Ecotoxicology and Environmental Safety 81 (2012) 49–54

Contents lists available at SciVerse ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Closer to reality — the influence of toxicity test modifications on the sensitivity of *Gammarus roeseli* to the insecticide imidacloprid

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ARTICLE INFO

Article history:
Received 26 January 2012
Received in revised form
9 April 2012
Accepted 14 April 2012
Available online 9 May 2012

Keywords:
Crustacea
Haber's rule
Neonicotinoid
Sensitivity differences

ABSTRACT

Laboratory toxicity test designs are far from reality and therefore extrapolations to field situations may be more difficult. In laboratory experiments with the amphipod *Gammarus roeseli* exposed to the insecticide imidacloprid it was investigated if test conditions closer to reality influences its sensitivity and if it is possible to extrapolate results from these laboratory tests to results from a stream mesocosm study. Experiments were run by varying medium, temperature, size, and seasonal origin of gammarids. Age and seasonal aspects had strongest effects with juveniles and animals taken from a spring population being most sensitive with an EC_{50} (96 h) of 14.2 $\mu g\,L^{-1}$ imidacloprid. The test designs closest to the conditions in the stream mesocosms reflected best the results in mesocosms study on basis of LOEC values. However, the EC_x extrapolation failed to predict the effects of short term imidacloprid pulses in the field.

1. Introduction

Gammarids (amphipoda, crustacea) can be found world-wide (Graça et al., 1994; Siegismund, 1988) and play a key role in freshwater lotic ecosystems. As shredders they are involved in the breakdown of coarse particulate organic matter such as leaves, and are also important as food source for fish (Cummins and Klug, 1979; Kelly et al., 2002). They can be found in freshwater in high abundance of up to 10,000 Ind. m^{-2} (Welton, 1979). A population decrease or breakdown, e.g., due to pollutants may therefore have severe consequences for the ecosystem (Wallace and Webster, 1996).

Gammarids are known to be sensitive to a wide range of pollutants (Ashauer et al., 2011; Borlakoglu and Kickuth, 1990). They can be affected by pesticides especially insecticides entering streams and rivers via run off and spray-drift (Schulz et al., 1995). Despite their important role in stream ecosystems, there is no standard laboratory toxicity test for gammarids so far since in risk assessment, the water flea *Daphnia sp.* (Cladocera) is considered as representative for crustaceans. Recently, Ashauer et al. (2011) found that the sensitivity of *Daphnia sp.* and *Gammarus sp.* towards insecticides is in general comparable but that gammarids are more sensitive to some chemicals such as neurotoxic neonicotinoid insecticides like imidacloprid. Also, sublethal endpoints for gammarids such as locomotion or shredding activity seem to be promising new endpoints for risk assessment (Kunz et al., 2010). Furthermore, gammarids as stream organisms are differently exposed to pollutants than, e.g., daphnids: they live on sediment and in detritus, which they also feed on and they show more complex behavior such as downstream drift (Beketov and Liess, 2008b; Maltby, 1992). Their life span with up to 17–23 months for females and 30 months for males (Welton and Clarke, 1980) is far longer than that of daphnids with a few weeks (Anderson and Jenkins, 1942). This different life cycle, feeding strategy and behavior may make them more vulnerable to pesticides under natural conditions in the long-term view. Indeed, the number of laboratory toxicity tests as well as field and cosm experiments using gammarids as test organisms has increased lately (Bundschuh et al., 2011; Adam et al., 2009; Bloor et al., 2005; Pascoe et al., 1994).

Stampfli et al. (2011) recently stated that the environmental context seems to be very important for ecotoxicological evaluations. However, in laboratory tests there is not much environmental context as e.g., clones are often used as test organisms and physico-chemical parameters are held optimal. On the other side, mesocosm studies are much closer to reality and are commonly used in higher tier risk assessment for pesticides and for the clarification of special risk assessment concerns. Among other studies, a comprehensive stream mesocosm experiment was conducted by the Federal Environment Agency in Germany in

