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Acute toxicity of organic chemicals to *Gammarus pulex* correlates with sensitivity of *Daphnia magna* across most modes of action

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ABSTRACT

We investigated the sensitivity of the freshwater crustacean amphipod *Gammarus pulex* towards organic xenobiotic compounds in comparison to the sensitivity of the crustacean cladoceran *Daphnia magna*. In addition we studied the influence of the chemical's mode of action on the relationship between the sensitivity of *G. pulex* and that of *D. magna*. We tested the acute toxicity of twelve compounds (Malathion, Aldicarb, Carbofuran, 2,4-dichloroaniline, 2,4-dichlorophenol, 1,2,3-trichlorobenzene, 4,6-dinitro-o-cresol, 2,4,5-trichlorophenol, Ethylacrylate, 4-nitrobenzyl-chloride, Sea-nine, Imidacloprid) with different modes of action and physicochemical properties towards the freshwater amphipod *G. pulex* in laboratory experiments. Additional toxicity data was collected from the peer-reviewed literature and databases (data pairs for 44 chemicals in total). The chemicals were assigned to seven mode of action groups. The relationship between the sensitivity of *G. pulex* (48 h-LC50s and 96 h-LC50s) and that of *D. magna* (48 h-EC50s) was investigated using regression analysis and correlation plots.

G. pulex is two to three orders of magnitude more sensitive towards neonicotinoids than *D. magna* ($P = 0.0046$, $n = 3$). For organophosphates we found that *D. magna* is more sensitive than *G. pulex* by approximately a factor of six ($P = 0.0256$, $n = 6$). There was no significant difference between the sensitivity of *D. magna* and that of *G. pulex* in any of the other mode of action groups; however chemicals with the same mode of action grouped together in the same area of the correlation plot.

Without the neonicotinoids 75% of all *G. pulex* toxicity data were within one order of magnitude of the *D. magna* data and 100% within two orders of magnitude. The regressions with all data and with all data minus neonicotinoids were both significant linear relationships with slopes around one and intercept around zero. Thus, *G. pulex* is generally equally sensitive towards organic xenobiotics as *D. magna*.

1. Introduction

1.1. Background

Environmental risk assessment of chemicals is generally based on toxicity tests with standard test organisms (van Leeuwen and Vermeire, 2007). One of these is the crustacean cladoceran *Daphnia magna,* which naturally occurs in lentic freshwater systems. In lower tiers of risk assessments of chemicals toxicity data on *D. magna* is combined with assessment factors to account, amongst other factors, for interspecies variation (Chapman et al., 1998). Thus, it is of interest how the sensitivity of *D. magna* and *Gammarus pulex*, a non-standard test organism, varies over a wide range of chemicals. The freshwater crustacean amphipod *G. pulex*, naturally occurring in lotic water bodies, has also been widely used in toxicity testing (Kunz et al., 2010) and plays an important role in detritus processing in streams (Maltby et al., 2002). More recently we have used *G. pulex* for developing models that can be used for risk assessment of fluctuating concentrations of chemicals (Ashauer et al., 2007a,b, 2010). Fluctuating concentrations and repeated pulses of pollutants are more likely encountered by long-lived stream dwelling organisms such as *G. pulex* compared to organisms that live in ponds and lakes and have a shorter life such as *D. magna*. For the integration of modeling approaches based on *G. pulex* into current risk assessment schemes it is necessary to know if there are systematic differences in sensitivity compared to the standard test organism of current risk assessment practice. Consequently the question has arisen whether *G. pulex* generally tends to be more, less or equally sensitive to organic xenobiotic toxicants compared to the closely related standard test organism *D. magna*.

The sensitivity of aquatic organisms and their relationships have been investigated by ranking species (Wogram and Liess, 2001;

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Rubach et al., 2010) or with regression models (Dyer et al., 2006; Raimondo et al., 2007, 2010). Both, the ranking approach (Rubach et al., 2010) as well as the regression modeling studies (Raimondo et al., 2010) have found a dependence of species sensitivity relationships on modes of toxic action.

1.2. Objective

The objective of this study was to investigate the sensitivity of the freshwater crustacean amphipod *G. pulex* towards organic xenobiotic compounds in comparison to the sensitivity of the crustacean cladoceran *D. magna*. In addition we studied the influence of the chemical's mode of action on the relationship between the sensitivity of *G. pulex* and that of *D. magna*.

1.3. Study outline

We tested the acute toxicity of twelve compounds (Malathion, Aldicarb, Carbofuran, 2,4-dichloroaniline, 2,4-dichlorophenol, 1,2,3-trichlorobenzene, 4,6-dinitro-o-cresol, 2,4,5-trichlorophenol, Ethylacrylate, 4-nitrobenzyl-chloride, Sea-nine, Imidacloprid) with different modes of action and physicochemical properties towards the freshwater amphipod *G. pulex* in laboratory experiments. Additional toxicity data for organic chemicals was collected from peer-reviewed literature and corresponding toxicity data for *D. magna* were collected from databases and peer-reviewed literature. The relationship between the sensitivity of *G. pulex* (48 h-LC50s and 96 h-LC50s) and that of *D. magna* (48 h-EC50s) was investigated using regression analysis and correlation plots.

