FINAL REPORT

PROJECT TITLE: Sublethal Effects of Chronic Exposure to Neonicotinoid Pesticides on Aquatic Organisms

PROJECT ID: DATCP2020-2



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PROJECT SUMMARY

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Project ID:	DATCP2020-2
Investigators:	Tisha King-Heiden, Professor, Department of Biology, UW-La Crosse Elisabeth Harrahy, Associate Professor, Department of Biology, UW- Whitewater
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Background/Need: Neonicotinoid insecticides were designed to specifically target the nervous system of invertebrate pests (specifically the nicotinic Acetylcholine receptor, nAChR) and deemed not overtly toxic to vertebrates. Initially hailed as an exceptional replacement for older insecticides, their reputation was quickly tarnished by their association with honeybee declines and impacts on other non-target terrestrial insects. Following decades of widespread use, neonicotinoids are now major contaminants of concern in aquatic ecosystems, and their impacts on the aquatic community are not fullyunderstood. Thiamethoxam (TM) and imidacloprid (IM) are found above ecological threshold levels in groundwater and surface waters in Wisconsin, particularly within the Central Sands Region. This poses a potential risk to aquatic invertebrates, and emerging evidence suggests that fish may be susceptible to adverse effects following chronic exposure to these insecticides.

Objectives: The overall objectives of our work were to better understand the chronic toxicity of TM and IM to key aquatic species within the Central Sands Region of Wisconsin, as well as an additional fish species that is a model organism in aquatic toxicology. Since neonicotinoids are commonly found in mixtures that are dominated by TM and IM, we also evaluated the toxicity of these compounds in mixtures.

Methods: We used standard laboratory assays to determine the acute toxicity of TM and IM singly and as a mixture to select aquatic invertebrates (*Ceriodaphnia dubia* water fleas, *Chironomus dilutus* midges, and *Gammarus pseudolimnaeus* amphipods), and the chronic toxicity of sublethal exposure of TM and IM singly and as a mixture to the water flea and amphipod. We also evaluated the toxicity of TM in the native fish fathead minnow (*Pimephales promelas*) embryos and larvae and zebrafish (*Danio rerio*) embryos, commonly used in toxicology studies. Static renewal toxicity tests were conducted in accordance with U.S. EPA and OECD guidelines. Mixture tests conducted with invertebrates utilized a toxic unit approach based on acute toxicity of each chemical alone, and mixture tests conducted with fish used specific ratios based on environmentally realistic concentrations. Endpoints measured included survival, growth, reproduction, and ecologically relevant behaviors (e.g., avoidance and foraging efficiency for fish). Finally, we used *in silico* approaches to estimate the binding affinity of TM and IM to the vertebrate nAChR in comparison to nicotine to better understand sublethal responses in fish.

Results and Discussion: We did not observe standard concentration-dependent mortality for invertebrates, but exposure to high concentrations of TM and IM increased mortality. Calculated LC/EC50 (lethal concentration/effective concentration for 50% of the individuals) values were well above concentrations of TM and IM typically found in streams and lakes. Amphipods were most sensitive to TM, while water fleas were most sensitive to IM. Reproduction of water fleas generally declined with increasing concentrations of TM and IM. Exposure to <6 μ g/L of TM did not impact total length of amphipods, but length of amphipods exposed to 25 μ g/L IM was significantly lower and growth was impaired over the course of the test. Mixture tests suggest that TM and IM act additively for invertebrates.

We observed no significant differences in survival for water fleas or amphipods. However, when we let the amphipod test continue beyond the standard duration (six days in clean water), survival was decreased in those exposed to the highest concentration of IM. We observed no significant effects of a mixture of TM and IM on reproduction of water fleas or growth of amphipods.

Chronic exposure to environmentally relevant concentrations of both TM and IM caused increased mortality by ~20% in fish larvae and caused subtle alterations in behaviors of survivors that could potentially impact their ability to reach adulthood. While computer modeling suggests that these insecticides can bind to the vertebrate nAChR, non-linear dose responses observed in our studies supports emerging findings that these compounds may cause toxicity in vertebrates through a different mode of action other than the nervous system - they may disrupt hormones like estrogen or thyroid hormones. Exposure to binary mixtures of TM and IM did not reveal a clear relationship with respect to their potential to act in an additive or synergistic fashion in fish, but we did observe slightly different toxic responses to mixtures compared to exposure to TM or IM singly.