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http://dx.doi.org/10.1016/j.ecoenv.2012.04.015

order to investigate the effects of repeated pulses of the insecticide imidacloprid on behavior of the gammarid *Gammarus roeseli*, in particular with regard to the endpoint macroinvertebrate drift (Mohr et al. 2012, pers. com. Rüdiger Berghahn, Umweltbundesamt). In this mesocosm study, the neonicotinoid insecticide imidacloprid was chosen since it can be found in high concentrations in water and sediment (Tennekes 2010a) and was known to affect the behavior of *Gammarus pulex* (Beketov and Liess 2008b). Mesocosm studies such as the stream mesocosm study of Mohr et al. (2012) are too costly and labor intensive to be used routinely in the risk assessment process. Laboratory studies with increased realism in design such as the inclusion of food, the use of organisms collected in the field rather than cultured organisms, and a natural temperature regime may help to bridge the gap between simplified laboratory and comprehensive mesocosm experiments. For comparison with the results of the stream mesocosm study on the effects of the insecticide imidacloprid by Mohr et al. (2012), six laboratory toxicity experiments with the amphipod *G. roeseli* were run with varying temperature, medium, test duration, size of gammarids, food availability, and seasonal origin of the tested animals. *Gammarus roeseli* was chosen as test organism for reason of comparison since it was the most abundant species in the stream mesocosm study (Mohr et al. 2012). *Gammarus roeseli*, a common species in German surface waters, originates from the Balkan area (Karaman and Pinkster, 1977) and has colonized several Western European countries in the course of the past century (Kley and Maier, 2006). In contrast to *G. pulex*, *G. roeseli* prefers slow-flowing and summer warm rivers and streams (Pöckl et al., 2003). So far, there are no toxicity data available for *G. roeseli* exposed to imidacloprid or other pesticides but for *G. pulex* and *G. fossarum* (Ashauer et al., 2011; Beketov and Liess, 2008b; Lukančič et al., 2010). However, as it is known that there are differences in toxicity between amphipods species (Alonso et al., 2010a; Maltby, 1995), toxicity data for e.g., *G. pulex* may not be transferable to *G. roeseli*.

In this toxicity study it should be investigated if test conditions closer to reality influences the sensitivity of *G. roeseli* and if it is possible to extrapolate results from these laboratory toxicity tests to results of the stream mesocosm study by Mohr et al. (2012).

2. Materials and methods

2.1. Experimental design

The acute toxicity of imidacloprid on *G. roeseli* was tested in six laboratory experiments with varying experimental conditions (Table 1). The experiments differed in medium, food availability, temperature, and size and life stages of the tested gammarids. It was intended to compare standard like test conditions using artificial medium and a common temperature regime (experiments 1and 2) with test conditions closer to the conditions in the stream mesocosm experiment (Mohr et al., 2012) with stream water and different seasonal origin of the test animals (experiment 3–6; animals from a spring population were exposed to imidacloprid pulses in the mesocosm study).

Table 1
Experimental design for the toxicity tests with varying test date, temperature, medium (AW: artificial fresh water, SW: stream water), food regime, and size. SD means standard deviation.

Experiment	Collecting date	Mean temperature in °C (SD)	Medium	Food	Size (mm)
1	17. Sep	17 (0.4)	AW	+	6
2	17. Sep	17 (0.4)	AW	–	6
3	05. Apr	12 (0.2)	SW	+	6
4	05. Apr	12 (0.2)	SW	+	9
5	05. Apr	12 (0.2)	SW	+	11
6	05. Apr	12 (0.2)	SW	–	11

A series of eight concentrations of imidacloprid (6, 12, 24, 48, 96, 192, 384, and 768 $\mu g\,L^{-1}$) was used in all 6 toxicity tests with ten replicates per concentration (one gammarid per replicate, ten gammarids per concentration) and twenty control beakers with one gammarid and medium only. The imidacloprid concentrations were prepared with the respective medium and the respective concentrations from a stock solution of 12 $mg\,L^{-1}$ imidacloprid. Because of the high water solubility of imidacloprid, no solvent was necessary (Tomlin, 2009).

As endpoint the immobility of *G. roeseli* was checked after 24 and 96 h of exposure. The animals were considered immobile when there was no flight reaction after a slight touch of their pleopods/uropods. Other endpoints like behavioral alterations were not determined since video tracking was not available and direct observations would have been too time consuming and may also have led to subjective misinterpretations. Exuviae were removed from the beaker during daily inspections. Test solutions were not renewed during the experimental period. The light regime was of 5:19 h (light/dark) in all experiments in order to reduce potential light stress of the animals (Lukančič, 2008) and to minimize photo degradation of imidacloprid via UV radiation (Moza et al., 1998). All used media were aerated before the experimental period but not during the experiment.

