Derivation of an aquatic benchmark for invertebrates potentially exposed to imidacloprid

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Water quality benchmarks are developed by many jurisdictions worldwide with the general goal of identifying concentrations that protect aquatic communities. Imidacloprid is a widely-used neonicotinoid insecticide for which benchmark values vary widely between North America and Europe. For example, the European Food Safety Authority (EFSA) and Dutch National Institute for Public Health and the Environment (RIVM) recently established chronic water quality benchmarks for imidacloprid of 0.009 and 0.0083 µg/L, respectively. In Canada and the United States (US), however, the current chronic water quality benchmarks – termed aquatic life benchmark by the United States Environmental Protection Agency (US EPA) – for freshwater biota are orders of magnitude higher, i.e., 0.23 and 1.05 µg/L, respectively. Historically, aquatic benchmarks for imidacloprid have been derived for invertebrates because they are the most sensitive aquatic receptors. To date, derivation of water quality benchmarks for imidacloprid have relied on the results of laboratory-based toxicity tests on single invertebrate species. Such tests do not account for environmental factors affecting bioavailability and toxicity or species interactions and potential for recovery. Microcosm, mesocosm and field studies are available for aquatic invertebrate communities exposed to imidacloprid. These higher tier studies are more representative of the natural environment and can be used to derive a chronic benchmark for imidacloprid. A water quality benchmark based on the results of higher tier studies is protective of freshwater invertebrate communities without the uncertainty associated with extrapolating from laboratory studies to field conditions. We used the results of higher tier studies to derive a chronic water quality benchmark for imidacloprid as follows: (1) for each taxon (family, subfamily or class depending on the study), we determined the most sensitive 21-day No Observed Effects Concentration (NOEC), (2) we fit the taxon NOECs to five distributions and determined the best-fit distribution, and (3) we determined the HC5

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from the best-fit distribution. The higher tier chronic HC5 for imidacloprid is 1.01 µg/L, which is close to the current US EPA chronic aquatic life benchmark of 1.05 µg/L.

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ABSTRACT

Water quality benchmarks are developed by many jurisdictions worldwide with the general goal of identifying concentrations that protect aquatic communities. Imidacloprid is a widely-used neonicotinoid insecticide for which benchmark values vary widely between North America and Europe. For example, the European Food Safety Authority (EFSA) and Dutch National Institute for Public Health and the Environment (RIVM) recently established chronic water quality benchmarks for imidacloprid of 0.009 and 0.0083 µg/L, respectively. In Canada and the United States (US), however, the current chronic water quality benchmarks – termed aquatic life benchmark by the United States Environmental Protection Agency (US EPA) – for freshwater biota are orders of magnitude higher, i.e., 0.23 and 1.05 µg/L, respectively. Historically, aquatic benchmarks for imidacloprid have been derived for invertebrates because they are the most sensitive aquatic receptors. To date, derivation of water quality benchmarks for imidacloprid have relied on the results of laboratory-based toxicity tests on single invertebrate species. Such tests do not account for environmental factors affecting bioavailability and toxicity or species interactions and potential for recovery. Microcosm, mesocosm and field studies are available for aquatic invertebrate communities exposed to imidacloprid. These higher tier studies are more representative of the natural environment and can be used to derive a chronic benchmark for imidacloprid. A water quality benchmark based on the results of higher tier studies is protective of freshwater invertebrate communities without the uncertainty associated with extrapolating from laboratory studies to field conditions. We used the results of higher tier studies to derive a chronic water quality benchmark for imidacloprid as follows: (1) for each taxon (family, subfamily or class depending on the study), we determined the most sensitive 21-day No Observed Effects Concentration (NOEC), (2) we fit the taxon NOECs to five distributions and determined the best-fit distribution, and (3) we determined the HC5 from the best-fit distribution. The higher tier chronic HC5 for imidacloprid is 1.01 µg/L, which is close to the current US EPA chronic aquatic life benchmark of 1.05 µg/L.

