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Relative chronic sensitivity of neonicotinoid insecticides to *Ceriodaphnia dubia* and *Daphnia magna*

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ABSTRACT

Neonicotinoid insecticides are a group of plant protectants frequently detected in surface waters at low concentrations. Aquatic invertebrates therefore have the potential to be exposed chronically to low concentrations of neonicotinoids. The cladocerans *Daphnia magna* and *Ceriodaphnia dubia* are among the most commonly used invertebrate test species in aquatic toxicology. Both species are known to be acutely insensitive to neonicotinoids, and while chronic toxicity has been characterized for *D. magna*, little research has been conducted with *C. dubia*. In the present study we conducted 7-d static-renewal life cycle tests for 6 neonicotinoids (acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam) with *C. dubia*, and a 21-d test with imidacloprid with *D. magna*. 7-d LC50s for *C. dubia* ranged from 8.42 mg L^{-1} for imidacloprid to > 100 mg L^{-1} for clothianidin; 7-d reproduction EC50s were 2.98 for thiacloprid, to > 67 mg L^{-1} for dinotefuran. *D. magna* were less sensitive than *C. dubia* to imidacloprid, by 4-fold for lethality and 1.5-fold for reproduction; however, acute-to-chronic ratios (ACRs) were similar. ACRs, based on 48-h acute LC50s and 7- or 21-d chronic reproduction EC10s, ranged from 5.4 for acetamiprid to 53.0 for imidacloprid (mean 36.6, CV = 51%). Chronic toxicity values for both species were orders of magnitude greater than concentrations reported in the environment, and thus hazard to these cladocerans is negligible.

1. Introduction

Neonicotinoid insecticides are a class of plant protectant pesticides used in both field and greenhouse agriculture as seed coatings, foliar sprays, and soil drenches (Jeschke et al., 2011), and in urban settings for lawn and garden care (Simon-Delso et al., 2015; Hladik et al., 2018). Neonicotinoids are highly water soluble and, as a result, are transported to receiving waters where they are frequently detected at concentrations in the ng L^{-1} range, with pulses in the low µg L^{-1} range (Hladik and Kolpin, 2016; Ontario Ministry of the Environment and Climate Change, 2016; Struger et al., 2017). Neonicotinoids are designed to target the nicotinic acetylcholine receptor (*n*AChR), found in insect nervous tissues, where they cause constant nerve stimulation leading to death (Matsuda et al., 2001; Morrissey et al., 2015). This mechanism of action is conserved between target pest species and non-target aquatic species, which, combined with exposure from agricultural and urban sources, leads to a potential hazard to aquatic communities. In response, water quality guidelines are currently being developed by jurisdictions around the world, including in Canada. Guidelines are typically developed for both acute (short-term) and chronic (long-term) exposure scenarios and are derived from corresponding acute and chronic toxicity data. Previous work in our laboratory evaluated the acute toxicity of six neonicotinoids using more than 20 aquatic invertebrates (Raby et al., 2018). Our data confirmed reports of large variations in acute toxicity between taxa, with five orders of magnitude separating the most sensitive species, typically insects, from the least sensitive, the cladocerans (Raby et al., 2018). Compared to acute data, less chronic data exists in the literature, especially for the infrequently studied neonicotinoids acetamiprid, thiacloprid, and dinotefuran.

Ceriodaphnia dubia and *Daphnia magna* are pelagic species that have the potential to be exposed to neonicotinoids in the water column of freshwaters receiving agricultural drainage. They are both commonly used in chronic studies, as their short life cycles allow for measurement of reproductive impairment over several broods within a time frame of one to several weeks. Here we present and compare relative chronic toxicities of six neonicotinoids (acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam) to the cladoceran *C. dubia*, and toxicity of one neonicotinoid, imidacloprid, to *D. magna*. Chronic data exists for *D. magna* for all six neonicotinoids, with 21 d NOEC or LOEC values for survival or reproduction ≥ 120 µg L^{-1}, and