Conclusions/Implications/Recommendations: Our laboratory data indicate that long-term impacts of neonicotinoid pesticides on aquatic communities warrant additional research. Exploration of potential modes of action for observed toxicity in vertebrates should be evaluated. We recommend increasing the observation period following exposure for invertebrates. In the case of *G. pseudolimnaeus* amphipods, this would require determining the best food for this species first. In the environment, neonicotinoid insecticides often enter streams and lakes in pulses. While we may not be able to capture short-term inputs of these chemicals, such short-term exposure may be enough to exert longer-term effects on survival, reproduction, growth and behavior of fish and aquatic invertebrates, and community-related impacts should be evaluated.

Current Publications and Awards:

S Victoria[†], M Hein^{*}, E Harrahy and TC King-Heiden. 2022. Potency matters: Impacts of embryonic exposure to nAChR agonists thiamethoxam and nicotine on hatching success, growth, and neurobehavior in larval zebrafish. J Toxicol Env Health, A. 85(18):767-782.

S Victoria[†], S Duffy^{*}, E Harrahy and TC King-Heiden. 2022. Embryonic exposure to thiamethoxam reduces survival and alters neurobehavior of fathead minnows. Env. Tox. and Chem. 41(5):1276-1285.

AJ Jeninga (MS student, UW-La Crosse) awarded Best Presentation Awards at (1) the Mississippi River Research Consortium Annual Meeting and (2) the Midwest Society of Environmental Toxicology and Chemistry annual meetings in 2022. S Victoria (MS, UW-La Crosse) won the best 3-min grad presentation (2021).

Key Words: Neonicotinoid, thiamethoxam, imidacloprid, fish, fathead minnow, zebrafish, aquatic invertebrate, water flea, chironomid, amphipod

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Final Report: A final report containing detailed information on this project is included in the <u>Groundwater Project Repository</u> of the Wisconsin Groundwater Research and Monitoring Program (WGRMP).

INTRODUCTION

Over the past two decades, neonicotinoid insecticides have become the most widely used insecticides in agriculture around the world (Simon-Delso et al., 2015; Casida and Durkin, 2013). At least 600 products registered for use on Wisconsin crops contain one or more neonicotinoid as active ingredients (DATCP, 2018; Kelly Products, Inc., 2017). Neonicotinoids function as neuroactive insecticides, over-stimulating nicotinic acetylcholine receptors (nAChR), leading to paralysis and death in target species. Since they have a relatively low affinity for mammalian nAChRs, they are considered a safer alternative to organophosphate, carbamate, and organochlorine pesticides (Simon-Delso et al., 2015; Tomizawa and Casida, 2005). Not surprisingly, non-target insects have been shown to be sensitive to exposure to low concentrations of these pesticides, and their unintended impacts on non-target species such as honeybees and other pollinators have garnered considerable attention (Goulson, 2013; Lundin et al., 2015). There is widespread concern regarding the potential large-scale risks they pose to ecosystem structure and function by impacting non-target species directly or indirectly by altering food webs (Chagnon et al., 2015; Gibbons et al., 2015). And while much is known about the toxicity of these insecticides to non-target terrestrial invertebrates (Goulson, 2013; Lundin et al., 2015), less is known about the toxicity of these pesticides to aquatic species (Anderson et al., 2015; Chagnon et al., 2015; Goulson, 2013).