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INTRODUCTION

Imidacloprid is a neonicotinoid insecticide used in agriculture to control a variety of pest insects, including aphids, Japanese beetles, lacebugs, leafhoppers, thrips, and others. It is widely used in row crops (e.g., cotton, potatoes), greenhouse vegetables, vine crops, citrus, stone fruit and pome orchards, bush berries, and tree nuts. Imidacloprid acts as a contact insecticide when applied to foliage or soil and is also systematically translocated through plants.

Imidacloprid is highly toxic to some classes of aquatic invertebrates including midges, mysids and mayflies (Gagliano, 1991; Ward, 1991; Roessink et al., 2013). As a result, various jurisdictions have based their water quality benchmarks for imidacloprid on the results of laboratory toxicity tests conducted with aquatic invertebrates.

Current chronic benchmarks that have the general goal of protecting freshwater aquatic biota vary widely despite all being based on laboratory toxicity data. The European Food Safety Authority (EFSA, 2014) recently established water quality benchmarks, known as Regulatory Acceptable Concentrations (RACs), for the European Union. The chronic RAC is 0.009 µg/L. In 2013, the Dutch National Institute for Public Health and the Environment (RIVM) revised their chronic water quality standard for imidacloprid to 0.0083 µg/L (RIVM, 2013). In Canada and the United States, however, the current chronic water quality benchmarks for freshwater biota are orders of magnitude higher, i.e., 0.23 and 1.05 µg/L, respectively (CCME, 2007; EPA, 2016). Using a species sensitivity distribution approach with laboratory toxicity data, Morrissey et al. (2015) recommended that concentrations of imidacloprid and other neonicotinoids need to be below 0.035 µg/L "to avoid lasting effects on aquatic invertebrate communities".

To date, chronic water quality benchmarks for imidacloprid have relied on laboratory toxicity tests conducted with single species. Laboratory studies generally follow strict regulatory guidelines and are performed under controlled conditions. However, laboratory conditions are not reflective of the real world. Higher tier studies (e.g., microcosms, mesocosms and field studies; hereafter "cosm" studies) are specifically designed to have exposure conditions that are representative of natural freshwater environments and consider species interactions, species recovery and other ecological factors. Additionally, higher tier studies can be designed to evaluate community-level effects, which is consistent with the protection goal of the water quality benchmark.

The objective of this paper was to use the best available, higher-tier toxicity data to develop a chronic water quality benchmark for imidacloprid that is protective of freshwater invertebrate communities.

Data relevance and data quality are critical aspects of deriving a water quality benchmark (Breton, 2014; Knopper et al., 2014). To ensure a scientifically defensible water quality benchmark for imidacloprid, we developed a data evaluation rubric to determine which higher tier cosm studies were acceptable, supplemental or unacceptable. Only acceptable studies were used in benchmark derivation.

METHODS

Data Evaluation

A data evaluation rubric was developed to assess the relevance and quality of aquatic invertebrate toxicity studies that have been conducted for imidacloprid. A total of 31 higher tier cosm studies were found and evaluated. Studies were obtained from the primary literature, registrant-sponsored studies following guidelines for Good Laboratory Practice (GLP), EPA's EcoTox database, existing water quality guideline documents, and grey literature studies. The study evaluation rubrics and evaluation results can be found in the Supplemental Information accompanying Whitfield-Aslund et al. (2016).

All studies were first evaluated for relevance and utility. Data relevance was assessed using five criteria: (1) Was the study community/ecosystem relevant (e.g., includes freshwater invertebrates)?; (2) Was imidacloprid the only active ingredient to which test organisms were exposed?; (3) Were test endpoints relevant to the population (e.g., mortality, growth or reproduction) or community level (e.g., richness, productivity) of organization?; (4) Was the exposure route relevant to what is expected in the environment?; and (5) Was the exposure duration consistent with potential chronic exposures in the field? For a study to be considered relevant, each relevance question had to be answered with a "yes", otherwise the study was deemed irrelevant and not considered further.

