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Toxicity of sediment-bound lufenuron to benthic arthropods in laboratory bioassays

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ABSTRACT

This paper deals with species sensitivity distributions (SSDs) for the lipophilic insecticide lufenuron and benthic arthropods based on sediment-spiked laboratory toxicity tests. This compound that inhibits chitin synthesis and moulting of arthropods persists in sediment. Using field-collected sediment, toxicity tests were conducted with three macro-crustaceans and six insects. The Hazardous Concentration to 5% of the tested species, the HC5 (and 95% confidence limit), derived from an SSD constructed with 10d-LC50's was 2.2 (1.2–5.7) μg/g organic carbon (OC) in dry sediment. In addition, HC5 values derived from SSDs constructed with 28d-LC10 and 28-d LC50 values were 0.13 (0.02–1.50) μg/g OC and 2.0 (1.3–5.5) μg/g OC, respectively. In 28d toxicity tests with *Chironomus riparius* and *Hyalella azteca,* a higher sensitivity was observed when using lufenuron-spiked field-collected sediment than in lufenuron-spiked artificial sediment. Overall, the non-biting midge *C. riparius* appeared to be a representative and sensitive standard test species to assess effects of lufenuron exposure in sediment. The Tier-1 (based on standard test species), Tier-2 (based on standard and additional test species) and Tier-3 (model ecosystem approach) regulatory acceptable concentrations (RACs) for sediment-spiked lufenuron did not differ substantially. The Tier-2 RAC was the lowest. Since to our knowledge this study is the first in the open literature that evaluates the tiered approach in the sediment effect assessment procedure for pesticides, we advocate that similar evaluations should be conducted for pesticides that differ in toxic mode-of-action.

1. Introduction

Lipophilic pesticides are frequently detected in sediments of edge-of-field freshwater ecosystems (e.g., Warren et al., 2003; Stehle and Schulz, 2015; Li et al., 2017; Wei et al., 2017), potentially leading to long-term toxic effects on benthic organisms and communities (e.g., Ding et al., 2010; Schäfer et al., 2011; McKnight et al., 2015; Hunt et al., 2016; Moran et al., 2017). These potential ecological risks of sediment-bound pesticides were confirmed by a few micro-/mesocosm experiments conducted with spiked sediment (Boyle et al., 2016; Brock et al., 2016; Rogers et al., 2016; Yin et al., 2018). To date, prospective sediment risk assessments for pesticides are mainly based on results of laboratory bioassays with a few standard test species such as larvae of the non-biting midge *Chironomus* (predominantly *C. dilutus* and *C. riparius*) and the amphipod *Hyalella azteca* (Deneer et al., 2013). These test species are recommended in test guidelines, guidance documents and opinion papers (e.g., USEPA, 2000; OECD, 2004; ASTM, 2010; Diepens et al., 2014; EFSA, 2015; Diepens et al., 2016). It is, however, uncertain whether the derivation of a regulatorily acceptable concentration in sediment (or a sediment quality standard) based on these standard benthic test species is in line with that based on a wider array of freshwater benthic organisms (e.g. species sensitivity distribution approach), or with that based on threshold concentrations for population-level effects observed in sediment-spiked microcosm or mesocosm tests (model ecosystem approach). In addition, in toxicity testing for sediment exposure to insecticides, predominantly pyrethroids received attention in the scientific literature (e.g., Fleming et al., 1998; Weston et al., 2009; Harwood et al., 2014; Boyle et al., 2016; Rogers et al., 2016; Li et al., 2017), while information on sediment ecotoxicology of other lipophilic insecticides is scarce.

We selected the benzoyl insecticide lufenuron as one of the benchmark substances to evaluate the prospective environmental effect assessment (ERA) procedure for sediment-associated pesticides as proposed by the European Food Safety Authority (EFSA, 2015). In the European Union prospective ERA for pesticides falls under the mandate of EFSA. ERA procedures developed by EFSA for pesticides – toxicants

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often characterised by a specific toxic mode-of-action – differ from those developed for industrial chemicals by the European Chemicals Agency (ECHA, 2017). For sediment ERA, EFSA published a detailed proposal how to apply the species sensitivity distribution approach based on sediment-spiked toxicity tests with benthic arthropods and pesticides (EFSA, 2015). Lufenuron is a benzoylurea compound inhibiting chitin synthesis and moulting of arthropods (Matsumura, 2010). It is used as an insecticide and as a veterinary medicine, including uses for flea and fish lice control. According to EFSA (2008) its $\log K_{OW}$ is 5.12 (25 °C) and in water-sediment test systems its degradation half-life (DT_{50}) ranges from 34 to 188 days. In the aquatic environment, lufenuron quickly partitions to the sediment and Brock et al. (2016) showed that in outdoor microcosms, on average 87.7% of the initial lufenuron concentration could still be detected in the sediment after 12 weeks.

