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Review

Neonicotinoid contamination of global surface waters and associated risk to aquatic invertebrates: A review

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ABSTRACT

Neonicotinoids, broad-spectrum systemic insecticides, are the fastest growing class of insecticides worldwide and are now registered for use on hundreds of field crops in over 120 different countries. The environmental profile of this class of pesticides indicate that they are persistent, have high leaching and runoff potential, and are highly toxic to a wide range of invertebrates. Therefore, neonicotinoids represent a significant risk to surface waters and the diverse aquatic and terrestrial fauna that these ecosystems support. This review synthesizes the current state of knowledge on the reported concentrations of neonicotinoids in surface waters from 29 studies in 9 countries world-wide in tandem with published data on their acute and chronic toxicity to 49 species of aquatic insects and crustaceans spanning 12 invertebrate orders. Strong evidence exists that water-borne neonicotinoid exposures are frequent, long-term and at levels (geometric means = 0.13 μg/L (averages) and 0.63 μg/L (maxima)) which commonly exceed several existing water quality guidelines. Imidacloprid is by far the most widely studied neonicotinoid (66% of the 214 toxicity tests reviewed) with differences in sensitivity among aquatic invertebrate species ranging several orders of magnitude; other neonicotinoids display analogous modes of action and similar toxicities, although comparative data are limited. Of the species evaluated, insects belonging to the orders Ephemeroptera, Trichoptera and Diptera appear to be the most sensitive, while those of Crustacea (although not universally so) are less sensitive. In particular, the standard test species *Daphnia magna* appears to be very tolerant, with 24–96 hour LC_{50} values exceeding 100,000 μg/L (geometric mean > 44,000 μg/L), which is at least 2–3 orders of magnitude higher than the geometric mean of all other invertebrate species tested. Overall, neonicotinoids can exert adverse effects on survival, growth, emergence, mobility, and behavior of many sensitive aquatic invertebrate taxa at concentrations at or below 1 μg/L under acute exposure and 0.1 μg/L for chronic exposure. Using probabilistic approaches (species sensitivity distributions), we recommend here that ecological thresholds for neonicotinoid water concentrations need to be below 0.2 μg/L (short-term acute) or 0.035 μg/L (long-term chronic) to avoid lasting effects on aquatic invertebrate communities. The application of safety factors may still be warranted considering potential issues of slow recovery, additive or synergistic effects and multiple stressors that can occur in the field. Our analysis revealed that 81% (22/27) and 74% (14/19) of global surface water studies reporting maximum and average individual neonicotinoid concentrations respectively, exceeded these thresholds of 0.2 and 0.035 μg/L. Therefore, it appears that environmentally relevant concentrations of neonicotinoids in surface waters worldwide are well within the range where both short- and long-term impacts on aquatic invertebrate species are possible over broad spatial scales.

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Contents

1. Introduction

1.1. Background on neonicotinoids

Neonicotinoids belong to the group of nitroguanidine systemic insecticides frequently applied to crops as soil and seed treatments at planting to protect seedlings from early-season root and leaf-feeding pests, as well as via later season foliar treatments. Imidacloprid-containing products now dominate the insecticide market and are registered for use on more than 140 different crops in 120 countries (Jeschke and Nauen, 2008). The neonicotinoid class of insecticides was first developed and registered in the early 1990s, partly in response to ongoing pest resistance, concerns over cumulative exposure from organophosphorous and carbamate insecticides, and increasing evidence linking impaired neural development in children to cholinesterase-inhibiting insecticides (Eskenazi et al., 1999). Following on the industry success of imidacloprid, development and sale of other neonicotinoid insecticides with similar chemistries rapidly followed after 2000, specifically acetamiprid, clothianidin, dinotefuran, nitenpyram, thiacloprid and thiamethoxam among others, under various trade names. Neonicotinoids now represent the largest selling class of insecticide and seed treatments on the global market (Jeschke et al., 2010).

Due to their systemic activity, improved rain fastness, and convenience of use as a seed treatment, neonicotinoids are extremely popular for pest control on a broad range of crops (Elbert et al., 2008; Main et al., 2014; USGS, 2012). However, they exhibit chemical properties that enhance environmental persistence and susceptibility to transport into aquatic ecosystems through runoff and drainage of agricultural areas (Armbrust and Peeler, 2002). Recent reports suggest toxic residues of imidacloprid and other neonicotinoids have been detected in water bodies and researchers in the Netherlands have found correlative links to reduced aquatic insect populations (Van Dijk et al., 2013) and insectivorous farmland birds (Hallmann et al., 2014). However, in most countries there is a general lack of systematic environmental monitoring data for neonicotinoids in surface waters and until recently, analytical procedures were often insufficient to report the low concentrations known to cause harm to aquatic invertebrates.

Neonicotinoids are successful insecticides largely because the acute toxicity to mammals is lower than its replacements, they are extremely toxic to most insect pests and can be conveniently used as a systemic seed or in furrow treatment to protect seedling crops from piercing-sucking and chewing insects. All neonicotinoids bind agonistically to the post-synaptic nicotinic acetylcholine receptors (nAChR) in the invertebrate central nervous system, thus competing with the natural neurotransmitter acetylcholine (ACh). Toxicity studies with arthropods suggest that the binding to these receptors is long-lasting (Tennekes, 2010a), and lethal effects are typically delayed (Beketov and Liess, 2008a) such that repeated or chronic exposure can lead to cumulative effects over time (Tennekes and Sánchez-Bayo, 2013). For many aquatic invertebrates with long larval aquatic stages, exposure to neonicotinoids is expected to be prolonged due to either repeated pulse events and/or low level chronic exposures. Many invertebrates are extremely sensitive to these compounds, including non-target aquatic species (Alexander et al., 2007; Beketov and Liess, 2008a; EFSA, 2013; Liess and Beketov, 2011; Pestana et al., 2009; Roessink et al., 2013; Sánchez-Bayo and Goka, 2006; Stoughton et al., 2008) and terrestrial pollinators such as bumble bees and honey bees (Decourtye and Devillers, 2010; Sanchez-Bayo and Goka, 2014; Whitehorn et al., 2012). Consequently, the persistence and movement of neonicotinoids into aquatic ecosystems could pose a risk to sensitive aquatic invertebrates upon which vertebrate wildlife depend for food (Gibbons et al., 2014; Goulson, 2013; Tennekes, 2010b). The objective of this review is to summarize the available data on different neonicotinoid concentrations in surface waters worldwide and to cohesively synthesize and compare these values to the growing body of data from laboratory, field and mesocosm studies on the concentrations observed to cause lethal and sub-lethal toxicity to aquatic invertebrates. Finally, based on probabilistic analyses, we provide recommended aquatic invertebrate effect thresholds to aid in the development of appropriate water quality reference values for the range of neonicotinoids.

1.2. Chemical properties and environmental fate

All neonicotinoids exhibit high water solubility that makes them amenable for use as systemic insecticides. In addition, they also have long half-lives in soil and in water, where they are resistant to hydrolysis at neutral or acidic pH and under anaerobic conditions; although some of them are subject to rapid photodegradation under favorable conditions (i.e. shallow waters with greater light penetration; Table 1). Their chemical properties, particularly their high water solubility and partitioning properties (low log K_{OW}) and low soil adsorption (log K_{OC}), promote movement of these insecticides through surface and subsurface runoff (CCME, 2007; EFSA, 2008) and result in extended persistence under simulated environmental conditions (Tisler et al., 2009). Local environmental conditions can modify the persistence of neonicotinoids in water (e.g., increasing pH and turbidity enhances persistence) (Sarkar et al., 2001). The major transport routes to aquatic ecosystems include surface runoff after rain events (Armbrust and Peeler, 2002), soluble or insoluble fractions transported via snowmelt (Main et al., 2014), leaching into groundwater (Lamers et al., 2011) with associated subsurface discharge into wetlands and other surface waters (PMRA, 2001), talc and graphite dust associated with seeding drills at the time of planting (Krupke et al., 2012; Nuyttens et al., 2013), decay of systemically treated plants in water bodies (Kreutzweiser et al., 2008), and deposition of