Relevant studies were further evaluated for data quality. The data quality evaluation focused on objectivity, clarity and transparency, and integrity. Data quality questions were weighted using a scoring rubric, whereby answers were scored from 0 (poor) to 3 (excellent). Questions that could be answered simply with a "yes" or "no" (e.g., was a concentration-response relationship observed?) were weighted lower in the overall study score and were given a 0 for "no" or 1 for "yes". The maximum score was 29 for cosm studies. Studies that scored 29-23 were rated as acceptable. Such studies followed scientifically-defensible guidelines, were considered relevant, and provided sufficient detail to fully reproduce the study. Supplemental (scored 22-13) and unacceptable (12-0) studies provided fewer details, had performance issues, and/or did not follow internationally recognized guidelines or scientifically-defensible protocols. Only acceptable studies were used for derivation of the higher tier chronic benchmark.

Chronic Benchmark Using Higher Tier Cosm Toxicity Data

The HC5 from a taxon sensitivity distribution (TSD) was used as the basis for the cosm-based chronic benchmark for imidacloprid. This approach is broadly consistent with that used by the United States Environmental Protection Agency (US EPA) in deriving water quality criteria (Stephan et al., 1985). Water quality criteria derived by the US EPA generally aim to protect 95% or more of aquatic biota (Stephan et al., 1985). The lowest NOEC was determined for each taxon, generally at the family or subfamily level of organization because NOECs were typically not available for species or genera. If multiple studies with acceptable endpoints were available for a taxon, the geometric mean was calculated. Ten cosm studies were found to be acceptable (Table 1). However, four of the acceptable studies only reported effects on overall invertebrate abundance and not taxon-specific endpoints (Hayasaka, 2012a,b; Kreutzweiser et al., 2009) or only reported endpoints for macrophytes and periphyton (Heimbach & Hendel, 2001). Thus, these studies could not be used to derive a water quality benchmark for aquatic invertebrates. The remaining acceptable cosm studies had varying exposure concentrations over time due to single or multiple applications, varying application intervals, and temporal decline following application as expected in the natural environment. Studies with a single imidacloprid application were conducted by Kreutzweiser et al. (2007, 2008). Studies with two applications and a 21-day retreatment interval were conducted by Ratte & Memmert (2003), Roessink et al. (2015), and Roessink & Hartgers (2014). The other exposure regime included four applications with a 14-day retreatment interval (Moring et al., 1992). Additionally, by extending the observation period beyond the final imidacloprid treatment, several cosm studies determined the potential for recovery of aquatic invertebrate populations (e.g., Moring et al., 1992; Ratte and Memmert, 2003). However, we did not consider recovery in selecting taxon NOECs for benchmark derivation.

To ensure that cosm-based NOECs were comparable, time-weighted average concentration estimates were determined for the reported no effect treatments. This approach helped to standardize results between different studies with varying exposure regimes. Time-weighted average concentration estimates were calculated using the degradation half-life (DT50) of 11.6 days reported by Roessink et al. (2015). Using this DT50 and assuming first-order elimination kinetics, time-weighted average concentrations were determined by averaging the daily estimated imidacloprid concentrations from the day of the first application to 21 days following the final application. The calculation period was limited to 21 days post final application as this duration corresponded to the most common application interval in the higher tier studies with multiple applications. Additionally, a consistent cutoff was required to ensure that exposure estimates were not severely underestimated in studies that had very long durations. The resulting time-weighted NOECs are reported in Table 1. The time-weighted NOECs include a range of population and community-relevant endpoints including density, abundance, emergence, mortality, and feeding rate. Unbounded data points (i.e., > or < values) were excluded.

If family or subfamily NOECs were not reported for a taxon, the data were grouped by subclass (e.g., Copepoda). Once grouped, a geometric mean of the lowest time-weighted NOEC from each study for each taxonomic group was calculated (Table 1). If only one study was available for a taxon, the lowest NOEC was used. SSD Master v3.0 software (Rodney et al., 2013) was used to derive the taxon sensitivity distribution (TSD). SSD Master fits up to five non-linear regression models (normal, logistic, extreme value, Weibull, and Gumbel) in log or arithmetic space to establish the best-fitting cumulative distribution function (CDF). Model fit was evaluated using the Anderson-Darling (AD) goodness-of-fit test statistic (A^2) and various graphical plots of model residuals to determine the best fit distribution for the TSD.

