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Multiple exposure routes of a pesticide exacerbate effects on a grazing mayfly

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ABSTRACT

Hydrophobic pesticides such as pyrethroid insecticides tend to occur in their soluble form mainly as transient pulses in streams. In addition, they are regularly detected in significant quantities adsorbed to stream sediments and other organic in-stream structures. Consequently, stream biota is likely subjected to pesticide exposure via multiple routes. In this study we aimed at investigating the influence of exposure routes for the pyrethroid insecticide lambda-cyhalothrin on the grazing mayfly *Heptagenia sulphurea*. Therefore, *H. sulphurea* was exposed to lambda-cyhalothrin via single- (water or biofilm) or biphasic exposure (water and biofilm) at environmentally realistic concentrations (0, 0.1, 1 $\mu g\,L^{-1}$) and exposure duration (2 h) in a full factorial design (n = 5). Mortality, moulting frequency, and biofilm accrual (proxy for feeding rate) were recorded subsequent to a 7 d post exposure period. Mortality significantly increased and moulting frequency significantly decreased with increasing concentrations of lambda-cyhalothrin in the water phase whereas exposure via biofilm prompted no significant effects on these endpoints ($\alpha = 0.05$). Effect predictions systematically underestimated and overestimated effects for mortality and moulting frequency, respectively. Similarly, mayfly feeding rate was significantly reduced by water phase exposure whereas pre-exposed biofilm did not significantly affect this variable. However, we found a significant but non-systematic interaction between water phase and biofilm exposure on mayfly feeding rate. Our results show that exposure to the same pesticide via multiple exposure routes may increase the magnitude of effects beyond the level predicted from single phase exposures which has clear implications for the aquatic risk assessment of hydrophobic pesticides. However, our results additionally reveal that interactions between pesticide exposure routes may vary between selected dependent variables. We emphasize that unravelling the underlying mechanisms causing these discrepancies in interactive effects between exposure routes is a major aspect that should receive further attention in future research.

1. Introduction

Organic pesticides constitute an integral part of conventional agricultural practice (Aktar et al., 2009; Farmer et al., 1995; Warren, 1998), and they are transported to adjacent freshwater systems especially via spray drift, surface runoff and flow through tile drains during heavy precipitation events (Schulz, 2004; Wauchope, 1978). As a consequence, agricultural streams belong – governed in part by their strong connectivity with their catchment (Stehle and Schulz, 2015) – to the most severely impacted ecosystems on earth (MEA, 2005).

In particular, the group of pyrethroid insecticides often dominates the ecotoxicological potential of pesticide mixtures detected in such streams (Mehler et al., 2011; Phillips et al., 2010; Rasmussen et al., 2015; Weston et al., 2013). Due to the high hydrophobicity of pyrethroids (Yang et al., 2006), they tend to occur in their soluble form mainly as transient pulses lasting a few hours following heavy precipitation events (Liess and Schulz, 1999) with concentrations ranging up to hundreds of $ng\,L^{-1}$ (Feo et al., 2010; Smalling and Orlando, 2011). Subsequently, pyrethroids can be regularly detected in significant quantities adsorbed to stream sediments and organic in-stream structures (e.g., macrophytes; Bennett et al., 2005; Hall et al., 2013; Hand et al., 2001) which significantly increases their environmental persistence (Gan et al., 2005). Consequently, it can be assumed that stream macroinvertebrates are subjected to chronic pyrethroid exposure via sediments or organic

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in-stream structures punctuated by pulse exposure during heavy precipitation events.

Multiple studies document that pulse exposure to sublethal and environmentally realistic concentrations of pyrethroids in the water phase can prompt significant short- and long-term effects on stream macroinvertebrates (see review by Rasmussen et al. (2013)). Moreover, pyrethroids adsorbed to sediments or leaf material may induce effects in stream macroinvertebrates documenting the importance of this exposure route for sediment feeders and shredders (Amweg et al., 2006; Bundschuh et al., 2013; Fleming et al., 1998; Lauridsen et al., 2006). In addition, epiphytic biofilm may act as a sink for hydrophobic pesticides constituting a potential risk for macroinvertebrate grazers (Lundqvist et al., 2012). However, to our knowledge, studies that address the potential role of freshwater biofilm as vector mediating the exposure of macroinvertebrate grazers to pyrethroids are lacking (but see for other toxicants Xie et al. (2010)).

Existing literature unanimously concludes that passive uptake of dissolved hydrophobic pesticides is more important compared to uptake of the same pesticide via ingested food for freshwater macroinvertebrates (see e.g., Gaskell et al., 2007; Lundqvist et al., 2012; Maund et al., 1998). Although the toxic potential of the same hydrophobic pesticide is not directly comparable between water phase and solid phase exposures, both are unequivocally environmentally relevant for stream macroinvertebrates. However, only very few studies have addressed multiple routes of pesticide exposure (water phase vs. ingested food) in freshwater ecotoxicological research; for pyrethroids in particular, only one study could be identified (Bundschuh et al., 2013). In line with our assumption, Bundschuh et al. (2013) revealed that the exposure towards the pyrethroid lambda-cyhalothrin via the water phase and ingested food (i.e., pre-exposed leaf material) may generate synergistic effects on stream macroinvertebrates. These insights point to the urgent need for understanding environmental risks of pyrethroids in agricultural stream induced by combinations of these exposure routes.

