative environmental risk within and between insecticide classes, we compared simulated peak environmental exposures for each insecticide to the US EPA level of concern ($0.5 \times LC50$) for each species. Both classes of these insecticides affect the nervous systems of target organisms; organophosphates inhibit acetylcholinesterase activity (Newman and Unger, 2002) and pyrethroid insecticides act on voltage-sensitive ion channels in the axonal membranes of neurons to prevent repolarization of action potentials (Soderlund et al., 2002). Our goals were to determine if individual insecticides within a chemical class pose similar threats to these arthropods, and if there are individual chemicals or classes that might pose a lower risk to these taxa if runoff events occur.

2. Methods

2.1. Study organisms

Two common macroarthropod predators were selected for this study. Both *P. alleni* and *B. flumineum* are ubiquitous in freshwater wetlands throughout Florida. *B. flumineum* occur throughout much of North America (Henry and Froeschner, 1988). While *P. alleni* are endemic to Florida (Jordan et al., 1996), the genus is widespread throughout southeastern North America, northern Central America and the northern Caribbean (Hobbs Jr., 1984), and *P. clarkii* have been introduced to every continent except Australia and Antarctica (Hobbs III et al., 1989). Juvenile *P. alleni* (10–43 mm total length) and adult *B. flumineum* (11–20 mm total length) were collected from a pond in Tampa, FL, located at 28°4.172′N, 82°22.665′W. This pond was located far from agricultural land and so was not likely to have been contaminated with agrochemicals with the exception of malathion, which is used ubiquitously throughout Hillsborough County, FL, for adult mosquito control. Individuals in the experiment were maintained separately in the lab in artificial spring water (ASW; Cohen and Neimark, 1980) at 22 °C, on a 14:10 photoperiod, and fed snails (*Physa* spp.) *ad libitum*. Artificial spring water had a pH of 6.8, dissolved oxygen of 6.1 mg/L, and specific conductance of 174.4 μS/cm.

2.2. Insecticides

Three organophosphate (chlorpyrifos, malathion, and terbufos) and three pyrethroid (esfenvalerate, λ-cyhalothrin, and permethrin) insecticides were selected for this study. With the exception of terbufos, all of these chemicals have been used extensively over at least the past two decades in this region (Stone, 2013; Thelin and Stone, 2013). We generated 150 simulated annual peak EEC values in ponds for each pesticide based on the manufacturer's recommended application rate, the physicochemical properties of the pesticide (acquired from the University of Hertfordshire's Pesticide Properties DataBase; 2013), and local abiotic conditions using the US EPA SWCC software (v1.106) and standard EPA scenarios for corn production in five US states (Illinois, Mississippi, North Carolina, Ohio, and Pennsylvania). Corn was used for all exposure scenarios to reduce variation associated with application recommendations for other crops and because applications for corn were included for each insecticide product label. The range of EEC values and the parameters used to calculate them are reported in Table S1.

We selected pesticide concentration ranges that included both the range of EECs and known LC_{50} concentrations for closely-related species and/or similar pesticides where data were available in the US EPA's Ecotox database (US EPA, 2014b). No toxicity data for *P. alleni* were available in the Ecotox database for any of the six insecticides used. However, toxicity data were available for other species of *Procambarus*. Toxicity data for these taxa are summarized in Table S2. No effect of malathion concentrations ranging from 130 to 460 μg/L was observed on *B. flumineum* mortality in mesocosm trials (Relyea and Hoverman, 2008). No other toxicity data were available for *B. flumineum* in the Ecotox database. However, 24-h LC50 concentrations of 15 and 60 μg/L were reported for an unidentified *Belostoma sp.* exposed to chlorpyrifos and parathion, respectively.

Technical-grade insecticides were used for all trials (purity > 98%; Chemservice, West Chester, PA, USA). Actual chemical concentrations applied to the replicates were confirmed using ELISA test kits for detection of organophosphates and pyrethroids (Abraxis, LLC, Warminster, PA, USA). ELISA assays were calibrated by using standards of known concentration for each insecticide. For any nominal concentrations below the limit of detection for the kit, we confirmed the concentration of the stock solution used for serial dilutions.

2.3. Experimental design

We used a static, nonrenewal (no water changes) dose–response design with 5 concentrations of each insecticide