Table 1
Chemical properties (solubility, log K_{OW} and K_{OC}) and environmental persistence (DT_{50} for soil and aqueous photolysis and hydrolysis) of neonicotinoid insecticides. Where available, field degradation studies were selected.[a]

Compound	Molecular Mass (Da)[b]	Water Solubility (mg/L) @ 20 °C	Lipophilicity (log K_{OW})	Soil Affinity (log K_{OC})	Soil Persistence (DT_{50} in days)[c]	Water Photolysis (DT_{50} in days)	Water Hydrolysis (DT_{50} in days)[d]
Acetamiprid	222.7	2950	0.80	2.3	2–20	34	Stable; 420 (pH 9)
Clothianidin	249.7	340	0.91	2.08	13–1386	<1	Stable; 14.4 (pH 9)
Dinotefuran	202.2	39,830	−0.55	1.41	50–100	<2	Stable
Imidacloprid	255.7	610	0.57	2.19–2.90	104–228	<1	Stable; >1 yr (pH 9)
Nitenpyram	270.7	590,000	−0.66	1.78	1–15	NA	Stable; 2.9 (pH 9)
Thiacloprid	252.7	184	1.26	3.67	9–27	10–63	Stable
Thiamethoxam	291.7	4100	−0.13	1.75	7–72	2.7–39.5	Stable; 11.5 (pH 9)

[a] Data sources: Pesticide Products Database (PPDB) University of Hertfordshire; 2006–2013 and Hazardous Substances Data Bank (HSDB) Accessed Feb. 5 2014. Available at: http://toxnet.nlm.nih.gov/cgi-bin/sis/htmlgen?HSDB.
[b] Da = Dalton = g/mol.
[c] Under anaerobic conditions, compounds are much more stable in water and soil.
[d] Under acidic or neutral pH conditions, compounds are stable to hydrolysis, whereas under alkaline conditions (pH 9) hydrolysis can occur.

treated seeds, soil or spray drift into water bodies or depressions. The majority of surface water contamination is expected to be through runoff after major precipitation events (Chiovarou and Siewicki, 2008).

Persistence in soil, and thus the likelihood of neonicotinoid movement into receiving waters, is largely dependent on factors such as application rate, pH, temperature, the presence or absence of crop or plant cover, crop rotation, soil type and organic content, and use of fertilizers. Field dissipation studies where imidacloprid was applied to various crops such as corn, tomatoes and turf at an application rate of 0.5 lb/acre report field half-lives in soil of 7, 53, and 61–107 days respectively (SERA, 2005), but half-lives up to 228 days have been reported (Miles Inc. 1992 in Fossen, 2006). Other neonicotinoids such as clothianidin can have half-lives in soil much longer (up to 1386 days) with residues persisting under some conditions for over 4600 days (DT_{90}) (PMRA, 2004). Scholz and Spiteller (1992) found that imidacloprid dissipation time was more rapid in soils with cover crops (48 days) than in bare soils (190 days). Interestingly, applications of fertilizer and use of formulated products have been reported to alter imidacloprid persistence in soil. For example, increases in soil organic carbon through application of organic fertilizers and manure can increase persistence (Rouchaud et al., 1994). Fertilizers have also been shown to decrease soil adsorption and further enhance the mobility and leaching of imidacloprid due to competition between the pesticide and organic matter for soil binding sites (Flores-Cespedes et al., 2002). In contrast, aged pesticide soil residues are more tightly bound leading to increased sorption and reduced transport down the soil profile, but may still move with particulates in solution to surface waters (Cox et al., 1998).

The features which influence soil retention and persistence are also known to influence leaching of neonicotinoids into groundwater. In the absence of light, neonicotinoids can persist in soil and be transported vertically into groundwater. Leachate concentrations may reach depths of 105 cm (Felsot et al., 1998) and concentrations of 0.005–1.32 μg/L (Gupta et al., 2008), 1–5 μg/L (Larsbo et al., 2013), and 100–400 μg/L (Felsot et al., 1998). Consequently, several studies have detected neonicotinoids in groundwater at maximum concentrations ranging from 1.93 μg/L (imidacloprid) to 8.93 μg/L (thiamethoxam) (Table A.1). Concentrations of thiamethoxam in irrigation water sourced from groundwater in a potato growing region of Wisconsin ranged from 0.31 to 0.58 μg/L, and state-wide sampling revealed noteworthy groundwater concentrations for clothianidin (0.21–3.43 μg/L), imidacloprid (0.26–3.34 μg/L), and thiamethoxam (0.20–3.34 μg/L) (Huseth and Groves, 2014). This suggests that shallow infiltration of neonicotinoids may move horizontally as groundwater and discharge into surface waters such as streams and wetlands.

When entering surface waters, neonicotinoids exhibit peak concentrations within 24 h post-application and breakdown following first-order kinetics: rapid initial loss over the first few days followed by a slower second phase (Armbrust and Peeler, 2002). Most field studies on the fate of neonicotinoids in water have focussed on experimental applications of imidacloprid in rice paddy plantations. Experimental applications at standard rates of 45 and 250 g/ha produced maximum paddy water concentrations of 0.18 μg/L (Kanrar et al., 2006) and 52.9 μg/L (La et al., 2014). At higher application rates of 10,000 g/ha, Thuyet et al. (2011) found that water concentrations peaked at similar levels for treatments applied before (30.2 μg/L) or after (3 μg/L) sowing crops. Rapid initial dissipation of imidacloprid in water in these field studies suggests losses through multiple pathways including dilution, infiltration, photolysis, microbial degradation, plant uptake and, to a much lesser extent, sorption to soil and sediment. The half-lives of imidacloprid in water generally appear to be relatively short (days) (Table 1), but measurable and ecotoxicologically relevant concentrations (0.1 or 0.2 μg/L), can still be detected up to a year after treatment (Kanrar et al., 2006; La et al., 2014), with prolonged persistence under specific environmental conditions such as low temperatures and low pH (Guzsvany et al., 2006) and with the use of the formulated products (Sarkar et al., 2001).

2. Evidence of surface water contamination

Our survey of the water monitoring literature suggests that of the 29 studies identified from 9 countries, neonicotinoids were detected in most surface waters sampled, including puddled water, irrigation channels, streams, rivers, and wetlands in proximity to, or receiving runoff from, agricultural cropland (Fig. 1, Table A.1). The concentrations of individual neonicotinoids from this dataset indicated a geometric mean for average surface water concentrations of 0.13 μg/L (n = 19 studies) and a geometric mean for peak surface water concentrations of 0.63 μg/L (n = 27 studies). Although pesticide monitoring data frequently reports means and maxima, these are usually from grab or spot samples which often underestimate peak concentrations by 1–3 orders of magnitude and average concentrations by 50% (Xing et al., 2013). Depending on the timing of water sampling, particularly in relation to rainfall events, this has major limitations for interpreting the actual peak and average concentrations that are relevant for estimating exposure to aquatic species.

About half of the available water monitoring studies reported detectable imidacloprid concentrations given its longer use history and breadth of applications. Detectable concentrations of imidacloprid ranged from 0.001 (>LOD) to 320 μg/L. Other neonicotinoids are detected at similar water concentrations ranging from 0.008 to 44.1 μg/L for acetamiprid, 0.003 to 3.1 μg/L for clothianidin, and 0.001 to 225 μg/L for thiamethoxam. Where water concentrations were higher, not surprisingly, detection frequencies were also higher. Some of the highest reported concentrations in aquatic systems include imidacloprid in Dutch agricultural surface waters at concentrations up to 320 μg/L (Van Dijk et al., 2013), and thiamethoxam and acetamiprid in playa wetlands of the Texas high plains of up to 225 μg/L (Anderson et al.,