2.2. Gammarus collection and acclimatization

Specimen of *G. roeseli* were collected in mid September (experiments 1 and 2) and beginning of April (experiments 3–6; Table 1) from the oligo- to slightly β-mesosaprobic stream Barolder Fließ (Lat: 51.99027°N, Lon: 14.21952°E, Brandenburg, Germany) by using straw bags as attraction devices (Mohr et al., 2012). After two weeks of exposure, colonized straw bags were transferred cool and humid to the laboratory using fine mesh cover bags. Gammarids were acclimated to the different test media and experimental conditions for at least five days. Gammarids were fed with preconditioned alder leaves (*Alnus glutinosa*; (Cummins et al., 1989). Three life stages were selected on the basis of body length: adults 11 mm (precopulatory pairs were rejected), early adult with 9 mm and juveniles with 6 mm (McCahon and Pascoe, 1988; Pöckl, 1992). The animals were visually selected by one person. After the experiment, each individual was measured with a binocular. Gammarids were not separated according to sex to prevent stress before the experiment. Animals, which were visibly affected by parasites, were not considered.

2.3. Artificial water experiment

Experiment 1 and 2 were conducted with gammarids of 6 mm length exposed to artificial water at a mean temperature of 17 °C (Tables 1 and 2). Active and externally undamaged individuals were placed randomly in 200 mL glass beakers which contained 100 mL of artificial water dosed with imidacloprid (AW medium, Table 2, (Naylor et al., 1989)). In experiment 1, animals were fed conditioned alder leaf disks of 1 cm diameter.

2.4. Stream water experiment

In experiments 3–6 stream water was used as test medium (SW medium, Table 2). SW medium was obtained from the control stream mesocosms of the Artificial Pond and Stream System of the German Federal Environment Agency (Berlin, Germany, http://www.umweltbundesamt.de/wasser-und-gewaesserschutz-e/fsa/index.htm), which had been biologically established at least 6 month prior to the start of these experiments. The stream water was filtered (Whatman: folded filter Grade 1573 ½) in order to remove plankton organisms. Main chemical characteristics of the SW medium are given in Table 2. The experiments 3–6 were run at 12 °C. Gammarids of 6 mm,

Table 2
Chemical and physical parameters of the artificial fresh water and stream water. Asterisk: randomly measured in four vessels per concentration every day. Data in brackets: standard deviation.

	Artificial fresh water	Stream water
Mean temperature in °C	17 (0.4)	12 (0.2)
Mean pH*	7.7 (0.09)	7.8 (0.17)
Mean electrical conductivity ($\mu S\,cm^{-1}$)*	687 (15.1)	505 (19.8)
Mean oxygen saturation (%)*	90.8 (0.9)	89.2 (1.1)
Mean alkalinity ($mmol\,L^{-1}$)	2.87	1.57
Cl^- ($mg\,L^{-1}$)	73.6	41.6
$SO4^{2-}$ ($mg\,L^{-1}$)	63.7	96.1
Mg^{2+} ($mg\,L^{-1}$)	16.1	6.7
Ca^{2+} ($mg\,L^{-1}$)	80.2	44.9
Na^+ ($mg\,L^{-1}$)	17.7	39.6
K^+ ($mg\,L^{-1}$)	3.0	2.4

9 mm and 11 mm size were used in experiment 3, 4, and 5, respectively, and fed alder leaf disks (Table 1). In contrast to experiment 5, the gammarids in experiment 6 were not fed during imidacloprid exposure.

2.5. Chemical and physical analysis

Oxygen saturation (fiber-optic microsensor, PreSens, Germany), temperature, electrical conductivity (Cond 340 with Tetra Con 325 sensor) and pH (pH 197-S with glass electrode, SenTix mic, each WTW, Germany) were measured randomly every day in 3 vessels for each concentration (Table 2). Major ion composition of the stream water (Ca, Mg, Na, K, SO_4, Cl, HCO_3) were measured by use of a combined titration and ion chromatography system (TitrIC; IC 861, anions with HCO_3-/CO_2-suppression, Metrohm Switzerland).

Imidacloprid was analyzed at the beginning and at the end of the toxicity test by use of SPE technique, derivatization and quantification by GC/MS (Mohr et al., 2012).

