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Aquatic toxicity of neonicotinoid insecticides

Acute Toxicity of Six Neonicotinoid Insecticides to Freshwater Invertebrates

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Abstract: Neonicotinoids are a group of insecticides commonly used in agriculture. Due to their high water solubility, neonicotinoids can be transported to surface waters and have the potential to be toxic to aquatic life. The present study assessed and compared the acute (48 or 96 h) toxicity of 6 neonicotinoids (acetamiprid, clothianidin, dinotefuran, imidacloprid, thiacloprid, and thiamethoxam) to 21 laboratory-cultured and field-collected aquatic invertebrates spanning 10 aquatic arthropod orders. Test conditions mimicked species' habitat, with lentic taxa exposed under static conditions, and lotic taxa using recirculating exposure systems. Lethal (LC50) and effective (immobility, EC50) concentrations were calculated and used to construct separate lethal- and immobilization-derived species sensitivity distributions (SSDs) for each neonicotinoid, from which 5th percentile hazard concentrations (HC5s) were calculated. Results showed the most sensitive invertebrates were insects from the orders Ephemeroptera (e.g. *Neocloeon triangulifer*) and Diptera (*Chironomus dilutus*), while cladocerans (e.g. *Daphnia magna*, *Ceriodaphnia dubia*) were the least sensitive. HC5s were compared to neonicotinoid environmental concentrations from Ontario monitoring studies. For all neonicotinoids except imidacloprid, the resulting hazard quotients indicated little to no hazard in terms of acute toxicity to aquatic communities in Ontario freshwater streams. For the neonicotinoid imidacloprid, a moderate hazard was found when only invertebrate immobilization, and not lethality, data was considered.

Keywords: Insecticides, Pesticides, Aquatic invertebrates, Species sensitivity distributions, Toxic effects

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INTRODUCTION

Neonicotinoids are a class of pesticides widely used in agriculture as plant protectants. All are water soluble, and can be applied as seed or spray treatments where they act systemically to protect the entire plant (Jeschke et al. 2011). They bind strongly to the nicotinic acetylcholine receptor (*n*AChR) found in the central nervous system of insects, causing constant nerve stimulation that eventually leads to death or other toxic effects (Morrissey et al. 2015).

The first neonicotinoid brought to market was imidacloprid in 1991, and six others followed in the next decade: acetamiprid (1995), nitenpyram (1995), thiamethoxam (1998), thiacloprid (2000), clothianidin (2002), and dinotefuran (2002) (Jeschke and Nauen 2008). Their systemic action, combined with low mammalian toxicity, effectiveness against pests, and low application volumes have made neonicotinoids the most popular class of insecticides worldwide (Jeschke et al. 2011; Simon-Delso et al. 2015). Due to their widespread use, high water solubility, and persistence, neonicotinoid residues are therefore frequently detected in surface waters that drain agricultural lands (Morrissey et al. 2015).

Transport of neonicotinoids to aquatic environments is typically driven by precipitation events (Hladik and Kolpin 2016; Struger et al. 2017), snowmelt (Main et al. 2016), and contaminated dust (Limay-Rios et al. 2016). Median neonicotinoid surface water concentrations are on the order of ng L^{-1}, with peak concentrations rising to low µg L^{-1}. Struger et al. (2017) detected the 3 most common neonicotinoids—imidacloprid, thiamethoxam, and clothianidin—in >90% of samples from over half of their fifteen Ontario surface water sites. Median concentrations in that study ranged from <0.00128 to

0.585 µg L^{-1}, and the maximum detection was 10.4 µg L^{-1} imidacloprid. Detections were correlated with registered uses; thiamethoxam and clothianidin were correlated with row crops, imidacloprid and acetamiprid with vegetable crops, greenhouses, and other agriculture, and thiacloprid with fruit production (Struger et al. 2017). In addition, neonicotinoid detections showed seasonality, with maximum concentrations occurring in the late spring and fall (Struger et al. 2017). Monitoring by the Ontario Ministry of the Environment and Climate Change (MOECC) in 2015 also showed the most common detections to be imidacloprid, thiamethoxam, and clothianidin, with median concentrations from 0.018 to 0.050 µg L^{-1} and maximum concentrations of 2.3 µg L^{-1} imidacloprid and 2.7 µg L^{-1} thiamethoxam (MOECC 2016). Environmental concentrations are similar on a global scale; Morrissey et al. (2015) surveyed 29 surface water neonicotinoid monitoring studies from 9 countries and reported mean surface water concentrations of 0.13 µg L^{-1}, with a geometric mean for peak concentrations of 0.63 µg L^{-1}.

Neonicotinoids are acutely toxic to aquatic macroinvertebrates at concentrations that span six orders of magnitude, with insects generally showing greater sensitivity than crustaceans (Morrissey et al. 2015). However, within the Insecta sensitivity varies widely. Roessink et al. (2013) reported 96-h median lethal concentrations (LC50s) for imidacloprid of between 6.68 µg L^{-1} for *Caenis horaria* (Ephemeroptera) to >10000 µg L^{-1} for *Notonecta* spp. (Hemiptera) and *Sialis lutaria* (Megaloptera). Reported 96-h LC50s for mayflies vary from 0.65 µg L^{-1} for *Epeorus longimanus* (Alexander et al. 2007) to 154 µg L^{-1} for *Cloeon dipterum* (Van den Brink et al. 2016). Such variability in toxicity also exists in the Crustacea, with 48-h L(E)C50s >10 000 µg L^{-1} reported for

Daphnia magna (Song et al. 1997; Hayasaka et al. 2012), compared to 48-h L(E)C50s of 2.07 µg L^{-1} (Chen et al. 2010) and 571.62 µg L^{-1} (Song et al. 1997) for *Ceriodaphnia dubia*. This wide range in sensitivity within and between groups highlights the need for testing across different aquatic invertebrate orders, including those outside the typical model species, to better understand the range of neonicotinoid toxicity and hence potential risks in aquatic ecosystems.

A small number of government regulatory bodies around the world have derived water quality guidelines for neonicotinoids, as summarized by Morrissey et al. (2015). In North America, the United States Environmental Protection Agency has published acute aquatic life benchmarks for six neonicotinoids, ranging from 10.5 µg L^{-1} for acetamiprid, to >484,150 µg L^{-1} for dinotefuran (United States Environmental Protection Agency 2017). The Canadian Council of Ministers of the Environment (CCME) has an interim water quality guideline for imidacloprid of 0.23 µg L^{-1}. This guideline is based on a 28-d lowest observed effect concentration (LOEC) for *Chironomus riparius* of 2.25 µg L^{-1} imidacloprid, with a 0.1 safety factor applied (Canadian Council of Ministers of the Environment 2007). Individual water quality guidelines for other neonicotinoids do not exist in Canada, in part, due to the lack of toxicity data and minimum data requirements (minimum of three aquatic invertebrates, ideally with one being a mayfly, caddisfly, or stonefly) stipulated by the CCME for constructing species sensitivity distributions (SSDs) (Canadian Council of Minister of the Environment 2007). In 2015, Morrissey et al. constructed an acute SSD using a combined dataset where toxicity data for all neonicotinoids was standardized and weighted by molecular mass to imidacloprid to establish a recommended short-term acute guideline of 0.2 µg L^{-1}. However, while a

useful interim approach, it is based on the assumption that the most toxic neonicotinoid across all aquatic species is imidacloprid which, as we show in the present paper, is not always the case.

The objective of the present study was to evaluate the acute toxicity of six neonicotinoid insecticides (imidacloprid, thiamethoxam, clothianidin, acetamiprid, thiacloprid, and dinotefuran) to a suite of aquatic invertebrate species (n=21), with the goal of better defining the range of sensitivity, creating a comprehensive dataset to characterize the potential risk of the six neonicotinoids, and develop rigorous short-term acute water quality guidelines. The invertebrates tested in the present study include both laboratory-cultured model aquatic invertebrates and field-collected species; represent both lotic and lentic habitats; have broad representation within the Arthropoda; and include several functional feeding groups (Merritt and Cummings 1996). All species tested are relevant to freshwaters near agricultural areas where neonicotinoids could be applied.

METHODS

Toxicity tests: 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), imidacloprid (99.9%, CAS 138261-41-3), thiacloprid (99.9%, CAS 111988-49-9), and thiamethoxam (99.6%, CAS 153719-23-4). Imidacloprid (99.8%) was also obtained from Bayer CropScience (Mississauga, ON, Canada) and thiamethoxam (technical grade, purity not stated) from Syngenta Crop Protection LLC (Guelph, ON, Canada). Stock

solutions were prepared by dissolving up to 100 mg of chemical per 1 L of dechlorinated municipal tap water (physicochemical properties are provided in Table S2) in Class A glassware, covered with tin foil to limit photodegradation, and stirring overnight. Stock solutions were stored in 1 L amber glass bottles at 4±2°C. Test solutions were prepared by either spiking the appropriate volume of stock into a known volume of dechlorinated water or by serial dilutions in which 5 to 7 test solutions were prepared for each test in a 50% or 70% geometric series. Each test included a negative control of dechlorinated water. Conductivity, pH, dissolved oxygen, and temperature were measured on at least the negative control and highest test concentration before and after each toxicity test. Temperature was monitored throughout the test.

