Sediment ecotoxicity of the insecticide lufenuron to benthic macroinvertebrates

Lucas Jollie
IBED-AEE
lucas_jollie@outlook.com

ABSTRACT
Pesticides such as lufenuron are widely used in agriculture to preserve crops and maximize harvests. The application of lufenuron might cause it to inadvertently end up in surrounding ecosystems and causing unwanted damage. To assess the potential ecological damage and the fate of lufenuron, sediment toxicity tests using spiked sediment were performed, as well as a sediment aging test. Chironomids appeared as the most sensitive species and the toxicity of lufenuron decreased faster than previously thought at 20°C. Lastly, very little lufenuron can easily cause an environmental risk after application.

Keywords
Lufenuron, pesticides, ecotoxicology, sediment toxicity tests, sediment aging tests, species sensitivity distribution, risk assessment

INTRODUCTION
Pesticides are widely used in agriculture in order to preserve crops and to maximize harvest yields. Benzoylureas are a class of pesticides used today as growth regulators through the inhibition of chitin synthesis in insects[24]. Lufenuron is an example of such a benzoylurea insecticide that targets chitin synthesis in insects[4,22,23]. Chitin is the second most abundant organic compound, after cellulose, and serves a similar structurally supporting function in arthropods, among others[,22,23]. When applied for agricultural purposes, pesticides will inadvertently end up in ecosystems surrounding farmlands, including aquatic habitats such as ditches. Due to its hydrophobic nature, lufenuron quickly partitions to the sediment and persists there[2].

Data on the half-life (DT_{50}) of lufenuron is very incoherent with estimations lying between 13 and 174 days depending on conditions[2,6,18,19]. Multiple metabolites are formed when lufenuron is broken down, differing in their toxicity to different species[11,27-30]. In addition, other data suggest that microbial life in the soil is an important factor in determining the rate of decay, with soil depleted of microbial life showing no signs of lufenuron degradation or formation of its metabolites[27]. These data indicate that lufenuron may persist in the environment and therefore data on the fate and effects of lufenuron are urgently needed in order to obtain a reliable risk assessment of its application.

Previous research showed a decline in the populations of several non-target macroinvertebrate species in outdoor mesocosm experiments with treated and untreated segments of ditches[2,3]. These species included benthic arthropods belonging to the genera *Chironomus*, *Caenis* and *Gammarus*, as well as the family Asselidae[2], These results have been verified for *Chironomus riparius* in a laboratory study using chronic toxicity tests[2].

Given the potential persistence of lufenuron in sediments and the first indications of adverse effects on non-target arthropods, the present project aimed to study the fate and effects of lufenuron associated sediments. To this purpose, missing lufenuron sediment toxicity data were generated and the effects of aging on lufenuron sediment toxicity were evaluated. Semi-chronic (10d) and chronic (28d) toxicity tests using spiked sediments with a range of lufenuron concentrations were conducted in order to assess the concentration at which 50% of the individuals undergo the effects (survival) of lufenuron (EC_{50}) of several benthic macroinvertebrates. For the sediment aging toxicity experiment, three batches of spiked sediment were kept out of cold storage for zero weeks (control) and eight weeks and used in 10d toxicity tests with *C. riparius*.

The present study made it possible to create a species sensitivity distribution (SSD) for lufenuron, which is useful for obtaining a reliable risk assessment. The selected test species share life-cycle traits with animals commonly found at the sites of lufenuron application, including the non-biting midge *Chironomus dilutus*, whose larvae are classified as a filter feeders that live in the sediment; the water louse *Asellus aquaticus* is an epi-benthic shredder, scraping organic matter of dropped leaves and the caddisfly *Sericostoma personatum*, which is considered a shredder as well[5,7,8,12-14,16,21,31,34,36]. These organisms were chosen due to their generally different sensitivity to pollutants, ranging from not very sensitive to very sensitive, respectively[14].
In the spiked sediment experiment, it was expected that all test species would be influenced negatively, especially at higher concentrations. *C. dilutus* was expected to be the least sensitive of the test species due to differences in feeding mechanisms compared to the other species, which depend on intake of (epi)-benthic organic matter to which lufenuron adheres more easily than water-bound carbon taken in through filter-feeding by *C. dilutus*.
In the sediment aging experiment, toxicity of the sediment was expected to decrease over time, albeit slow due to the lack of aerobic conditions.