2.6. Data analysis

EC_{50} (based on immobility) values of *G. roeseli* for 24 h and 96 h and their respective 95% confidence intervals were calculated for imidacloprid for the experiments 1–6. A log-logistic dose-response model with variable slope was fitted to the survival data with GraphPad Prism (Version 4.03, GraphPad Software Inc., USA) using the average of the nominal exposure concentrations from the different sampling times for each treatment. The parameters top and bottom were fixed to 100% and 0%.

For technical reasons it was not possible to measure immobility of *G. roeseli* after 12 h of exposure in the study at hand. However, for comparison with the results of the mesocosm study, in which 12 h pulses of 12 μgL^{-1} imidacloprid were set (Mohr et al. 2012), Haber's rule was used to extrapolate 12 h EC_{50} values from the 24 h and 96 h EC_{50} values. In the basic linear model of Haber's rule the toxicity depends on the product of concentration and time (Hommen et al., 2010).

Haber's rule: $k = c * t$ where c is the concentration, t is the exposure time, and k is the constant.

To check if Haber's rule is applicable to the data, extrapolations were done by (a) extrapolating the 24 h EC_{50} value to 12 h and 96 h EC_{50} values and by (b) extrapolating the 96 h EC_{50} value to 12 h and 24 h EC_{50} values. If Haber's rule is applicable, the both extrapolations (a) and (b) should give the same or very similar EC_x values.

3. Results

In all tests, the measured imidacloprid concentrations at the end of the experiments varied less than 1% to the start and nominal concentrations. Therefore nominal concentrations were used for data evaluation. In all experiments with both media the oxygen saturation was never less than 74% and the pH ranged from 7.6 to 7.8.

3.1. Effects of imidacloprid on different size classes of G. roeseli

Although the differences in EC_{50} and EC_{10} values after an imidacloprid exposure of 24 and 96 h between the three tested size classes of *G. roeseli* (Fig. 1) were small, they followed the pattern: the smaller the gammarids the higher the sensitivity. Small gammarids were more sensitive by a factor of 1.2 after 24 h, respectively, 96 h. The lowest EC_{50} (based on immobility) with 1.9 $\mu g\,L^{-1}$ (C.I. 95%: 0.1–33.6 $\mu g\,L^{-1}$) was found for the 9 mm size class after 96 h exposure, but the more reliable EC_{50} value of 14.2 μgL^{-1} (C.I. 95%: 6.4–31.2 μgL^{-1}) was found for the 6 mm size class. For all size classes, the EC values decreased with increasing exposure time (Fig. 1).

3.2. Influence of a standard and a more realistic test design on the toxicity of imidacloprid for G. roeseli

The AW and SW medium were similar in terms of pH and oxygen but differed in temperature, conductivity and major ion composition. While the AW medium was dominated by calcium and chloride ions the sulfate and carbonate (alkalinity) was more prominent in the SW medium (Table 2). *Gammarus roeseli* was far more sensitive exposed in the SW medium at 12 °C as compared to the AW medium at 17 °C by a factor of 3.6 (24 h) and 9.2 (96 h) (Fig. 2). The lowest EC_{50} and EC_{10}values of 14.2 μgL^{-1} and 1.42 $\mu g\,L^{-1}$ imidacloprid with C.I. 95% of 6.4–31.2 $\mu g\,L^{-1}$ and 0.2–12.8 $\mu g\,L^{-1}$ were found for the SW medium, respectively. Confidence intervals were wider in the AW medium as compared to the SW medium (Fig. 2). Again, toxicity effects became more pronounced after 96 h regardless the test design.

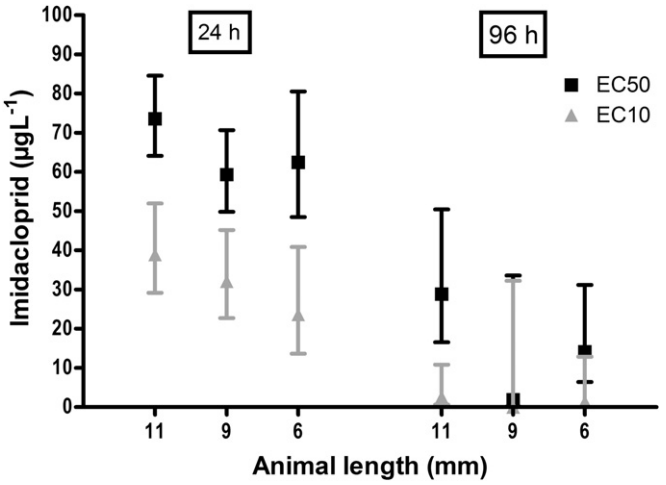

Fig. 1. EC_{50} and EC_{10} values with confidence intervals (C.I. 95%) for *G. roeseli* of different sizes in artificial water with food after 24 h and 96 h exposure to imidacloprid (experiment 3, 4, 5).