Test species

Laboratory-cultured species. Acute toxicity tests were performed with seven laboratory-cultured species: *Ceriodaphnia dubia*, *Chironomus dilutus*, *Daphnia magna*, *Hexagenia* spp., *Hyalella azteca*, *Lumbriculus variegatus* and *Neocloeon triangulifer*. Culturing methods are outlined in Supplemental Data, Table S1. Test methods were based on standard MOECC and literature-derived methods (Rodrigues and Kaushik 1984; Environment Canada 2007a; Environment Canada 2007b; Soucek and Dickinson 2015; MOECC Aquatic Toxicology Unit 2016). Tests were conducted with 5 or 10 organisms per replicate, with 3 to 5 replicates per treatment using a regression design. Test parameters are described in Table 1. Endpoints of immobility and mortality were observed at 96±4 h; obvious mortalities (i.e. individuals starting to decompose) during the tests were counted and removed daily. Mortality was classified as complete cessation of organism movement even after gentle prodding with a pipette and was confirmed

under a dissecting microscope (2x to 4x magnification). Immobilization responses included lethargy, limited swimming behaviours and muscle spasms, as described by Camp and Buchwalter (2016). In preliminary tests, we observed that immobile individuals that did not respond to prodding were alive when observed under a microscope, often with mouthparts, legs, and/or gills moving. We therefore confirmed all mortality under a microscope, and only scored individuals as dead when there was complete cessation of all organism movement. Other studies observing effects of neonicotinoids on aquatic invertebrates have generally counted what we considered to be immobility as death, and this should be kept in mind when comparing the results of the present study to other studies. For example, it is possible that LC50s reported in other literature where immobility was taken as death are equivalent to the EC50s reported here. Organisms were considered immobile if the customary swimming response or movement was not observed in a period of ≥15 s after gentle agitation of the solution or gentle stimulation with a probe. Tests with laboratory-cultured species were considered invalid if control mortality exceeded 10%, except for *Hexagenia* spp. where the acceptability criterion was 20% mortality due to inherent increased variability when testing these sediment-burrowing mayflies in water-only exposures.

Field-collected species. Test organisms were collected from ponds and streams around Guelph, ON and Ottawa, ON (Table 1). Grab samples taken from one of the ponds and the Eramosa River (Guelph, ON) during one of the organism collecting events showed that all neonicotinoids were less than the method detection limit (ranging from 5 – 10 ng L^{-1}, as shown in Table 2).

Toxicity tests were conducted with 14 field-collected taxa from the orders Ephemeroptera (*Caenis* sp., *Cloeon* sp., *Ephemerella* sp., *Isonychia bicolor*, *McCaffertium* sp.), Trichoptera (*Cheumatopsyche* sp., *Micrasema* sp.), Coleoptera (*Stenelmis* sp., *Gyrinus* sp.), Isopoda (*Caecidotea* sp.), Hemiptera (*Trichocorixa* sp.), Odonata (*Coenagrion* sp.), Diptera (*Aedes* sp.), and Plecoptera (*Agnetina* sp. and *Paragnetina* sp.). *Agnetina* sp. and *Paragnetina* sp. were tested together and treated as a single test unit due to low collection numbers for the individual genera. All field-collected taxa were nymphs/juveniles, with the exception of *Caecidotea* sp., *Trichocorixa* sp., *Gyrinus* sp., and *Stenelmis* sp., which were tested as adults. An attempt was made to collect and test species within a similar visual size range. Animals were collected using a kick-sampling technique and a 500 μm D-net, or careful removal from the underside of stream rocks. After collection, organisms were transferred to aerating dechlorinated water and maintained at 12–20°C, dependent on the water temperature at the collection site, for one to 5 d prior to testing. A selection of aquatic vegetation and/or leaf litter was provided for food and substrate during the holding period. Species were chosen to represent a diverse set of lentic and lotic invertebrate taxa known to occur in agricultural landscapes in Ontario.

Tests with pond (lentic)-dwelling species were conducted under static conditions as stated (*Laboratory-cultured species*). Tests were conducted with 7 to 10 organisms per treatment, with one replicate per treatment using a regression design (*Concentration-response models*). Tests with stream (lotic)-dwelling species were conducted in recirculating exposure systems that mimic stream flow (Rodrigues and Kaushik 1984). Details concerning the construction of the flow-through exposure system are provided in

the Supplemental Data, S7. Tests were conducted with 7 to 20 organisms per treatment in each simulated stream (flow rate 25 ± 6 mL/s), with one replicate per treatment using a regression design (*Concentration-response models*). Water depth in the artificial streams was maintained at approximately 2 cm using a glass partial wall and 280 µm Nitex screen on the lower end of the ramp. Test temperatures were 12 to 15 (±2) C to mimic collection conditions and reduce thermal stress. In all tests, endpoints of immobility and mortality were observed and recorded as described above. Organisms were removed from the stream apparatus and examined under microscopy to confirm mortality at test completion, or when mortality was obvious (i.e. decomposition).

Test acceptability criteria was set at 20% mortality due to higher inherent variability in field-collected species. Any tests with >20% mortality in the controls were rejected except for clothianidin toxicity to *Aedes* sp., where the control mortality was 2/9 = 22%.

Chemical analysis

Samples from the exposure treatments were collected and analysed to confirm nominal concentrations using liquid chromatography/tandem mass spectrometry (LC-MS/MS). 40 mL samples of one to 4 exposure treatments per test were collected at time of initial organism exposure and stored in amber glass bottles at 4±2°C prior to analysis. Across all toxicity tests, a total of 149 samples were collected to measure actual concentrations. Complete details of chemical analysis are provided in the Supplemental Data, S3.

Statistical analysis

Concentration-response models. Endpoints were tallied as percent of total organisms in each treatment exhibiting mortality or immobilization. The number of treatment replicates for each test varied depending on organism availability; tests with laboratory-cultured organisms used a minimum of 3 replicates, while tests with field-collected organisms used one replicate due to the logistical constraint of collecting large numbers of organisms in the field. All tests were conducted using a regression design, with 5-7 treatments per test in a dilution series. Nominal concentrations were corrected using the mean percent difference between the nominal and measured concentrations taken from each test and the corrected concentrations used to model the concentration-response relationships.

Concentration-response relationships were modeled for datasets containing one or more partial values using a 4-parameter log-logistic regression model (LL.4) (Equation 1) in R v3.3.3 with the *drc* package (Ritz and Streibig 2005). The lower limit was fixed to 0, and the upper limit to 100, representing the minimum and maximum possible effects,

respectively. The variables b, c, d, and e represent the relative slope around the LC/EC value, the lower and upper limits, and the LC/EC value, respectively.

$$Mortality\ or\ immobilization = c + \frac{d-c}{1+exp\{b(\log(concentration)-\log(e))\}} \quad (1)$$

Datasets containing no partial values were analysed using the binomial method (Environment Canada 2007c). Lethal and effective concentrations for the 10th and 50th percentile (LC10/LC50 and EC10/EC50, respectively) and associated 95% confidence intervals were calculated from regression models. L/EC10s were not calculated for binomial distributions.

Species sensitivity distributions and hazard assessment. The dataset of 112 neonicotinoid/species combinations was used to construct short-term species sensitivity distributions (SSDs) for each neonicotinoid. These SSDs allow for comparison of toxicity between aquatic taxa and between the 6 neonicotinoids. SSDs were constructed according to CCME Type A guidelines (Canadian Council of Minister of the Environment 2007). Data from the present study was used to fulfill the dataset requirement for aquatic invertebrate species, and data from the ECOTOX database (http:/www.epa.gov/ecotox/) was used to fulfill requirements for fish (3 species, at least one salmonid and one non-salmonid), suggested plant and amphibian species requirements, and to strengthen the invertebrate dataset from the present study. To be included in the SSDs, external data from the ECOTOX database had to be derived from tests using the active ingredient and not a formulated product and have an acute exposure duration (≤4 d for invertebrates, and

≤5 d for plants). If multiple entries were present for the same species, the lowest value was included. If multiple exposure durations were present for the same species from the same study, the duration closest to 4 d was chosen to maintain consistency with the present study exposure duration. Fish, plant, and invertebrate data was available for all neonicotinoids, but amphibian data was only available for imidacloprid. SSDs were generated using CCME SSD V3 (2010) software (Rodney et al. 2013). Two short-term SSDs were generated for each neonicotinoid; one with invertebrate LC50 values (lethality-derived), and one with invertebrate EC50 values (immobilization-derived). Both SSDs used external, ECOTOX database EC/IC50s for plant data and LC50s for fish and amphibian data. LC50 or EC50 values that fell below the smallest concentration in a test (e.g. <600 µg L^{-1}) were not used in the dataset. LC50 or EC50 values that were above the largest concentration in a test (e.g. >9 600 µg L^{-1}) were included as-is (e.g. 9 600 µg L^{-1}) in a conservative approach. Any species with LC50 or EC50 values ≥100 000 µg L^{-1} were considered tolerant, and were used to calculate plotting positions but were not fit to the model. Although less environmentally-relevant, LC50s and EC50s were chosen due to the inherent increased statistical robustness and the greater abundance of LC50s and EC50s compared to the application of LOEC, NOEC or L(E)C*x* values.

