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COMPREHENSIVE CHARACTERIZATION OF THE ACUTE AND CHRONIC TOXICITY OF THE NEONICOTINOID INSECTICIDE THIAMETHOXAM TO A SUITE OF AQUATIC PRIMARY PRODUCERS, INVERTEBRATES, AND FISH

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Abstract: Thiamethoxam is a neonicotinoid insecticide used widely in agriculture to control a broad spectrum of chewing and sucking insect pests. Recent detection of thiamethoxam in surface waters has raised interest in characterizing the potential impacts of this insecticide to aquatic organisms. We report the results of toxicity testing (acute and chronic) conducted under good laboratory practices for more than 30 freshwater species (insects, molluscs, crustaceans, algae, macrophytes, and fish) and 4 marine species (an alga, a mollusc, a crustacean, and a fish). As would be anticipated for a neonicotinoid, aquatic primary producers and fish were the least sensitive organisms tested, with acute median lethal and effect concentrations (LC50/EC50) observed to be $\geq$80 mg/L in all cases, which far exceeds surface water exposure concentrations. Tested molluscs, worms, and rotifers were similarly insensitive (EC50 $\geq$ 100 mg/L), except for *Lumbriculus* sp., with an EC50 of 7.7 mg/L. In general, insects were the most sensitive group in the study, with most acute EC50 values $<$ 1 mg/L. However, the crustaceans *Asellus aquaticus* and Ostracoda exhibited a sensitivity similar to that of insects (acute EC50 $<$ 1 mg/L), and the midge larvae *Chaoborus* sp. were relatively insensitive compared with other insects (EC50 $>$ 5.5 mg/L). The most sensitive chronic response was for *Chironomus riparius*, with a 30-d no-observed-effect concentration (NOEC; emergence) of 0.01 mg/L. Observed toxicity to the tested marine organisms was comparable to that of freshwater species. We used the reported data to construct species sensitivity distributions for thiamethoxam, to calculate 5% hazard concentrations (HC5s) for acute data (freshwater invertebrates), and compared these with measured concentrations from relevant North American surface waters. Overall, based on acute toxicity endpoints, the potential acute risk to freshwater organisms was found to be minimal (likelihood of exceeding HC5s $<$ 1%). *Environ Toxicol Chem* 2017;36:2838–2848. © 2017 The Authors. Environmental Toxicology and Chemistry published by Wiley Periodicals, Inc. on behalf of SETAC.

Keywords: Insecticide Aquatic toxicology Species sensitivity distributions Neonicotinoid Thiamethoxam

INTRODUCTION

The insecticide thiamethoxam was first approved for use in agriculture by the US Environmental Protection Agency (USEPA) in 1999. It is now used around the world to control a wide range of insect pests on major agricultural crops. Approved uses in the United States include seed treatments, foliar and soil applications. As a neonicotinoid insecticide, thiamethoxam acts selectively on insects by interfering with nicotinic acetylcholine receptors [1]. Globally, neonicotinoid insecticides comprise the most widely used insecticide class in agriculture [2] and have largely replaced a variety of older chemistries (e.g., organophosphates, carbamates, and organochlorine pesticides). This is because of their broad-spectrum control of numerous insect pests (e.g., aphids, whiteflies, flea beetles, thrips, and wireworms), versatile use pattern (e.g., crops such as maize, canola, sugar beet, and cotton), reduced risk profile to human health and the environment, and resulting agronomic benefit [1].

With their increased use, neonicotinoid insecticides have increasingly been included in surface water monitoring programs, with a number of publications citing detections of these compounds. Morrissey et al. [3] recently reported that 29 studies from 9 countries have published detectable concentrations of neonicotinoid insecticides in puddles, streams, rivers, wetlands, and irrigation channels. Government monitoring appears to be expanding in recent years, with surveillance programs now reporting findings from Canada and the United States [4–7]. While not systematically monitored, the presence of neonicotinoid insecticides in marine environments has also been reported [8]. These detections have led to suggestions that aquatic ecosystems may be impacted by neonicotinoid insecticides [9,10].

To quantify this potential impact, there is a need to first understand the potential for direct impacts on aquatic organisms. As highlighted by Anderson et al. [9] and Pisa et al. [11], there are relatively few published reports of the effects of neonicotinoid insecticides on freshwater and marine species. Furthermore, most publications have focused on the neonicotinoid imidacloprid [3], with fewer than 10 published, peer-reviewed experimental studies of freshwater organisms identified to date for thiamethoxam [12,13], and none for marine species.

Although not published as traditional peer-reviewed literature, a significant amount of aquatic toxicity data for neonicotinoid insecticides has been generated as part of regulatory packages for registration of these pesticides. Regulatory risk assessment follows a tiered approach, which

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applies high levels of conservatism at its base, and increasing realism at higher tiers. At the lowest tier, assessment factors are applied to toxicity estimates (e.g., median lethal concentrations [LC50s]) in the risk characterization to give conservative threshold concentrations, which, if not exceeded, are considered to present an acceptable risk. If preliminary estimates of exposure indicate that the threshold concentrations may be exceeded, subsequent testing and analysis may be employed to refine the understanding of risk, including the creation of species sensitivity distributions (SSDs). Initial studies early in the process typically focus on single-species acute and chronic laboratory toxicity assays with indicator species. Subsequent higher-tier studies may include testing additional species and/or testing under more realistic conditions, such as community-level studies in aquatic mesocosms.

Because these tests are required for regulatory purposes to support the registration of an active ingredient, they are typically generated using standardized procedures (e.g., following guidelines of the Organisation for Economic Co-operation [OECD], the USEPA, and ASTM International), by laboratories accredited under the OECD's Good Laboratory Practice (GLP) program [14], resulting in a substantial body of high-quality data. It should be noted that much of the ecotoxicology data published in the peer-reviewed literature is generated from studies that do not follow the rigors and quality control associated with GLP procedures. With the current level of interest in understanding neonicotinoid insecticide toxicity to aquatic organisms, the aim of the present study was to compile previously unpublished data from a suite of laboratory toxicity assays conducted with the neonicotinoid insecticide thiamethoxam for freshwater and marine primary producers, invertebrates, and fish. We did not address in detail the aquatic risk assessment for thiamethoxam, as this is clearly dependent on locale and duration-defined exposure profiles and specific protection goals that are highly context-dependent. We did create SSDs to better understand the general risk to aquatic species under currently reported concentrations of thiamethoxam in freshwater ecosystems. Overall, the extensive collection of high-quality data we report will assist those seeking to conduct formal ecological risk assessments for thiamethoxam and those aiming to gain a greater understanding of the potential impact of detected concentrations of thiamethoxam in surface waters.

METHODS

In total, 30 separate laboratory toxicity studies (24 freshwater, 6 marine) were conducted, with up to 12 single-species tests per study (Table 1). The total freshwater dataset includes 5 tests of aquatic primary producers, 34 tests with aquatic invertebrates (29 acute and 5 chronic), and 6 tests of fish (4 acute and 2 chronic). Experiments with marine species included 1 algal test, 1 mollusc test, 2 tests with a marine crustacean (1 acute and 1 life-cycle), and 2 tests with fish (1 acute and 1 chronic early life stage). All tests were conducted by GLP-accredited laboratories and, with the exception of 2 studies conducted with nonstandard invertebrate species (studies 11 and 12), all tests followed published standard guidelines, with few deviations (as noted in Supplemental Data, Tables S1–S7). We have summarized all relevant test information (e.g., replication, exposure concentrations, and assay conditions) in tabular form, as noted in the following sections, for each test. Species were selected based on availability of widely accepted protocols, organism accessibility, and a desire to better understand the range of possible responses to a variety of nontarget organisms. The names of some species are different in the report titles from those referred to in this paper. This is due to changes in taxonomic identification since the original studies were performed.