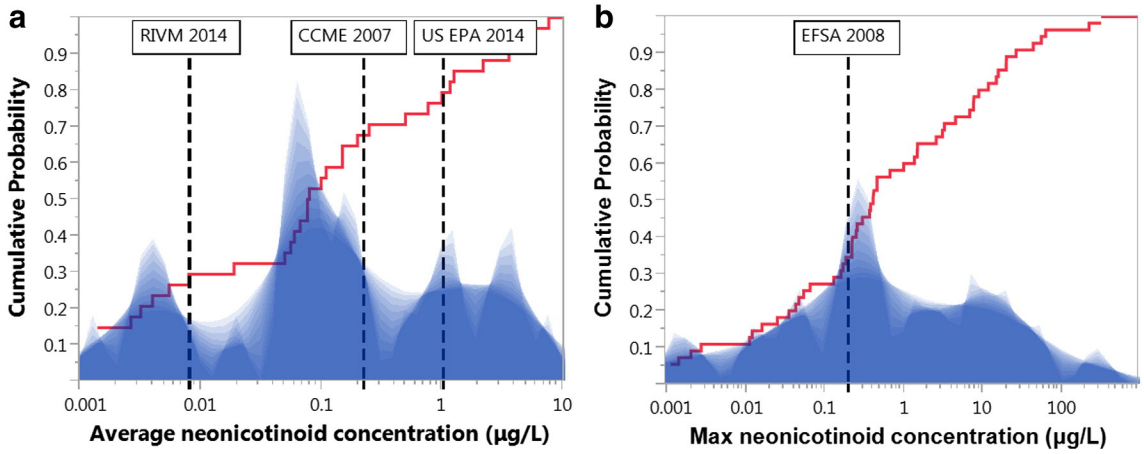

Fig. 1. Shadow histogram of a) average and b) maximum individual neonicotinoid concentrations (log scale, µg/L) reported from water monitoring studies. Overlaid is the cumulative distribution probability (red ascending line) using all available surface water monitoring data showing proportion of data below any given neonicotinoid concentration. Vertical dashed lines illustrate multiple ecological quality reference values set for average imidacloprid water concentrations (RIVM, 2014: 0.0083 µg/L, CCME, 2007: 0.23 µg/L, and US EPA: 1.05 µg/L) or for maximum imidacloprid water concentrations (EFSA, 2008: 0.2 µg/L).

2013). Water samples collected since the mid-1990s from Eastern Canada revealed that imidacloprid was increasingly detected in stream waters draining potato fields after rainfall events, reaching concentrations up to 11.9 µg/L (Denning et al., 2004 in CCME, 2007). In Sydney, Australia, rivers draining horticulture and vegetable growing regions contained five different neonicotinoids with detections in 27–93% of samples and concentrations reaching 4.6 µg/L (imidacloprid) and 1.4 µg/L (thiacloprid) after rainfall events (Sanchez-Bayo and Hyne, 2014). In California, 89% of surface water samples collected from agricultural regions contained imidacloprid with concentrations of up to 3.29 µg/L (Starner and Goh, 2012). Main et al. (2014) found that wetlands in the Canadian Prairies sampled four times over a one year period had maximum concentrations detected in early summer (3.1 µg/L clothianidin and 1.5 µg/L thiamethoxam) and detection frequencies of 36–91%. While not formally considered water bodies, puddles collected on the surface of neonicotinoid seed-treated corn fields in Quebec, Canada have also been found to contain maximum concentrations of 55.7 µg/L clothianidin and 63.4 µg/L thiamethoxam (Samson-Robert et al., in press).

Although no regional patterns were apparent for neonicotinoid detections, wetlands and rivers directly draining or receiving runoff from agricultural crops appear most susceptible. Neonicotinoids, however, have also been frequently detected in water draining urban environments at similar concentrations (Sanchez-Bayo and Hyne, 2014). Importantly, multiple neonicotinoids have been detected in single water samples (Main et al., 2014; Sanchez-Bayo and Hyne, 2014) and often outside of the growing season (Main et al., 2014; Starner and Goh, 2012) suggesting long-term persistence, repeated transport to surface water bodies, or degradation to persistent metabolites (ie. thiamethoxam to clothianidin).

Existing water monitoring data are too scarce to make inferences about the fate of surface water contamination from neonicotinoids in relation to land use and water body features. Frequent detection in water is predicted given the unique properties of this class of insecticides which are highly water soluble, stable to hydrolysis and often slowly degraded. As for other pesticides, water concentrations will be determined by the abiotic and biotic features of the water body and the surrounding land which facilitates transport, retention and degradation (Goldsborough and Crumpton, 1998; Sarkar et al., 1999). Main et al. (2014) reported no statistical differences in average concentrations of neonicotinoids in wetlands surrounded by different cereal and canola crops, although wetlands near canola fields had a higher detection frequency and all contained significantly higher concentrations than wetlands surrounded by grassland. In Texas, playa wetlands in or near grasslands were contaminated with acetamiprid at levels comparable to the cropland, although the frequency of detection was lower (Anderson et al., 2013). As neonicotinoid use increases and more monitoring is conducted, the frequency of detection and the peak and average concentrations of neonicotinoid residues are expected to rise. Equally, as sensitive analytical methods become more widely available, the detection limits also come more in line with toxicity thresholds which, for many sensitive aquatic invertebrates, are typically in the part per billion (µg/L) or part per trillion (ng/L) range (Sánchez-Bayo et al., 2013).

3. Aquatic invertebrate toxicity

3.1. Acute and chronic toxicity of neonicotinoids to aquatic invertebrates

Although the acute toxicity of neonicotinoids to mammals, fish, and birds is generally reported as being lower than for many other insecticides (but see Mineau and Palmer, 2013), extremely low concentrations appear to exert measurable toxicity to a wide range of arthropods, especially insects and some crustaceans. The neonicotinoids have been selected for their specific ability to bind, and activate, the post-synaptic nicotinergic acetylcholine receptors (nAChR) in the insect central nervous system. The neonicotinoid molecule remains bound to the nAChR in insects, holding the channel open and effectively causing continuous nervous system stimulation. In mammals and other vertebrates, the lesser affinity of neonicotinoids for their nAChR appears to be related to the different configuration of the subunits that make up this receptor, so the insecticide binding is weak and/or does not last as long as in insects (Yamamoto et al., 1995). Receptor binding affinity and specificity to the nAChR appears equivalent among different neonicotinoids and is known to be highly conserved across several insect species examined (Zhang et al., 2000). Therefore, differences in toxicity among terrestrial insect species and neonicotinoids have been attributed largely to molecule structure. Neonicotinoid molecules contain either an electronegative nitro- (e.g. imidacloprid, clothianidin, dinotefuran, thiamethoxam) or cyano- (e.g. acetamiprid thiacloprid) substituted heterocyclic group that confers a higher detoxification potential of the latter as reported in bees (Iwasa et al., 2004). Differences in hydrophobicity of the compounds may also affect uptake (penetration across the cuticle and membrane) and thus insecticidal activity (Yamamoto et al., 1998), but this may not be as critical to aquatic invertebrate species. Receptor binding in invertebrates appears to be near irreversible; thus, permanent effects are cumulative with exposure time (Tennekes, 2010a; Tennekes and Sánchez-Bayo, 2011) (but see response by Maus

and Nauen (2010) and rebuttal by Tennekes (2011)), and may therefore exhibit delayed toxicity (Beketov and Liess, 2008a). This trait, in combination with high among-species variability in neonicotinoid toxicity, suggests that current toxicological endpoints commonly used in the regulatory process (i.e., 48-h acute tests for single species) may be inappropriate for this class of insecticides and will lead to an underestimation of the true toxic potential of these insecticides (Beketov and Liess, 2008a; Brock and Wijngaarden, 2012; Tennekes and Sánchez-Bayo, 2011). However, short-term tests still dominate the toxicity literature.

Here, we reviewed over 214 toxicity tests, including acute and chronic tests for imidacloprid, acetamiprid, clothianidin, dinotefuran, thiacloprid, and thiamethoxam with 49 different aquatic arthropod species spanning 12 orders (Table A.2). We conducted a full review of toxic endpoints for aquatic invertebrates following on and updating the work of Goulson (2013), Mineau and Palmer (2013) and Vijver and van den Brink (2014) among others, through searches on the ISI Web of Science for published peer-reviewed studies, but also included industry studies and government reports. Studies included tests with six different neonicotinoids, but predominantly imidacloprid (66%, n = 141 tests), acute studies of ≤96 h duration (83%, n = 178 tests), and (sub)chronic studies of 7 to 39 days duration (17%, n = 36 tests). We only included toxicity tests reporting LC_{50} values (64%, n = 137 tests) and EC_{50} values (36%, n = 77 tests) and excluded those reporting only No Observable Effect Concentrations (NOECs) or Lowest Observable Effect Concentrations (LOECs) because of inconsistency in interpretation. We further considered 16 additional chronic, multi-species field or mesocosm studies to incorporate field-realistic effects on aquatic invertebrate communities (Table A.3). Toxicity data, where combined for the different neonicotinoids are presented as molar equivalents (μmol/L) given the known differences in molecular weights. Back-conversions to concentrations (μg/L) may be approximated by multiplying the molar concentration by the molecular mass of the compound shown in Table 1.