To construct the SSDs, the datasets were fit to 4 models: normal, logistic, extreme value, and Gumbel. Models were assessed for goodness-of-fit using mean squared error (MSE) for the entire dataset and for the lower tail using the Anderson-Darling (A^2 statistic) test and visual inspection. The model with the lowest MSE for the lower tail, A^2 statistic, and best visual inspection was chosen. If a model failed a visual inspection and the Anderson-Darling test for goodness-of-fit, it was not chosen; if all models failed both

tests, that SSD was not included in the hazard assessment. The chosen model was plotted in R with ggplot2 (v. 2.2.1) package. The 5th percentile of the distribution, termed the HC5 (hazardous concentration, 5%), and its associated 95% confidence intervals were calculated from the model.

HC5s for each neonicotinoid were compared to environmental concentrations of associated neonicotinoids in Ontario surface waters in a hazard assessment. To assess environmental concentrations, monitoring data from 3 Ontario studies spanning years 2012 – 2016 (see Supplemental Data, S14, for details on each study) with 2790 observations between the 6 neonicotinoids were compiled. Due to the highly left-censored nature of the data (i.e. values below method detection or quantitation limits, or non-detects) coupled with multiple detection limits, the non-parametric Kaplan-Meier method was used to model the data as empirical cumulative distribution functions (ECDFs) using the 'NADA' package (v. 1.6-1) in R (v. 3.3.2) (Helsel 2012). ECDFs were estimated for environmental monitoring data for clothianidin, imidacloprid, and thiamethoxam, but not estimated for acetamiprid, dinotefuran, and thiacloprid due to low numbers of detections (>98% of observations were censored). ECDF summary statistics are shown in Table 4, and ECDF plots are presented in the Supplemental Data, Figure S15.

Hazard quotients (HQs) were calculated for each neonicotinoid where HQ = exposure ÷ effects. 'Exposure' was either the maximum environmental concentration (acetamiprid, dinotefuran, and thiacloprid, where ECDFs could not be calculated due to too few detections) or the 99% percentile of the ECDF (clothianidin, imidacloprid, and

thiamethoxam). 'Effects' was the associated HC5s calculated for each compound from SSDs.

RESULTS AND DISCUSSION

Solution chemistry

Water chemistry parameters in exposure solutions were not affected by addition of neonicotinoids. For all tests at test initiation, measured values of pH ranged from 7.9 – 8.4, conductivity ranged from 240–360 µS/cm, and dissolved oxygen ranged from 8.1–10.5 mg/L, or within 90–110% saturation at test temperatures varying < 2°C (<3°C for *Trichocorixa* sp.), from the intended temperatures (range 11–25°C, depending on test). For all tests at test completion, pH ranged from 7.5–8.8, conductivity ranged from 260–450 µS/cm, and dissolved oxygen ranged from 4.5–11.2 mg/L, or within 60–110% saturation at for temperatures relative to test initiation. Summarized water chemistry parameters for all tests at test termination are available in the Supplemental Data, Table S8.

Mean percent difference between measured and nominal neonicotinoid concentrations for the 149 water samples is summarized in Table 2. Measured concentrations were generally within 20% of nominal concentrations.

Toxicity

Many studies have assessed the acute toxicity of neonicotinoid insecticides but most have focused on only one or two neonicotinoids and generally involved individual or a small number of species. The novel contributions of the present study are that we assessed and compared the acute toxicity of 6 neonicotinoid insecticides to aquatic invertebrates; included species from ten aquatic arthropod orders, many of which have

not previously been tested; and constructed individual SSDs for each compound. A total of 112 neonicotinoid/species combinations were tested; derived lethal (LC) and effective (EC) concentrations and control mortality are presented in Table 3.

The cladocerans *Daphnia magna* and *Ceriodaphnia dubia* were the least sensitive taxa, with effects only observed at concentrations >40 000 µg L^{-1}. Insects were among the most sensitive taxa tested with 63% of calculated LC50s, and 91% of calculated or threshold estimated EC50s ≤1000 µg L^{-1}. The most sensitive species for both lethality and immobilization endpoints were the midge *Chironomus dilutus* and the mayfly *Neocloeon triangulifer*, with LC50s < 65 µg L^{-1} and as low as 1.7 µg L^{-1}, and EC50s <40 µg L^{-1} and as low as 0.8 µg L^{-1}, respectively. The amphipod *Hyalella azteca* also showed high sensitivity to clothianidin and acetamiprid, with LC50s and EC50s around 5 µg L^{-1}. These results are consistent with published studies in which Diptera, especially *Chironomus* spp., and Ephemeroptera have been reported to be the most sensitive insect taxa to neonicotinoids (Roessink et al. 2013; Morrissey et al. 2015; Smit et al. 2015; Camp and Buchwalter 2016; Cavallaro et al. 2017). The greater sensitivity of insect taxa relative to non-insect taxa was not unexpected since neonicotinoids are designed to target and bind strongly with the insect nicotinic acetylcholine receptor (*n*AChR).

While the insects were, on average, most sensitive, toxicity values spanned 4 orders of magnitude indicating wide variation in relative sensitivity within this group. Variation in the sensitivity of aquatic organisms to pesticides is a function of the toxicokinetic and toxicodynamic relationship between the organism and the pesticide (Rico and Van den Brink 2015). Inter-species differences in uptake rate and transport to the site of action, the structure of the *n*AChR, and metabolism or detoxification

mechanisms all have the potential to introduce variation in the expression of toxic effects. For example, uptake of the pesticide chlorpyrifos has been shown to be a function of insect body size and respiratory strategy, with small insects that rely on aqueous gas exchange to respire, such as Ephemeroptera, Trichoptera, and some Diptera, typically showing greater uptake (Buchwalter et al. 2002). Further, and specific to neonicotinoids, highly sensitive mayfly species such as *Neocloeon triangulifer* have been shown to bioconcentrate more imidacloprid per mass than less sensitive stoneflies or gastropods; uptake rates also increased with temperature (Camp and Buchwalter 2016). In the present study, species that were small at test initiation, and tested at higher temperatures (23 to 25°C) such as *Neocloeon triangulifer* and *Hyalella azteca*, showed greater sensitivity relative to similar taxa (e.g. *Cloeon* sp., and *Caecidotea* sp., respectively). Many of these small, sensitive species were laboratory-cultured and showed greater sensitivity than field-collected species, which were likely collected as later developmental stages (instars were not determined) which could be more resilient. As a result of differences in developmental stage and testing temperature, there is the potential in the present study for toxicity to field-collected species to be underestimated, and toxicity to laboratory-cultured species to be overestimated. Differential binding affinity at the *n*AChR is another potential source of variation. Neonicotinoids are thought to be highly selective in their binding to insect versus mammalian *n*AChRs due to structural differences in the receptor site that lead to changes in binding affinity (Tomizawa and Casida 2003). Within insects, variation in the type and number of *n*AChR subunits leads to differences in the way agonists, such as neonicotinoids, interact with the receptor (Jones et al. 2007). Conversely, different neonicotinoids have been shown to act differently at the same

*n*AChR (Brown et al. 2006). For example, imidacloprid has been shown to act as a partial agonist, and clothianidin as a super-agonist, on native *Drosophila n*AChRs (Brown et al. 2006).

