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Structural and functional effects of a short-term pyrethroid pulse exposure on invertebrates in outdoor stream mesocosms

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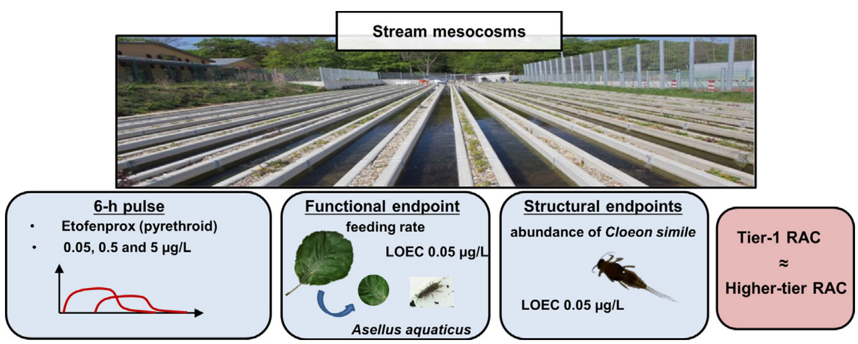
HIGHLIGHTS

- A stream-typical pyrethroid pulse exposure was simulated using stream mesocosms.
- Effects on structural and functional variables were observed slightly above the PEC.
- Higher tier RAC is in line with the official tier 1 RAC.
- Functional endpoints as a supportive concept for higher-tier approaches

GRAPHICAL ABSTRACT



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ABSTRACT

Agricultural land-use frequently results in short pulse exposures of insecticides such as pyrethroids in river systems, adversely affecting local invertebrate communities. In order to assess insecticide-induced effects, stream mesocosms are used within higher tier aquatic risk assessment. Regulatory acceptable concentrations (RACs) derived from those studies are often higher compared with tier 1 RACs. Hence, the present mesocosm study evaluates this aspect using a pulse exposure scenario typical for streams and the pyrethroid insecticide etofenprox. A 6-h pulse exposure with measured concentrations of 0.04, 0.3 and 5.3 μg L^{-1} etofenprox was used. We considered abundance, drift and emergence of invertebrates as structural endpoints and the in situ-measured feeding rates of the isopod *Asellus aquaticus* as functional endpoint. Most prominent effects were visible at 5.3 μg L^{-1} etofenprox which caused adverse effects of up to 100% at the individual and population level, as well as community structure alterations. Transient effects were observed for invertebrate drift (effect duration ≤24 h) and for the invertebrate community (9 days after exposure) at 0.3 μg L^{-1} etofenprox. Furthermore, 0.04 μg L^{-1} etofenprox affected the abundance of the mayfly *Cloeon simile* (decrease by 66%) and the feeding rate of *A. aquaticus* (decrease by 44%). Thus, implications for the functional endpoint leaf litter breakdown in heterotrophic ecosystems may be expected. A hypothetical RAC derived from the present mesocosm study (0.004 μg L^{-1}) is in line with the official tier 1 RAC (0.0044 μg L^{-1}) and thus shows that the present mesocosm study did not result in a higher RAC.

1. Introduction

Regulatory risk assessment uses a tiered approach based on laboratory standard tests (tier 1) and micro- and mesocosm tests (higher

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tier risk assessment) to assess adverse effects of insecticides on aquatic ecosystems (EFSA, 2013). The current higher tier risk assessment approach often uses pond mesocosms with static test conditions and rather long exposure durations of days or weeks, which is typical for lentic surface waters. However, such pond systems are – in contrast to stream mesocosms – not designed to mimic running water-typical pulse exposures of few hours as reported for small agricultural streams (Spurlock et al., 2005; Rasmussen et al., 2013; Stehle et al., 2013; Stehle and Schulz, 2015). Pyrethroid insecticides in particular have been detected at ecologically relevant concentrations in agricultural surface waters worldwide (Stehle and Schulz, 2015). Due to their high lipophilicity, pyrethroids are expected to rapidly adsorb to organic matrices (Hill, 1989) resulting in longitudinal decreases of maximum concentrations with increasing stream lengths (Bennett et al., 2005). Thus, the exposure of aquatic organisms towards pyrethroids water-phase concentrations may be very short, but indirect uptake via pyrethroids adsorbed to food sources is in turn possible (Hill, 1989). Due to the rapid uptake of pyrethroids by aquatic organisms (Coats et al., 1989; Tang and Siegfried, 1995) and their fast mode of action adversely affecting the nervous system (Farmer et al., 1995; Antwi and Reddy, 2015) brief pulse exposures of few hours can already trigger adverse effects (Schulz and Liess, 2000). For example, pyrethroid pulse exposures may induce catastrophic drift of distinct populations (Lauridsen and Friberg, 2005; Heckmann and Friberg, 2005; Beketov and Liess, 2008) and cause mortality of invertebrates in experimental studies and under field conditions (Jergentz et al., 2004; Bereswill et al., 2013). Furthermore, ecosystem functions such as leaf breakdown, which are the basis of heterotrophic food webs, may be adversely affected as a consequence of pyrethroid exposure (Rasmussen et al., 2013).