Table 1 **Data used to derive the chronic taxon sensitivity distribution (TSD) using results from cosm studies for imidacloprid.**

Taxon (Family, Subfamily, Subclass)	***NOEC (µg/L)***	***Geometric Mean NOEC (µg/L)***	***Time-weighted Average NOEC (µg/L)***	***Reference***
Baetidae	0.6	0.816	0.581	Ratte & Memmert, 2003
	2			Moring et al., 1992
	1.52			Roessink and Hartgers, 2014

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Taxon (Family, Subfamily, Subclass)	*NOEC (µg/L)*	*Geometric Mean NOEC (µg/L)*	*Time-weighted Average NOEC (µg/L)*	*Reference*
	0.243			Roessink et al., 2015
Chironominae	0.6	1.90	1.48	Ratte & Memmert, 2003
	6			Moring et al., 1992
Caenidae	2	2	1.87	Moring et al., 1992
Hydrophilidae	2	2	1.87	Moring et al., 1992
Hydroptilidae	2	2	1.87	Moring et al., 1992
Chaoboridae	3.8	3.8	2.47	Ratte & Memmert, 2003
Naididae	3.8	3.8	2.47	Ratte & Memmert, 2003
Orthocladiinae	3.8	3.8	2.47	Ratte & Memmert, 2003
Copepoda	6	7.51	5.85	Moring et al., 1992
	9.4			Ratte & Memmert, 2003
Daphniidae	9.4	9.4	6.12	Ratte & Memmert, 2003
Glossiphoniidae	9.4	9.4	6.12	Ratte & Memmert, 2003
Planorbidae	9.4	9.4	6.12	Ratte & Memmert, 2003
Tipulidae	12	12	6.84	Kreutzweiser et al., 2007
Tanypodinae	20	13.7	10.7	Moring et al., 1992
	9.4			Ratte & Memmert, 2003
Pteronarcyidae	12	24	13.7	Kreutzweiser et al., 2007
	48			Kreutzweiser et al., 2008

RESULTS

The cosm-based chronic TSD was fit to time-weighted NOECs representing 15 taxa. Time-weighted average effects concentrations ranged from 0.581 to 13.7 µg/L (Table 1). The Gumbel distribution in log space (Equation 1) was the best-fitting model.

$$f(x) = e^{-e^{\frac{(\mu - x)}{b}}} \qquad \textbf{Equation 1}$$

where, $f(x)$ = proportion of taxa affected, x = log concentration (µg/L), μ = location parameter, and s = scale parameter (always positive). The AD goodness-of-fit test statistic ($A^2 = 0.612$, $p > 0.05$) indicated good model fit as confirmed by visual inspection of the residuals and the distribution and the data (Figure 1).