All tests were conducted with technical-grade thiamethoxam provided by Syngenta Crop Protection, with purity greater than 98% (Supplemental Data, Tables S1–S7). No organic solvents were used in the preparation of test solutions, except for the chronic test with *Chironomus riparius* (study 17), where acetone was used as a vehicle for sediment treatment. A solvent control was employed in this test, and no significant differences from the negative control were found for any endpoint (Supplemental Data, Table S4). All studies included analytical confirmation of thiamethoxam concentrations at multiple time-points by high-performance liquid chromatography, except for study 18, where analysis was by liquid chromatography–tandem mass spectrometry. Measurements of general water quality included pH, dissolved oxygen, temperature, light level, and in certain studies (as indicated), ammonia, salinity, and total organic carbon in sediment. Details of each study methodology are provided (Supplemental Data, Tables S1–S7). Concerns about thiamethoxam degrading via photolysis to clothianidin (a neonicotinoid), and confounding toxicity have been raised. When the formation of this metabolite has been observed, it is not a major metabolite (typically $<1\%$); and for some studies under field conditions, is not observed at all [15]. However, to address this issue, many of the tests conducted were flow-through or static renewal.

Tests with freshwater primary producers

Assessment of effects of thiamethoxam on primary producers included 2 tests with the green alga *Raphidocelis subcapitata* (formerly *Selenastrum capricornutum* and then *Pseudokirchneriella subcapitata*), 1 test with the filamentous cyanobacteria *Anabaena flos-aquae*, 1 test with the diatom *Navicula pelliculosa*, and 1 test with the macrophyte *Lemna gibba*. All tests assessed sublethal effects (i.e., exposure durations of 72 or 96 h for algae, 7 d for *L. gibba*). Endpoints were growth rate and biomass based on cell density at the start and end of the study (algae), or based on frond count and frond dry weight (*L. gibba*). For each study, no-observed-effect concentration (NOEC) and lowest-observed-effect concentration (LOEC) values were calculated by Dunnett's multiple comparison test, and effective concentration (EC*x*) values were calculated by logit analysis or linear interpolation of the response (the inhibition concentration [ICp] method of Norberg-King [16]). Details of each test, including duration, source of organisms, test concentrations, replication, test conditions, statistics, and validity of the test are described in Supplemental Data, Table S1.

Tests with freshwater invertebrates

Twenty-nine acute toxicity tests were conducted with freshwater aquatic invertebrates for 21 different organisms (insects, molluscs, and aquatic worms). All acute tests were 24- or 48-h, water-only, static tests, with endpoints of immobilization and/or mortality. The NOEC values were typically determined empirically. The EC50 values (concentration causing an increase in immobilization by 50% compared with controls) and LC50 values (concentration causing an increase in mortality by 50% compared with controls) were calculated according to the maximum likelihood method using the logit or probit model. It should be noted that the organism's specific life stage (i.e., instar) was not determined for some tests, which may increase uncertainty around the resulting estimates of toxicity. Details of each test, including duration, source of organisms, test

Table 1. Reference study ID, authors, year, species tested, and original report title as submitted to regulatory agencies and referenced in the present study

Study ID	Authors, year	Species	Title
Freshwater			
1	R. Grade, 1996	*Raphidocelis subcapitata*	Growth Inhibition Test of CGA 293343 tech. to Green Algae (*Selenastrum capricornutum*) in a Static System
2	R. Grade, 1998	*Raphidocelis subcapitata*	Growth Inhibition Test of CGA 293343 tech. to Green Algae (*Selenastrum capricornutum*) Under Static Conditions
3	M. Staggs, 2014	*Anabaena flos-aquae*	Thiamethoxam—96-Hour Toxicity Test with the Cyanobacterium, *Anabaena flos-aquae*
4	M. Staggs, 2014	*Navicula pelliculosa*	Thiamethoxam—96-Hour Toxicity Test with the Freshwater Diatom, *Navicula pelliculosa*
5	R. Grade, 1998	*Lemna gibba*	Acute Toxicity Test of CGA 293343 tech. to the Duckweed *Lemna gibba* G3 under Semi-Static Conditions
6	L. Sayers, 2008	*Procambarus clarkii*	Acute Toxicity to Red Swamp Crayfish (*Procambarus clarkii*), Under Static-Renewal Conditions
7	C. Neumann, 1996	*Daphnia magna*	Acute Toxicity Test of CGA 293343 to the Cladoceran *Daphnia magna* Straus Under Static Conditions
8	K. Knauer, 2000	*Gammarus* sp.	Acute Toxicity Test of CGA 293343 tech. to *Gammarus* sp. Under Static Conditions
9	K. Knauer, 2000	*Daphnia pulex* Leydig, *Thamnocephalus platyurus*, *Brachionus calyciflorus*	Acute Toxicity Test (24 h) of CGA 293343 tech. to Three Invertebrate Species *Daphnia pulex* Leydig, *Thamnocephalus platyurus*, and *Brachionus calyciflorus* Under Static Conditions
10	K. Knauer, 2000	Ostracoda, *Chaoborus* sp., *Lymnea stagnalis*, *Radix peregra*	Acute Toxicity Test of CGA 293343 tech. to Individual Invertebrate Species and Molluscs from a Natural Pond Assemblage Under Static Conditions
11	J. Ashwell and R. Dark, 2002	*Asellus aquaticus*, Copepoda, *Cloeon dipterum*, *Chaoborus crystallinus*, *Chironomus riparius*, Dytiscidae, *Crangonyx pseudogracilis*, *Coenagrionidae*, *Lymnea stagnalis*, Erpobdellidae, *Lumbriculus sp.*, Planariidae	Acute Toxicity to Aquatic Insects
12	J. Pickervance, R. Dark, and J. Ashwell, 2003	*Asellus aquaticus*, *Cloeon dipterum*, *Chironomus riparius*, Dytiscidae, *Crangonyx pseudogracilis*	CGA293343 (Thiamethoxam technical) and CGA322704 (Thiamethoxam metabolite) Acute Toxicity to a Range of Aquatic Invertebrates
13	K. Knauer, 2000	*Cloeon* sp.	Acute Toxicity Test of CGA 293343 tech. to the Ephemeroptera *Cloeon* sp. Under Static Conditions
14	M. Mank and H. Kruegar, 1998	*Chironomus riparius*	CGA 293343 Technical: A 48-hour Static Acute Toxicity Test with the Midge (*Chironomus riparius*)
15	C. Neumann, 1997	*Daphnia magna*	*Daphnia magna* Reproduction Test: Effects of CGA 293343 on the Reproduction of the Cladoceran *Daphnia magna* Straus in a Semi-Static Laboratory Test
16	R. Grade, 2002	*Chaoborus* sp.	Toxicity Test of CGA 293343 tech. on *Chaoborus* sp. (Invertebrate, Insect) Under Static Conditions in a Sediment-Water-Test System
17	R. Grade, 1998	*Chironomus riparius*	Toxicity Test of CGA 293343 tech. on Sediment-Dwelling *Chironomus riparius* (syn. *Chironomus thummi*) Under Static Conditions
18	M. Bradley, 2015	*Chironomus dilutus*	10-day Toxicity Test Exposing Midge (*Chironomus dilutus*) to Thiamethoxam Applied to Sediment Under Static-Renewal Conditions
19	H. Rufli, 1996	*Oncorhynchus mykiss*	Acute Toxicity Test of CGA 293343 tech. to Rainbow Trout (*Oncorhynchus mykiss*) in the Flow-Through System
20	H. Rufli, 1997	*Oncorhynchus mykiss*	Acute Toxicity Test of CGA 293343 tech. to Rainbow Trout (*Oncorhynchus mykiss*) Under Flow-Through Conditions
21	K. Drottar and J. Swigert, 1996	*Lepomis macrochirus*	A 96-hour Flow-Through Acute Toxicity Test with the Bluegill (*Lepomis macrochirus*)
22	S. Maynard, 2003	*Cyprinus carpio*	Thiamethoxam (CGA 293343 technical): Acute Toxicity to Mirror Carp (*Cyprinus carpio*)
23	H. Rufli, 1997	*Oncorhynchus mykiss*	Prolonged Toxicity Test of CGA 293343 tech. to Rainbow Trout (*Oncorhynchus mykiss*) in the Flow-Through System
24	K. Drottar, W. Graves, and J. Swigert, 1997	*Oncorhynchus mykiss*	An Early Life-Stage Toxicity Test with the Rainbow Trout (*Oncorhynchus mykiss*)
Marine			
25	M. Staggs, 2014	*Skeletonema costatum*	Thiamethoxam—96-hour Toxicity Test with the Marine Diatom, *Skeletonema costatum*
26	K. Drottar and J. Swigert, 1997	*Crassostrea virginica*	CGA-293343: A 96-Hour Shell Deposition Test with the Eastern Oyster (*Crassostrea virginica*)
27	L. Sayers, 2015	*Americamysis bahia*	Thiamethoxam—Life-Cycle Toxicity Test with Mysids (*Americamysis bahia*)
28	K. Drottar and J. Swigert, 1997	*Americamysis bahia*	CGA-293343: A 96-Hour Flow-Through Acute Toxicity Test with the Saltwater Mysid (*Mysidopsis bahia*)
29	K. Drottar and J. Swigert, 1997	*Cyprinodon variegatus*	CGA-293343: A 96-Hour Flow-Through Acute Toxicity Test with the Sheepshead Minnow (*Cyprinodon variegatus*)
30	L. Sayers, 2015	*Cyprinodon variegatus*	Thiamethoxam—Early Life-Stage Toxicity Test with Sheepshead Minnow, *Cyprinodon variegatus*