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most ≥ 2000 μg L^{-1} (Jemec et al., 2007; Pavlaki et al., 2011; Ieromina et al., 2014; United States Environmental Protection Agency, 2017). In contrast, the chronic toxicity of only imidacloprid has been studied in *C. dubia*. In 2010, Chen et al. reported a 48 h LC50 for imidacloprid (as formulation) of 2.1 (95% confidence intervals 1.1–3.4) μg L^{-1}, and an 8 d NOEC for reproduction of 0.305 (95% CI 0.058–0.670) μg L^{-1}; however, only one concentration, equivalent to the LC25, was tested in this chronic study.

Although cladocerans are relatively insensitive to neonicotinoids compared to insects (Morrissey et al., 2015; Raby et al., 2018), they remain significant indicators of ecosystem health through their use as regulatory toxicity test organisms (e.g. Ontario Regulation 63/95 under the Ontario Environmental Protection Act, and Whole Effluent Toxicity (WET) testing under the Clean Water Act section 402 in the United States). It is therefore important to characterize cladoceran responses to neonicotinoid insecticides and to include these species in the derivation of water quality guidelines.

2. Methods

2.1. Test solution preparation

Toxicity tests were conducted with six technical grade neonicotinoids purchased from Sigma-Aldrich (Oakville, ON, Canada): acetamiprid (99.9%, CAS 135410-20-7), clothianidin (99.9%, CAS 210880-92-5), dinotefuran (98.6–98.8%, CAS 165252-70-0), and thiacloprid (99.9%, CAS 111988-49-9). Imidacloprid (99.8%, CAS 138261-41-3) was obtained from Bayer CropScience (Mississauga, ON, Canada) and thiamethoxam (98.5%, CAS 153719-23-4) was obtained from Syngenta Crop Protection LLC (Guelph, ON, Canada). Highest test concentrations were prepared by dissolving 50 mg (for thiacloprid), or 100 mg of chemical (for all other neonicotinoids) per 1 L of dechlorinated municipal tap water (representing the highest nominal test concentration) in Class A glassware, covering with tin foil to limit photodegradation, and stirring overnight. Physicochemical properties of dechlorinated municipal tap water are in Supplemental data, Table S2. Seven concentrations of each neonicotinoid were prepared by serial dilution in a 50% geometric series from the highest test concentration. Nominal concentrations were 100, 50, 25, 12.5, 6.25, 3.12, 1.56 mg L^{-1} for dinotefuran, clothianidin, imidacloprid, and thiamethoxam; and 50, 25, 12.5, 6.25, 3.12, 1.56, 0.78 mg L^{-1} for acetamiprid and thiacloprid. Each test included a negative control of dechlorinated water for a total of eight treatments. All test solutions were prepared at the start of the test and stored in the dark at room temperature (21 ± 2 °C). Conductivity, pH, dissolved oxygen, and temperature were measured on all test concentrations at test initiation and termination, and at each solution change. Temperature was monitored throughout the test.

2.2. Toxicity tests

2.2.1. Ceriodaphnia dubia

Static renewal life-cycle tests with *C. dubia* were conducted according to Environment Canada (2007). Each of the 8 treatments had 10 replicates consisting of a 30-mL flat-bottomed glass tube containing 15 mL of test solution, 0.6 mL total food, and 1 *C. dubia* individual. Tests were initiated with < 24 h old neonates from a continuous culture of *C. dubia*. Culturing was performed according to Environment Canada (2007); a brief summary is provided in the Supplemental data, Table S1. Test solutions were renewed daily by preparing new replicates and transferring the adult daphnid from old to new test solution. Mortalities and the number of neonates produced were counted at this time. After transfer, each replicate was fed 0.5 mL concentrated *Raphidocelis subcapitata* algae (~ 10^8 cells/mL) and 0.1 mL yeast-cereal grass-trout chow (YCT; 1.8 g dw L^{-1}; filtered through 35 μm screen). Tubes were incubated at 25 ± 1 °C under 500–1000 lx cool-white fluorescent light with a 16:8 light: dark cycle. Tests were considered complete after ≥ 60% of negative control replicates had produced 3 broods; this always occurred within 7 d. Water chemistry parameters (pH, conductivity, dissolved oxygen, temperature) were measured at test initiation, after each solution renewal, and at test termination.