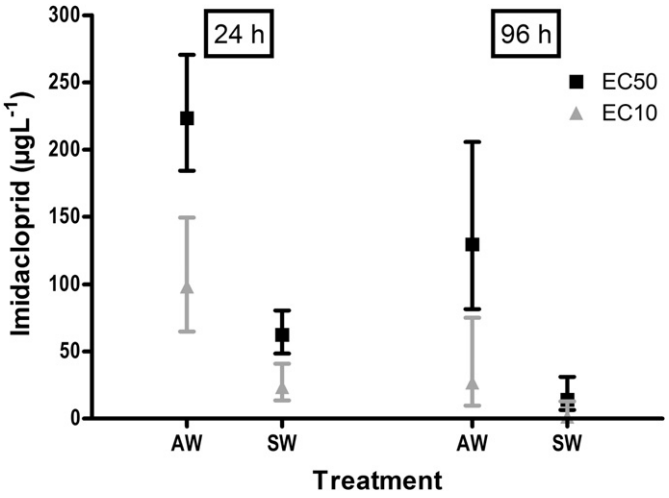

Fig. 2. EC_{50} and EC_{10} values with confidence intervals (C.I. 95%) for *G. roeseli* in artificial water (AW, 17 °C) and stream water (SW, 12 °C) after 24 h and 96 h exposure to imidacloprid (experiment 1, 3).

3.3. Influence of food on the toxicity of imidacloprid for G. roeseli

For both media, *G. roeseli* was more sensitive if no food was available, though the effects were far less pronounced in the treatments with SW medium (Fig. 3). Again, independent of food availability, EC_{50} and EC_{10} values for *G. roeseli* were lower with SW medium and with longer exposure time.

3.4. Extrapolations to 12 h EC_{50} values using Haber's rule

In experiment 1, the extrapolations from 96 h and from 24 h did not show similar results as indicated in Fig. 4. The extrapolations failed in four of six experiments. For experiment 3, Haber's

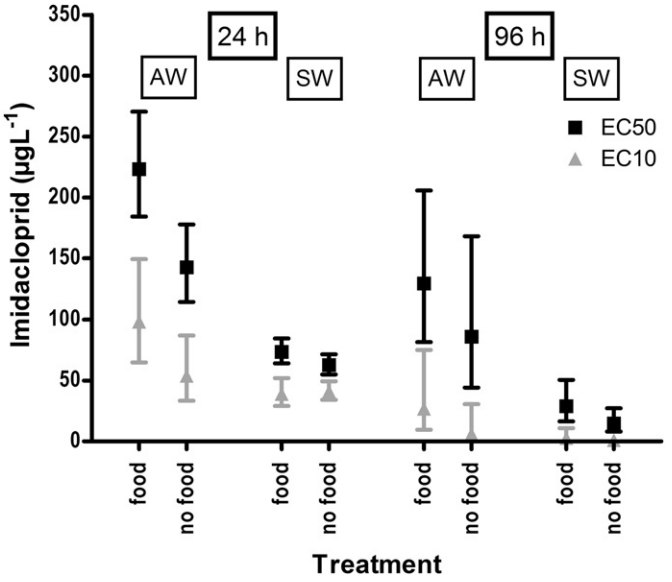

Fig. 3. EC_{50} and EC_{10} values with confidence intervals (C.I. 95%) for *G. roeseli* with and without food in artificial water (AW, 6 mm body length, 17 °C) and stream water (SW, 11 mm body length, 12 °C) after 24 h and 96 h exposure to imidacloprid (experiment 1, 2, 5, 6).

