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Comparing the impacts of sediment-bound bifenthrin on aquatic macroinvertebrates in laboratory bioassays and field microcosms

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ABSTRACT

We conducted two laboratory bioassays and two field microcosm exposures with bifenthrin (a synthetic pyrethroid) in order to evaluate the capacity of single-species laboratory bioassays to predict lethal and sublethal impacts on aquatic invertebrates in microcosms. For the laboratory species, *Chironomus tepperi*, larval survival was reduced by 24% at 53.66 μg/g OC, while adult emergence was reduced at concentrations of 33.33 μg/g OC and higher, with a 61% decrease at 77.78 μg/g OC and no emergence at 126.67 μg/g OC. The abundance of several other microcosm taxa was reduced in the microcosms at a similar concentration range (33.33 μg/g OC and above), however there was no impact on the abundance of the congeneric species, *Chironomus oppositus*. The differences in impacts between test systems were potentially due to both differing species sensitivity and the interaction of ambient temperature with bifenthrin toxicity. Bifenthrin also was associated with early emergence of *Chironomus* sp. in both test systems, at concentrations of 10 μg/g OC and higher (laboratory) and 43.90 μg/g OC (microcosm), and with a significant decrease in the proportion of *C. oppositus* males in a microcosm. These findings indicate that while laboratory bioassays accurately predict many impacts in the field, there are some limitations to the predictive capacity of these tests.

1. Introduction

Laboratory bioassays, used to predict the impact of chemicals on aquatic ecosystems, are required for the registration of new chemicals, and provide data for risk assessment tools such as species sensitivity distributions (SSDs) and multiple substance potentially affected fractions (msPAFs) (Smetanová et al. 2014). Laboratory bioassays are used because they are rapid and low cost, and allow toxicant effects to be measured in the absence of confounding variables. However, the restricted biotic and abiotic conditions of these tests mean that they also have the capacity to under- or overestimate toxicant impacts (Kimball and Levin, 1985; Chapman 2002).

Field microcosms retain some advantages of laboratory bioassays, including replicability and the use of uncontaminated controls, which may not be possible for field studies (Musset, 2006; Diepens et al., 2014). Although the degree of environmental realism they represent may be incomplete, they can provide a more realistic indication of how toxicants might impact on ecosystems (Musset, 2006). Microcosm exposures can be used alongside laboratory bioassays as an additional source of information to be used in decision-making, as a means of evaluating the accuracy of laboratory exposures, and to evaluate the consistency of a biological signature in response to toxicant exposure (Musset, 2006; Volatier et al., 2009).

Studies comparing laboratory bioassay to microcosm approaches have found varying degrees of concurrence between the two testing methods. Some comparisons suggest consistency (e.g. Kimball and Levin, 1985; Pascoe et al., 2000; Schroer et al., 2004) whereas in others there has been less agreement between the approaches (Delous et al., 2008). Comparisons of laboratory bioassay and field data also point to limitations in the predicative capacities of laboratory bioassays. Smetanová et al. (2014) compared msPAFs derived from laboratory bioassays to field data from pesticide-contaminated sites, and found that threshold values for impacts in the field were between 2 and 1000 times lower than the model predicted.

The outcome of laboratory bioassays and microcosm exposures may differ for many reasons. The limited range of test species used in laboratory bioassays may not adequately represent the range of sensitivities of field species (Chandler et al., 1997). No organism is sensitive to all toxicants (Burton, 1991). Laboratory bioassays may

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expose organisms for only part of the life cycle (Volatier et al., 2009), for example, sediment toxicity tests using chironomids often do not test embryos or first instar larvae. Single-species tests may fail to account for mitigating effects of a more diverse biota (Chapman, 2002; Delous et al., 2008), and so may overestimate toxicant effects. Laboratory bioassays ignore potential community-level impacts, such as trophic cascades (Fleeger et al., 2003). Such tests are also usually carried out under optimal density and food conditions, so they may underestimate toxicant effects that are additive to environmental stressors (Chapman, 2002).

Similarly, the absence of realistic abiotic conditions also means that laboratory bioassays may not accurately predict toxicant effects. For example, hydrophobic chemicals rapidly leave the water column in test systems and bind to sediment and macrophytes, but may remain in the water column for longer in laboratory systems (de Kermoysan et al., 2013). Laboratory experiments are generally conducted at set temperature (21–25 °C), whereas in the field water temperatures may be much lower (<20 °C), delaying development in invertebrates, and lengthening the exposure period (Airas et al., 2008). Low temperatures may also affect the toxicity and degradation rate of several contaminants, including synthetic pyrethroids (Harwood et al., 2009). While laboratory bioassays remain a valuable tool, several authors have recommended a tiered approach to toxicity testing, using both laboratory and microcosm exposures (Volatier et al., 2009; Diepens et al., 2014).