MATERIALS & METHODS
Test compound
The tested compound was lufenuron, a chitin production-inhibiting benzoylurea pesticide (chemical formula: $C_{17}H_8C_{l2}F_8N_2O_3$, CAS: 103055-07-8), with a log K_{OW} of 5.12 at 25°C[2,11]. Toxicity tests were conducted using

sediment spiked with concentrations of µg lufenuron per gram organic carbon per kg dry sediment (referred to as µg from this point on) as shown in table 1.

Table 1. Lufenuron concentrations in the spiked sediment subjected to toxicity testing, given in µg.

Regular sediment	*Sericostoma* sediment
0.00 (control)	0 (control)
0.15	0.032
0.50	0.094
1.5	0.32
4.5	0.94
15	2.85
45	8.54
135	-

Test species

For this study, *C. dilutus*, *A. aquaticus* and *S. personatum* were selected as test species. *C. dilutus* and *A. aquaticus* were cultured at IBED at the University of Amsterdam and *S. personatum* was collected from the Seelbeek near Heveadorp, The Netherlands.

Sediment toxicity tests

Methods adapted from Brock *et al.* (2016) were used for the performance of the toxicity tests, following OECD guideline 218[2,26]. Sediment used for *S. personatum* was diluted with quartz sand to provide a more suitable habitat (table 1). Dried nettle powder was mixed into all sediments (0.25% of dry weight of the sediment). Five replicates per concentration, per species were used by filling vessels (300mL) with 1.5cm of sediment, topped off with 250mL of Dutch Standard Water. For *C. dilutus* and *S. personatum*, ten and five larvae were placed in each replicate, respectively, and for *A. aquaticus*, ten young individuals were used. Animals were exposed for ten days or 28 days in the semi-chronic and chronic toxicity tests respectively. After exposure, surviving individuals were counted and the collected data were used to determine EC_{50}-values for survival per species. The same methods used for 10d toxicity tests were used in the sediment aging tests using *C. riparius* with sediment exposed to air for 0w and 8w at 20°C.

Data analysis

Data analysis was done according to Haanstra *et al.* (1985), using the formula below[17]:

$$y = \frac{c}{1 + e^{b(\log(x) - \log(a))}}$$

In which y is the measured survival, x is the exposure concentration, a is the EC_{50}, b is the slope of the logistic curve and c is the average survival in the control. Comparisons between EC_{50}-values were made using a χ^2 goodness of fit test. The DT_{50} of lufenuron toxicity was calculated by fitting the proportions of remained toxicity through the following equations:

$$C_t = e^{-kt} \qquad DT_{50} = \frac{\ln(2)}{k}$$

in which C_t represents the proportion of the toxicity after aging the sediment, k is a constant and t is the time the sediment was left to age.

SSD and risk assessment

An SSD was created using presently and previously obtained EC_{50}-values and fitting a line through these data using an SSD generator[35]. The resulting SSD can be used for risk assessment, using the Predicted No Effect Concentration and the Predicted Environmental Concentration ratio (PNEC:PEC-ratio). The PNEC is derived from the concentration at which 5% of exposed species is expected to experience adverse effects of lufenuron (HC_5) and an assessment factor (AF)[9]. Using the following formula, the PNEC was calculated:

$$PNEC = \frac{HC_5}{AF}$$

RESULTS

Chronic toxicity tests performed with *A. aquaticus* and *S. personatum* yielded EC_{50}-values of 8.1 µg and 7.9 µg respectively (figure 1).

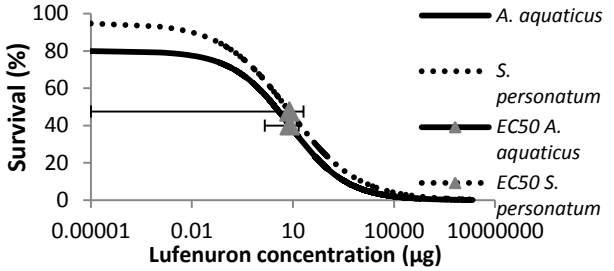

Figure 1. Fitted concentration-response curves of survival (% of initial animals) of *A. aquaticus* and *S. personatum* after 28d exposure to lufenuron (µg) concentrations in spiked sediment. EC_{50} error bars represent the 95%-confidence interval. Control concentrations (0 µg) are altered to 0.00001 µg.