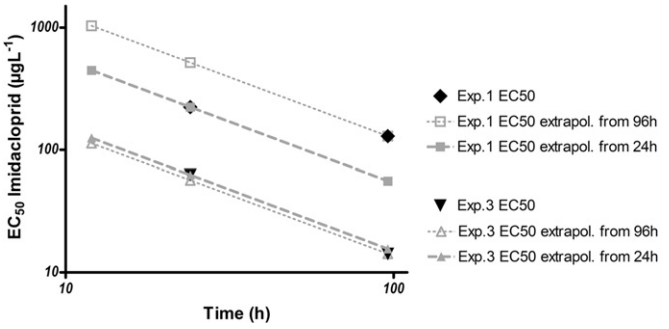

Fig. 4. EC_{50} values for 12 h, 24 h, and 96 h extrapolated from the experimental derived EC_{50} values from experiment 1 and 3 (Exp.1, Exp.3, black symbols) after (24 h) and after EC_{50} (96 h) using Haber's rule. In Exp. 1 is no correlation between extrapolation from 24 h and from 96 h. In Exp. 3 is a correlation between extrapolation from 24 h and from 96 h. Axis are in double logarithmic scale.

rule was applicable and the values were in extrapolation (a) and (b) very similar. For experiment 3, the resulting extrapolated EC_{50} 12 h value is 119 µg L^{-1} ± 6 (range) and the extrapolated EC_{10} (12 h) is 29 µg L^{-1} ± 18 (range).

4. Discussion

4.1. Influence of various experimental conditions

In this study, the EC_{50} (96 h) for *G. roeseli* varied considerable from 1.9 to 129.5 µg L^{-1} imidacloprid depending on the different experimental conditions and developmental stages of the tested animals. Experimental conditions with food, stream water and animals from spring population closely to mesocosm study resulted in highest sensitivity.

The fact that experimental conditions as well as the chosen endpoints can strongly influence the sensitivity of organisms has recently been criticized by Jager (2011) and makes it difficult to compare results of this study with other gammarid studies. Lukančič et al. (2010) found an EC_{50} (24 h) for *G. fossarum* of 70 µg L^{-1} imidacloprid in a similar range as in this study with AW medium, food and 11 mm sized gammarids but far higher LC_{50} (48 h) of 800 µg L^{-1}. Other authors found also high LC_{50} (96 h) values of 270 µg L^{-1} (Beketov and Liess, 2008b) and 131 µg L^{-1} (Ashauer et al., 2011) for *G. pulex*. For *Hyalella azteca* an LC_{50} (96 h) of 0.065 mg L^{-1} (Stoughton et al., 2008) and 0.526 mg L^{-1} (SERA-Syracuse Environmental Research Associates, 2005) has been found. Other crustacean species were less sensitive to imidacloprid than gammarids. For the standard test organism *Daphnia magna* an EC_{50} (24 h) of 97.9 mg L^{-1} (Tišler et al., 2009) and EC_{50} (48 h) 56.6 mg L^{-1} (Jemec et al., 2007) was reported. In conclusion, *G. roeseli* seems to be as good as other amphipod species like *G. pulex* and *G. fossarum* as test organism for toxicity testing also with regard to its increasing dispersal over Europe (Kley and Maier, 2006).

Some studies demonstrated that neonates and juveniles of amphipods/invertebrates reacted more sensitive to stressors as compared to adult stages (Adam et al., 2010; Naylor et al., 1990; Pastorinho et al., 2009). Especially, a greater ventilation rate of the juveniles (Maltby, 1995), a higher molting rate (Pöckl, 1995), and a larger surface area to volume ratio and therefore a greater capacity for the exchange with toxicants, as well as a higher rate of metabolism may facilitate the uptake of pollutants (Buikema and Benfield, 1979). Although it was not possible to test neonates and early life stages of *G. roeseli* in this study, there are indications for increasing sensitivity with decreasing size for *G. roeseli*. In contrast to this, (Maltby, 1995) reported a greater tolerance of juvenile gammarids to hypoxia and ammonia stress. These natural stressors may have been compensated by higher ventilation rates in the juveniles. With regard to toxicity testing, the use of juveniles should be preferred since they seem more sensitive to stressors and less sensitive to oxygen stress and ammonium which may accumulate during the test when the test medium is not renewed and several animals are tested in one test vessel. As juveniles may not be available according to the season as in this study, establishing standardized gammarid culture techniques could help to overcome this problem.