Among the 7 mayfly species tested, response to neonicotinoids was highly variable with LC50s spanning 4 orders of magnitude. *Neocloeon triangulifer* was the most sensitive mayfly, while the least sensitive in terms of lethality was *Hexagenia* spp., and the field-collected *Cloeon* sp. and *McCaffertium* sp., with LC50s >890 µg L^{-1}; however, these species were immobilized at much lower concentrations, with most EC50s <100 µg L^{-1}. In general, Ephemeroptera and Diptera exhibited immobility at concentrations much lower than those that caused lethality, as illustrated by the difference between median LC50 and EC50 estimates (Figure 1). An extreme example of this disparity occurred in the mayfly *Hexagenia* spp., which had EC50s of <40 µg L^{-1} for all neonicotinoids, but a concentration-response relationship for lethality could not be established even in concentrations 2 to 3 orders of magnitude greater. While *Hexagenia* spp. was the only sediment-dwelling mayfly studied, all exposures were water-only therefore negating the influence of sediment on toxicity. Rubach et al. (2010, 2011) found some freshwater invertebrates that also showed a large sensitivity difference between immobility and lethality for the pesticide chlorpyrifos; through studying uptake and elimination kinetics, they showed these species had high uptake rates and bioconcentration factors, and suggested differences in biotransformation or compensatory gene-regulation abilities as likely sources of variability (Rubach et al. 2010; Rubach et al. 2011). Extrapolating to the present study, *Hexagenia* spp. may have bioconcentrated neonicotinoids to a lesser degree than the other mayflies, or may have been able to

biotransform neonicotinoids once accumulated so to keep internal residues low, leading to constant immobility but not lethality.

Macroinvertebrates that become immobile as a result of neonicotinoid exposure may be subject to drift (in a lotic system). Berghahn et al. (2012) showed repeated 12 h pulses of 12 µg L^{-1} imidacloprid caused drift of insect larvae and young *Gammarus*. Immobile individuals may also be subject to increased predation due to reduced escape behaviours. Pestana et al. (2009) showed exposure to a formulation of imidacloprid altered behaviour in response to predation in the insects *Chironomus riparius* (Diptera) and *Sericostoma vittatum* (Trichoptera). Both effects could reduce or remove these organisms from the community and make the endpoint of immobilization functionally equivalent to mortality. Endpoints that consider immobility, such as the EC50s in the present study, may therefore be a more relevant measure of real-world toxicity.

Recent studies have shown neonicotinoid toxicity is influenced by temperature and seasonality. Camp and Buchwalter (2016) found a positive relationship between temperature, imidacloprid uptake, and toxicity for aquatic insects, most notably for mayflies. Van den Brink et al. (2016) also found a slight increase in toxicity at higher temperatures, as well as differences in sensitivity to imidacloprid between summer and overwintering generations of the mayfly *Cloeon dipterum*. When tested at the same temperature, summer generations were more sensitive in terms of immobilization, but not lethality, to imidacloprid than overwintering generations (Van den Brink et al. 2016). The present study used early summer (May) populations for lentic mayflies, and overwintering (November to December) populations for lotic mayflies. Assuming that the seasonal results of Van den Brink et al. (2016) are indicative of other mayfly species, the

results for lotic mayflies in the present study could be under-protective. All field-collected organisms were tested at temperatures of 12 to 15 (±2)°C with the goal of mimicking field collection conditions in the laboratory. When compared to laboratory-cultured species, which were cultured and tested at 23 to 25 (±2)°C, field-collected species' toxicity could be underestimated due to lower testing temperatures, and associated lower uptake and toxicity. Additional studies characterizing neonicotinoid toxicity to the most sensitive taxa (e.g. the mayfly *Neocloeon triangulifer*) are needed to understand how neonicotinoid toxicity is affected by temperature. This is especially needed for spring and early summer temperatures when neonicotinoid exposure is potentially at peak in streams, as transport to, and occurrence in, aquatic systems has been shown to be driven by crop planting and precipitation events (Hladik et al. 2014), both of which occur in spring and early summer.

With few exceptions, the data given in the present study is typically within ≤1 order of magnitude compared to neonicotinoid toxicity data in the literature. In general, our median effective and lethal concentrations were greater than those from other studies, which may partially reflect our stricter criteria for the confirmation of lethality and use of technical rather than formulated product. There were some exceptions where our data indicated toxicity that was >1 order of magnitude compared to published data. These include a reported 96-h LC50 for *Cloeon dipterum* of 26.3 µg L^{-1} imidacloprid (Roessink et al. 2013), a 44-fold difference compared to our value of 1152 µg L^{-1}; and a reported 48-h LC50 for *Ceriodaphnia dubia* of 2.11 µg L^{-1} (Chen et al. 2010), a 34 000-fold difference compared to our value of 72 124 µg L^{-1}. Both of these studies used formulated products. Many neonicotinoid toxicity studies in the literature use formulated products

(e.g. Alexander et al. 2007; Beketov and Liess 2008; Stoughton et al. 2008; Pestana et al. 2009; Roessink et al. 2013), perhaps rationalizing that the formulated product is what is applied in crop protection and therefore a more realistic assessment of possible exposure in the environment. We used technical grade neonicotinoids in our study to facilitate comparison of the relative potency between neonicotinoids without the possible confounding toxicity of formulants. It is unclear whether there is a difference between technical and formulated neonicotinoid products as there have been few comparative studies. Stoughton et al. (2008) found a 3.75-fold difference in 96-h LC50s between technical imidacloprid and the formulation Admire® for *H. azteca*; however, LC25s and LOECs were similar. The authors indicated the difference in toxicity at higher concentrations may have been due to toxicity at higher concentrations of formulants, or combined toxicity between the formulants and the active ingredient (Stoughton et al. 2008). The same experiment with *C. tentans* found no difference in toxicity between the technical and formulated product. In a separate study, a comparison of toxicity to *D. magna* found the commercial liquid formulation Confidor SL 200 (imidacloprid) to be 4x more toxic (by lowest observable lethal concentration) compared to the active ingredient (Jemec et al. 2007). Further studies are needed to better understand difference between formulated and technical neonicotinoid insecticides.

Hazard assessment

A hazard assessment was conducted to compare acute toxicity data to the maximum or 99th percentile of neonicotinoid surface water detections in Ontario. This assessment conservatively approximates short-term exposures such as pulse exposures. HC5s determined from both lethality and immobilization-derived SSDs are presented in

Table 4. SSDs (Figure 2) yielded the following order of neonicotinoid toxicity based on the more robust lethality-derived SSDs: acetamiprid ≈ thiacloprid < imidacloprid ≈ clothianidin ≈ dinotefuran ≈ thiamethoxam. Hazard quotients (HQ), calculated as the ratio of the HC5 to environmental concentrations for lethality-derived datasets for each neonicotinoid insecticide, indicated no acute hazard (HQ < 0.1) for dinotefuran, thiacloprid and thiamethoxam, or low acute hazard (0.1 < HQ < 1.0) for acetamiprid, clothianidin, and imidacloprid. Immobilization-derived datasets (see SSDs in Supplemental Data, Figure S13) indicated low acute hazard for acetamiprid, dinotefuran, thiacloprid and thiamethoxam, and moderate acute hazard (HQ > 1.1) for imidacloprid. Hazard could not be determined for clothianidin, as none of the immobilization-derived SSDs constructed passed a goodness-of-fit test or visual inspection. Guidelines for interpreting HQs were based on (Lemly 1996). When considering the more robust, but less sensitive endpoint of lethality, this hazard assessment indicates a low or no acute hazard situation for 95% of the species tested exposed to 99th percentile or the maximum of the Ontario surface water concentrations detected. Alternatively, when considering the less robust, but more sensitive and perhaps more environmentally-relevant endpoint of immobilization, this hazard assessment indicates a moderate hazard for the neonicotinoid imidacloprid. For the other neonicotinoids acetamiprid, dinotefuran, thiacloprid, and thiamethoxam, a conclusion of low or no acute hazard remained; for clothianidin, hazard could not be determined due to ill-fitting species sensitivity distribution models.

The results of this hazard assessment are comparable to other recent neonicotinoid hazard or risk assessments for aquatic invertebrates that are based on single-neonicotinoid SSDs. A recent probabilistic ecological risk assessment for imidacloprid by Whitfield-

Aslund et al. (2017), which considered both acute lethal and immobility data, derived an acute HC5 of 1.73 µg a.i. L^{-1}; when compared to exposure distributions for multiple use patterns, they categorized the risk to aquatic invertebrates as *de minimis*. Similarly, Finnegan et al. (2017) constructed an acute SSD for thiamethoxam and derived HC5s of 5.1 and 22.9 µg L^{-1} using EC50s and LC50s, respectively. When compared to neonicotinoids in North American surface water samples, the risk was found to be low, with a ~0.5% likelihood of exceeding the HC5. In contrast to these studies, an earlier assessment by Morrissey et al. (2015) derived a HC5 of 0.63 µg L^{-1}, which is lower than the results presented here and by others [e.g. 45,46].