Similarly, semi-chronic toxicity tests using *C. dilutus* and *S. personatum* yielded EC_{50}-values of 6.5 µg and 54.0 µg, respectively (figure 2).

None of the found EC_{50}-values differed significantly between species or between exposure duration due to large confidence limits ($p > 0.05$; figure1; figure 2).

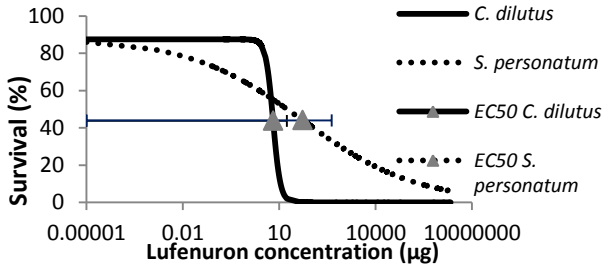

Figure 2. Fitted concentration-response curves of survival (% of initial animals) of *C. dilutus* and *S. personatum* after 10d exposure to lufenuron (µg) concentrations in spiked sediment. EC_{50} error bars represent the 95%-confidence interval. Control concentrations (0 µg) are altered to 0.00001 µg.

The SSD that was created following these and other tests is shown in figure 3. From the SSD, the HC_5 was determined to be 0.3 µg. Next, the PNEC was calculated with the AF being 10, resulting in a PNEC of 0.03 µg[9].

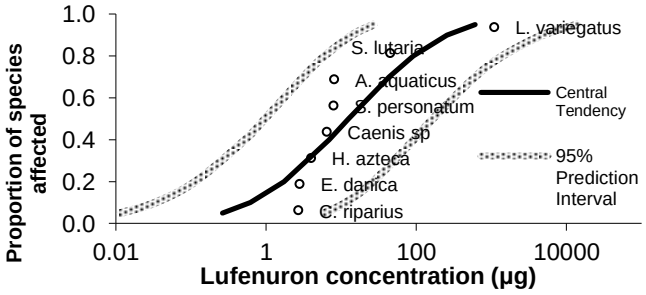

Figure 3. SSD for lufenuron. X-axis displays lufenuron concentrations in µg, whereas the Y-axis displays the proportion of species affected. The solid line represents EC_{50}'s, whereas the dotted lines represent the two-tailed 95% confidence intervals. Data points represent the tested species and their corresponding EC_{50}-values for lufenuron. Curves were fitted with the US EPA SSD generator[35].

Semi-chronic toxicity tests performed with *C. riparius* on sediment aged for 0w (control) and 8w showed a significant decrease in toxicity (EC_{50}'s 8.9 µg and 42.5 µg, respectively; $p < 0.001$; figure 4). Using these calculated EC_{50}-values, the proportion of remaining lufenuron toxicity was calculated and used to determine the DT_{50} for lufenuron toxicity, which resulted in a DT_{50} of 24.7 days.

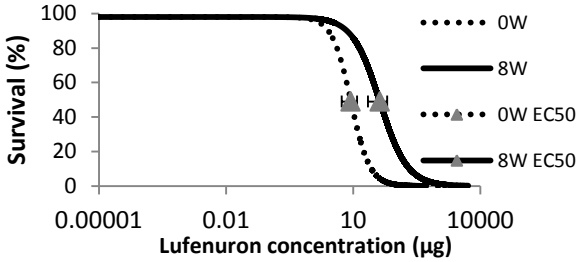

Figure 4. Fitted concentration-response curves of survival (% of initial animals) of *C. riparius* after 10d exposure to lufenuron (µg) concentrations in spiked sediment aged for 0w and 8w. EC_{50} error bars represent the 95%-confidence interval. Control concentrations (0 µg) are altered to 0.00001.