Newly implemented monitoring programs suggest that aquatic organisms are at risk from exposure to neonicotinoid pesticides because their chemical properties make them susceptible to transport into surface waters via runoff and/or groundwater movement, and regular use enhances their persistence within the environment (Anderson et al., 2015; Armbrust and Peeler, 2002; Sánchez-Bayo et al., 2016; CCME, 2007; EFSA, 2008; Tisler et al., 2009). A study conducted by the Wisconsin Department of Agriculture, Trade and Consumer Protection (DATCP) recently detected neonicotinoids in surface waters and groundwater across the state, which were particularly high within the Central Sands Region (DATCP, 2018). Thiamethoxam, for which there are limited toxicity data available, was frequently detected, ranging in concentration from ~ $0.05 - 0.30 \mu g/L$ and concentrations of imidacloprid ranged from ~ $0.05 - 0.09 \mu g/L$ (DATCP, 2018). These concentrations exceed established U.S. EPA benchmarks for chronic exposure of invertebrates to imidacloprid, but not thiamethoxam ($0.01 \mu g/L$ and $0.74 \mu g/L$ (US EPA, 2017), although there is considerably less information about the toxicity of thiamethoxam to support these target concentrations (Anderson et al., 2015). Concentrations in this area also exceeded the ecological thresholds recommended by Morrissey et al. (2015) for chronic exposure ($0.035 \mu g/L$) and acute exposure ($0.2 \mu g/L$) of aquatic invertebrates to neonicotinoids.

Evaluating the potential ecological risks of these neonicotinoids within the Central Sands Region ecosystem will require careful examination of the effects of chronic exposure to sublethal concentrations on non-target aquatic species. Prolonged exposure to sublethal concentrations of neonicotinoids is of particular concern for invertebrates that have long aquatic life stages (Morrissey et al., 2015), and while less sensitive than invertebrates, there is evidence that these compounds can have both direct and indirect impacts on fish as well (Gibbons et al., 2015; DeCant and Barrett, 2010; Cox, 2001). Because more than one neonicotinoid may be found together in groundwater and in surface waters, it is also important to examine the potential for these insecticides to have additive or even synergistic effects when organisms are exposed to mixtures.

Given the limited amount of empirical data on sublethal effects of chronic exposure to thiamethoxam, as well as the impact of co-exposure to more than one neonicotinoid, this project examined the effects of chronic exposure to sublethal concentrations of neonicotinoid insecticides (thiamethoxam or imidacloprid, as well as mixtures of thiamethoxam + imidacloprid) to two aquatic invertebrates (*Ceriodaphnia dubia* and *Gammarus pseudolimnaeus*) and to a fish species (*Pimephales promelas*) found in aquatic ecosystems of the Wisconsin Central Sands Region and Wisconsin. Given the lack of toxicity

data for thiamethoxam, we also ran toxicity tests using another model fish (zebrafish, *Danio rerio*) for comparison. Acute toxicity tests were conducted with *Chironomus dilutus*, *Ceriodaphnia dubia*, and *Gammarus pseudolimnaeus* to determine LC50 (lethal concentration for 50% of the population) or EC50¹ (effective concentration for 50% of the population) values and to better determine concentrations to be used in the chronic toxicity tests conducted with these invertebrates. Acute exposure studies were not performed for fish since LC50s are predicted/known to be at an order of magnitude greater than measured environmental concentrations. Endpoints for chronic studies focused on sublethal responses that could contribute to population declines.

PROCEDURES AND METHODS

Preparation and Confirmation of Test Solutions: Thiamethoxam (TM) and imidacloprid (IM) were purchased from Sigma-Aldrich (>98% purity) and test solutions were made in the appropriate dilution water for each species (e.g., moderately hard reconstituted water for the invertebrates). Samples of test solutions were stored in amber glass bottles with Teflon lids and stored frozen until analysis. Concentrations were confirmed by high-performance liquid chromatography with tandem mass spectrometry (HPLC/MS/MS) performed by the Lumigen Instrument Center at Wayne State University. In all experiments, test solutions were 100% renewed each day, for the duration of exposure.

Aquatic Invertebrate Studies: Water fleas (*Ceriodaphnia dubia*) and midges (*Chironomus dilutus*) were cultured in the laboratory and amphipods (*Gammarus pseudolimnaeus*) were collected from Bluff Creek in Whitewater, WI. Culture and toxicity test methods followed standard guidelines (US EPA, 2002a; US EPA, 2002b; US EPA, 2002c; US EPA, 2000; Buikema and Cairns, 1980). All tests were conducted in environmental chambers with a 16 h light/8 h dark cycle and a temperature of 25°C (water fleas and chironomids) or 17°C (amphipods). Water quality parameters were measured daily in every test and included temperature, dissolved oxygen, conductivity and pH. Hardness and alkalinity were measured in the moderately hard reconstituted dilution water.