2.2.2. Daphnia magna

A 21-d static-renewal life-cycle test was conducted with *Daphnia magna* according to the Organization for Economic Cooperation and Development (2012) method. The test consisted of 7 concentrations of imidacloprid and a negative control with 10 replicates each. As chronic data for *D. magna* exists in the literature, only imidacloprid was tested to provide a point of comparison to *C. dubia*. Replicates consisted of a 50-mL glass test tube containing 50 mL test solution, 1.0–1.5 mL concentrated algae, and 1 *D. magna* individual. Tests were initiated with < 24 h old neonates from a continuous culture of *D. magna*. Culturing was performed according to Ontario Ministry of the Environment and Climate Change Aquatic Toxicology Unit (2014), and is summarized in Supplemental data, Table S1. Test solution was renewed 3 ×/ week on non-consecutive days by pipetting the adult daphnid into a temporary holding chamber and then pouring the tube contents through a fine screen to isolate neonates. Fresh solution was poured into the tube, and the adult daphnid transferred into the tube. After renewal, each replicate tube was fed concentrated algae (~ 10^8 cells/mL); only *R. subcapitata* was fed on days 0 and 2, and an approximately 1:1 (based on cells/mL) mixture of *R. subcapitata* and *Chlorella fusca* was fed thereafter, with a total of 1.0–1.5 mL algae fed at each solution exchange. Tubes were incubated at 20 ± 1 °C under 400–800 lx cool-white fluorescent light with a 16:8 light: dark cycle. Water chemistry parameters were measured at test initiation, before and after each solution renewal, and at test termination.

2.3. Chemical analysis

Test solutions were collected and analyzed by liquid chromatography/tandem mass spectrometry (LC-MS/MS) to confirm nominal concentrations. Due to constraints on the number of samples we could analyze, 1 or 2 prepared concentrations per neonicotinoid (40-mL sample) was collected at time of initial organism exposure and stored in amber glass vials at 4 ± 2 °C for up to 9 weeks prior to analysis. Method detection limits were 0.000005–0.00001 mg L^{-1}, as described in Table 1. Complete details regarding chemical analysis are provided in the Supplemental data, S2.

2.4. Statistical analysis

Two endpoints were analyzed: lethality and reproduction. Lethality was tallied as percent of individuals per treatment (n = 10) dead at test termination. Reproduction was tallied as total number of neonates produced for each replicate in either the first three broods for *C. dubia*, or over the total 21 d for *D. magna*. For both species' tests, if a daphnid died before producing a brood, the number of neonates produced was recorded as zero. If a daphnid died after producing young, the number of neonates was still used in the analysis (Environment Canada, 2007).

Significant differences between control and neonicotinoid treatments within a test were assessed for reproduction. The assumptions of normality were assessed with a Shapiro-Wilk test, and the assumption of equal variance were assessed using a Spearman rank correlation between the residuals and dependent variable. If assumptions of normal and equal variance passed, a one-way analysis of variance (ANOVA) with $\alpha = 0.05$ and a post-hoc Tukey's test was conducted. If assumptions failed, a Kruskal-Wallis ANOVA, with $\alpha = 0.05$, and a post-hoc Dunn's test was used. ANOVAs were conducted in SigmaPlot version 12.5 (Systat Software Inc., San Jose, CA, USA). If significant differences were found between treatments and the control, concentration-response relationships were modeled in R v3.3.3 with the *drc* v3.0-1 package (Ritz and Strebig, 2005). Lethality and reproduction datasets