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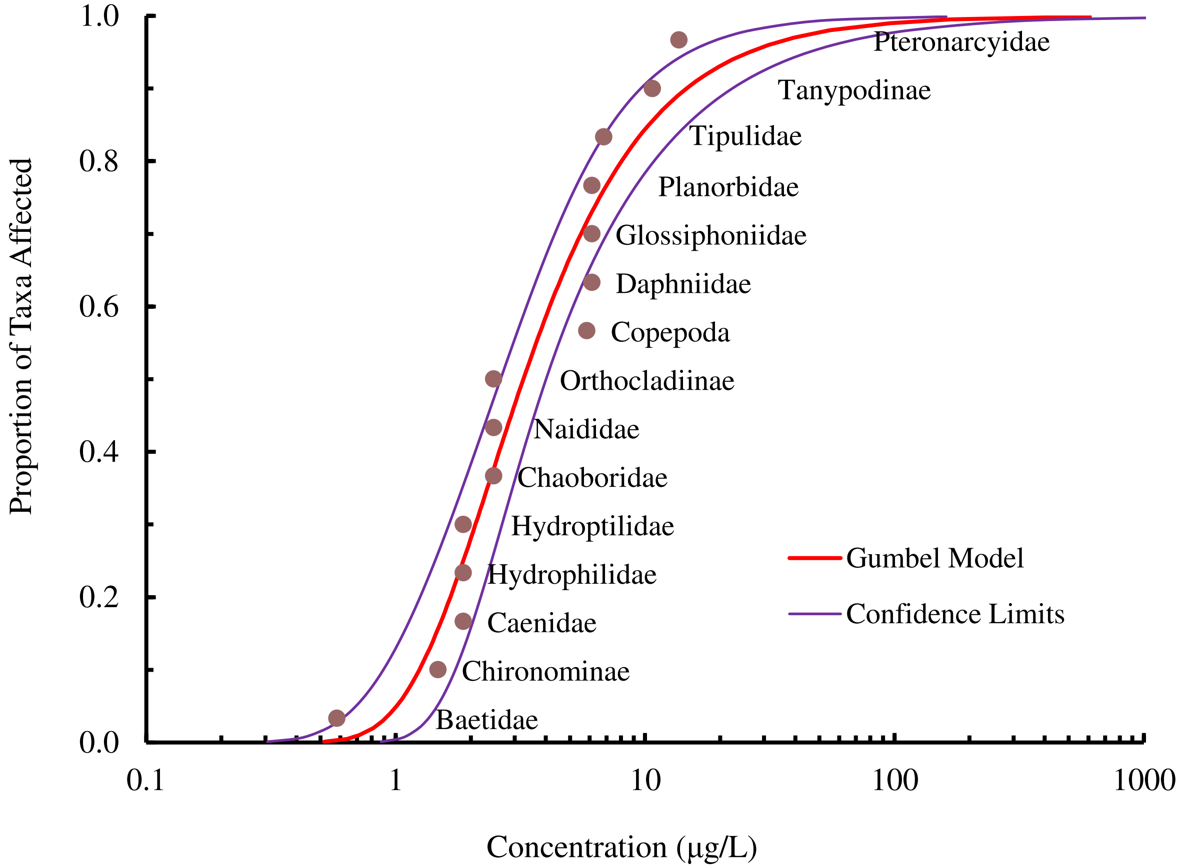

Figure 1 **Chronic taxon sensitivity distribution (TSD) for imidacloprid with 95% confidence limits for family, subfamily and subclass level data extracted from cosm studies.**

The fitted location and scale parameters were 3.38 and 0.347, respectively, for chronic toxicity data reported in log ng/L (the results were subsequently converted to µg/L). The HC5 was 1.01 µg/L, with approximate 95% confidence limits of 0.692 and 1.47 µg/L.

DISCUSSION

In this paper, we derived a chronic water quality benchmark for imidacloprid using the best available data from higher tier cosm studies. The studies underwent detailed evaluations for relevance and quality (see supplemental information in Whitfield-Aslund et al., 2016 for evaluations), and only data of acceptable quality were used to derive the water quality benchmark.

Although a laboratory-based water quality benchmark for imidacloprid can consider a broad range of taxa through the use of the species sensitivity distribution (e.g., Morrissey et al. 2015), it does not account for the more realistic environmental conditions that occur outside the laboratory, reduced fitness due to stress from laboratory confinement, or indirect effects including changes in food, habitat availability, and interspecies interactions. Mesocosm, semifield and field studies explicitly account for many of these factors and generally provide data that match the goal of protection of the aquatic invertebrate community. Further, concentrations of imidacloprid are temporally variable in the environment, as they were in the cosm studies, but not in standard toxicity tests conducted in the laboratory. Given the limitations of laboratory-based water quality benchmarks with regard to extrapolating to natural aquatic invertebrate communities, we recommend adopting the chronic water quality benchmark for imidacloprid derived using the higher tier toxicity data from acceptable cosm studies, i.e., 1.01 µg/L. In the discussion that follows, we provide further rationale for this recommendation.