CONCLUSION

The present study contributes novel information to support the growing knowledge of neonicotinoid toxicity to aquatic invertebrates, especially for lesser-studied aquatic invertebrates and poorly documented neonicotinoids such as acetamiprid, thiacloprid, and dinotefuran. The acute toxicity data presented here is relevant to short-term pulses of neonicotinoids in aquatic environments, which typically occur during heavy precipitation events and during spring freshet. Importantly, our observation of immobilization but not lethality for many species over the exposure periods applied in the present study suggests that recovery may be possible following pulse exposure scenarios in the field. Future work in our lab will test this hypothesis. Further, we found that sensitivity for many other species was often less than indicated in the literature. The derived HC5s, therefore, are greater than those estimated in previous work and may represent a more realistic assessment of acute neonicotinoid toxicity.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.xxxx.

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Data Availability—Data pertaining to this manuscript are deposited in *figshare* at DOI:xxxx.

This article includes online-only Supplemental Data.

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Figure 1. Distribution of median lethal (LC50) and effective (EC50) concentrations. Lower and upper threshold data (e.g. >9 600 µg L^{-1}) were treated as-is (e.g. 9 600 µg L^{-1}). Data for 6 neonicotinoids are pooled.

Figure 2. Species sensitivity distributions (SSDs) of acute (≤5 d) toxicity data for 6 neonicotinoids constructed using lethality (LC50) values for aquatic invertebrates generated from the present study and external literature data mined from ECOTOX database. Closed (black) shapes represent data from the present study (internal) and open (white) shapes represent external data. Circles represent aquatic invertebrates, squares represent fish, triangles represent plants, and diamonds triangles represent amphibians. For data from the present study, all field-collected species (indicated with an asterisk [*]) was derived from tests with a single replicate; remaining internal data were lab-cultured species tested with a minimum of 3 replicates. Data were fit to regression models (red line) with 95% confidence band for the regression (dashed line). Data for clothianidin, imidacloprid and thiamethoxam fit to Gumbel models; acetamiprid to the extreme value model; dinotefuran to the logistic model; and thiacloprid to the normal model. Data for imidacloprid did not pass Anderson-Darling goodness-of-fit test for any of the four possible models, but passed visual inspection. The vertical dotted line illustrates the 5th percentile hazardous concentration (HC5), with 95% CI. SSDs were modeled in CCME V3 Master software and plotted in R with *ggplot2*. Model parameters are in Supplemental Data, Table S10.

Table 1. Summary of tested species and associated test conditions used to assess the acute toxicity of 6 neonicotinoid insecticides

Species	*Ceriodaphnia dubia*	*Daphnia magna*	*Chironomus dilutus* *Hexagenia* spp. *Hyalella azteca* *Lumbriculus variegatus*	*Neocloeon triangulifer*	*Caecidotea* sp. *Gyrinus* sp. *Caenis* sp. *Cloeon* sp.	*Trichocorixa* sp. *Aedes sp.*	*Coenagrion* sp.	*McCaffertium* sp. *Ephemerella* sp. *Isonychia bicolor* *Cheumatopsyche* sp. *Micrasema* sp. *Stenelmis* sp. *Agnetina/Paragnetina* sp.
Origin	MOECC laboratory culture	MOECC laboratory culture	MOECC laboratory culture	MOECC laboratory culture	Field-collected in ponds in Guelph, ON, *Gyrinus* sp. Lac Gerard, QC	Field-collected in quiet eddies of Speed River and ponds in Guelph, ON	Field-collected in pond in Guelph, ON	Field-collected in Speed River, Eramosa River, Guelph, ON
Test protocol	(Environment Canada 2007a)	(Environment Canada 2007b)	(Ontario Ministry of the Environment and Climate Change Aquatic Toxicology Unit 2016)	(Soucek and Dickinson 2015)	Adapted from (Ontario Ministry of the Environment and Climate Change Aquatic Toxicology Unit 2016)	Adapted from (Ontario Ministry of the Environment and Climate Change Aquatic Toxicology Unit 2016)	Adapted from (Ontario Ministry of the Environment and Climate Change Aquatic Toxicology Unit 2016)	Adapted from (Rodrigues and Kaushik 1984)
Life stage	<24 h old	<24 h old	*Chironomus*: 3rd instar, as measured by mean head capsule width (0.33-0.45 mm) *Hexagenia:* mean wet weight ~4-6 mg *Hyalella:* 2-9 d old, within 2 d of each other *Lumbriculus:* age-synchronized to 7 d	<24 h old	*Caecidotea* sp.: adults *Gyrinus* sp.: adults *Caenis* sp.: nymphs *Cloeon* sp.: nymphs	*Trichocorixa* sp.: adults *Aedes sp.*: larvae	nymphs	All nymphs, except *Stenelmis* sp.
Exposure duration (hrs)	48 ± 2	48 ± 2	96 ± 4	96 ± 4	96 ± 4	48 ± 2	96 ± 4	96 ± 4
Exposure temp. (°C)	25 ± 2	20 ± 2	23 ± 2	25 ± 2	15 ± 2	14 ± 2, except thiamethoxam to *Trichocorixa* sp., 18 ± 2	13 ± 3	15 ± 2
Light intensity (lux)	All tests conducted at 500 – 1000 lux, 16:8 light: dark photoperiod							
Test vessel	15 mL solution in 30 mL flat-bottom glass tube	50 mL solution in 50 mL glass tube	200 mL solution in 250 mL glass beaker	200 mL solution in 250 mL glass beaker	200 mL solution in 250 mL glass beaker	200 mL solution in 250 mL glass beaker	20 mL solution in 30 mL flat-bottom glass tube	Recirculating apparatus
Minimum no. of treatments	8	8	8	8	8	8	6	6
No. of test replicates	10	10	3	4	1	1	10	1
No. animals per vessel/replicate	1	1	10	5	10	10	1	10

Feeding during test	0.5 mL *P. subcapitata*, 0.1 mL YCT	0.5 mL *P. subcapitata*, 0.5 mL *Chlorella* sp.	*Chironomus*: 1.25 mL 3:2 ratio cereal grass: ground Nutrafin *Hexagenia:* None *Hyalella:* 2 mg ground Nutrafin *Lumbriculus:* None	0.3 mL *Navicula* sp. free diatoms	None	None	None	None
Test substrate	None	None	*Chironomus*: 20 mL silica sand *Hexagenia:* glass tubes *Hyalella:* 5x5 cm cotton gauze *Lumbriculus:* None	None	None	None	3x1 cm 500 µm Nitex screen	280 µm Nitex screen

Table 2. Summary of percent difference between nominal and measured neonicotinoid concentrations

Neonicotinoid	Method performance[a]	Sample analysis		
	Method detection limit (μg L^{-1})	Mean percent difference	SD	N
Acetamiprid	0.010	11.0	7.1	20
Clothianidin	0.010	9.6	6.5	32
Dinotefuran	0.010	1.5	15.8	16
Imidacloprid	0.010	8.5	5.8	32
Thiacloprid	0.005	12.9	8.4	20
Thiamethoxam	0.010	16.0	7.5	29

[a] Estimated uncertainty for neonicotinoid analysis method is 9 – 13%.

Table 3. Summary of acute toxicity values for 6 neonicotinoids (LC*x* and EC*x* (immobilization) values with 95% confidence intervals in parentheses). All estimates are based on concentrations directly measured or calculated using a correction factor based on the percent difference between nominal and measured concentrations.