DISCUSSION

Sediment ecotoxicity of lufenuron

In the present study, the lufenuron spiked sediment clearly affected all test species, after (semi-)chronic exposure. In contrast to expectations, *S. personatum* was observed to be equally sensitive as *A. aquaticus* during chronic exposure to lufenuron (EC_{50}'s 7.9 µg and 8.1 µg, respectively), whilst being expected to be the most sensitive. Moreover, in the 10d toxicity tests *C. dilutus* was the most sensitive species, whereas *S. personatum* was very insensitive after 10d exposure (EC_{50}'s 6.5 µg and 54.0 µg, respectively). The EC_{50} determined for *S. personatum* during the semi-chronic toxicity test, however, is unreliable due to low mortality at the highest test concentrations. The observed higher sensitivity of *C. dilutus* might be due to the use of first instar larvae[15,25]. Consequently, individuals went through at least one moult during the exposure, on which the chitin production inhibiting mechanism of lufenuron could act, whereas most *S. personatum* larvae were near the end of their life-cycle and therefore not readily producing chitin[4,33]. In addition, instar duration reported for *S. personatum* was at least 26 – 28d depending on instar, temperature and light:dark ratios, resulting in a total life-cycle duration of at least one year[37]. In the present study, it was only during chronic exposure, when incomplete pupation was observed for some of the tested individuals, where lufenuron could exhibit its specific mode of action. These results imply that a proper analysis of the effects of lufenuron on *S. personatum* should be done with toxicity tests lasting at least 28d and using younger instar larvae.

Interestingly, the EC_{50} for *C. dilutus* after 10d exposure was lower than that of *A. aquaticus* after 28d exposure, whereas toxicity tends to increase with increasing exposure time, implying that *C. dilutus* is more sensitive to lufenuron than *A. aquaticus*[31,32,38]. This might partially be explained by the fact that *C. dilutus* larvae live in the sediment and therefore are more readily exposed to lufenuron, as opposed to the epi-benthic *A. aquaticus* younglings. Performing chronic toxicity tests with *C. dilutus* and semi-chronic toxicity tests with *A. aquaticus* might provide further insight into the actual differences in sensitivity to lufenuron of both species.

Lufenuron SSD

From the created SSD, the resulting PNEC is quite low, implying that a PEC higher than 0.03 µg would imply an environmental risk[9]. Due to the unavailability of a PEC, a proper risk assessment cannot be made. A PEC should be generated through the use of statistical models or data on environmental concentrations of lufenuron in application areas. Until then, careful application of lufenuron is needed due to the easily exceeded PNEC value.

Effects of aging on lufenuron sediment toxicity

Assuming toxicity of lufenuron and its metabolites did not change significantly over time, these findings indicate that the biologically available lufenuron concentrations lowered over time proportionally to the decreased measured toxicity. Thus, based on toxicity was therefore not as persistent as previously noted based on chemical analysis showing that lufenuron met the REACH regulation standards of (very) persistent chemicals[2,10]. The discrepancy between previously found DT_{50}-values might be due to different test conditions, but also due to a decrease in toxicity whilst lufenuron still persisted in the soil[1]. In addition, a study using lead and arsenic observed that a decrease in bioaccessibility leads to a decrease in ecotoxicity as well, which might be applicable here too[22]. Further research into degradation kinetics and decreasing bioaccessibility of lufenuron might provide more insight into the exact degradation of lufenuron and its ecotoxicity under different conditions.

CONCLUSION

From the present study, it can be concluded that chironomid larvae are overall more sensitive to lufenuron exposure than *A. aquaticus* younglings and near-adult *S. personatum* larvae. Furthermore, it can be concluded that the decrease in toxicity of lufenuron proceeds faster under anaerobic conditions at 20 °C than previously thought. Lastly, lufenuron should be applied very carefully, as the very low PNEC-value may easily be exceeded, potentially causing an environmental risk.

ROLE OF THE STUDENT

I worked on my bachelor's project under the supervision of dr. Arie Vonk and dr. Michiel Kraak, in cooperation with dr. Theo Brock and dr.ir. Ivo Roessink of the University of Wageningen. The overall design of the experiment was made by Theo Brock, whereas the execution of the experiments and the data analyses were performed by me. The design of the aging experiment was done together with my supervisors.

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