concentrations, replication, test conditions, statistics, and validity of the test, are described in Supplemental Data, Tables S2–S5.

Assessments of chronic toxicity were conducted for 4 aquatic invertebrates—the crustacean *Daphnia magna*, and the insect larvae *Chaoborus* sp., *Chironomus riparius*, and *Chironomus dilutus*. The larval tests included a sediment phase, and toxicity to *C. riparius* was assessed for both sediment- and water-phase applications of thiamethoxam. Endpoints for *D. magna* were mortality and immobilization of adults and number of young produced, endpoints for *Chaoborus* sp. and *C. riparius* were based on emergence (number, rate), and endpoints for *C. dilutus* were survival and growth of the larvae. Details of each test, including duration, source of organisms, test concentrations, replication, test conditions, statistics, and validity of the test, are described in Supplemental Data, Table S5.

Tests with freshwater fish

Four tests for assessment of acute toxicity (96 h) were conducted with freshwater fish, including 2 flow-through tests with the rainbow trout *Oncorhynchus mykiss*, 1 flow-through test with the bluegill sunfish *Lepomis macrochirus*, and 1 static test with the common carp, *Cyprinus carpio*. For all acute tests, the endpoint was mortality, and LC50s were estimated by visual interpretation. In addition, an assessment of chronic toxicity to juvenile *O. mykiss* was conducted (28-d exposure), along with an assessment of early life stage toxicity (88-d exposure from embryo stage). Chronic test endpoints included a variety of sublethal effects. Details of each test including duration, source of organisms, test concentrations, replication, test conditions, endpoints, statistics, and validity of the test are described in the Supplemental Data, Table S6.

Tests with marine species

Marine species tested include a diatom (*Skeletonema costatum*), the Eastern oyster (*Crassostrea virginica*), the opossum shrimp (*Americamysis bahia*), and the sheepshead minnow (*Cyprinodon variegatus*). Acute (96-h) tests were conducted for all species, along with a life-cycle test (28-d) with *A. bahia*, and an early life stage toxicity test (33 d) with *C. variegatus*. Acute test endpoints were based on measurements of cell density (*S. costatum*), shell growth (*C. virginica*), or mortality (*A. bahia, C. variegatus*). Chronic test endpoints included survival, reproduction, and growth-based metrics (organism length, weight). Details of each test are provided in the Supplemental Data, Table S7.

Preliminary assessment of the acute risk from thiamethoxam

To begin placing the data reported in the present study in a broader ecological context, we performed a preliminary risk assessment focusing on acute toxicity data for freshwater invertebrates, as these are the most sensitive organisms and have the greatest amount of data. We did not examine risk via chronic exposure using SSDs for 2 primary reasons. First, it can be reasonable to assume that a point measurement of exposure can be reflective of acute exposure. This is not necessarily the case with chronic exposure, which requires more continuous monitoring and is highly site specific. Second, we lack data on a sufficient number of organisms to construct a robust SSD for chronic responses at this time.

For the effects assessment, SSDs were created as described by Solomon et al. [17] and 5% hazard concentrations (HC5s) were calculated from the resulting distributions. These HC5s describe the concentration estimated to produce an adverse impact according to the assessed endpoint in 5% of species. Because a minimum of 6 reported responses is recommended for HC calculations, the datasets for 24- to 48-h EC50 (immobility) and 48-h LC50 estimates were chosen as the basis for the constructed SSDs. No distinctions were made as to the relative quality of individual responses; rather, the lowest reported value for each species was utilized to be conservative. Data were plotted as a cumulative frequency distribution using a

Table 2. Regression coefficients, intercepts, and concentrations estimated to cause effects in 5% of species (HC5) for thiamethoxam acute toxicity species sensitivity distributions as calculated using the Weibull equation[a]