Not surprisingly, neonicotinoid insecticides can exert significant lethal and sub-lethal effects on many aquatic invertebrate populations. In general, acute and chronic toxicity of the neonicotinoids varies greatly among aquatic arthropods (i.e., LC_{50} values range from <1 to >100,000 μg/L, 6 orders of magnitude), with species belonging to the class Insecta typically being the most sensitive (e.g. Alexander et al., 2008), and with cladocerans (Branchiopoda) having the broadest range of sensitivity (Fig. 2). In particular, the Ephemeroptera, Trichoptera and several Diptera, particularly the Chironomidae (midges), were consistently the most sensitive taxa. Many of these species exhibit short-term lethal effects at water concentrations often below 1 μg/L. Sub-lethal endpoints in chronic studies were frequently an order of magnitude or more below the acute tests. For example, Beketov and Liess (2008b) found that downstream drift of aquatic invertebrates (an ecologically relevant endpoint) occurred at concentrations at least nine times lower than corresponding LC_{50} values.

The most widely tested species, *Daphnia magna*, represented 34 studies, or 16% of all neonicotinoid toxicity tests reviewed. This is largely because *D. magna* is considered the global industry standard invertebrate species for most (82%) chemicals tested (Sanchez-Bayo, 2006). However, several authors Ashauer et al. (2011), Beketov and Liess (2008a) and Jemec et al. (2007) reported that this species is by far the least sensitive test species for acute and chronic neonicotinoid studies (Fig. 2). The short-term L[E]C50 for *D. magna* ranges from 4100 to >1,000,000 μg/L, with a geometric mean of 43,927 μg/L (175.8 μmol/L), a value that is at least two to three orders of magnitude higher than the geometric means for most other aquatic invertebrate species (Fig. 2, Table 2). By comparison, Roessink et al. (2013) examined acute and chronic toxicity of imidacloprid to a comprehensive range of aquatic insects and other crustaceans and found that mayflies (Ephemeroptera) and caddisflies (Trichoptera) were the most sensitive species in both acute and chronic tests, with LC_{50} and EC_{50} values in the range of 0.1–0.3 μg/L; other studies have shown midges (Chironomidae) and some other Diptera also to have similar sensitivity (Fig. 2, Table A.2).

While LC_{50} values dominate the hazard assessment for these compounds and allow for direct comparisons of sensitivity among species, several sub-lethal endpoints (growth, reproduction, immobility, feeding, swimming behavior, and emergence) are all responsive to neonicotinoid exposures. Alexander et al. (2007) found that short (12 h) exposure pulses of ≥1 μg/L imidacloprid caused feeding inhibition in mayflies. Even pulse exposures as low as 0.1 μg/L affected the size of adults at emergence (Alexander et al., 2008). Feeding inhibition from imidacloprid exposure similarly appeared to be responsible for decreases in growth and body size for the shredder, *Gammarus pulex* (Ashauer et al., 2011). Immobility of mayflies and caddisflies after a 96 hour exposure to imidacloprid was reported at concentrations in the range of 0.1 to 0.2 μg/L (Roessink et al., 2013). Beketov and Liess (2008b) reported increased downstream drift of macroinvertebrates in a stream microcosm within 2–4 h of exposure to thiacloprid, imidacloprid and acetamiprid. Downstream drift appears to be a sensitive and ecologically relevant measure of imidacloprid effects to several aquatic invertebrate species (Berghahn et al., 2012).

3.2. Relative toxicity of different neonicotinoids and mixtures

Consistent with reported water monitoring data, most of the toxicity research to date has focused on imidacloprid, with relatively few published studies on other neonicotinoids. Based on limited data, however,

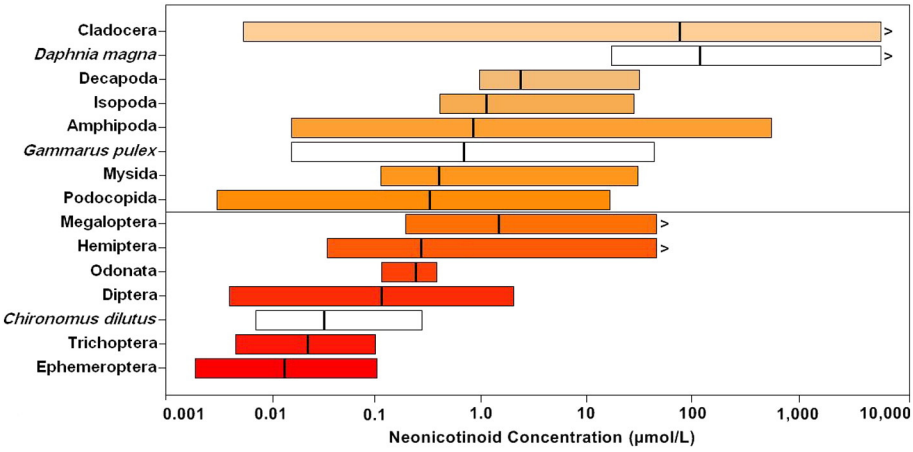

Fig. 2. Range of neonicotinoid toxicity (L[E]C_{50}: 24–96 h in μmol/L) among all tested aquatic invertebrate orders. For context, three of the most common test species (open bars) for the orders Cladocera (*Daphnia magna*), Amphipoda (*Gammarus pulex*) and Diptera (*Chironomus dilutus*) are shown to illustrate differences in sensitivity by species. Vertical lines within bars represent geometric means of test values (see also Table 2).

Table 2
Geometric means by concentration (in μg/L) and by molecular weight (μmol/L) derived from acute toxicity test values (24–96 h L[E]C_{50}) by taxonomic group and by neonicotinoid active ingredient in order of increasing relative toxicity.

Order	Taxa	Geometric mean (μg/L)	Geometric mean (μmol/L)	Active Ingredient	Geometric mean (μg/L)	Geometric mean (μmol/L)
Crustaceans	Cladocera	23,690.0	94.2	Dinotefuran	37,753.1	186.7
	Daphnia magna	43,926.5	175.8	Thiamethoxam	8864.5	30.4
	Decapoda	1562.2	6.87	Acetamiprid	1271.4	5.71
	Isopoda	464.8	1.83	Clothianidin	842.3	3.37
	Amphipoda	235.8	0.93	Thiacloprid	614.8	2.43
	Gammarus pulex	258.7	1.02	Imidacloprid	587.0	2.30
	Mysida	106.2	0.42			
	Podocopida	73.6	0.29			
Aquatic Insects	Megaloptera	711.3	2.78	Dinotefuran	229.8	1.14
	Hemiptera	64.9	0.25	Thiamethoxam	44.8	0.15
	Odonata	55.2	0.22	Acetamiprid	44.4	0.20
	Diptera	32.9	0.13	Imidacloprid	26.8	0.11
	Chironomus dilutus	9.3	0.04	Clothianidin	25.3	0.10
	Trichoptera	6.9	0.03	Thiacloprid	9.6	0.04
	Ephemeroptera	3.9	0.02			

it appears that differences in relative toxicity among the various individual neonicotinoids are minor. For example, the overlap in toxicity ranges of the different neonicotinoids is considerable, and differences among taxonomic groups are greater than those observed among different neonicotinoids (Fig. 3). Therefore, we combined the toxicity L[E]C_{50} values of individual neonicotinoids into a single dataset.