2. Material and methods

2.1. Acute toxicity tests with G. pulex

Adult *G. pulex* were collected several times during 2008/2009 from a small headwater stream in the Itziker Ried (coordinates: E 702150, N 2360850), ca. 20 km southeast of Zürich, Switzerland. After collection, the test organisms were acclimatized for a minimum of five days to the experimental conditions (13 °C, 12 h:12 h light:dark). Experiments were carried out in Pyrex® beakers, each containing 500 mL of pre-aerated artificial pond water (APW, Naylor et al., 1989). Each beaker contained ten organisms at the start of the experiment and they were fed ad libitum with a minimum of three horse-chestnut leaf discs (diameter 20 mm) inoculated with the fungi *Cladosporium herbarum* (Naylor et al., 1989).

A geometric dilution series of seven concentrations was used in each toxicity test, with two replicate beakers per concentration (i.e. 20 organisms per concentration) and one solvent control beaker and one blank control beaker. Dosing stocks were made by dilution in acetone from a mixture of ^{14}C-labelled and unlabelled material except for Malathion, Aldicarb and Carbofuran where only ^{14}C-labelled material was used. After spiking the dosing solution directly into the test medium, beakers were carefully stirred, sealed with parafilm and kept at 13 °C under a 12 h:12 h light:dark regime.

Live/dead organisms were counted after 24, 48, 72 and 96 h by gently prodding and observation of movement of appendages. Organisms were counted as dead if none of the appendices were moving after prodding for three times. Dead organisms were removed. At the same times 1 mL of the test solution was sampled from each beaker and chemical concentrations were quantified using liquid scintillation counting. Exposure concentrations were measured for all compounds, except for Aldicarb and Carbofuran, where nominal concentrations were used.

Table 1 lists the tested chemicals. The supporting information contains more details on collection dates, purities and ^{14}C-label position of chemicals, sample processing and quantification of radioactivity.

A log-logistic dose-response model with variable slope was fitted to the survival data with GraphPad Prism (v. 4.03, GraphPad Software Inc., USA) using the averages of the measured exposure concentrations from the different sampling times for each treatment. The parameters top and bottom were fixed to 100% and 0%.

2.2. Collection of additional data from literature

Additional LC50 data for *G. pulex* were collected from the peer-reviewed literature (Table 2). Corresponding 48 h-EC50 data for *D. magna* was collected from the FOOTPRINT database (http://www.eu-footprint.org/ppdb.html) where available. The FOOTPRINT database is based on quality controlled and reviewed data used for registration of pesticides (FOOTPRINT, 2009) and contains data of the highest quality available. The remaining toxicity data for *D. magna* were collected from the US-EPA ECOTOX AQUIRE database (http://cfpub.epa.gov/ecotox/), where the median was used in case of multiple entries, and from the peer-reviewed literature. Thus we collected all data pairs for acute toxicity of organic xenobiotics for these two species that were available through our study, peer-reviewed literature and the two electronic databases (FOOTPRINT and ECOTOX AQUIRE).

2.3. Data analysis

All toxicity values were log transformed to obtain normally distributed data and then analyzed with linear regression and by means of correlation plots to investigate the relationship between sensitivity of *D. magna* and *G. pulex*. As the toxicity data for *G. pulex* consists of mainly 48 h-LC50 and 96 h-LC50 values, the data with different test durations were first analyzed separately. The regression of the 48 h-EC50 *D. magna* vs. the 48 h-LC50 *G. pulex* ($n = 27$) was compared with that of the 48 h-EC50 *D. magna* vs. the 96 h-LC50 *G. pulex* ($n = 34$). As the slopes and intercepts of these two regressions did not differ significantly (see Supplementary information) all the *G. pulex* toxicity data with different test durations were pooled and analyzed together. We also added two data pairs where only 24h-LC50s were available. The total sample size is 44 data pairs (Table 2).

Each chemical was assigned a mode of action based on its molecular structure and its classification in the FOOTPRINT database (FOOTPRINT, 2009). Seven modes of action were assigned (Table 2): baseline toxicity, pyrethroids, organophosphates, carbamates, neonicotinoids, effects on nervous system (other than organophosphates, carbamates, neonicotinoids and pyrethroids), effects on energy production (which combine uncoupling and inhibition of energy transduction). Not all chemicals were assigned a mode of action and only modes of action with at least three data pairs were further analyzed.

Model II least squares regressions (Deming regression) were applied, as there is random error in both variables and were fitted to log transformed data using GraphPad Prism (v. 4.03, GraphPad Software Inc., USA). Correlation plots of all data and for each mode of action group were used to identify mode of actions, which differ from the whole dataset in that either *G. pulex* or *D. magna* are clearly more sensitive than the other.

3. Results

3.1. Acute toxicity data for G. pulex

The acute toxicity data measured for the twelve compounds in this study and the parameters of the fitted dose-response model are