The influence of food on the sensitivity of the test organism *Gammarus* has also been demonstrated in other studies (Alonso et al., 2010b; Geffard et al., 2010). With food deficiency as an additional stressor, *G. roeseli* reacted more sensitive especially in the treatments with AW medium. The use of food in toxicity tests has pros and cons. Treatments with food simulate more realistic conditions but food may also affect the water quality due microbial decomposition and higher concentration of fecal pellets. Tested chemicals with a high log K_{ow} may also absorb to the food and increase the chemical availability via food uptake (Gobas, 1993; Steen and Karickhoff, 1981). However, food uptake and shredder activity may be also interesting functional endpoints in a toxicity test (Kunz et al., 2010) and increase the realism of the test design. Furthermore, food was used as coverage by the animals especially in treatments with high pesticide concentrations. This indicates the need for test standardization in order to simulate a more natural environment (e.g., sand, leave disks, fake coverage) and thereby reduce experimental stress and allow for natural behavior.

In this study, *G. roeseli* was far more sensitive to imidacloprid in stream water medium at 12 °C than in artificial water medium at 17 °C. It cannot be ruled out that differences in temperature may have had an effect on the sensitivity of *G. roeseli*. However, both temperatures are within the temperature optimum of this species (lowest mortality for juvenile at 12 °C and for adults at 16 °C; Pöckl, 1995). Furthermore, the molting intervals increase with temperature, which would have made the animals even more vulnerable in the 17 °C treatments (Pöckl, 1995) and not less sensitive as shown in this study. The molting frequency should be considered a relevant factor in lab studies even in short term experiments.

The different media may have been also responsible for the sensitivity differences of *G. roeseli*. Stephenson (1983) reported water hardness to have a significant influence on the sensitivity of *G. pulex*. So far, there is no standard test medium for gammarid testing. Several studies used slightly different media like artificial pond water (Ashauer et al., 2010; Naylor et al., 1989), M7 medium (Beketov and Liess, 2008a), and standard water acc. DIN-EN-ISO-6341 (1996) (Lukančič et al., 2010) to investigate the toxicity of imidacloprid to *G. pulex* and *G. fossarum*. However, all media were quite similar in the main parameters such as conductivity, pH, and major ion composition. In contrast to the stream water used here, some differences in major ion composition were obvious compared to the AW medium (Table 2), but no strong impact on the sensitivity of *G. roeseli* for imidacloprid would be expected, especially if considering the variations of these compounds in the field.

A further explanation for the different sensitivity of *G. roeseli* in the treatments with AW and SW medium may be the seasonal origin of the test animals. Gammarids in the treatment with SW medium at 12 °C were taken from the field during spring while the animals exposed to AW medium were taken from the same source in autumn at 17 °C. It is known that the physiological condition of gammarids may be influenced by seasonal changes (Krog, 1954). For instance, Sroda and Cossu-Leguille (2011) found a lower lipid content in gammarids in spring/summer than in winter. They assumed that organisms could be more vulnerable in spring/summer when their energy reserves are low as a consequence of reproduction and lower food availability. Therefore, the higher sensitivity of *G. roeseli* in the SW medium treatment may be due to weaker physiological conditions after winter pause and nutrient deficiencies in spring rather than due to medium or temperature differences. Indeed, the mean dry weight (standard deviation) of 6 mm sized *G. roeseli* taken in autumn was 2.18 ($\pm$ 0.83) mg while only 0.9 ($\pm$ 0.4) mg for the gammarids of the same size class taken in spring.

This finding is also underlined when comparing results from experiments with and without food for the different mediums. While the sensitivity of *G. roeseli*, taken in autumn from the field when there was sufficient litter available, increased without food in the AW medium, there were only very little differences with or without food in the treatments, in which animals from the spring season were used. A plausible explanation would be that the animals were already weakened and food supply in the treatments or during the adaptation phase of 5–7 days prior the experiments did not compensate for these nutritional deficiencies.

In most toxicity studies, gammarids were collected from the field (Adam et al., 2009; Alonso et al., 2010a; Beketov and Liess, 2008a; Beketov and Liess, 2008b; McCahon and Pascoe, 1988) with main reason been the lack of methods for long term maintaining and culturing gammarids. Pascoe et al. (1994) and Kelly et al. (2002) are one of the rare references who had successfully cultivated gammarids over a longer time period. Taking animals from the field has some disadvantages: the influence of natural and chemical stressors on the animals cannot be ruled out, and age of adult gammarids is difficult to determine since gammarids can live up to 350 days (Pöckl, 1995). Using cultured animals would make it easier to better determine the age and nutritional status of the animals and juveniles would be available all over the year. Furthermore, infections with parasites can be avoided. Often, populations from natural sources do have quite a high infectious rate with parasites (Poulton and Pascoe, 1990). If it is not possible to culture the animals or if it is too time consuming, origin and nutritional status of the tested animals should be described in more detail. So far, this very important information has not frequently been mentioned in published literature. Besides different experimental conditions, the seasonally dependent fitness of gammarids may therefore be a further important factor to explain the high variability of EC_{50} values even within the same species.