Synthetic pyrethroids (SPs) are amongst the most common pollutants detected by environmental monitoring (e.g. Weston et al., 2009; Wang et al., 2012). Field and laboratory exposures have shown that exposure to SPs through the water column results in mass mortality amongst aquatic invertebrates (Sibley and Kaushik, 1991; Conrad et al., 1999). However, these pesticides are highly hydrophobic, so rapidly adsorb to sediment particles. Liu et al. (2004) found that less than 3% of the spiked concentrations of sediments spiked with bifenthrin and permethrin were freely available in water. Once bound to sediment, synthetic pyrethroids can remain in aquatic ecosystems for several months (Gan et al., 2005). The effects of synthetic pyrethroid exposure via contaminated sediment, as opposed to in water, are less clear. Some authors suggest that benthic invertebrates experience minimal effects of exposure through sediment, due to the pesticides' low bioavailability in this form (eg. Åkerblom et al., 2008), However, other research has demonstrated impacts of sediment-bound SPs on larval chironomids, including effects on mortality (Conrad et al., 1999), mobility, growth rate and larval dry mass (Maul et al., 2008).

We aimed to compare the abundance and life cycle impacts of sediment-bound bifenthrin in two laboratory-bioassay and two microcosm exposures. More specifically, our aim was to compare the threshold concentrations for these endpoints across the four exposures, to see whether the threshold concentrations derived from laboratory bioassays produced comparable effects in the microcosms, ie. on a more diverse biota and under variable environmental conditions. This was to evaluate the capacity of laboratory bioassays to predict impacts in the field.

The laboratory bioassays were conducted with *Chironomus tepperi*. Chironomid species (Family Chironomidae, Order Diptera) are used worldwide in ecotoxicological testing, as they are widespread and abundant, of high ecological importance, and are suitable for culture in the lab (Armitage et al., 1995). The microcosm technique we employed has previously been validated against field data (Pettigrove and Hoffmann, 2005).

2. Methods and materials

The two laboratory exposures and two microcosms measured different endpoints. Laboratory bioassay 1 measured larval endpoints, while laboratory bioassay 2 measured adult endpoints. The microcosm exposures were conducted at different times of the year to evaluate the effects of bifenthrin under a range of environmental conditions. Chironomids (the predominant microcosm taxa) emerge and breed year-round, however emergence is most rapid and prolific during warmer months (Armitage et al., 1995). Consequently, microcosm 1 (spring-summer) measured the impacts of bifenthrin exposure on adult chironomids, as warm temperatures meant most individuals emerged during the exposure, while microcosm 2 (autumn-winter) measured impacts on larval chironomids, as most individuals did not emerge. For both laboratory bioassay 2 and microcosm 2, the concentration range and maximum dose of bifenthrin were increased, compared to laboratory bioassay 1 and microcosm 1, as complete mortality did not occur at the concentrations used in the latter exposures.

2.1. Sediment collection

Field sediment was collected from an unpolluted site (Glynns wetland, near Warrandyte, Victoria, Australia) The site has been tested regularly for contaminants including pesticides, herbicides, fungicides, metals, PAHs and nutrients (Supplementary Tables 1–4). Sediment from the top 2 cm was collected with a shovel and filtered through a 64 μm nybolt mesh net into a plastic bucket. Filtration was necessary to ensure a uniform particle size across replicates. Filtered sediment was stored at 4 °C for one week to allow sediment to settle out, then the overlying water was discarded. The sediment was homogenized with a paint mixer and transferred to glass jars; a subsample was sent to a commercial laboratory for analysis of percent total organic carbon (TOC), using the LECO method (Australian Laboratory Services, Springvale, Melbourne, Australia). Moisture content was also determined.

2.2. Laboratory bioassays

Laboratory bioassay 1 was conducted in October 2011 and laboratory bioassay 2 in April 2013. The set-up and maintenance of both exposures followed the same protocol, with some exceptions, as detailed below. Acetone and ethanol were obtained from Sigma-Aldrich (USA) and bifenthrin was obtained from Chem Service (USA). All chemicals used were reagent grade.

Chironomus tepperi larvae were from the CAPIM in-house culture, and were cultured as described elsewhere (Jeppe et al., 2014). Larvae were fed ground tropical fish flakes (Tetramin ®) three times per week with a ration of 0.25 g per tank.

For laboratory bioassay 1, filtered sediment was homogenized, then spiked to nominal concentrations of 12.5, 25 and 50 μg/kg, normalized to measured TOC. The concentrations were obtained by sequential dilution with an acetone solvent for each concentration, at a concentration in sediment of 50 mL/kg (wet weight). Unspiked sediment was reserved as a control treatment, while the same concentration of acetone was added to unspiked sediment as a solvent control. Sediment was stored in the dark at 4 °C.

All jars of sediment were placed on a rolling machine for four hours each day for three days after spiking, then once a week for four weeks until the start of the experiment, to homogenise the bifenthrin and evaporate the acetone. Approximately 100 g of sediment from each treatment was analyzed for bifenthrin concentration by a commercial laboratory (ACS laboratories, Kensington, Melbourne, Australia). Measured concentrations were 10.73, 15.61 and 53.66 μg/g OC. We will refer to measured concentrations throughout the remainder of the article. The concentration of bifenthrin in the control and solvent control were both below the limit of detection. The TOC was 2.05%, while the moisture content was 54%.