Up to now, knowledge of effects on invertebrates following pyrethroid pulse exposures was mainly based on laboratory and static microcosm approaches (Rasmussen et al., 2013). Although stream mesocosm are generally being used more frequently, most setups focus on low or moderately lipophilic insecticides (Liess and Beketov, 2011; Mohr et al., 2012), fungicides (Bayona et al., 2015a, 2015b) and/or herbicides (Mohr et al., 2007; Magbanua et al., 2013; Wieczorek et al., 2017) and used exposure durations of ≥12 h. Exposure durations of few hours as expected for pyrethroids in natural streams were not represented by these study designs. Therefore, the present study simulated a field relevant 6-h pulse exposure scenario using the pyrethroid ether etofenprox as model insecticide. Etofenprox is registered in several European countries (Lewis et al., 2016) and used worldwide e.g. during rice farming. This compound was measured in Asian streams at concentrations between 0.04 and 0.2 μg L^{-1} (Tanabe et al., 2001; Añasco et al., 2010) for up to 7 h (Tanabe and Kawata, 2009). As there is no literature on measured field concentrations for the EU, this study used the predicted environmental concentration in surface waters (PEC_{sw}) of 0.024 μg L^{-1} etofenprox determined in the EU regulatory risk assessment (EFSA, 2008).

Since the tier 1 regulatory acceptable concentration (RAC) of the EU regulatory risk assessment of 0.0044 μg active substance (a.s.) L^{-1} (based on *Daphnia* 48-h EC_{50} for the formulation Trebon 30EC; EFSA, 2008) is up to two orders of magnitude below the measured field concentrations, adverse effects cannot be excluded. A logical next step to assess the risk of these field concentrations would be a refinement of the RAC using higher tier studies. However, the existing higher tier mesocosm study could not be used to refine the EU RAC due to lacking information on population recovery and high uncertainty (EFSA, 2008). Van Wijngaarden et al. (2015) demonstrated that the majority (>90%) of tier 1 and tier 2 RACs of insecticides were lower (and thus more conservative) compared with the higher tier RACs derived from micro- and mesocosm studies. Hence, the present study using 45-m outdoor stream mesocosm evaluates if the same holds true using a pulse exposure scenario typical for streams and the pyrethroid insecticide etofenprox. Thereby, the present study aims at providing additional data on the ecological effects of etofenprox using a 6-h pulse exposure with concentrations between 0.04 and 5.3 μg L^{-1} and a hypothetical stream mesocosm RAC. Ecotoxicological effects were assessed via the structural endpoints abundance, drift and emergence of invertebrates as well as the functional endpoint of in situ-measured feeding rates of *Asellus aquaticus* in order to uncover potential effects on the invertebrate mediated decomposition of allochthonous organic matter.

2. Material and methods

2.1. Experimental design

The study was conducted at the Landau Stream Mesocosm Facility (LSMF) at the University of Koblenz-Landau, Campus Landau (Germany). The test facility consists of 16 independent channels (length = 45 m, width = 0.4 m and average water depth 0.26–0.27 m; $n = 4$ replicate streams each; 16 streams in total). The streams were run in recirculation conditions except for the application phase (3 h prior to and 48 h following etofenprox application), during which flow-through conditions were used. During flow-through conditions water from an adjacent storage pond was used (flow velocity ≈ 1 cm s^{-1}). Both, stream channels and the storage pond were supplied with chlorine-free municipal tap water. Invertebrate colonization via drift from the storage pond was restricted to small or juvenile invertebrates as the spillway was covered with Polyester mesh screen (mesh size = 1 mm; Schulz, 2005). Further information on the LSMF is provided in Elsaesser et al. (2013).

The experimental period started in October 2013 and lasted until September 2014. Non-sterile artificial substratum and aquatic macrophytes (collected from non-polluted streams in the Palatinate region and tributaries of the river Rhine) were introduced at the beginning of October 2013. The substratum (height approx. 0.08 m) consisted of medium to coarse sand (grain size = 50% 0–0.5 mm, 50% 0.2–1.0 mm) and in total 5% vol. white peat, which has thus been approximated to the substratum composition described in the guideline OECD 219. Two 7.5 m sampling areas SA1 (5 m below the water inlet) and SA2 (35 m below the water inlet) were planted with both western waterweed (*Elodea nuttallii* (Planch.) H. St. John) and Eurasian watermilfoil (*Myriophyllum spicatum* L.) (Fig. 1). We used *E. nuttallii* due to its widespread presence in German surface waters and *M. spicatum* as it is a well-studied macrophyte species in ecotoxicological studies (Maltby et al., 2010). Comparable to agricultural streams not shaded by riparian tree vegetation, the vegetation coverage of the sampling areas was in the range of 50 to 100%. The first 5 m below the inlet were kept free of macrophytes in order to enable a homogeneous distribution of etofenprox in the water phase within all channels.

2.2. Stream water quality

The water quality parameters temperature, pH, oxygen saturation, and conductivity were measured once a week at 9 a.m. and 4 p.m. with the WTW Multi 340i (WTW GmbH, Weilheim, Germany) in all 16 channels. Mean values are presented in the supplemental data for the sampling dates (Fig. A.1). Additionally, nitrate (NO_3^-), nitrite (NO_2^-), ammonium (NH_4^+), phosphate (PO_4^{3-}), sulfate (SO_4^{2-}) and total hardness (°dH) were measured twice on the day of the etofenprox application and one week after the last invertebrate sampling (Table A.1) using Visocolor test-kits (Macherey-Nagel, Düren, Germany).

2.3. Etofenprox application and monitoring

Etofenprox (IUPAC name 2-(4-ethoxyphenyl)-2-methylpropyl 3-phenoxybenzyl ether, CAS: 80,844-07-1) is an insecticide belonging to the pyrethroid ethers. Due to the very low water solubility of the active substance and to simulate field realistic conditions we used the commercial formulation Trebon 30EC (287.5 g (a.s.) L^{-1}; Mitsui Chemicals Agro, Inc.) as used in the agricultural praxis. Furthermore, the use of