	$y = ax + b$[b]						% of species impaired at median	% of species impaired at 75th percentile	% of species impaired at 90th percentile	% of species impaired at 95th percentile	% of species impaired at 99th percentile
Distribution	*a*	*b*	r^2	No.[c]	HC5 (μg/L)	Likelihood of exceeding HC5 (%)	0.0125 (μg/L)	0.0250 (μg/L)	0.0320 (μg/L)	0.0538 (μg/L)	0.4000 (μg/L)
All invertebrate acute 24–48-h EC50s	0.550	–2.035	0.95	22	5.1	0.15	0.103	0.178	0.214	0.314	1.211
Insect acute 48-h EC50s	0.727	–1.732	0.94	8	1.3	0.51	0.092	0.189	0.241	0.397	2.161
Invertebrate (excluding insect) acute 24–48-h EC50s	0.471	–2.314	0.92	14	26.3	0.01	0.066	0.108	0.127	0.180	0.619
All invertebrate acute 48-h LC50s	0.604	–2.466	0.97	13	22.9	0.01	0.015	0.030	0.038	0.061	0.340
Insect acute 48-h LC50s	0.758	–2.303	0.96	6	7.4	0.12	0.009	0.022	0.029	0.055	0.460
Invertebrate (excluding insect) acute 48-h LC50s	0.684	–3.473	0.96	7	470.2	<0.001	0.001	0.0002	0.0003	0.0007	0.0090

[a]The percentage of species impaired by exposure to thimethoxam based on an exposure distribution at specific percentiles are also provided. Where multiple responses for the same species and time point were available, the lower concentration was used.
[b]These values are transformed into units of log and probit for the purposes of regression and back-transforms were used to calculate the intercepts. The distribution units were in μg/L.
[c]Number of data points used in the ranking.

probability scale as a function of the log concentration. Plotting positions were expressed as percentages and calculated using the Weibull formula with a total of 6 SSDs created (Table 2). Three distributions were constructed each for LC50s and EC50s and consisted of all invertebrates, insects only, and invertebrates excluding insects. Separate distributions were created to be as conservative as possible in the identification of sensitive groups of organisms. In addition, we calculated HC5s using the USEPA CADDIS Species Sensitivity Distribution Generator Ver 1 software for the same acute insect EC50 and LC50 data in order to test results using a different model [18].

For the exposure assessment, a review of the published literature was conducted to compile a broad dataset of measured environmental concentrations. Peer-reviewed literature, published reports, and online databases were searched for thiamethoxam analyses of freshwater habitats (or potential habitats) within the United States and Canada as of November 2016. If not published, raw data were requested from the authors. Duplicated values were identified and removed using a search formula that matched latitude, longitude, sampling date, and sampling time. In cases in which multiple samples were obtained within a 24-h period at a site, the greatest value was retained in the dataset as a conservative method. Only studies or databases that reported limits of detection or quantitation were retained. Samples with nondetectable concentrations were assigned a value of one-half of that sample's respective limit of detection (LOD) or limit of quantitation (LOQ), whichever was greater. The LOD/LOQs ranged from 1 to 50 ng/L across all data sources. The peer-reviewed sources consulted are given in the *References* section [4,5,19–22]. A list of the online or unpublished data sources consulted is provided in the Supplemental Data, Table S8 and the raw data used in the distribution are provided as a separate Excel file. In total, 6906 data points were assembled, of which 1322 (19.1%) reported thiamethoxam concentrations at or above the LOD.

Once the HC5s were calculated, the likelihood of observing a concentration that would impair at least 5% of species was calculated. In addition, the percentages of species that could be impaired at the median, 75th, 90th, 95th, and 99th percentiles of the exposure distribution were determined for each constructed SSD.

RESULTS

Test concentrations

Results of analytical confirmations of test concentrations, along with LOQ/LOD values, are provided in Supplemental Data, Tables S1–S7. Except for final concentrations in chronic static tests, measured concentrations were typically 80 to 120% of nominal, which fall within guideline recommendations for use in statistical analyses. All measured concentrations in control units were below the test LOD/LOQ, except where noted (study 7: *D. magna*). Statistical analyses are based on nominal or mean measured concentrations, as described in Supplemental Data, Tables S1–S7.

Effects on freshwater primary producers

All measured responses (raw data) for primary producers are provided in Supplemental Data, Tables S9–S13. In the 96-h test with *R. subcapitata*, exponential growth occurred in controls, and conditions for validity of the test were met according to the primary guideline method [23]. Similarly, conditions for validity were met in the test with *L. gibba* (10-fold increase in biomass in 7 d) [24]. For tests with *A. flos-aquae* and *N. peliculosa*, 2 of the 5 OECD [25] conditions for validity of tests with freshwater algae and cyanobacteria were not met (coefficients of variation for mean yield and section-by-section growth rate exceeded requirements). However, results were considered typical of those species, because growth is more variable than for the unicellular algae used to define the criteria [26]. Conditions for validity of the tests are described in the Supplemental Data, Table S1.

Estimated NOEC, LOEC, and EC50 values for primary producers are presented in Table 3. First-tier algal and plant studies demonstrate that, as expected for an insecticide, thiamethoxam has low toxicity to algae and aquatic macrophyte species. Estimated 72-h and 96-h EC50 values were all greater

Table 3. No-observed-effect concentrations (NOECs), lowest-observed-effect concentrations (LOECs), and median effect/lethal concentration (EC50/LC50) estimates for freshwater primary producers exposed to thiamethoxam[a]

Organism	Test type	Study ID	Endpoint	NOEC (mg/L)	LOEC (mg/L)	ECx_x (mg/L)
Raphidocelis subcapitata	72 h (static)	1	Biomass ($_b$) and growth rate ($_r$)	$NOEC_r/NOEC_b$: 81.8[b]	>81.8	$EC50_{r/b}$: >81.8
	96 h (static)	2	Biomass ($_b$) and growth rate ($_r$)	$NOEC_r/NOEC_b$: 100[b]	>100	$EC50_{r/b}$: > 100
Anabaena flos-aquae	96 h (static)	3	Avg. specific growth rate ($_r$); biomass (area under growth curve) ($_b$); yield ($_y$)	72-h $NOEC_{r,b,y}$: 97[b] 96-h $NOEC_{r,b,y}$: 47	72-h $LOEC_{r,b,y}$: >97 96-h $LOEC_{r,b,y}$: 97	72-h $EC10_b$: 51 (ne – 61) 72-h $EC20_b$: 62 (ne – 74) 72-h $EC10_y$: 53 (ne – 64) 72-h $EC20_y$: 64 (ne – 87) 72-h $EC10_{yr}$: 88 (32 – ne) 72-h $EC20_{yr}$: >97 72- and 96-h $EC50_{r/b/y}$: > 97
Navicula pelliculosa	96 h (static)	4	Avg. specific growth rate ($_r$); biomass (area under growth curve) ($_b$); yield ($_y$)	72-h $NOEC_{r,b,y}$ 98[b] 96-h $NOEC_{r,b,y}$: 98[b]	72-h $LOEC_{r,b,y}$: >98 96-h $LOEC_{r,b,y}$: >98	72-h $EC10_b$: 66 (16 – ne) 72-h $EC20,50_b$: >98 72-h $EC10,20,50_{r/y}$: >98 96-h $EC50_{r/b/y}$: >98
Lemna gibba	7 d (semistatic)	5	Inhibition of frond number (growth rate ($_r$) and biomass ($_b$) as area under the growth curve); frond dry weight	Frond number $NOEC_{r,b,y}$: 90.2[b] Weight NOEC: 90.2[b]	>90.2	Frond number $EC50_{r/b}$: >90.2

[a]Species, test durations, and endpoints as indicated.
[b]Greatest tested concentration.
ne = not estimable.

than the greatest concentrations tested (≥81.8 mg thiamethoxam/L). In all but 1 case, NOEC values were equivalent to the highest concentration tested, and the minimum reported NOEC was 47 mg thiamethoxam/L (96-h growth rate, biomass, and yield for *A. flos-aquae*).