Adverse effects observed in laboratory studies with singles aquatic invertebrate species are not necessarily translated to the community level of organization because adverse effects to one or a few sensitive species may be offset by increases in functionally similar but more tolerant species (Rosenfeld, 2002). Thus, overall community structure and function are not necessarily affected by adverse effects to one or a few sensitive species. In short, the effects of a pesticide such as imidacloprid are not, as a rule, transmitted to higher levels of organization. This statement is one of the foundations of hierarchy theory as proposed by Allen & Starr (1982). There are many examples of aquatic invertebrate communities exhibiting functional redundancy or compensation (e.g., Boersma et al., 2014; Schriever & Lytle, 2016). At some level, all species are unique, but overlap in resource use is common in freshwater food webs (Ehrlich & Walker, 1998). Thus, there are often multiple species present for each of the major functional roles of aquatic invertebrates in freshwater ecosystems, e.g., leaf shredders, suspension feeders, scrapers, detritivores and others that are critical to overall production, nutrient cycling, decomposition and energy flow (Covich et al., 1999). In highly stressed aquatic ecosystems, e.g., those with low functional richness and functional redundancy, the loss of a taxon is likely to have a greater impact on community functioning than in less stressed systems (Suarez et al., 2016). Thus, there are limits to the role that functional redundancy plays in preserving community structure and function. Functional redundancy likely partially explains why the overall aquatic invertebrate community is more resilient to imidacloprid exposure in cosm studies than would be predicted by laboratory studies on single species (Whitfield-Aslund et al., 2016).

Rather than assuming exposure to a constant concentration of imidacloprid, the higher tier cosm studies accounted for varying exposure concentrations over time due to multiple applications, varying application intervals, and temporal decline following application as expected in the natural environment. Cosm studies also had more realistic exposure conditions by, for example, including sediment (Moring et al., 1992; Ratte & Memmert, 2003; Roessink and Hartgers, 2014; Roessink et al., 2015), and carrying out the studies in open air environments with natural lighting and weather fluctuations (Moring et al., 1992; Ratte & Memmert, 2003). Some of these factors may have reduced bioavailability and/or toxicity, e.g., declining concentrations allow for detoxification. In all likelihood, functional redundancy and more realistic peak exposure conditions both contributed to the cosm-based chronic benchmark of 1.01 µg/L for imidacloprid being much higher than the laboratory-based chronic benchmarks derived by EFSA (2014), RIVM (2013) and Morrissey et al. (2015).

The cosm-based chronic benchmark for imidacloprid is conservative because the NOECs used in the benchmark derivation did not consider that many aquatic invertebrates are capable of rapid recovery following cessation of exposure. For example, in Moring et al. (1992), the test system was observed for three months following the final application of imidacloprid. Although a number of macroinvertebrate families (e.g., Baetidae, Caenidae, Hydroptilidae, Hydrophilidae, and Libellulidae) experienced declines in abundance during exposure to the treatment with an initial concentration of 6 µg/L, full recovery of all taxa was observed within eight weeks of the final treatment. During the exposure period, the most sensitive NOEC in this study was an initial concentration of 2 µg/L (time-weighted average concentration = 1.87 µg/L); the corresponding time-weighted NOEC was used in our benchmark derivation (Table 1). Moring et al. (1992), however, recommended that the next highest treatment (initial treatment concentration = 6 µg/L) be adopted as the regulatory NOEC because effects were transient in this treatment and recovery occurred after exposure ceased. Similar results were observed by Ratte & Memmert (2003), who noted complete recovery of Baetidae and Chironominae within eight weeks of the last application. Had recovery been considered in this study the most sensitive initial concentration NOEC of 0.6 µg/L (Table 1) would have increased to ≥9.4 µg/L.

CONCLUSIONS

Higher-tier studies (i.e., mesocosm, microcosm and field studies) should be used when available to derive water quality benchmarks because they offer a level of realism not attainable with standard laboratory toxicity tests. We derived a chronic cosm-based benchmark for imidacloprid for the protection of freshwater invertebrates using relevant and high quality toxicity data. The cosm-based water quality benchmark (1.01 µg/L) supports the current US EPA chronic aquatic life benchmark (1.05 µg/l) as being protective of aquatic invertebrate communities. Although the cosm-based benchmark is higher than the laboratory-based benchmarks adopted in Europe and Canada for imidacloprid, our benchmark accounts for potential effects under more realistic conditions. Functional redundancy and the more realistic exposure conditions used in cosm studies likely explain this difference.

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