In the acute toxicity tests, organisms were exposed to high concentrations for short durations to determine the effects of TM or IM singly, or as a mixture, on survival. In the single insecticide toxicity tests, organisms were exposed to a control and seven or eight concentrations of thiamethoxam or imidacloprid using a 60% dilution series for 48 h (water fleas and chironomids) or 96 h (amphipods). Nominal concentrations of test solutions ranged from 56 to 2000 or from 28 to 1000 µg/L. In the mixture toxicity tests, organisms were exposed to a control, a TM alone treatment, an IM alone treatment, and four mixture treatments, using a toxic unit approach, where one toxic unit was equal to the nominal LC/EC50 value for TM or IM for the test species, based on results of the single insecticide acute toxicity tests. Three of the mixture treatments were ratios of the two insecticides that should have added up to a total of one toxic unit (e.g., 0.5 LC50 for TM plus 0.5 LC50 for IM), and one of the mixture treatments was equal to two toxic units (1.0 LC50 for TM plus 1.0 LC50 for IM). The TM alone and IM alone treatments were equal to the LC/EC50 value for that chemical, for the test species. The toxic unit study design can allow us to determine if two chemicals act in an additive, synergistic, or antagonistic manner (Hepditch et al., 2020). For example, if the combined effects of the two insecticides in a given treatment result in greater than 50% mortality (i.e., > one toxic unit), then the interaction may be synergistic (greater than additive). For all experiments, there were five water fleas (<24 h old) in each of four replicates, ten chironomids (2nd instar) in each of four replicates, or one amphipod (3-6 mm) in each of 20 replicates pertreatment. Mortality was used to calculate LC/EC50 values using the trimmed Spearman-Karber method (Hamilton

¹EC50 values are used for very small organisms like water fleas and midges where it might be difficult to determine death. Immobilization is used as an endpoint instead. We did examine all organisms under a microscope in these tests to confirm mortality, but follow standard reporting of an EC50.

et al. 1977), and analysis of variance (ANOVA) and Tukey's multiple comparison test were used to determine if there were significant differences in survival among treatments.

In the <u>chronic toxicity tests</u>, organisms were exposed as for acute studies to lower concentrations for longer durations (seven days for water fleas or ten days for amphipods) to determine the effects of TM or IM singly, or as a mixture, on reproduction of water fleas and growth of amphipods. In the water flea tests, there was one water flea in each of ten replicate glass beakers to better follow reproduction for each water flea. Nominal concentrations in the single insecticide tests ranged from 5.4 to 200 (water fleas) and 0.93 to 20 (60% dilution; amphipods) or 0.02 to 20 (10% dilution; amphipods), based on results of the acute toxicity tests. In the chronic mixture tests, a toxic unit was equal to 0.5 LC/EC50 instead of 1.0 LC/EC50 (based on nominal concentrations). Water fleas were fed YTC (yeast, cerophyl, trout chow) and algae (*Selenastrum capricornutum*), and amphipods were fed stream-conditioned leaves. Water flea reproduction was measured as number of neonates produced. All surviving amphipods were collected, preserved in 70% ethanol and photographed under a microscope. Image J software was used to measure total length as a measure of growth. ANOVA and Tukey's multiple comparison tests were used to determine if there were significant differences in survival, reproduction, or growth among treatments.