Effects on freshwater invertebrates

All measured responses (raw data) are provided in the Supplemental Data, Tables S14–S33. In all acute tests with aquatic invertebrates, <10% response (immobility/mortality) was observed in control units, indicating that criteria for validity were met according to standard guidelines [27]. Similarly, all criteria for validity were met in chronic tests (Supplemental Data, Table S5).

Among tested crustaceans, a wide range of sensitivities were observed (Table 4). Thiamethoxam had low toxicity to daphnids (*D. magna* and *Daphnia pulex*) in both acute and chronic tests and to Copepoda (acute tests only were conducted), with estimated EC50 values all exceeding 100 mg thiamethoxam/L. For other species tested (*Asellus aquaticus, Gammarus* sp., Ostracoda, *Thamnocephalus platyurus, Procambarus clarkii*), 48-h EC50 estimates ranged from 0.084 mg thiamethoxam/L (*A. aquaticus*) to 3.0 mg thiamethoxam/L (*P. clarkii*).

With the exception of the phantom midge larvae *Chaoborus* sp. (48-h EC50s of 5.5 and 7.3 mg thiamethoxam/L), aquatic insects were the most sensitive species tested, with 48-h EC50 estimates for each species reported below 1 mg thiamethoxam/L (Table 5). Values ranged from 0.014 mg thiamethoxam/L (*Cloeon* sp.) to 0.98 mg thiamethoxam/L (Coengrionidae). Among insects tested under chronic conditions, *C. riparius* was the most sensitive, with a 30-d NOEC (emergence) of 0.01 mg/L.

Other aquatic invertebrates tested include 2 snails, a rotifer, and 3 worms. With the exception of the blackworm, *Lumbriculus* sp. (48-h EC50 = 7.7 mg/L), all EC50 estimates were >100 mg/L (Table 6).

Effects on freshwater fish

In all tests with fish (acute and chronic), conditions for validity of the test were met (Supplemental Data, Table S6). Acute tests demonstrated that all tested species (*O. mykiss*, *L. macrochirus*, *C. carpio*) were relatively insensitive to thiamethoxam (Table 7). All NOEC values in the 96-h tests were equivalent to the maximum tested concentration (≥100 mg thiamethoxam/L). Endpoints included mortality for all species, as well as sublethal symptoms for *O. mykiss* (swimming behavior, loss of equilibrium, respiratory function, exophthalmos, pigmentation). Chronic-duration tests were conducted with *O. mykiss*, including a 28-d prolonged toxicity test and an early life stage test (28-d hatch time, 60-d post hatch time). The NOEC values in these tests were also equivalent to the greatest concentration tested (100 mg thiamethoxam/L and 20 mg thiamethoxam/L, respectively). Endpoints in the 28-d test were mortality, growth rate (length and weight), food conversion efficiency, and sublethal effects (feeding activity, swimming behavior, respiratory movement, pigmentation,

Table 4. No-observed-effect concentrations (NOECs), lowest-observed-effect concentrations (LOECs), and median effect/lethal concentration (EC50/LC50) estimates for freshwater crustaceans exposed to thiamethoxam[a]

Organism	Common name	Study ID	Test type	NOEC/LOEC (mg/L)	EC50 (mg/L; 95% CL)	LC50 (mg/L; 95% CL)
Acute studies						
Asellus aquaticus	Water louse	11	48 h (static)	—	0.084 (0.044–0.16)	2.3 (0.82–7.32)
		12		—	>0.32 (ne)	—
Copepoda	N/A	11	48 h (static)	—	>100 (ne)	>100 (ne)
Daphnia magna	Water flea	7	24 h (static)	—	>100	
			48 h (static)	NOEC = 32	>100	—
Daphnia pulex	Water flea	9	24 h (static)	—	>100 (ne)	—
Gammarus sp.	N/A	8	24 h (static)	—	15 (10– 23)	—
			48 h (static)	—	2.8 (1.7–4.1)	—
Ostracoda	Seed shrimp	10	24 h (static)	—	0.24 (0.20–0.29)	—
			48 h (static)	—	0.18 (0.15–0.22)	—
Thamnocephalus platyurus	Fairy shrimp	9	24 h (static)	—	>100 (ne)	—
Procambarus clarkii	Crayfish	6	24 h (static renewal)	—	3.7 (2.7–5.1)	110 (13–ne)[c]
			48 h (static renewal)	—	3.0 (2.1–4.6)	17 (6.0–14 000)[c]
			72 h (static renewal)	—	2.8 (2.0–3.9)	12 (4.9–580)[c]
			96 h (static renewal)	NOEC = 0.65	2.3 (1.6–3.2)	10 (4.5–360)[c]
Chronic studies						
Daphnia magna (parent and juvenile)	Water flea	15	14 d (semi-static)	Reproduction (no. of young/female): NOEC = 100[b]; LOEC = >100	—	—
			21 d (semi-static)	Reproduction (no. of young/female); adult length; time to first brood: NOEC = 100[b]; LOEC = >100	Reproduction (no. of young/female); immobilization: >100[b]	—

[a]The endpoint for all NOEC and EC50 values is immobilization, except for study 6 (NOEC for immobility and mortality combined); study 12 (immobilization and mortality combined), and study 15 (as indicated). The LOEC endpoints are as indicated.
[b]Greatest tested concentration.
[c]LC50 calculated by probit analysis, but response considered insufficient (max. 50% mortality) to produce adequate effect estimate.
ne = not estimable.

Table 5. No-observed-effect concentrations (NOECs), lowest-observed-effect concentrations (LOECs), and median effect/lethal concentration (EC50/LC50) estimates for freshwater aquatic insects[a]

