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# Effects of sediment-spiked lufenuron on benthic macroinvertebrates in outdoor microcosms and single-species toxicity tests

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ABSTRACT

Sediment ecotoxicity studies were conducted with lufenuron to (i) complement the results of a water-spiked mesocosm experiment with this lipophilic benzoylurea insecticide, (ii) to explore the predictive value of laboratory single-species tests for population and community-level responses of benthic macroinvertebrates, and (iii) to calibrate the tier-1 effect assessment procedure for sediment organisms. For this purpose the concentration-response relationships for macroinvertebrates between sediment-spiked microcosms and those of 28-d sediment-spiked single-species toxicity tests with *Chironomus riparius*, *Hyalella azteca* and *Lumbriculus variegatus* were compared. Lufenuron persisted in the sediment of the microcosms. On average, 87.7% of the initial lufenuron concentration could still be detected in the sediment after 12 weeks. Overall, benthic insects and crustaceans showed treatment-related declines and oligochaetes treatment-related increases. The lowest population-level NOEC in the microcosms was 0.79 μg lufenuron/g organic carbon in dry sediment (μg a.s./g OC) for Tanytarsini, Chironomini and *Dero* sp. Multivariate analysis of the responses of benthic macroinvertebrates revealed a community-level NOEC of 0.79 μg a.s./g OC. The treatment-related responses observed in the microcosms are in accordance with the results of the 28-d laboratory toxicity tests. These tests showed that the insect *C. riparius* and the crustacean *H. azteca* were approximately two orders of magnitude more sensitive than the oligochaete *L. variegatus*. In our laboratory tests, using field-collected sediment, the lowest 28-d $EC_{10}$ (0.49 μg a.s./g OC) was observed for *C. riparius* (endpoint survival), while for the standard OECD test with this species, using artificial sediment, a NOEC of 2.35 μg a.s./g OC (endpoint emergence) is reported. In this particular case, the sediment tier-1 effect assessment using the chronic $EC_{10}$ (field-collected sediment) or chronic NOEC (artificial sediment) of *C. riparius* and an assessment factor of 10, seems to be protective for the treatment-related responses observed in the sediment-spiked microcosms.



## 1. Introduction

Sediments act as sink for lipophilic pesticides that may persist there, potentially leading to toxic effects on benthic organisms (Warren et al., 2003; Pettigrove and Hoffmann, 2005; Schäfer et al., 2011; Diepens et al., 2014; McKnight et al., 2015; Hunt et al., 2016; Nowell et al., 2016; Diepens et al., 2016). One of these lipophilic pesticides is the benzoylurea insecticide lufenuron. In experimental ditches of 40 m length, and sprayed with this insecticide for, respectively, 0, 33, 67 and 100% of their surface area, it was observed that (i) lufenuron dissipated quickly from the water column (dissipation $DT_{50}$ of 1.9–2.3 days), (ii) caused direct toxic effects on pelagic and benthic arthropod populations, and (iii) recovery was not observed for several sediment-associated arthropods in the sprayed ditch sections in particular (López-Mancisidor et al., 2008; Brock et al., 2009, 2010). Although sediment exposure to lufenuron was not studied, Brock et al. (2010) hypothesized that these long-term effects on sediment-associated arthropods in the sprayed ditch sections were caused by long-term sediment exposure to lufenuron. To complement the water-spiked ditch study we selected lufenuron

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as benchmark compound to further explore the impact of sediment exposure on benthic organisms. Moreover, lufenuron has been detected in lake sediments as well (Chiaia-Hermandez et al., 2014), and benzoylurea insecticides hardly have not received attention in sediment ecotoxicology (see e.g. Deneer et al., 2013; Nowell et al., 2016).

In European prospective environmental risk assessment (ERA) for pesticides, the first tier sediment effect assessment is based on chronic 'no observed effect concentrations' (NOECs) or 'effect concentrations to 10% of the test organisms' ($EC_{10}$'s) for a few benthic standard test species and the application of an assessment factor (AF) of 10 to derive a regulatory acceptable concentration for sediment ($RAC_{sed}$) (EFSA, 2013, 2015). The tier-1 data requirements for sediment organisms concern chronic toxicity tests for the insect *Chironomus* spp. and/or the oligochaete worm *Lumbriculus variegatus* (EC, 2013). Currently the Aquatic Guidance Document of the European Food Safety Authority (EFSA) is used in prospective sediment risk assessment for pesticides. This document recommends using the 28d $EC_{10}$ and/or NOEC of *Chironomus* spp. in tier-1 sediment ERA for compounds with insecticidal activity and that of *L. variegatus* for compounds with fungicidal activity (EFSA, 2013). For a future update of the Aquatic Guidance Document, however, a recent scientific opinion of EFSA proposes to use the lowest 28d $EC_{10}$ value obtained for two benthic arthropods, the insect *Chironomus* spp. and the crustacean *Hyalella azteca*, in tier-1 sediment ERA for insecticides, preferably by using sediment-spiked toxicity tests (EFSA, 2015). It is, however, uncertain whether the current tier-1 approach and its proposed update are sufficiently protective for benthic invertebrates in general, since sediment-spiked toxicity data for a wider array of typical benthic species and insecticides is lacking (Deneer et al., 2013). Published microcosm and mesocosm experiments with a focus on sediment exposure to pesticides and treatment-related responses of benthic invertebrate populations are scarce. The design of microcosm and mesocosm experiments usually is based on spiking the water layer with the contaminant and not on the introduction of contaminant-spiked sediment (Fletcher et al., 2001; EFSA, 2015; Diepens et al., 2016). For these reasons, an evaluation of the protectiveness of the tier-1 effect assessment approach for insecticides by using threshold levels of population-level and/or community-level effects observed in semi-field studies as done for water exposure and aquatic organisms (Van Wijngaarden et al., 2015; Brock et al., 2016) is not yet possible for sediment exposure and typical benthic organisms.