Fish Studies: Exposure concentrations for assessing the toxicity of TM or IM alone encompassed environmental concentrations found in the Central Sands Region of Wisconsin: 0.02, 0.02, 0.2, or 2 µg/L, as well as higher concentrations, 20 or 200 μ g/L. For mixtures studies, we used 1:1 ratios of IM:TM to assess potential additive responses or environmentally relevant ratios of 1:3, 1:4 or 1:5 IM:TM. Zebrafish embryos were exposed to TM beginning just after fertilization through larval development (5 days), while fathead minnows were exposed to TM for 8 days as embryos (beginning just after fertilization through larval development) or as larvae (beginning at post-hatch). Fathead minnow embryos were exposed to IM or mixtures of TM and IM for 8 days. In addition to monitoring survival and general health (hatching success and signs of malformations in developing fish), we assessed behaviors that rely on proper activation of the nAChR such as embryonic motor activity (spontaneous movement of the tail before the fish hatches from its chorion), the "C-start response" (an innate swimming response used to avoid predators), and/or foraging efficiency (timed ability to capture prey items). Finally, we used computer modeling software (Maestro, Schrödinger, LLC) to predict the strength/ability of both TM and IM to bind to a vertebrate nAChR in comparison to nicotine to better interpret our findings in fish. For all studies, we used Kaplain-Meier survival analysis to evaluate treatment-related effects on survival, and ANOVA and Tukey's post hoc or ANOVA on Ranks with Dunn's post hoc to evaluate treatment related impacts on growth and behavior. Chi Square analyses and linear regression analyses were also performed to gain additional insight into observed effects.

RESULTS AND DISCUSSION

Aquatic Invertebrate Studies: Results of the single insecticide acute toxicity tests are summarized in Table 1. Our results can be compared to those published in the literature and presented in Table 1 in Appendix B. We were able to calculate LC/EC50 values for both TM and IM, with the exception of *C. dilutus* midges for which we were unable to calculate an EC50 for IM. In that test, survival was significantly lower than the control, starting at a concentration of 390 μ g/L IM. In some of the acute toxicity tests, we observed non-monotonic dose responses, in which survival was higher for individuals exposed to higher concentrations than for those exposed to moderate concentrations. Raby et al. (2018c) and Raby et al. (2018a) also observed "poor concentration-response relationships" in examining the effects of imidacloprid and thiamethoxam on select aquatic invertebrates including *C. dilutus*. In some tests, there was high variability in response among individuals within a treatment, which led to broader 95% confidence intervals around the LC/EC50 values. And where tests were repeated, there was some

variability in LC/EC50 values calculated for the same chemical for the same species, even when actual (versus nominal) concentrations were taken into account.

Table 1. Impacts of	acute exposure to thiamethoxam or	r imidacloprid on survival of a	aquatic invertebrates.			
LC50 (lethal concent	tration for 50% of the population; amp	phipods) or EC50 (effective con	centration for 50% of			
the population; midg	es and water fleas) values and 95% co	onfidence intervals were calcula	ated using a trimmed			
Spearman-Karber me	ethod following 48 hours (midges and	l water fleas) or 96 hours (amph	ipods) exposure.			
Chemical Species LC50/EC50 95% C						
	_	μg/L	μg/L			
Thiamethoxam	Chironomus dilutus	134	107 - 168			
		373	304 - 458			
<i>Ceriodaphnia dubia</i> 146 1.36 - >1,000						
223 155 - 3						
Gammarus pseudolimnaeus48.825.3 - 9						
		127	74.8 - 215			
Imidacloprid	Chironomus dilutus	EC50 not calculable.				
_		Survival sig < control at				
		390				
	Ceriodaphnia dubia	31.7	8.77 - 114			
		EC50 not				
		calculable.				
		Survival sig < control at				
		98.8				
	Gammarus pseudolimnaeus	189	120 - 298			
	-	145	112 - 188			

Our 48 h EC50 values for *C. dubia* water fleas exposed to IM (31.7 μ g/L) and TM (146 and 223 μ g/L) were significantly lower than those found in the literature for IM, while our 48 h EC50 values for *C. dilutus* exposed to TM (134 and 373 μ g/L) were significantly higher (less sensitive) than those reported in the literature (see Table 1, Appendix B). We were unable to calculate a 48 h EC50 value for *C. dilutus* exposed to IM because survival remained at 60% or higher, even at the highest concentration tested. However, survival was significantly reduced, compared to the control, at a concentration of 390 μ g/L. Many of the chironomids exposed to IM and TM in our test were immobilized (barely moving under the microscope), or impaired (exhibited muscle spasms). In the wild, immobilized or impaired individuals may be more likely to die through inability to forage or eat, increased drift and increased predation. Relative sensitivity among the species differed by chemical. *G. pseudolimnaeus* amphipods were most sensitive to TM, while *C. dubia* water fleas were most sensitive to IM. This is in contrast to studies in the literature that have shown water fleas to be less sensitive than amphipods to IM (Raby et al., 2018a; Finnegan et al., 2017). However, to the best of our knowledge, we are the first to examine the effects of neonicotinoid insecticides on *G. pseudolimnaeus*, and less is known about the sensitivity of this species of amphipod.