Aquatic semi-field experiments and laboratory toxicity tests conducted with water-spiked (see e.g., EFSA, 2008; Brock et al., 2009, 2010) and sediment-spiked (Brock et al., 2016) lufenuron showed that this insecticide had direct toxic effects on aquatic arthropods (insects and crustaceans) in particular. Considering this specific toxic mode-of-action, we decided to conduct additional sediment-spiked laboratory toxicity tests with a wider array of benthic arthropods to further evaluate the tiered sediment effect assessment scheme proposed for insecticides by EFSA (2015). Hence, the focus of this paper is on 10-day and 28-day sediment-spiked laboratory toxicity tests with lufenuron using standard and additional benthic test species (Tier-2 according to EFSA, 2013, 2015). This paper complements the study of Brock et al. (2016), that focussed on concentration-response relationships for lufenuron in sediment-spiked microcosms and in 28-day laboratory bioassays with the standard test species *C. riparius*, *H. azteca* and *Lumbriculus variegatus*. The aim is to present 10-day and 28-day toxicity estimates from sediment-spiked laboratory bioassays with benthic arthropods belonging to different taxonomic groups (Diptera, Ephemeroptera, Trichoptera, Megaloptera, Isopoda and Amphipoda) that can be used to derive a Tier-2 regulatory acceptable concentration (RAC) for the sediment compartment. We used field-collected sediment of the same type that was used in the sediment-spiked outdoor microcosms experiment to facilitate the comparison of treatment-related responses. Furthermore, in this paper we compare the sensitivity of the standard benthic test species *C. riparius* and *H. azteca* between bioassays conducted with field-collected and artificial (OECD) sediment. Finally, the consistency of the tiered effect assessment scheme proposed by EFSA (2015) will be discussed using these data.

2. Materials and methods

2.1. Sediment and sediment spiking

Sediment was collected from experimental ditches at the Wageningen Environmental Research (Alterra) outdoor facility the Sinderhoeve near Renkum, the Netherlands. The ditches from which the sediment was collected were used in the past for basic ecological research (focus on nutrient transfer between water and sediments) and never treated with pesticides. The sediment was sieved through a 1 mm mesh sieve, mixed and stored at −18 °C. The final field-collected sediment contained 5.3% organic matter (OM) dry weight and 2.4% of the dry weight of the sediment was measured to be organic carbon (OC) (using the method described in Walinga et al., 1992). This field-collected sediment was used in sediment toxicity tests for all benthic test species (see Tables 1 and 2).

To compare the sensitivity between bioassays using field-collected and artificial sediment for the benthic standard test species *H. azteca*, an additional 28-day sediment-spiked toxicity test was performed using artificial OECD sediment. This sediment was prepared in accordance to OECD (2010), having an OC content of approximately 2.5% (dry weight). Results of a 28-day sediment-spiked test with lufenuron, artificial sediment and the standard test species *C. riparius* reported in EFSA (2008) were provided by Syngenta.

In our experiments, the active substance (a.s.) lufenuron was applied as the formulated product Match®, as in previous semi-field experiments and laboratory sediment toxicity tests (Brock et al., 2009; Brock et al., 2016). By using Match®, the plant protection product actually used in agriculture, additional organic solvent when spiking the sediment was not required.

The procedure used to prepare the dosing solutions for spiking the sediment followed Brock et al. (2016). These dosing solutions were used to obtain lufenuron concentrations ranging from 0.08 to 59.70 µg a.s./g OC in dry sediment (see Tables 1 and 2). To spike the field-collected sediment, portions of approximately 20 L wet sediment were placed in concrete mixers that continuously mixed the sediment during the drip-wise application of one of the dosing solutions. The mixing of sediment continued 30 min post-application. Spiking was conducted from low to high concentrations to avoid cross-contamination. The spiked sediment was stored per dose in a clean container, and mixed again for 15 min the next day. Then the batches of spiked field-collected sediments were stored in a freezer (−20 °C) in portions of approximately 5 L. Before use of these batches of sediment in the laboratory bioassays they were placed in a climate room of 20 ± 2 °C for 7 days (ageing period).

Spiking of artificial sediment was performed in smaller volumes than those of field-collected sediment. For each test concentration portions of approximately 4 kg of wet artificial sediment (constructed following the procedure described in OECD, 2010) were dosed using a specific dosing solution (see above) and manually mixed with a hand-held electric cement mixer for 10 min. After spiking, the artificial sediment was aged for 7 days before use in the toxicity test.

Lufenuron concentrations in the sediment were measured in subsamples of each sediment batch to confirm dosing.

2.2. Sediment lufenuron analysis

To analyse the lufenuron concentrations in the sediment, the procedure described in Brock et al. (2016) was followed. Sediment concentrations and toxicity estimates were expressed in µg a.s./g OC/kg dry sediment. The limits of detection and quantification of lufenuron in sediment were approximately 0.008 and 0.024 µg a.s./g OC/kg dry sediment, respectively. In quality control samples, the average recovery of lufenuron (n = 9) was 92% (standard deviation 13%).

2.3. Test species

Since a water-spiked mesocosm experiment (Brock et al., 2009), a sediment-spiked microcosm experiment and sediment-spiked laboratory toxicity tests with the standard benthic test species *C. riparius*, *H. azteca* and *L. vulgaris* (Brock et al., 2016) indicated that aquatic arthropods are sensitive in particular, the benthic test species selected comprised macro-crustaceans and insects. The selection of the test species was based on their availability in laboratory cultures at Wageningen University and Research and the University of Amsterdam, and, in the case of field-collected species, their availability in sufficient numbers in nearby freshwater ecosystems (see Tables 1 and 2). An additional criterion was to perform tests with benthic arthropods belonging to different taxonomic groups (families or genera). Three taxa of the genus *Chironomus* were selected as test species since they often dominate the benthic arthropod community in freshwater sediments, and OECD guidelines for conducting sediment-spiked tests with these taxa are available.

The selected benthic test species comprised of three macro-crustaceans (the isopod *Asellus aquaticus*; the amphipods *Gammarus pulex* and *Hyalella azteca*) and larvae of seven insects (the dipterans *Chironomus dilutus*, *Chironomus riparius* and *Chironomus* gr. *thummi*; the ephemeropterans *Caenis horaria* and *Ephemera danica*; the trichopteran