Species	LC10[a] (µg L^{-1})	LC50[a] (µg L^{-1})	Control Mort.[b]	EC10[c] (µg L^{-1})	EC50[c] (µg L^{-1})	Control Immob.[b]
Acetamiprid						
Insecta						
Ephemeroptera						
Caenis sp. †	270.2 (106.6 – 433.8)	782.8 (499.3 – 1066.2)	0%	NC	<138.8	0%
Cloeon sp. †	201.0 (–0.5 – 402.5)	2369.7 (805.0 – 3934.4)	0%	<16.6	<16.6	0%
Ephemerella sp. †	29.1 (–6.3 – 64.5)	158.2 (76.6 – 239.8)	0%	NC	<56.1	0%
Hexagenia spp.	NC	>35600.0	0%	NC	1.8 (1.3 – 2.6)	0%
Isonychia bicolor†	NC	>9600.0	10%	NC	<600.0	10%
McCaffertium sp. †	NC	>890.0	0%	NC	<56.1	0%
Neocloeon triangulifer	NC	1.7 (1.2 – 2.2)	0%	NC	1.6 (1.2 – 2.2)	0%
Odonata						
Coenagrion sp.	5439.1 (345.8 – 10532.3)	24392.9 (13151.1 – 35634.7)	10%	<5625.0	<5625.0	10%
Hemiptera						
*Trichocorixa sp.** †	359.9 (97.0 – 622.8)	1515.2 (758.6 – 2271.8)	0%	NC	63.5 (44.9 – 89.9)	0%
Trichoptera						
Cheumatopsyche sp. †	134.8 (47.5 – 222.1)	403.8 (245.9 – 561.6)	10%	NC	<56.1	10%
Coleoptera						
Gyrinus sp. †	165.3 (43.2 – 287.3)	686.5 (311.9 – 1061.1)	0%	NC	88.2 (–35.0 – 248.7)	0%
Stenelmis sp. †	152.8 (100.0 – 205.6)	238.3 (182.2 – 294.4)	10%	63.6 (38.2 – 88.9)	104.6 (79.3 – 129.8)	10%
Diptera						
Chironomus dilutus	1.6 (1.2 – 2.0)	2.8 (2.4 – 3.2)	10%	1.4 (1.1 – 1.8)	2.7 (2.3 – 3.2)	10%
Aedes sp.* †	64.5 (30.0 – 99.0)	159.6 (106.5 – 212.8)	11%	NC	NC	NC
Crustacea						
Caecidotea sp. †	146.7 (–118.3 – 411.7)	2129.6 (634.0 – 3625.1)	0%	NC	<556.0	0%
*Ceriodaphnia dubia**	NC	>33500	0%	NC	NC	NC
Hyalella azteca	2.3 (1.6 – 2.9)	4.8 (4.0 – 5.7)	10%	2.0 (1.4 – 2.6)	4.4 (3.6 – 5.2)	10%
Oligochaeta						
Lumbriculus variegatus	21.6 (19.4 – 23.8)	26.5 (24.7 – 28.3)	0%	14.6 (11.9 – 17.4)	15.6 (10.7 – 20.6)	0%
Clothianidin						
Insecta						
Ephemeroptera						
Caenis sp. †	52.3 (25.2 – 79.3)	122.1 (82.9 – 161.3)	10%	NC	NC	40%
Cloeon sp. †	357.0 (9.1 – 704.9)	3939.2 (1044.9 – 6833.5)	0%	NC	<16.4	0%
Ephemerella sp. †	NC	586.9 (415.0 – 830.0)	0%	NC	18.5 (13.3 – 25.7)	0%
Hexagenia spp.	NC	>17400.0	10%	0.9 (0.4 – 1.5)	5.5 (3.9 – 7.0)	13%
Isonychia bicolor†	NC	>1740.0	0%	NC	<108.8	0%
McCaffertium sp. †	396.0 (120.7 – 671.3)	1328.3 (653.9 – 2002.7)	10%	NC	<108.8	10%

Neocloeon triangulifer	NC	3.5 (2.5 – 5.0)	0%	NC	3.5 (2.5 – 5)	0%
Odonata						
Coenagrion sp.	3336.9 (–197.5 – 6871.4)	14556.3 (7632.8 – 21479.9)	10%	<5918.8	<5918.8	10%
Plecoptera						
Agnetina, *Paragnetina* sp. †	697.0 (287.0 – 1107.0)	1714.8 (1105.3 – 2324.2)	11%	NC	<300.5	11%
Hemiptera						
*Trichocorixa sp.** †	5.5 (–0.3 – 11.4)	34.8 (17.1 – 52.5)	0%	5.1 (0.5 – 9.7)	21.3 (11.7 – 30.9)	0%
Trichoptera						
Cheumatopsyche sp. †	235.8 (13.0 – 458.6)	1281.0 (423.1 – 2138.8)	0%	NC	<108.8	0%
Coleoptera						
Gyrinus sp. †	33.4 (18.8 – 48.1)	62.6 (45.4 – 79.8)	0%	23.0 (13.6 – 32.5)	41.2 (30.2 – 52.1)	0%
Stenelmis sp. †	81.2 (32.6 – 129.7)	208.0 (136.5 – 279.4)	0%	NC	84.9 (60.0 – 120.0)	0%
Diptera						
Chironomus dilutus	0.6 (–0.1 – 1.2)	11.6 (6.5 – 16.8)	0%	NC	3.4 (2.7 – 5.5)	0%
Aedes sp.* †	10.3 (3.6 – 17.1)	28.5 (17.5 – 39.6)	22%	NC	NC	NC
Crustacea						
Caecidotea sp. †	2368.7 (260.7 – 4476.8)	16085.8 (2636.6 – 29534.9)	0%	108.5 (–17.8 – 234.8)	537.2 (248.0 – 826.3)	0%
*Ceriodaphnia dubia**	NC	>100000.0	0%	NC	NC	NC
Hyalella azteca	3.1 (2.4 – 3.8)	5.2 (4.4 – 5.9)	0%	2.7 (2.0 – 3.3)	4.8 (4.1 – 5.6)	0%
Oligochaeta						
Lumbriculus variegatus	NC	177.1 (145.3 – 207.5)	0%	NC	41.7 (34.9 – 49.8)	0%
Dinotefuran						
Insecta						
Ephemeroptera						
Caenis sp. †	1524.2 (500.3 – 2548.1)	5466.5 (3134.4 – 7798.6)	0%	NC	<212.2	0%
Cloeon sp. †	NC	>13250.0	0%	NC	<25.2	0%
Ephemerella sp. †	82.0 (–53 – 217.1)	395.5 (119.4 – 671.6)	0%	NC	<344.3	0%
Hexagenia spp.	NC	>27400.0	20%	8.4 (6.0 – 10.9)	19.0 (15.6 – 22.5)	0%
McCaffertium sp. †	NC	>5500.0	0%	NC	<344.3	0%
Neocloeon triangulifer	3.9 (2.4 – 5.3)	9.4 (7.1 – 11.6)	0%	3.2 (2.2 – 4.1)	6.0 (4.8 – 7.2)	0%
Odonata						
Coenagrion sp.	1193.8 (–2284.1 – 4671.7)	12347.9 (29.3 – 24666.5)	20%	NC	<5187.5	20%
Hemiptera						
*Trichocorixa sp.** †	15.8 (7.6 – 24)	36.9 (25.1 – 48.8)	20%	2.0 (–0.5 – 4.6)	10.1 (4.3 – 15.9)	20%
Trichoptera						
Cheumatopsyche sp. †	NC	>5500.0	10%	NC	419.5 (344.3 – 687.5)	10%
Coleoptera						
Gyrinus sp. †	54.7 (–66.1 – 175.5)	1790.6 (306.6 – 3274.5)	0%	NC	<163.0	0%
Stenelmis sp. †	123.8 (7.8 – 239.8)	555.6 (299.5 – 811.8)	10%	153.1 (71.1 – 235.1)	335.6 (230.6 – 440.7)	10%
Diptera						
Chironomus dilutus	6.4 (3.9 – 8.9)	23.5 (18.0 – 29.0)	0%	5.8 (4.2 – 7.4)	12.4 (10.2 – 14.5)	0%
Aedes sp.* †	134.8 (21.3 – 248.3)	741.5 (137.9 – 1345.1)	0%	NC	NC	NC
Crustacea						
Caecidotea sp. †	NC	>27300.0	0%	NC	580.5 (426.6 – 853.1)	0%
*Ceriodaphnia dubia**	NC	>87000.0	0%	NC	NC	NC
Hyalella azteca	36.2 (29.9 – 42.5)	57.8 (52.0 – 63.7)	0%	32.8 (28.1 – 37.5)	44.5 (40.9 – 48.2)	0%
Oligochaeta						