The experiments described in this paper aimed to (i) assess effects of sediment-spiked lufenuron on benthic macroinvertebrates in outdoor microcosms to complement the water-spiked mesocosm study described above, (ii) facilitate the interpretation of the direct and indirect effects observed in the microcosms by conducting sediment-spiked toxicity tests with *Chironomus riparius*, *H. azteca* and *L. variegatus* in field-collected sediment that was also used in the microcosms, and (iii) to explore the protectiveness of the prospective tier-1 effect assessment procedure for sediment organisms as currently described in the EFSA Aquatic Guidance Document (EFSA, 2013) and recently proposed by EFSA (EFSA, 2015). This latter objective is done by comparing the NOEC of the most sensitive population- or community-level endpoint in the sediment-spiked microcosm experiment with the $EC_{10}$ and/or NOEC of the most sensitive benthic tier-1 test species. Prospective aquatic ERA for pesticides, as developed by EFSA, follows a tiered approach. A tiered ERA system as a whole needs to be appropriately protective, internally consistent and address the problem with a higher accuracy and precision when going from lower to higher tiers. Consequently, if these criteria apply, lower tiers can be validated by higher tiers, but the information of lower tiers can also be used to explain the direct and indirect effects observed in higher tiers (Fig. 1).

## 2. Materials and methods

### 2.1. Test chemical and chemical analysis

Lufenuron is a benzoylurea compound inhibiting chitin synthesis and moulting of arthropods in particular (Reynolds, 1987; Graf, 1993; Matsumura, 2010) and is used as insecticide and as veterinary medicine, including uses for flea and fish louse control. According to EFSA (2008) its $\log K_{OW}$ is 5.12 (25 °C) and its degradation half-life ($DT_{50}$) ranges from 34 to 188 days (two different laboratory water-sediment test systems).

In our experiments, lufenuron was applied as the formulated product Match®. We used this formulated product since it was also used in the previous water-spiked experimental ditch experiment that we aimed to complement. By using Match®, the plant protection product actually used in agriculture, additional solvent when spiking the sediment was not required. To spike the sediment three different stock solutions were made by dissolving the formulated product in tap water to obtain intended concentrations of 4.31, 43.10 and 431.03 mg lufenuron/L. The product remained in solution in all stock solutions prepared. To verify that the product remained in the stock solution and the lufenuron concentrations in stock and dosing solutions a subsample (1 mL) was diluted with 4 mL methanol (80% methanol), and analysed on an Agilent 1200 LC equipped with Agilent 6410 triplequad MS/MS (high concentration solutions were diluted before injection). The mean measured concentrations of the active substance (a.s.) lufenuron in the three stock solutions ranged from 104.1 to 115.6% of the intended concentrations.

The method detection limit of lufenuron in water was 0.1 μg a.s./L (López-Mancisidor et al., 2008).

To analyse lufenuron in sediment, 30 mL of acetone was added to a well-mixed wet sediment sample of 50 g and shaken for 2 h (175 movements per minute). This sample was centrifuged for 10 min at 2000 rpm. The supernatant was carefully transferred to a glass tube and evaporated under a gentle air flow until it was dry. First, 1600 μL methanol was added, sequentially vortexed and placed in an ultrasonic bath for 5 min. Then, 400 μL Milli-Q water was added, sequentially vortexed and placed in an ultrasonic bath for 5 min. Since lufenuron was not commercially available as internal standard, the bezoylurea compound novaluron that resembles the chemical structure of lufenuron, was used for this purpose. As internal standard, novaluron (2 μL, concentration 20 μg/mL) was added to 2 mL extract. Extracts were analysed on an Agilent 1200 LC equipped with Agilent 6410 triplequad MS/MS, by injecting a sample of 100 μL in a Agilent Analytical Zorbax Eclips XDB-C18 column. Concentrations of lufenuron were calculated from the calibration line of the standard solution with the use of Agilent MassHunter Software. By this software the concentration of lufenuron in the sediment samples was corrected for the signal of the MS/MS to the internal standard novaluron.

Since we used field-collected sediment in our experiments, and to facilitate comparison of toxicity estimates with published literature data, we expressed both the sediment concentrations and the toxicity estimates in terms of μg lufenuron/g organic carbon in dry sediment (in this paper indicated as μg a.s/g OC). Limit of detection of lufenuron in sediment was approximately 0.008 μg a.s./g OC and limit of quantification 0.024 μg a.s./g OC. Average recovery of the a.s. lufenuron (n = 9) in quality control samples was 92% with a standard deviation of 13%.

In our experiments we focussed on lufenuron concentrations in whole sediment and not on lufenuron concentrations in pore water