Organism	Common name	Study ID	Test type	NOEC/LOEC (mg/L)	EC50 (mg/L; 95% CL)	LC50 (mg/L; 95% CL)
Acute studies						
Chaoborus cristallinus	Glassworm (phantom midge larvae)	11	48 h (static)	—	7.3 (5.4–10)	11 (7.9–17)
Chaoborus sp.	Glassworm (phantom midge larvae)	10	24 h (static)	—	6.9 (5.7–8.3)	—
			48 h (static)	—	5.5 (4.4–6.6)	—
Chironomus riparius	Bloodworm (harlequin fly larvae)	14	24 h (static)	—	0.061 (0.050–0.075)	—
			48 h (static)	NOEC = 0.013	0.035 (0.030–0.041)	—
		11	48 h (static)	—	0.045 (ne)	0.26 (0.13–0.52)
		12	48 h (static)	—	0.071 (0.034–0.194)	—
Cloeon dipterum	Species of mayfly (larvae)	11	48 h (static)	—	0.021 (ne)	0.053 (0.038–0.073)
		12	48 h (static)	—	0.044 (0.042–0.045)	—
Cloeon sp.	Species of mayfly (larvae)	13	24 h (static)	—	0.019 (0.016–0.023)	—
			48 h (static	—	0.014 (0.011–0.017)	—
Coengrionidae	Species of damselfly (larvae)	11	48 h (static)	—	0.98 (ne)	1.6 (0.82–2.9)
Crangonyx pseudogracilis	None	11	48 h (static)	—	0.42 (0.20–0.87)	20 (7.28–96)
		12	48 h (static)	—	1.491 (1.029–2.403)	—
Dytiscidae	Predacious diving beetle	11	48 h (static)	—	0.069 (ne)	0.34 (0.17–0.62)
		12		—	0.047 (0.022–0.094)	—
Chronic studies						
Chaoborus sp.	Glassworm (phantom midge larvae)	16	34 d (static; with sediment; water application)	NOEC: total emergence: 0.64; development rate: 1.28; LOEC: total emergence: 1.28; development rate: >1.28	Total emergence: 0.48 (ne) Development rate: ne	—
Chironomus dilutus	Species of nonbiting midge	18	10 d (static renewal; (sediment application)	NOEC: survival: 1.3; growth (ash-free dry wt): 0.60 mg/kg sediment dry wt LOEC: survival: 2.6; growth: 1.3 mg/kg sediment dry wt	Growth: >2.6 (ne) mg/kg sediment dry wt	2.0 (1.9–2.1) mg/kg sediment dry wt
Chironomus riparius	Bloodworm (harlequin fly larvae)	17	30 d (static) (sediment application)	NOEC: emergence and development rate: 0.10 mg/kg sediment dry wt LOEC: emergence: 0.020 development rate: >0.010[b]	Emergence: 0.11 Development rate: >0.10 mg/kg sediment dry wt[b]	-
			30 d (static) (water application)	NOEC: emergence and development rate: 0.010 LOEC: emergence: 0.020 development rate: >0.010[b]	Emergence: 0.0114 Development rate: >0.010[b,c]	-

[a]Endpoint for all NOEC and EC50 values is immobilization, except for studies 12 and 14 (immobilization and mortality), and chronic studies (as indicated). LOEC endpoints as indicated.
[b]Greatest concentration where emergence occurred.
[c]Because of a 100% effect on emergence at 0.02 and above, the effect on development rate could not be calculated.
ne = not estimable.

Table 6. No-observed-effect concentrations (NOECs) and median effect/lethal concentration (EC50/LC50) estimates for other freshwater aquatic invertebrates[a]

Organism	Common name	Study ID	Test type	NOEC (mg/L)	EC50 (mg/L; 95% CL)	LC50 (mg/L; 95% CL)
Lymnaea stagnalis	Great pond snail	10	48 h (static)	100[b,c]	>100 (ne)	—
		11	48 h (static)	—	>100 (ne)	>100 (ne)
Radix peregra	None (pond snail)	10	48 h (static)	100[c]	>100 (ne)	—
Brachionus calyciflorus	None (planktonic rotifer)	9	24 h (static)	100[c]	>100 (ne)	—
Erpobdellidae	None (leech)	11	48 h (static)	—	100[c] (ne)	>100 (ne)
Lumbriculus sp.	Blackworm	11	48 h (static)	—	7.7 (ne)	>32 (ne)
Planariidae	Flatworm	11	48 h (static)	—	>100 (ne)	>100 (ne)

[a]The NOEC/EC50 endpoint was immobilization. LOEC values were not estimated.
[b]Greatest concentration with 10% or less immobilization because this amount is considered acceptable for control performance.
[c]Greatest tested concentration.
ne = not estimable.

Table 7. No-observed-effect concentrations (NOECs), lowest-observed-effect concentrations (LOECs), and median lethal concentration estimates (LC50) for freshwater fish

Organism	Common name	Study ID	Test type	Endpoint	NOEC (mg/L)	LOEC (mg/L)	LC50 (mg/L)
Acute studies							
Oncorhynchus mykiss	Rainbow trout	19	96 h (flow-through)	Mortality; sublethal symptoms (swimming behavior, loss of equilibrium, respiratory function, exophthalmos, pigmentation)	125[a]	—	24, 48, 72, 96 h: >125
		20		Mortality; sublethal symptoms (swimming behavior, loss of equilibrium, respiratory function, exophthalmos, pigmentation)	100[a]	—	24, 48, 72, 96 h: >100
Lepomis macrochirus	Bluegill sunfish	21	96 h (flow-through)	Mortality	114[a]	—	24, 48, 72, 96 h: >114
Cyprinus carpio	Common carp	22	96 h (static)	Mortality	120[a]	—	3, 24, 48, 72, 96 h: >120
Chronic studies							
Oncorhynchus mykiss	Rainbow trout	23	Prolonged toxicity 28 d (flow-through)	Mortality; growth rate (length and weight); food conversion efficiency; sublethal effects: feeding activity, swimming behavior, respiratory movement, pigmentation, exophthalamus, loss of equilibrium, reaction to external stimulus	100[a]	>100	—
		24	Early life stage 88 d (flow-through)	Hatching success and time to hatch, time to swim up, larvae and fry survival, growth (d 31 length, d 31 and d 60 length and weight)	20[a]	>20	—

[a]Greatest tested concentration.

Table 8. No-observed-effect concentrations (NOECs), lowest-observed-effect concentrations (LOECs), and median effect/lethal concentration estimates (EC50/LC50) for marine organisms

Organism	Common name	Study ID	Test type	Endpoints	NOEC (mg/L)	LOEC (mg/L)	EC_x (mg/L; 95% CL)	LC50 (mg/L; 95% CL)
Skeletonema costatum	None	25	96 h (static)	Avg. specific growth rate ($_r$); biomass (area under growth curve) ($_b$); yield ($_y$)	72- and 96-h $NOEC_{r,y}$: 99[b]72- and 96-h $NOEC_b$: 48	72- and 96-h $NOEC_{r,y}$: >99 72- and 96-h $NOEC_b$: 99[b]	72-h $EC10_b$: 8.7 (4.0–35) 72-h $EC20_b$: 28 (ne–87) 72-h $EC10_y$: 8.8 (ne–75) 72-h $EC20_y$: 30 (ne) 72-h EC10, 20_r: >99 72-h and 96-h $EC50_{r/b/y}$ > 99	N/A
Crassostrea virginica	Eastern oyster	26	96 h (flow-through)	Inhibition of shell growth	119[b]	—	96-h EC50: >119	—
Americamysis bahia	Opossum shrimp	27	28 d (flow-through)	Postpairing survival ($F_{0f,m}$); overall 28-d survival (F_{0all}); F1 96-h survival (F_1); no. of offspring; male and female length ($L_{m,f}$) and weight ($W_{m,f}$)	Survival: $F_{0f,m}$: 1.1 F_{0f}: 3.9[b] F_{0all}: 0.56 F_1: 2.0[b] No. of offspring: 2.0 $L_{m,f}$ and $W_{m,f}$: 3.9[b]	Survival: $F_{0f,m}$: 2.0 F_{0f}: >3.9 F_{0all}: 1.1 F_1: >2.0 No. of offspring: 3.9 $L_{m,f}$ and $W_{m,f}$: >3.9	—	—
Americamysis bahia	Opossum shrimp	28	96 h (flow-through)	Mortality	—	—	—	24 h: >16 48 h: >14 (11–25)[a] 72 h: 9.3 (7.6–12) 96 h: 6.9 (5.8–8.4)
Cyprinodon variegatus	Sheepshead minnow	29	96 h (flow-through)	Mortality	—	—	—	24, 48, 72, 96 h: >111
Cyprinodon variegatus	Sheepshead minnow	30	33 d (flow through)	Hatching success (Hs); Live, normal hatch (Hn); Larval survival (S_L); Length (L); Wet and dry wt ($W_{w,d}$)	Hs,n, S_L, $W_{w,d}$: 9.9[b]L: 1.7	Hs,n, S_L, $W_{w,d}$: >9.9 L: 4.1	Hs,n, S_L, $W_{w,d}$,L: >9.9	Hs,n, S_L, $W_{w,d}$,L: >9.9

[a]Concentration–response relationship not demonstrated over a reasonable range (<37 to >63% dead).
[b]Greatest tested concentration.
ne = not estimable; N/A = not available.