4.2. Extrapolation to field situation and implications for risk assessment

In the mesocosm study by Mohr et al. (2012), repeated 12 h pulses of 12 $\mu g\,L^{-1}$ imidacloprid were set in spring and summer in order to investigate effects of imidacloprid pulses on abundance and behavioral endpoints. The chosen imidacloprid concentration was an environmentally realistic concentration, which can reach streams after correct use via spray drift (CCME, 2007; Mohr et al., 2012). In review of the mesocosm study, repeated 12 h pulses of imidacloprid did not have effects on the abundance of *G. roeseli* (Mohr et al., 2012), but strong sublethal effects could be observed for behavioral endpoints such as drift (Mohr et al., 2012, pers. com. R. Berghahn, Umweltbundesamt) even after a single pulse.

For the extrapolation of the toxicity results of this study with the findings in the mesocosm study, results from experiments 3–5 in stream water medium with animals from a spring population and food represented closest the conditions in the mesocosm study. In both, the toxicity tests and the mesocosm study, juveniles reacted most sensitive. In the laboratory, only 10–20% of test animals showed adverse effects at the test concentration of 12 $\mu g\,L^{-1}$ imidacloprid after 24 h of exposure. These adverse effects most likely would have been even less pronounced after 12 h of exposure and the laboratory results would then be comparable to the results found in the mesocosm study. So, in both the mesocosm and the laboratory study, 12 $\mu g\,L^{-1}$ imidacloprid can be considered as low effect concentration (LOEC) for a 12 h pulse exposure for *G. roeseli*.

Similar to the LOEC value, an EC_{10} value can be considered as a concentration with low effects on the test animals. However, in this study the extrapolated 12 h EC_{10} values (experiment 3 for which Haber's rule was applicable) of 29 $\mu g\,L^{-1}$ was higher than the LOEC concentration of 12 $\mu g\,L^{-1}$. Therefore, it is doubtful if repeated 29 $\mu g\,L^{-1}$ imidacloprid pulses would have resulted in comparable effects as the 12 $\mu g\,L^{-1}$ imidacloprid pulses in the stream mesocosm study. Furthermore, Haber's rule was not applicable to the EC_{50} values in 4 of 6 cases. Therefore, the EC_x approach at fixed exposure times seemed to be unsuitable for extrapolating effects that may occur at other exposure times. As implication for the risk assessment, more sophisticated toxicokinetic and toxicodynamic models may help to overcome this dilemma as proposed recently by Tennekes (2010b) and Jager (2011). The implementation of "time to effect" models in risk assessment would be especially important for the risk prediction of stream organisms, which are often confronted with short term chemical pulses or for organisms.

5. Conclusions

Age as well as seasonal aspects had the strongest influence on the sensitivity of *G. roeseli* towards imidacloprid. Nutritional deficiencies of *G. roeseli* obtained from a spring population of a natural stream strongly increased its sensitivity towards imidacloprid. When comparing the results from the laboratory toxicity test with results of the mesocosm study by Mohr et al. (2012), the test designs closest to the conditions in the stream mesocosms reflected best the conditions in the stream mesocosms on basis of LOEC values. However, the EC_x evaluation using a simple linear model failed to predict the effects of short term imidacloprid pulses in the stream mesocosms. For predictions of short term pulse effects in streams, the implementation of toxicokinetic and toxicodynamic models in risk assessment may be a better solution to better predict effects of stream organisms in the field than the EC_x approach. Another possible tool for testing the toxicity of

pollutants to stream invertebrates may be an establishment of a standardized in-situ test procedure.

Acknowledgments

We are grateful to the colleagues of the department IV 2.5 for the analytical support and to Bonny Alscher, Anika Günther, and Jennifer Samer for their technical assistance. The manuscript benefited from critical remarks by Michael Feibicke, Rüdiger Berghahn and two anonymous reviewers.

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