Lumbriculus variegatus	NC	2404.2 (1700.0 – 3400.0)	0%	NC	601.0 (425.0 – 850.0)	0%
Imidacloprid						
Insecta						
Ephemeroptera						
Caenis sp. †	NC	<21.8	0%	NC	<21.8	0%
Cloeon sp. †	126.3 (9.5 – 243.1)	1152.0 (513.1 – 1790.8)	0%	NC	23.1 (16.2 – 33.2)	0%
Ephemerella sp. †	16.1 (2.9 – 29.4)	68.2 (33.1 – 103.3)	10%	NC	10.6 (7.5 – 15.0)	10%
Hexagenia spp.	427.7 (47.7 – 807.6)	9320.5 (3757.2 – 14883.8)	0%	NC	NC	20%
Isonychia bicolor†	113.2 (–18.1 – 244.4)	715.2 (319.3 – 1111.0)	0%	31.4 (16.9 – 45.8)	60.4 (43.2 – 77.7)	0%
McCaffertium sp. †	738.7 (314.9 – 1162.5)	1810.2 (1018.2 – 2602.3)	10%	NC	10.6 (7.5 – 15.0)	0%
Neocloeon triangulifer	2.9 (2.0 – 3.7)	5.2 (4.2 – 6.2)	5%	1.9 (1.4 – 2.4)	3.1 (2.6 – 3.7)	5%
Odonata						
Coenagrion sp.	762.7 (–1835.1 – 3360.6)	3462.7 (–2046.6 – 8972.0)	20%	NC	<5437.5	20%
Hemiptera						
*Trichocorixa sp.** †	139.4 (51.4 – 227.4)	450.4 (274.0 – 626.7)	0%	NC	63.1 (44.6 – 89.2)	0%
Trichoptera						
Cheumatopsyche sp. †	48.9 (–3.2 – 101.0)	324.5 (72.1 – 576.8)	20%	48.6 (12.2 – 85.1)	176.4 (99.7 – 253.1)	20%
Micrasema sp. †	7.0 (3.8 – 10.1)	14.6 (11.0 – 18.2)	5%	NC	<6.4	5%
Coleoptera						
Gyrinus sp. †	79.6 (47.4 – 111.8)	132.2 (99.9 – 164.5)	0%	28.0 (14.9 – 41.2)	57.5 (40.5 – 74.5)	0%
Stenelmis sp. †	35.1 (–20.8 – 91.1)	365.7 (107.1 – 624.2)	10%	44.6 (17.6 – 71.7)	99.2 (66.9 – 131.6)	10%
Diptera						
Chironomus dilutus	1.7 (0.7 – 2.7)	11.8 (8.3 – 15.4)	0%	1.4 (1.1 – 1.8)	2.5 (2.1 – 2.8)	0%
Aedes sp.* †	18.9 (99.4 – 28.5)	40.8 (27.9 – 53.6)	0%	NC	NC	NC
Crustacea						
Caecidotea sp. †	NC	>15600.0	0%	98.0 (–6.1 – 202.2)	320.8 (162.0 – 479.6)	0%
*Ceriodaphnia dubia**	NC	72124.9 (51000.0 – 102000.0)	0%	NC	NC	NC
*Daphnia magna**	NC	>102000.0	0%	NC	NC	NC
Hyalella azteca	114.0 (73.1 – 155.0)	363.2 (301.3 – 425.1)	0%	77.2 (51.4 – 102.9)	176.9 (149.4 – 204.4)	0%
Oligochaeta						
Lumbriculus variegatus	42.6 (9.9 – 75.4)	45.4 (30.6 – 60.1)	0%	30.4 (8.5 – 52.3)	32.4 (26.7 – 38.0)	0%
Thiacloprid						
Insecta						
Ephemeroptera						
Caenis sp. †	46.4 (1.6 – 91.3)	231.4 (120.7 – 342.1)	0%	NC	<66.3	10%
Cloeon sp. †	856.3 (232.7 – 1479.9)	3882.6 (1948.5 – 5816.7)	0%	NC	23.1 (16.2 – 33.2)	0%
Ephemerella sp. †	24.4 (–13.1 – 61.9)	190.6 (78.3 – 302.8)	0%	NC	<58.0	0%
Hexagenia spp.	NC	>9300.0	0%	NC	<1.3	0%
McCaffertium sp. †	NC	>920.0	0%	NC	10.6 (7.5 – 15.0)	0%
Neocloeon triangulifer	NC	1.9 (1.4 – 2.6)	0%	NC	1.9 (1.4 – 2.6)	0%
Odonata						
Coenagrion sp.	2377.4 (939.0 – 3815.9)	5647.2 (3762.42 – 7531.96)	0%	NC	<2500.0	0%
Hemiptera						
*Trichocorixa sp.** †	45.1 (15.1 – 75.1)	135.3 (84.7 – 185.9)	0%	NC	<39.7	0%
Trichoptera						
Cheumatopsyche sp. †	NC	>920.0	0%	NC	162.6 (115.0 – 230.0)	0%
Coleoptera						

Gyrinus sp. †	NC	180.9 (139.5 – 279.0)	0%	40.8 (23.4 – 58.3)	72.3 (51.5 – 93.1)	0%
Stenelmis sp. †	99.4 (55.5 – 143.2)	183.6 (133.1 – 234.1)	0%	67.0 (33.8 – 100.2)	129.0 (92.2 – 165.9)	0%
Diptera						
Chironomus dilutus	0.3 (0.2 – 0.5)	1.6 (1.2 – 2.0)	3%	0.4 (0.3 – 0.5)	0.8 (0.7 – 0.9)	3%
Aedes sp.* †	10.0 (0.8 – 19.2)	53.4 (28.0 – 78.8)	0%	NC	NC	NC
Crustacea						
Caecidotea sp. †	NC	NC	0%	NC	<136.8	0%
*Ceriodaphnia dubia**	NC	>41500	0%	NC	NC	NC
Hyalella azteca	17.3 (10.9 – 23.6)	55.0 (43.0 – 67.0)	0%	13.2 (9.6 – 16.9)	26.9 (22.3 – 31.5)	0%
Oligochaeta						
Lumbriculus variegatus	29.9 (9.2 – 50.5)	33.8 (23.0 – 44.6)	0%	NC	11.5 (8.1 – 16.2)	0%
Thiamethoxam						
Insecta						
Ephemeroptera						
Caenis sp. †	61.6 (6.2 – 117.1)	381.9 (185.0 – 578.8)	0%	NC	<23.3	0%
Cloeon sp. †	815.5 (169.3 – 1461.7)	4633.6 (1835.8 – 7431.3)	0%	NC	44.1 (31.2 – 62.4)	0%
Ephemerella sp. †	45.1 (–13.4 – 103.8)	334.9 (135.9 – 533.9)	0%	NC	<59.0	0%
Hexagenia spp.	NC	>30800.0	7%	1.0 (-0.2 – 2.2)	35.8 (14.1 – 57.4)	10%
Isonychia bicolor†	NC	>7120.0	0%	NC	<445.0	0%
McCaffertium sp. †	NC	>920.0	0%	NC	81.7 (58.0 – 115.0)	0%
Neocloeon triangulifer	NC	5.5 (3.9 – 7.8)	0%	NC	5.5 (3.9 – 7.8)	0%
Odonata						
Coenagrion sp.	1290.4 (–2327.2 – 4908.0)	15061.8 (619.0 – 29504.6)	20%	NC	<4187.5	20%
Plecoptera						
Agnetina, Paragnetina sp. †	NC	>7120.0	0%	NC	<445.0	0%
Hemiptera						
*Trichocorixa sp.** †	126.2 (–18.5 – 270.9)	1473.1 (176.3 – 2769.9)	0%	NC	56.3 (34.3 – 68.6)	0%
Trichoptera						
Cheumatopsyche sp. †	53.1 (–8.7 – 114.8)	170.1 (78.6 – 261.6)	14%	NC	118.5 (108.8 – 218.0)	14%
Micrasema sp. †	23.2 (16.7 – 29.8)	32.8 (26.4 – 39.2)	10%	NC	18.5 (13.1 – 26.2)	10%
Coleoptera						
Gyrinus sp. †	NC	31.0 (21.9 – 43.8)	0%	4.3 (0.6 – 8.0)	14.0 (7.6 – 20.4)	0%
Stenelmis sp. †	86.0 (50.7 – 121.3)	148.0 (109.6 – 186.4)	10%	86.0 (50.7 – 121.3)	148 (109.6 – 186.4)	10%
Diptera						
Chironomus dilutus	16.0 (9.5 – 22.5)	61.9 (45.4 – 78.4)	0%	13.7 (9.3 – 18.2)	36.8 (29.4 – 44.3)	0%
Aedes sp.* †	23.2 (8.6 – 37.8)	67.4 (42.2 – 92.5)	0%	NC	NC	NC
Crustacea						
Caecidotea sp. †	NC	>35600	0%	1512.0 (566.6 – 2457.4)	4775.4 (2976.3 – 6574.6)	10%
*Ceriodaphnia dubia**	NC	>80000	0%	NC	NC	NC
*Daphnia magna**	NC	>80000	0%	NC	NC	NC
Hyalella azteca	183.8 (105.0 – 262.7)	801.0 (518.7 – 1083.3)	7%	151.1 (102.4 – 199.7)	391.0 (312.1 – 469.9)	7%
Oligochaeta						
Lumbriculus variegatus	1762.5 (1406.4 – 2118.6)	3438.2 (3025.5 – 3850.9)	0%	1908.0 (607.1 – 3208.8)	2035.1 (1699.7 – 2370.6)	0%

[a] LC*x* = lethal concentration at *x*%; [c] EC*x* = effective concentration at *x*%; NC = Not Calculable

[b] Control mortality/immobilization = percentage of negative control individuals exhibiting mortality/immobilization

†Number of experimental replicates = 1. For all other tested species, replicates ≥ 3.

*Test duration 48 h. All other test durations 96 h.