We used a model system representing trophic interactions at the bottom of photoautotrophic food webs to study effects of the pyrethroid lambda-cyhalothrin using single- and biphasic exposures. In more detail, the grazing mayfly *Heptagenia sulphurea* was exposed to lambda-cyhalothrin via water and/or epiphytic biofilm using environmentally realistic concentrations (i.e., 0, 0.1, 1.0 $\mu g\,L^{-1}$) and exposure duration (i.e., 2 h) in a full factorial design. Moulting frequency and mortality in *H. sulphurea* were registered following a 7 d post exposure observation period. Moreover, algal biomass accrual was recorded as a measure for feeding activity of the test animals. We hypothesized that i) moulting frequency decreases and mortality increases with increasing exposure concentrations, ii) algal biomass accrual increases with increasing exposure concentrations due to reduced feeding activity, iii) effects of water phase exposure on moulting frequency, mortality, and algal biomass accrual will exceed those of biofilm exposure, and iv) biphasic exposure (i.e., via both water and biofilm) increases effects more than expected compared to single phase exposures.

2. Materials & methods

2.1. Pyrethroid

Lambda-cyhalothrin (PESTANAL®, 99.8% purity, analytical grade) purchased from Sigma Aldrich (Selze, Germany) was used as model pesticide. The half-life (DT_{50}) of lambda-cyhalothrin in a water-sediment system is 15.1 d (Pesticide Properties DataBase, http://sitem.herts.ac.uk/aeru/iupac/Reports/415.htm, accessed on 7/7-2016). A dilution series of lambda-cyhalothrin was produced in 96% EtOH 1 h before exposure, and the exposure volume was transferred to exposure beakers using a glass pipette. One composite water sample (composed of 0.5 L from the mayfly and the biofilm exposure each) representing each exposure concentration was collected at the initiation of the experiment. The collected samples were stored frozen (−20 C°) immediately after exposure until analysis.

Table 1
The number of aquarium replica in the experimental treatments.

		Biofilm exposure ($\mu g\,L^{-1}$)		
	Water exposure ($\mu g\,L^{-1}$)	0	0.1	1.0
+H. sulphurea	0	8	5	5
	0.1	5	5	5
	1.0	5	5	5
−H. sulphurea		4	4	4

Lambda-cyhalothrin was verified following purification by solid phase extraction (SPE column: Chromabond C18, 500 mg, 6 mL; conditioning solvent: MeOH) by injecting 20 μL of each sample to a LC–MS system. Briefly, the LC system consisted of a Combipal autosampler (CTC Analytics, Zwingern, Switzerland) connected to a U-HPLC system consisting of an Accela Pump and a Hypersil Gold C18 column (40 × 2.1 mm, particle size 1.9 μm) (both Thermo Fisher Scientific, Dreieich, Germany). The MS was a benchtop orbitrap system (Exactive, Thermo Fisher Scientific, Dreieich, Germany) run in full scan mode (scan range: 100–2000 *m/z*). The compound was chromatographically separated using MilliQ water and methanol, both containing 0.1% formic acid and 4 mM ammonium format (both Sigma Aldrich, Seelze, Germany, puriss. p.a. grade) with the following eluent composition: 0–2 min 5% B; 2–7.01 min, 100% B; 7.01–8.5 min, 5% B. Compound identification and quantification were carried out using accurate masses of the compounds (*m/z*: 467.1339) and external calibrations ranges from 0.5 to 40 $\mu g\,L^{-1}$. Limits of quantification and detection were 20 and 10 $ng\,L^{-1}$ according to DIN (2008). The analysis revealed measured concentrations deviated by less than 10% from the nominal concentrations (0.091 and 0.944 $\mu g\,L^{-1}$ measured in the 0.1 and 1 $\mu g\,L^{-1}$ treatments) justifying the use of the latter throughout the manuscript.

2.2. General experimental design

A full-factorial test design was used to assess the effects of three lambda-cyhalothrin concentrations (0, 0.1, and 1 $\mu g\,L^{-1}$) on *H. sulphurea* via single- (water or biofilm) or biphasic exposures (water and biofilm; Table 1). The study was conducted in a climate chamber at 10 °C, resembling the stream water temperature at the mayfly sampling site, with a diurnal light/darkness cycle of 14/10 h. Exposure of *H. sulphurea* was performed in glass beakers containing 2 L artificial freshwater (AFW) (294 $mg\,L^{-1}$ $CaCl_2{\cdot}2H_2O$, 123.25 $mg\,L^{-1}$ $MgSO_4{\cdot}7H_2O$, 64.75 $mg\,L^{-1}$ $NaHCO_3$, and 5.75 $mg\,L^{-1}$ KCL). All specimens of *H. sulphurea* intended for treatments with similar exposure concentrations were collectively exposed. In contrast, biofilms established on tile stones (width, length, and height were 8, 8, and 3 cm, respectively) were exposed individually in glass beakers containing 2 L AFW containing 0, 0.1, or 1 $\mu g\,L^{-1}$ lambda-cyhalothrin. Total EtOH concentrations in all exposure media were 1‰, and untreated controls received an equivalent amount of EtOH to account for potential effects of EtOH. The exposure duration for both *H. sulphurea* and biofilms was 2 h. Subsequently, mayflies and biofilms were gently rinsed in clean AFW and transferred to the respective experimental aquaria (1.5 L, n = 60) to assess pesticides effects during a 7 d post exposure period.

Subsequent to this post exposure period, effects in *H. sulphurea* induced by single phase exposure (*H. sulphurea* exposed via water