Mineau and Palmer (2013) contend that any apparent differences among neonicotinoids are likely artifacts of data availability rather than any real differences in toxicity. For two species of crustaceans, *Americamysis bahia* and *G. pulex*, and one insect species, *Chironomus riparius*, LC_{50} values are available for multiple neonicotinoids, although not necessarily from the same lab or research group nor identical test conditions. For *A. bahia*, the relative order of toxicity was thiacloprid (LC_{50} = 31–50 μg/L) ≥ clothianidin (LC_{50} = 51 μg/L) ≥ imidacloprid (LC_{50} = 34–159 μg/L) ≥ acetamiprid (LC_{50} = 66 μg/L) > dinotefuran (LC_{50} = 790 μg/L), >thiamethoxam (LC_{50} = 6900 μg/L). Some differences were also apparent for *G. pulex* where relative toxicity ordered acetamiprid (LC_{50} = 50 μg/L) > imidacloprid (LC_{50} = 350 μg/L) ≥ thiacloprid (LC_{50} = 190–9520 μg/L). While some differences in toxicity among neonicotinoids appear to exist for these two crustaceans, in reviewing data for an insect species, *C. riparius*, the data show fewer differences: imidacloprid (LC_{50} = 20 μg/L) ≥ clothianidin (EC_{50} = 22 μg/L) > thiamethoxam (EC_{50} = 35 μg/L). Differences in molecular weights of the various neonicotinoids range from 202.2 to 291.7 Da (Table 1), which may account for some apparent differences in the relative toxicity for certain aquatic species. For example, for *C. riparius*, the above effect levels expressed as molar concentrations are even more similar across neonicotinoids 0.08–0.12 μmol/L.

Neonicotinoids are known to be additively or synergistically toxic when they occur together, or when combined with certain fungicides that are potent cytochrome P450 monooxygenase enzyme inhibitors (Andersch et al. 2010; Iwasa et al., 2004). For example, the combination of clothianidin and the fungicide trifloxystrobin (as in the canola seed treatment formulation PROSPER™) resulted in a 150-fold increase in kill rate to leaf beetle (Phaedon) larvae over clothianidin alone (Wachendorff-Neumann et al., 2012). Bayer Crop Science has patented several combinations of two neonicotinoids demonstrating synergism of insecticidal activity. For example, individual treatments with 0.8 ppm of thiacloprid or 0.8 ppm clothianidin destroyed 25% and 0% of aphid populations after 6 days, but combined at the same doses, the kill rate rose to 98% (Andersch et al. 2010). Binary mixtures of imidacloprid and thiacloprid have been tested on *D. magna* where effects on reproduction, growth and survival most closely followed patterns of synergism or concentration addition (Pavlaki et al., 2011). Neonicotinoids also may interact synergistically with other pesticides, or other inert formulation ingredients commonly present in aquatic systems in agricultural areas (Alexander et al., 2013; Chen et al., 2010; LeBlanc et al., 2012; Vijver and van den Brink, 2014). In contrast, the influence of prior exposure to other xenobiotics, including common-use herbicides, has been shown to provide mosquitos (*Aedes aegypti*) greater co-tolerance to imidacloprid (Riaz et al., 2009), through up-regulation of the P450 monooxygenase genes (CYP enzymes) involved

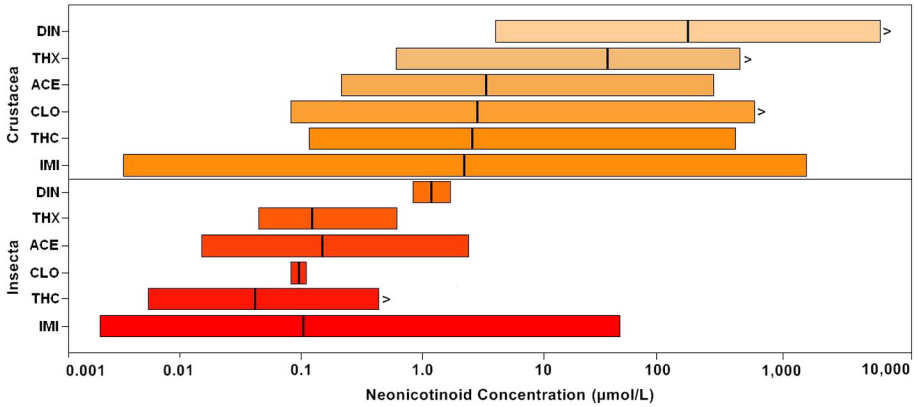

Fig. 3. Range of neonicotinoid toxicity (L[E]C_{50}: 24–96 h in μmol/L) among crustaceans (upper) and aquatic insects (lower) for six different neonicotinoid active ingredients: dinotefuran (DIN), thiamethoxam (THX), acetamiprid (ACE), clothianidin (CLO), thiacloprid (THC), and imidacloprid (IMI). The width of each bar represents the range of standard L[E]C_{50} values (μg/L) and vertical lines within bars represent the geometric mean of the tests (see also Table 2). Note that data are more limited for compounds other than imidacloprid and thiacloprid.

in detoxification (Daborn et al., 2002). Tolerance may also occur at a community level through survival of only the resistant species — known as pollution-induced community tolerance (PICT) (Blanck, 2002).

We compared the species sensitivity distribution curves of imidacloprid (slope = 0.75) to that of thiacloprid (slope = 0.94) and all other neonicotinoids (slope = 0.97). Although a reduction in slope was apparent for imidacloprid, the other neonicotinoids were near parallel and the overall curve shapes were very similar (Fig. 4). Differences in slopes should ideally be less than 10% to assume the same mode of action and an additivity model (de Zwart and Posthuma 2005), but we noted that this subtle difference was influenced by the right-weighting of the upper end of the imidacloprid curve by the large number of studies on the insensitive *D. magna*. In a comprehensive review of mixtures in aquatic environments, Rodney et al. (2013) determined that the concentration addition of individual compounds is typically recommended. This is further supported by Deneer (2000) who found that in 90% of pesticide mixture studies, concentration additivity accurately predicted effect concentrations within a factor of two. Therefore, given the existing limited data showing a high degree of overlap in toxicity among neonicotinoids and the fact that the mechanism of action of different neonicotinoids is the same, we speculate that toxicity thresholds should be reasonably similar and predicted to be at least additive when in mixtures.

3.3. Toxicity of neonicotinoid metabolites

Degradation of neonicotinoids in water through photolysis and hydrolysis produces primary and secondary metabolites that may also exert toxic effects. Most published data on degradation and toxicity of metabolites are for imidacloprid. Although relatively stable to hydrolysis, the major metabolite of imidacloprid in water from hydrolysis is 1-[(6-chloro-3-pyridinyl) methyl]-2-imidazolidone (Zheng and Liu, 1999). Photolysis is the main degradation pathway and has been shown to produce up to nine different metabolites in water. The five most prominent include a cyclic guanidine derivative, a cyclic urea, an olefinic cyclic guanidine, and two fused ring products. In a radiotracer study following 2h of radiation, these five metabolites together accounted for 48% of the radio carbon label and the parent compound accounted for 23% of the radio label (Roberts and Hutson, 1999).

It appears that for those metabolites tested, their relative toxicity to aquatic invertebrates is typically lower than that of the parent compounds, at least under acute 24-h or 48-h exposure conditions (Malev et al., 2012) (Table A.4). The only exception is for thiamethoxam which readily breaks down to clothianidin, an active ingredient itself in several formulated products exhibiting high toxicity to sensitive aquatic taxa (Fig. 3). Most of our knowledge of metabolite toxicity to invertebrates is from bee studies which indicate that some neonicotinoid metabolites can contribute to the observed toxicity (Decourtye et al., 2003; Nauen et al., 2001; Suchail et al., 2001) with the exception of acetamiprid which has no reported toxic metabolites (Iwasa et al., 2004). Most studies for bees have been conducted on metabolites of imidacloprid demonstrating that those with a nitroguanidine-group (olefin-, hydroxy-, and dihydroxy-imidacloprid) were more toxic (oral LD_{50}) than the urea-metabolite and 6-chloronicotinic acid (Nauen et al., 2001). Only three aquatic test species have been used to evaluate toxicity of neonicotinoid metabolites. *D. magna* was tolerant to a range of metabolites, *C. riparius* was somewhat sensitive to the clothianidin metabolite thiazolylnitroguanidine (TMG) (28 day LC_{50} < 18 ug/L) (USEPA OPP Pesticide Ecotoxicity Database) while *Gammarus fossarum* exhibited effects on behavior (24-h LOEC ≤ 62.8) and antioxidant enzyme activity from imidacloprid's degradation product, 6-chloronicotinic acid (6-CNA) (24-h LOEC ≤ 157.7 μg/L) (Malev et al., 2012). Thiamethoxam's metabolite, N-(2-chlorothiazol-5-ylmethyl)-N′-methyl-N′-nitroguanidine (CGA-322704), also exhibits a relatively low NOEC of 0.67 μg/L in a 28-d toxicity test with *C. riparius* (European Commission, 2006).