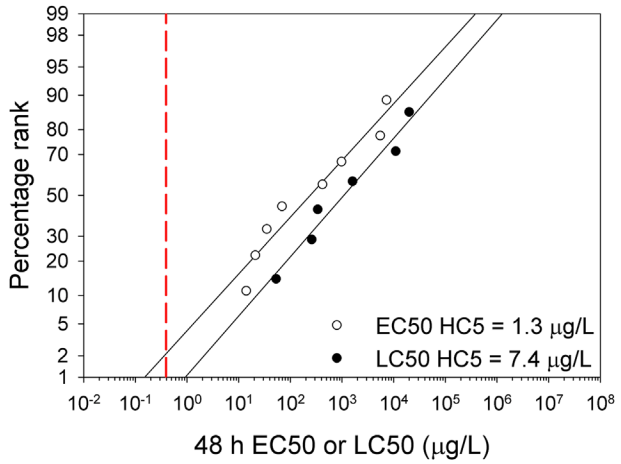

Figure 1. The species sensitivity distributions for insect 48-h median effective concentrations (EC50s) and median lethal concentrations (LC50s). The red line represents the 99th centile of exposure to thiamethoxam (derived from Figure 3). HC5 = 5% hazard concentration.

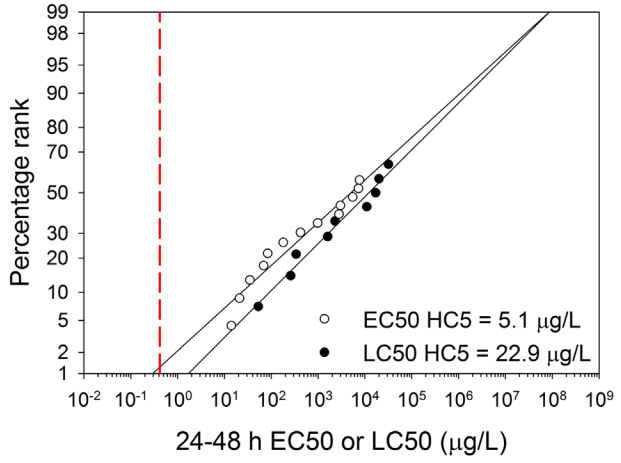

Figure 2. The species sensitivity distributions for invertebrates, including insect, 24- and 48-h median effective concentrations (EC50s) and 48-h median lethal concentrations (LC50s). The red line represents the 99th centile of exposure to thiamethoxam (derived from Figure 3). HC5 = 5% hazard concentration.

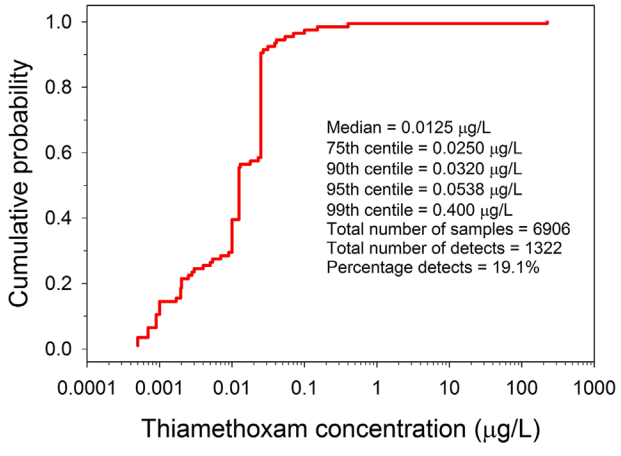

Figure 3. Cumulative probability of all surface water monitoring data for thiamethoxam. The cumulative probability distribution includes all data ($n = 6906$), including detects and nondetects. Nondetects were treated as one-half of the respective limit of detection or limit of quantitation. Inset table contains statistics that characterize the dataset.

exophthalmos, loss of equilibrium, reaction to external stimulus). Endpoints in the early life stage test were hatching success, time to swim up, larvae and fry survival, and growth (31-d length, 31-d and 60-d length and weight).

Effects on marine organisms

All measured responses (raw data) are provided in the Supplemental Data, Tables S38–S46. When provided in the test guideline, all criteria for validity of the marine tests were met (Supplemental Data, Table S7). As with freshwater species, the marine diatom, mollusc, and fish were insensitive to acute exposure to thiamethoxam (96-h EC/LC50 values exceeded 99 mg/L for all species tested; Table 8). Estimated EC50 values in the early life stage test with *C. variegatus* also exceeded the greatest concentration tested (9.9 mg/L) for all measured endpoints (hatching success, normal appearance at hatch, larval survival 28-d posthatch, and 28-d larval length and weight). The most sensitive marine organism was the opossum shrimp (*A. bahia*), with a 96-h LC50 of 6.9 (95% confidence interval [CI], 5.8–8.4) mg/L. In the life cycle test, males and females were paired after 13 d of exposure (following brood appearance), and the following endpoints were assessed: postpairing survival, overall 28-d survival, F1 96-h survival, number of offspring/female, and 28-d male/female length and weight. The LOEC values ranged from 1.1 mg/L (overall 28-d survival) to >3.9 mg/L (female postpairing survival, male/female length and weight).

Assessment of the acute risk from thiamethoxam

The resulting SSDs, HC5s, and likelihood of exceeding specific centiles of exposure can be found in Table 2 and the representative SSDs in Figures 1 and 2. The resulting exposure distribution from surface water samples collected from North America can be found in Figure 3. It is unlikely (<~0.5%) that current concentrations of thiamethoxam found in these aquatic environments will exceed the HC5 for any of the 6 distributions.

Using the USEPA CADDIS SSD software [18], the HC5 (with 95% CIs) derived for 48-h acute EC50 insect data was 3.27 (0.51, 21.14) µg/L thiamethoxam, with an r^2 of 0.924. The HC5 (with 95% CIs) derived for 48-h acute LC50 insect data was 21.06 (4.4, 99.97) µg/L thiamethoxam, with an r^2 of 0.956. These HC5 estimates are greater than those reported in Table 2, with likelihoods of exceedance for our North American exposure distribution of <0.2% and <0.02%, respectively.