Table 4. Estimated Kaplan-Meier ECDF summary statistics, and hazard assessment for neonicotinoid concentrations in Ontario surface waters from select monitoring studies

Neonicotinoid	N	Percent censored	Maximum (µg L^{-1})	ECDF median (µg L^{-1})	ECDF mean (95% CI) (µg L^{-1})	ECDF Stdev (µg L^{-1})	99% percentile	Endpoint: immobilization			Endpoint: lethality		
								HC5 (95% CI) (µg L^{-1})	HQ	Hazard	HC5 (95% CI) (µg L^{-1})	HQ	Hazard
Acetamiprid	348	98.3	0.100	*Not calculated*				0.47 (0.16 – 1.41)	0.21	Low	0.48 (0.21 – 1.07)	0.21	Low
Clothianidin	544	60.3	0.970	0.020	0.052 (0.044 – 0.060)	0.093	0.44	0.14 (0.01 – 2.93)	*No model passed goodness-of-fit test or visual inspection*		4.13 (3.32 – 5.15)	0.11	Low
Dinotefuran	348	99.4	0.019	*Not calculated*				0.86 (0.17 – 4.43)	0.020	Low	7.81 (4.67 – 13.06)	<0.005	None
Imidacloprid	544	79.8	11.000	N/A[a]	0.091 (0.037 – 0.145)	0.645	2.70	1.08 (0.82 – 1.43)	1.92	Moderate	3.71 (2.82 – 4.87)	0.73	Low
Thiacloprid	462	99.8	0.002	*Not calculated*				0.30 (0.07 – 1.25)	0.0070	Low	0.90 (0.60 – 1.34)	<0.005	None
Thiamethoxam	544	63.4	2.700	0.012	0.077 (0.058 – 0.097)	0.230	1.20	6.09 (3.07 – 12.08)	0.20	Low	12.29 (9.90 – 15.25)	0.010	None

[a] ECDF median is not applicable for imidacloprid due to probability of detection > 0.5

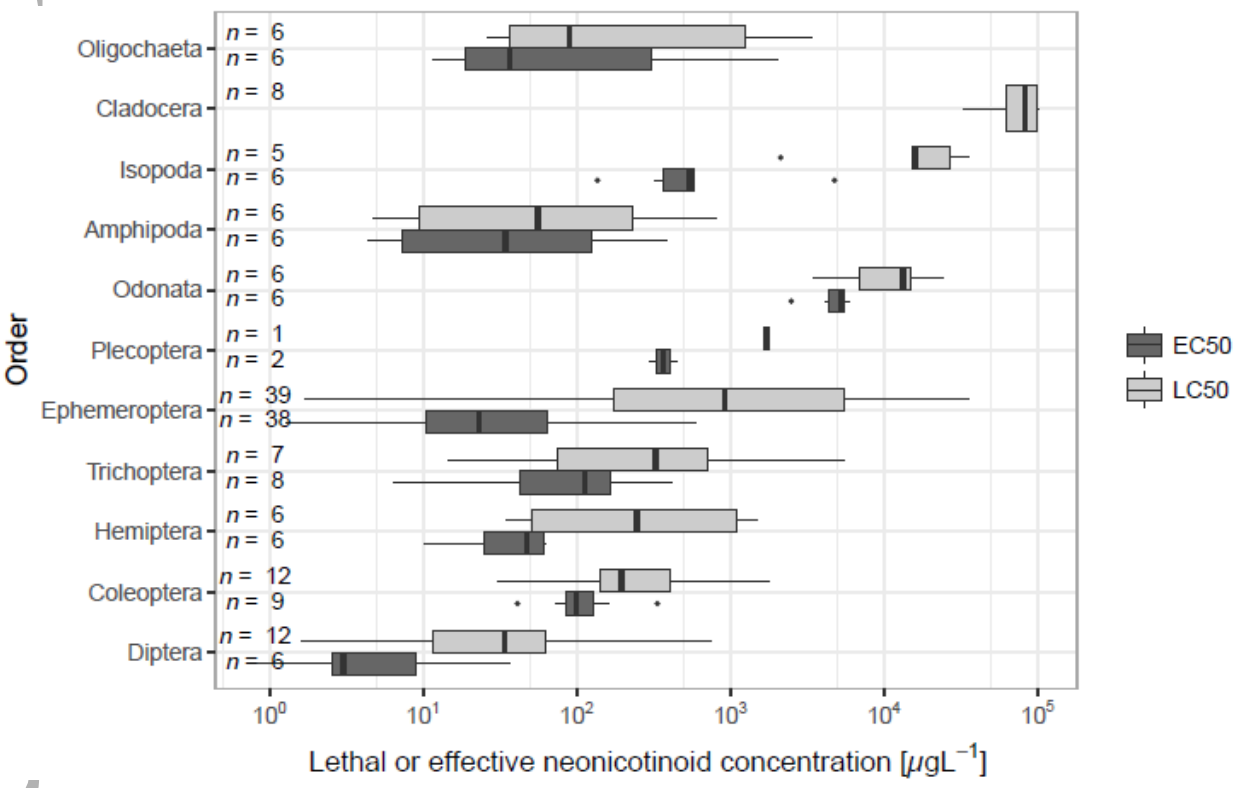

Figure 1

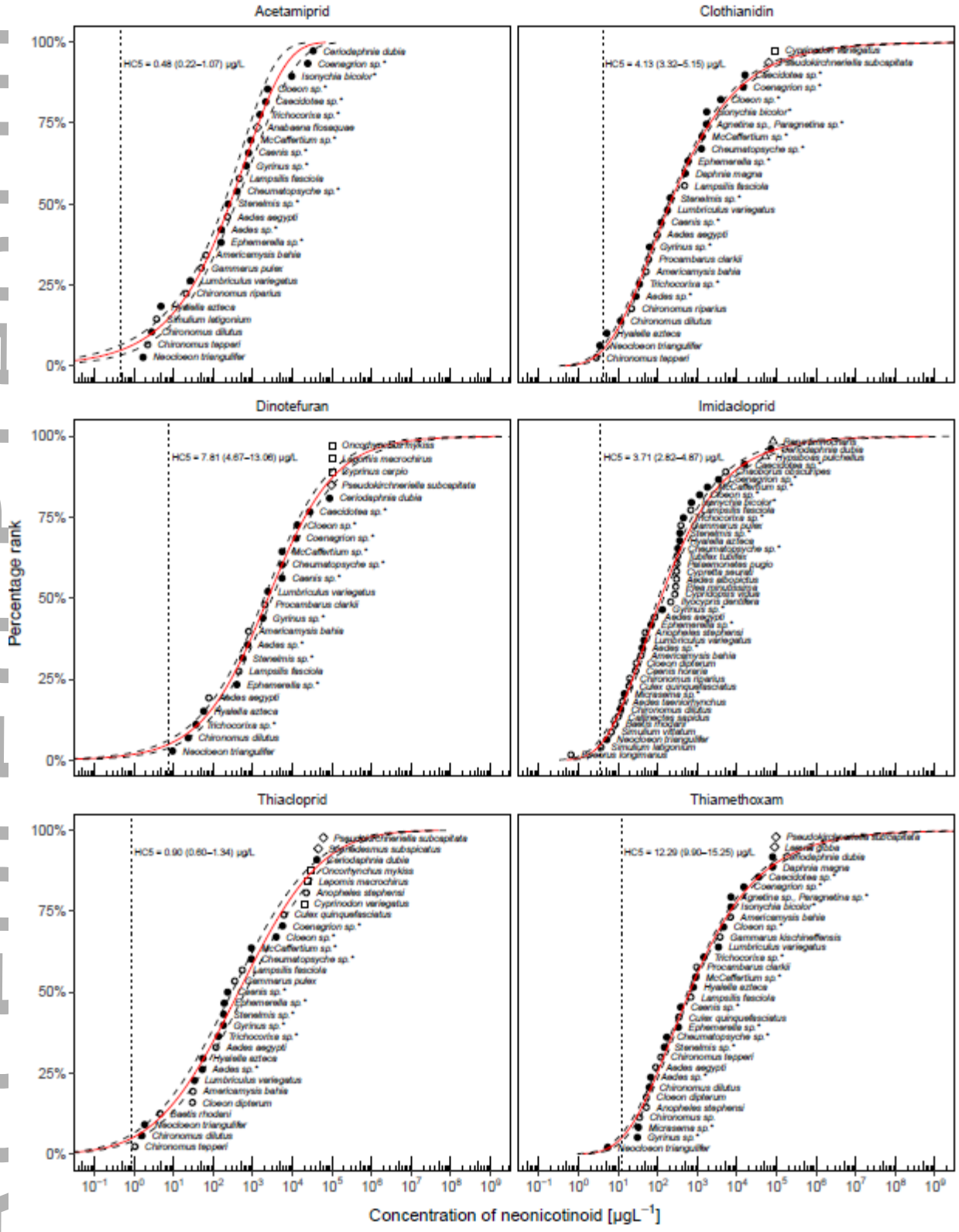

Figure 2