Neonicotinoid metabolites therefore represent a potentially lower, although still relevant, toxicity concern, however current water monitoring data do not routinely quantify these metabolites in their analyses. Therefore, we have little information on the prevalence or persistence of these metabolites for exposure assessments. Although toxicity data for aquatic invertebrates are also limited, our current understanding is that, with the exception of thiamethoxam breakdown to the toxic metabolite clothianidin, other neonicotinoid metabolites in water probably contribute relatively less to ecotoxicological effects compared to the parent compounds.

3.4. Analysis of species sensitivity distributions

The use and validation of the SSD approach and HC_5 calculation for neonicotinoid insecticides can be found in Liess and Beketov (2012) and Mineau and Palmer (2013), among others. Here, we used a traditional approach of only including data with similar endpoints of population relevance (LC_{50} or EC_{50}) and only single species laboratory studies. Many other similar analyses reported in the literature have included a mixture of field, mesocosm and laboratory studies, as well as a variety of endpoints (EC_{50}) plus NOECs and LOECs within the same analysis. Other authors, including Maltby et al. (2005), have previously compared single-species acute toxicity data for other pesticides with effects observed in mesocosm studies. They concluded that the lower confidence interval of the HC_5 derived from a SSD based on acute laboratory LC_{50} data was generally protective for aquatic communities in mesocosms, whereas the median HC_5 would require the application of an assessment factor. Guy et al. (2011), in another review of pesticide mesocosm studies, found that one-tenth of the crustacean HC_5 was usually low enough to prevent widespread mortality of different invertebrate taxa. In general, we caution that in extrapolating from a SSD based on laboratory data, an appropriate assessment factor may still be necessary to ensure that no deleterious effects on the ecosystem will occur, particularly for the persistent neonicotinoids demonstrating some level of cumulative action. For example, based on the study of Liess and Beketov (2011), long-term alterations of aquatic community structure were observed at 0.1 μg/L using $SPEAR_{mesocosm}$. This concentration was seven times below the HC_5 threshold identified as a relevant endpoint from a SSD based on acute laboratory LC_{50} information for thiacloprid (Beketov and Liess, 2008a). Presently, no clear standard on the application of assessment factors exists, particularly for SSDs that use only sensitive species (as in the aquatic insects and crustaceans), but typically these range from 3 to 6 as outlined in the European Union surface water guidance document (EFSA, 2013) or from 3 to 10 (RIVM, 2014).

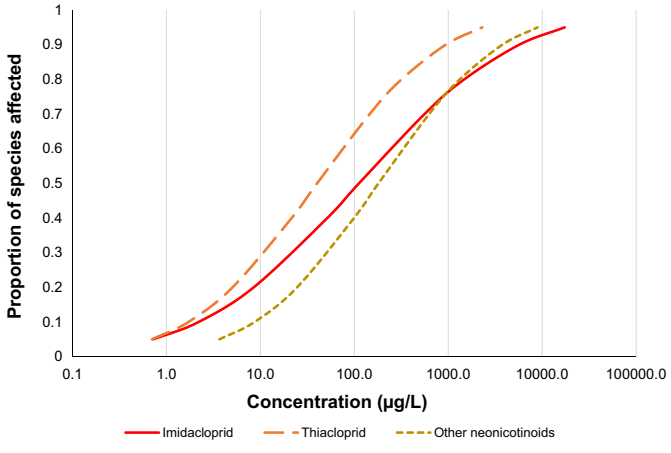

Fig. 4. Comparison of acute LC_{50} species sensitivity distribution curves of imidacloprid with thiacloprid and other neonicotinoids (acetamiprid, clothianidin, dinotefuran, thiamethoxam) combined. Data were insufficient to compare all individual neonicotinoids separately.

Here, we applied the species sensitivity distribution (SSD) approach to examine neonicotinoid toxicity among aquatic arthropods using the CADDIS Species Sensitivity Distribution Generator v.1 software (US EPA http://www.epa.gov/caddis/da_software_overview.html). Consistent with several other researchers, we calculated HC_5 (Hazardous Concentration) levels at the 5% tail of a log-normal SSD (Postuma et al., 2002) (Fig. 5). Using the acute toxicity data available for all individual neonicotinoids (standardized and weighted by molecular mass to imidacloprid) on 42 different species (geometric mean of multiple tests by species) and based only on lethality as an endpoint (LC_{50} values), we fitted a SSD ($r^2 = 0.95$) which yielded an HC_5 of 0.63 μg/L or 0.002 μmol/L (95% CI: 0.20–2.20 μg/L; 0.001–0.008 μmol/L) (Fig. 5a). The results of the chronic toxicity SSD with 18 test species used in studies of 7–28 days duration where the endpoints included lethality (LC_{50}), and any other sub-lethal endpoints (EC_{50}) such as growth, reproduction, immobility, or emergence ($r^2 = 0.92$) yielded an HC_5 of 0.146 μg/L or 0.001 μmol/L (95% CI: 0.035–0.61 μg/L; 0.00014–0.002 μmol/L) (Fig. 5b). We propose that the lower confidence limit of each of the two HC_5 values would be appropriate acute and chronic exposure thresholds, above which ecologically relevant population-level effects on sensitive aquatic invertebrate species, are likely to occur. Sublethal and community-level effects could still occur during short-term (acute) exposure at concentrations below 0.63 μg/L (HC_5 of acute SSD). Based on short-term toxicity tests reporting sublethal EC_{50} values for 26 species, we estimated an $HC_5 = 0.395$ μg/L or 0.002 μmol/L (95% CI: 0.073–2.13 μg/L; 0.0003–0.008 μmol/L). Thus in setting regulatory thresholds, regulators may need to consider both short-term sublethal effects in addition to lethality under acute and chronic neonicotinoid exposure scenarios to prevent impacts to aquatic communities.

3.5. Impacts on aquatic communities and ecosystems: mesocosm and field studies

Limitations of extrapolating effects from laboratory studies with single species to possible effects in the field have prompted several researchers to assess neonicotinoid effects on multi-species communities. However, such field and mesocosm studies usually suffer from a lack of control over species composition, contaminant exposure, and the role of different environmental variables, thereby limiting their reproducibility. Regardless, more environmentally relevant multi-species community effects are often observed at neonicotinoid concentrations well below single species toxicity thresholds. Our review suggests stream mesocosms exposed to imidacloprid or thiacloprid produced effects on a range of invertebrate taxa at environmentally relevant water concentrations of 0.01 to 24.1 μg/L (e.g. Pestana et al., 2009; Mohr et al., 2012; Berghahn et al., 2012, Boettger et al. 2013); rice mesocosm experiments revealed similar community-level effects at water concentrations ranging from <0.01 to 240 μg/L (Daam et al., 2013; Hayasaka et al., 2012a,b; Jinguji et al., 2013; Sánchez-Bayo and Goka, 2006) (Table A.3). The insect groups most commonly affected belong to the orders Ephemeroptera, Trichoptera and Diptera, as generally predicted by their sensitivity in single species tests. Emergence and other sublethal effects such as growth appear to be a more sensitive endpoint than abundance (Alexander et al., 2008; Mohr et al., 2012).