DISCUSSION

As would be anticipated for a neonicotinoid insecticide, aquatic primary producers and fish were the least sensitive organisms tested, with acute median effect concentrations exceeding the greatest tested exposure concentrations (≥80 mg/L) in all cases. The tested molluscs, worms, and single rotifer species were similarly insensitive; among these, only *Lumbriculus* sp. exhibited a median effect concentration lower than 100 mg/L. In general, insects were the most sensitive species, with the majority of EC50 values < 1 mg/L. However, the freshwater crustaceans *A. aquaticus* and Ostracoda exhibited a similar sensitivity, while the midge larvae *Chaoborus* sp. were relatively insensitive compared with other insects (EC50 > 5.5 mg/L).

To date, relatively few assessments of the toxicity of thiamethoxam to aquatic species have been published in the peer-reviewed literature. Here we compare the thiamethoxam dataset generated by Syngenta for product registration (as described in the present study) with values reported in the

literature to date, and find that there is general agreement in the observed responses.

In our search, and to the best of our knowledge, no peer-reviewed studies examining thiamethoxam toxicity to any marine species, freshwater primary producers, rotifers, worms, or fish have been published. Two published studies have examined effects of thiamethoxam on crustaceans. As in Syngenta study 6, Barbee and Stout [28] assessed 96-h toxicity of thiamethoxam to crayfish (*P. clarkii*). Both tests followed ASTM standard E729-96, "Standard practice for conducting acute toxicity tests with fishes, macroinvertebrates, and amphibians" (a static-renewal method) [29]. Barbee and Stout [28] reported 96-h LC50s of 0.967 (95% CI = 0.879–1.045) mg thiamethoxam/L. Although mortality response in study 6 was not sufficient to produce statistically robust LC50 values (maximum 50% mortality observed; Table 3), the EC50 value based on immobility was estimated as 2.3 mg (95% CI, 1.6–3.2) thiamethoxam/L. Acute toxicity to the amphipod *Gammarus kischineffensis* was tested by Ugurlu et al. [30], with reported LC50s for 24-h and 48-h exposures of 75.6 and 23.5 mg thiamethoxam/L, respectively. That study used the pesticide formulation, rather than the technical grade product, and 50% water changes were completed daily to renew exposure solutions. In study 8, mortality was not recorded separately from immobility. Estimates of 24-h and 48-h EC50s (immobility) were 15 (95% CI, 10–23) mg/L, and 2.8 (95% CI, 1.7–4.1) mg/L, respectively, for *Gammarus* sp. tested under static conditions, with technical-grade thiamethoxam.

Three published studies reporting 24-h or 48-h acute effects to insects were identified. Riaz et al. [31] examined toxicity to larvae of the mosquito *Aedes aegypti* and reported a 24-h LC50 of 0.183 (95% CI, 0.162–0.205) mg thiamethoxam/L. Similarly, Stevens et al. [32] determined a 24-h LC50 for *Chironomus tepperi* larvae of 0.121 (95% CI, 0.108–0.136) mg thiamethoxam/L. Finally, van den Brink et al. [33] assessed toxicity of thiamethoxam to *Cloeon dipterum* nymphs over 96 h and reported a 24-h EC50 (immobility) of 0.092 (95% CI 0.085–0.099) mg/L. Among our studies, 24-h toxicity of thiamethoxam to insect larvae was determined for the midge larvae *Chaoborus* sp. (study 10) and *C. riparius* (study 14), and the mayfly larva *Cloeon* sp. (study 13). The LC50 estimates were not separately determined, but 24-h EC50 values based on immobility were estimated at 6.9 (95% CI, 5.7–8.3), 0.061 (95% CI, 0.050–0.075), and 0.019 (95% CI, 0.016–0.023) mg thiamethoxam/L, respectively. Our assessments of 48-h toxicity to *Cloeon* sp. (LC50 of 0.053 mg/L [study 11] and EC50 [immobility] of 0.044 mg/L [study 12]) were also similar to those reported in van den Brink et al. [33], with an EC50 (immobility) of 0.049 (95% CI, 0.038–0.064) mg/L. These data demonstrate an overall agreement in acute toxicity estimates within insect species groups.

Two chronic duration tests with insects were found in the peer-reviewed literature. Van den Brink et al. [33] assessed the toxicity of thiamethoxam to *C. dipterum* nymphs after a 28-d exposure. The test was conducted under a static-renewal method with analytical confirmation of test concentrations. The reported EC50 for immobility after 28 d was 0.00068 mg/L (95% CI, 0.00038–0.0012 mg/L). Cavallaro et al. [13] conducted a full life cycle assessment using the midge *C. dilutus*, under a static-renewal method with analytical confirmation of test concentrations. That study reported an EC50 value for emergence after 40 d of 0.00413 (95% CI, 0.00353–0.00476) mg thiamethoxam/L. Among the reports in our study, a 30-d life cycle test was conducted with *C. riparius* larvae, with an EC50 for emergence of 0.0114 mg/L (95% CI not estimated), based on nominal concentrations. However, this test was conducted under static conditions (study 17), and by day 30, all concentrations were lower than the LOD (Supplemental Data, Table S31). A 34-d life cycle test was also conducted with the midge larvae *Chaoborus* sp. under static conditions, with an EC50 for emergence of 0.48 mg/L, based on nominal concentrations. Again, however, concentrations of thiamethoxam in the water phase declined substantially over the test, and were <38% of nominal concentrations by test termination.

No readily comparable toxicity estimates for molluscs were found to be available. However, Prosser et al. [12] assessed toxicity of thiamethoxam to the snail *Planorbella pilsbryi* after 7-d exposures, as well as to a freshwater mussel, *Lampsilis fasciola,* after 48-h exposure. For *P. pilsbryi*, the 7-d LC50 value was estimated at 6.195 (95% CI, 2.9078–9.4822) mg thiamethoxam/L, and 48-h EC50 estimates based on viability for *L. fasciola* exceeded the highest concentration tested (0.691 mg/L). Our studies included only 48-h tests with 2 different snail species (*Lymnaea stagnalis* and *Radix peregra*), and EC50 (immobility) values were estimated to exceed the highest test concentration (100 mg/L).

Our preliminary risk assessment reveals little likelihood of acute toxicity at current environmental concentrations for freshwater invertebrates, even using conservative models and including the most sensitive responses. Although our methods did not include an assessment of quality in the exposure concentration dataset, this approach should be included in HC5 calculations for more formal purposes [34]. Based on results of our studies and those reported previously in the literature, primary producers and fish (acute and chronic) are not sensitive to thiamethoxam, and current environmental concentrations pose no risk to these organisms. Invertebrates, and specifically insects, are significantly more sensitive, but current environmental concentrations are unlikely to exceed our calculated HC5s. Although the sizeable dataset assembled in the present study allows for the calculation of freshwater invertebrate HC5s based on acute endpoints (immobility and mortality), additional assessments of toxicity for sensitive insects (and potentially some crustacean species) should be generated to develop more complete SSDs, especially for chronic exposures. These resulting acute and chronic HC5 estimates should then be further confirmed through mesocosm studies.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.3846.

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Conflict of Interest—The toxicity tests were funded by the registrant of thiamethoxam (currently Syngenta). Baxter and Hanson were compensated for assisting with the writing of the manuscript and had full access to all data, as well as editorial control.

Data availability—The raw data behind the toxicity values are provided in the Supplemental Data. Full GLP reports are available upon request from the contact author.

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