When considering the ecological effects of pesticides, sensitive community and ecosystem processes and functions, such as important trophic interactions and leaf litter breakdown rates, need to be considered. At concentrations of ~1.0 μg/L, neonicotinoids have been observed to alter predator–prey interactions in experimental aquatic communities (Hayasaka et al., 2012b; Sánchez-Bayo and Goka, 2006). For example, Englert et al. (2012) observed reduced leaf consumption and increased carnivorous behavior by *G. fossarum*, an important shredder species, at thiacloprid concentrations above 0.5–1.0 μg/L. Significant reductions in leaf feeding activity of *G. pulex* have also been observed at concentrations of imidacloprid above 30 μg/L ($EC_{50} = 5.34$ μg/L) with lasting effects on feeding behavior even at the lowest exposure concentrations of 0.81 to 9.0 μg/L (Agatz et al., 2014). Kreutzweiser et al. (2008) found that leaves from maple trees treated with imidacloprid at realistic field concentrations (3–11 mg/kg in trees) did not affect survival of aquatic leaf-shredding insects or litter-dwelling earthworms. However, adverse sub-lethal effects from these exposures were detected; specifically feeding rates of aquatic insects and earthworms were reduced, leaf decomposition (mass loss) was decreased, measurable weight losses occurred among earthworms, and aquatic and terrestrial microbial decomposition activity was significantly inhibited.

Of particular concern for field relevance is that toxic effects may be amplified at concentrations lower than observed in short-duration laboratory experiments, and that they may be delayed until after exposure ceases thereby delaying population recovery (Beketov and Liess, 2008a; EFSA, 2013; Song et al., 1997). For example, in a multi-generation microcosm study, populations of the mosquito larvae *Culex pipiens* exposed to thiacloprid pulses were found to decline and failed to recover in the presence of the more pesticide tolerant competitor, *D. magna* (Liess et al., 2013). Also, in a multi-year mesocosm study, Liess and Beketov (2011) found that species with low intrinsic sensitivity to thiacloprid showed only short-term effects at 100 μg/L, but species with high intrinsic sensitivity showed effects at 3.3 μg/L, and particularly sensitive univoltine (1 brood/yr) species showed long-term effects at 0.1 μg/L, with several species disappearing from the community. These effect levels were up to 70 times below the lowest laboratory, short-term LC_{50} for single species. Three processes may be responsible for this mismatch. First, in the field, additional natural and anthropogenic stressors are widely known to lower effective thresholds for toxicants. Liess and Beketov (2011) concluded that those species characterized by vulnerable traits in the presence of natural stressors (e.g., intra- and interspecific competition), were affected more strongly by thiacloprid than non-stressed species. Thus, sensitivity was more than an order of magnitude greater when additional stress was present. Second, field exposure scenerios generally include repeated pulses of neonicotinoids or other chemical stressors. Such sequential pulses of neonicotinoids may act cumulatively to exert stronger effects than single exposures (Liess et al., 2013). Third, the persistence of neonicotinoids in water under certain field conditions, such as high turbidity, acidity, depth, and filamentous algal or other shading, will increase chemical persistence thereby increasing the duration of aquatic organism exposure. This suggests that even with short-term pulse exposures, standard laboratory toxicity tests may not capture the range of lethal or sub-lethal effects that can continue to occur and thus impede population and community recovery. Chronic or repeated neonicotinoid exposure conditions appear more probable in nature than single acute exposures, and natural environmental conditions and stressors can inherently enhance toxicity.

3.6. Water quality reference values for protection of aquatic life

Current ecological water quality guidelines vary widely by country and several are presently under review (Table 3). Despite the controversy over this class of insecticides, few water quality reference values presently exist for (ecologically) acceptable levels of neonicotinoids in surface waters; these are predominantly limited to the most widely studied compound, imidacloprid (Table 3). Recommended water quality reference values have been derived primarily from available acute and chronic laboratory toxicity tests with standard test organisms using a mixture of LC_{50}s, EC_{50}s, NOECs and LOECs as toxicity endpoints. Few have considered multispecies or field-realistic long-term exposure scenarios beyond standard 48 to 96 h, and 14 to 28 day tests. The most recent reference values (e.g. Netherlands (RIVM, 2014; Smit et al., 2014)) were derived using a probabilistic (SSD) approach and incorporated a large range of toxicity data, including mesocosm and field studies, to obtain a reference value of 0.0083 μg/L for imidacloprid. By contrast, the U.S. EPA (2014) has set the "Aquatic Life Benchmark"

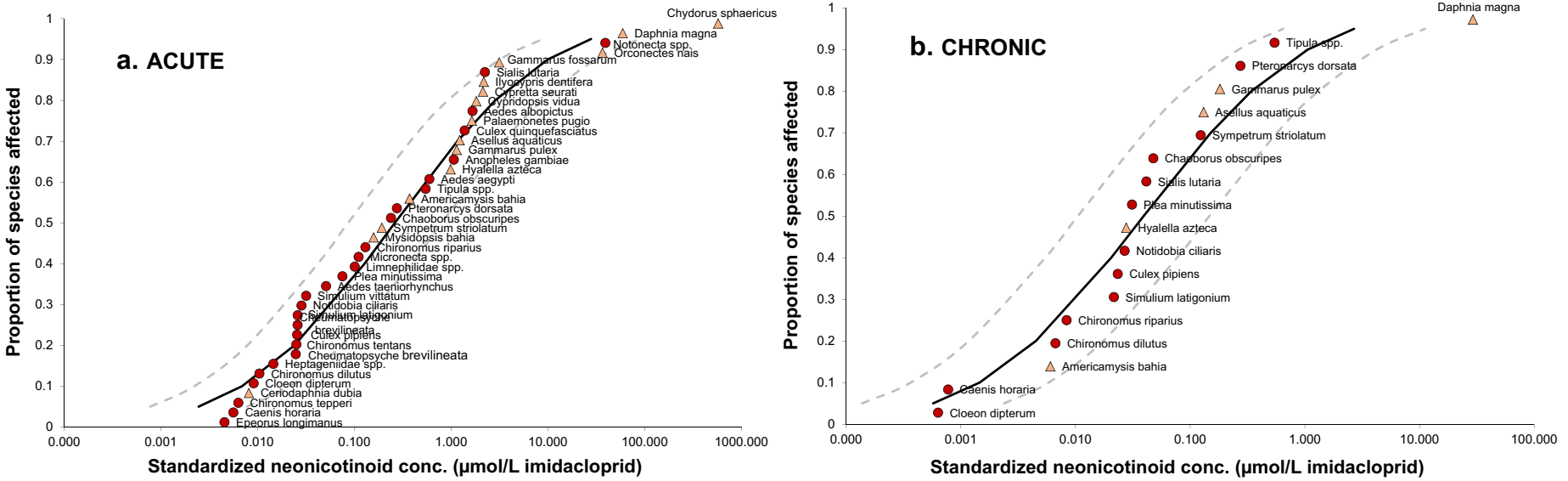

Fig. 5. Species sensitivity distributions for a) 137 acute (LC_{50}; 24–96 h) laboratory toxicity tests with 42 different aquatic invertebrate species, and b) 36 chronic ($L[E]C_{50}$; 7–39 day) laboratory toxicity studies with 18 aquatic invertebrate species. Red circles represent aquatic insects and orange triangles represent crustaceans. Distributions are based on test values of multiple neonicotinoid compounds with concentrations standardized to imidacloprid molecular mass. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 3
Summary of published ecological quality reference values for neonicotinoids (imidacloprid except this review) in freshwater environments against which average (chronic or long-term) or maximum (acute or peak) exposure concentrations are to be compared.

Source	Reference value (μg/L)	Justification
EPA (2014) (USA)	1.05 (average) 35.0 (maximum)	Aquatic life benchmark — methodology uncertain
CCME (2007) (Canada)	0.23	EC_{15} for the most sensitive of two freshwater species tested with assessment factor of 10 applied.
EFSA (2008) (Europe)	0.2 (maximum)	No Observable Effect Concentration (NOEC) (0.6 μg/L) from a 21 d German microcosm study to which an assessment factor of 1–3 has been applied based on expert deliberations
RIVM (2008) (Netherlands)	0.067 (average)	Maximum permissible concentration (MPC) for long term exposure derived from the lowest NOEC value for chronic toxicity studies with assessment factor of 10 applied.
RIVM (2014) (Netherlands)	0.0083 (average)	Updated MPC for long-term exposure derived from chronic studies using species sensitivity distribution (SSD) approach and Hazard Concentration (HC_5) applied to NOEC/LC_{10}/EC_{10} values with assessment factor of 3 applied.
Mineau and Palmer (2013)	0.0086 or 0.029 (average)	The higher of two empirically-determined acute–chronic ratios applied to the most sensitive of 8 aquatic species tested to date; or HC_5 from SSD applied using NOECs from chronic studies of 7 single species and 1 species assemblage.
This review	0.035 (average) 0.2 (maximum)	Lower confidence interval of HC_5 from SSDs generated using 137 acute (LC_{50}) and 36 chronic (L[E]C_{50}) toxicity tests considering all neonicotinoid compounds weighted and standardized to imidacloprid and all available test species.

for imidacloprid at 1.05 μg/L for invertebrate chronic (average) exposure and 35 μg/L for acute (maximum) exposure using methods that are unclear, though likely based on species such as *D. magna*. Canada has published a single value for imidacloprid of 0.23 μg/L as a "Water Quality Guideline for the Protection of Aquatic Life" (CCME, 2007). Under the European Water Framework Directive, a Maximum Permissible Concentration (MPC) of 0.067 μg/L is used for chronic or average imidacloprid concentrations, while a Maximum Acceptable Concentration (MAC) of 0.20 μg/L is used for short-term or peak concentrations (RIVM, 2008). Until recently, the lowest reference value reported is that of the Dutch regulatory body which has adopted a Maximum Permissible Risk (MPR) level for protection of ecosystems of 0.013 μg/L. In 2014, the Netherlands released an update recommending that the MPC for imidacloprid be lowered to 0.0083 μg/L, while the MAC would remain at 0.2 μg/L (RIVM, 2014). Fig. 1 demonstrates how many of the water monitoring data (means and maxima) reported worldwide would exceed these published reference values. For example, 79% (15/19) of studies reported "average" neonicotinoid concentrations that would have exceeded the most recent RIVM (2014) threshold of 0.0083 μg/L, while 81% (22/27) of studies reporting "peak" neonicotinoid concentrations found levels that would have exceeded the 0.2 μg/L imidacloprid reference value set by EFSA (2008).

Reference values for other neonicotinoids in surface waters are not well established although, consistent with our findings, Mineau and Palmer (2013) suggested that guidelines for other neonicotinoids should be similar to that for imidacloprid. Currently, the US EPA has established one acute benchmark for thiamethoxam of 17 μg/L and 18.9 μg/L for thiacloprid; however, derivation methods for these values and for their imidacloprid value (1.05 μg/L) are unclear and insufficiently protective given the available evidence and the lack of inclusion of chronic exposure data. We note that considerable variability exists in the reference values themselves and in how they are derived (Table 3). In some cases, acceptable levels are derived from single species and/or mesocosm studies using the lowest L[E]C_{50}, others add assessment factors of 3–10, and still others have applied SSDs to derive the HC_5 or HC_{15} followed by a range of assessment factors. The wide discrepancy in water quality reference values is not unique to imidacloprid or the neonicotinoid insecticides more generally. Guy et al. (2011) reported several examples of widely divergent reference values and argued that the majority were insufficiently protective, at least based on field and mesocosm data. Here we take the approach that, based on a very large number of neonicotinoid studies using consistent LC_{50} and EC_{50} endpoints and applying the lower confidence interval of our HC_5 calculation, threshold values of 0.2 μg/L for maximum (peak, short-term) neonicotinoid concentrations and 0.035 μg/L for average (longer-term) neonicotinoid concentrations represent minimally protective thresholds for sensitive aquatic invertebrates to which safety factors might need to be applied as we further elucidate mechanisms of cumulative action, field level responses, and recovery patterns for this class of insecticides.

3.7. Proposed approaches for addressing neonicotinoid mixtures in water

The toxicity of these compounds is predicted to be additive in nature through cumulative agonistic binding at the same receptor type. However, to our knowledge, this assumption has not been tested formally using binary or mixture aquatic toxicity studies. Based on this assumption that all neonicotinoids have the same mechanism of action, relatively equivalent toxicity, and predicted additive toxicity, we pooled all toxicity data for different neonicotinoid compounds weighted and standardized to imidacloprid molecular mass when estimating our HC_5 values. In doing so, we propose that where multiple neonicotinoids are present in water, the sum of all neonicotinoid concentrations corrected for molecular mass (total neonicotinoids) may be used as an approximation for predicting additivity of toxic effects. Other approaches may be more appropriate, such as standardizing individual neonicotinoid concentrations to imidacloprid based on toxic equivalency. However, this would require more detailed knowledge of the comparative toxicity of the different neonicotinoids. Ignoring neonicotinoid mixtures may greatly underestimate the threshold exceedances and thus we currently advocate for the simple molar concentration summation approach as an approximation until further experimental work using neonicotinoid mixtures confirms a more mechanistic or comprehensive method. Ultimately, the reference values proposed here based on individual compound exposures and assuming equivalent neonicotinoid toxicity may need to be revised when new mixture and comparative neonicotinoid data becomes available.

4. Conclusions and recommendations

We conclude based on comprehensive species sensitivity distribution analysis of 214 toxicity tests of 48 species that any long-term neonicotinoid concentrations in water exceeding 0.035 μg/L or short term peak exposures exceeding 0.2 μg/L can affect sensitive aquatic invertebrate populations. By comparison, this 0.035 μg/L value is consistent with the Vijver and van den Brink (2014) suggested threshold of 0.013–0.067 μg/L for imidacloprid, but higher than that proposed by Mineau and Palmer (2013) (0.0086 μg/L) and by the Netherlands MPC (RIVM, 2014) (0.0083 μg/L) (Table 3). Given the uncertainty of the ecological safety of these pesticides and their long-term persistence in the natural environment, we concede that additional safety factors may be appropriate. Our analysis shows that 74% (14/19) of surface water studies reporting average individual neonicotinoid residues exceeded 0.035 μg/L. Furthermore, exceedance of our proposed 0.2 μg/L peak

threshold would thus occur for 81% (22/27) of monitoring studies reporting maximum water concentrations of individual neonicotinoids. That exceedance would be expected to increase if multiple neonicotinoids were summed during monitoring and presented as "total neonicotinoids" given the likelihood of additive effects.

A recent surge in the number of published toxicity studies with neonicotinoid insecticides and aquatic invertebrates has produced a mass of new and useful data, but often with confusing results. This appears to be largely due to 1) vast differences in species sensitivity of test organisms which ranges several orders of magnitude, 2) differences in species, duration, conditions and reporting of toxicity tests, and 3) apparent differences between laboratory studies and field or mesocosm studies representing varying levels of field realism. This can often impede the ultimate goal of setting regulatory threshold concentrations that are protective. Generally speaking, environmental risk assessments that follow a tiered approach of increasing complexity and environmental relevance have received considerable support (Brock and Wijngaarden, 2012; EFSA, 2013) and are recommended for the different neonicotinoids. In addition, environmental monitoring data suggest that multiple neonicotinoids are frequently and repeatedly transported into water bodies, or are persisting for durations well beyond the commonly used 48 to 96 hour duration of acute toxicity tests. Given this exposure profile, chronic studies (28 days or longer) and mesocosm studies should be the primary tests guiding regulatory decision making. Equally, many of the mayfly (Ephemeroptera), caddisfly (Trichoptera), and midge (Diptera) species that are critical for supporting numerous aquatic and terrestrial food webs, appear highly sensitive to neonicotinoids, but are not as extensively tested as some standard test species (e.g., *D. magna*) that appear to be up to 100,000 times less sensitive. Adverse indirect effects of imidacloprid on food webs including insectivorous birds have already been reported from areas draining Dutch farmlands (Hallmann et al., 2014).

Despite the ongoing advances in technology, extensive experimental toxicity data and strict regulation of pesticides in many developed countries, recent data for rivers in the European Union suggests that pesticides (not including neonicotinoids) still account for 87% of organic pollutant exceedances of acute risk thresholds based on aquatic invertebrates (Malaj et al., 2014). The neonicotinoid insecticides represent a significant additional pesticide threat to surface and ground waters because of their broad use, high water solubility, environmental persistence and very high non-target toxicity, and thus require scientifically robust approaches to accurately determine risk. Existing information presented here suggests that stricter regulations and use of neonicotinoid insecticides are warranted to protect aquatic ecosystems and the broader biodiversity they support.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.envint.2014.10.024.

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