LC/EC50 values were all well above concentrations typically found in surface waters (Metcalfe et al., 2019; Hladik and Kolpin, 2016) and groundwater (Bradford et al., 2018). But having the acute toxicity data was useful in determining the best concentrations to use in the single chemical chronic tests and in the acute and chronic mixture tests. These data can also be useful in the case of a spill, and in calculating water quality criteria and secondary values.

Results of the <u>single insecticide chronic toxicity tests</u> are summarized in Figure 1. The number of neonates produced by the water fleas generally declined with increasing concentrations of IM and TM. Reproduction was significantly different from the control at 175 μ g/L IM and 82 μ g/L TM. These are much lower effect concentrations than those determined by others (Table 1, Appendix B). The decrease in reproduction was not significant until the concentration of IM was higher than the EC50 value for lethality (31.7 μ g/L), but the lack of significance at lower concentrations may be due to variability within a treatment. There seems to be a good deal of intraspecific variability in sensitivity. In addition, the dose response curve in the acute test for water fleas exposed to IM was atypical, with higher survival at higher concentrations than at moderate concentrations. There was no significant difference in total length among amphipods exposed to seven concentration for that treatment was only 6.0 μ g/L, and this may not have been high enough to elicit an effect. Total length of amphipods exposed to 25 μ g/L IM (nominal of 20 μ g/L) was significantly lower than length of amphipods in the control, and amphipods in this treatment did not significantly grow over the course of the 10-d test compared to an initial sample. There was no significant difference in length of amphipods exposed to an initial sample. There was no significant difference in length of amphipods in the control, and amphipods in this



Figure 1. Effects of chronic exposure to thiamethoxam or imidacloprid on reproduction and growth of aquatic invertebrates. Effects of the neonicotinoid insecticides thiamethoxam or imidacloprid on reproduction (as number of neonates) of *Ceriodaphnia dubia* water fleas (A and C, respectively) and growth (as length) of *Gammarus pseudolimnaeus* amphipods (B and D, respectively). Duration of exposure was 7 days (water fleas) or 10 days (amphipods). Endpoints were compared among treatments using analysis of variance and Tukey's multiple comparison procedure. Treatments with the same letter are not significantly different from each other. Amphipods in treatments with an "*" had an average length that was significantly greaterthan those in the initial sample. Error bars are +1 standard error.

While G. pseudolimnaeus is an ecologically and locally important species, it proved difficult to culture in the laboratory. Survival of our wild caught amphipods did not allow for longer tests (survival of the control amphipods declined below 80% between ten and 14 days, likely because they were not getting enough nutrition). It is possible we would have seen greater differences in length among treatments, and in overall growth compared to initial size had we run the tests for a longer duration, but such studies would require improved culturing techniques or use of other species that are easier to maintain in the lab (e.g., Hyalella azteca; Bartlett et al., 2019). Feeding studies also proved difficult for this species. While most amphipods are considered to be shredders, and other researchers have been able to conduct longterm studies that examined the effects of neonicotinoid insecticides on feeding of leaves (e.g., Nyman et al., 2013), we observed a high level of intraspecific variability in feeding in our G. pseudolimnaeus. Some individuals consumed the leaves we provided them, and other individuals consumed only portions or none of the leaves. In fact, we ran a test to compare their preference for four different species of leaves collected from the same stream from which we obtained the amphipods: cut-leaf water parsnip (Berula erecta), watercress (Nasturium officinale), curly-leaf pondweed (Potamogeton crispus) and Canadian waterweed (Canadian waterweed). While each leaf species was eaten by some individuals, the cut-leaf water parsnip, seemed to be favored (unpublished data). This was not surprising, given we find more of the amphipods living on and around this plant species in the stream. We also tried feeding the amphipods sugar maple leaves that had been stream-conditioned in Bluff Creek for 30 days in leaf packbags. Regardless, no one leaf species was regularly consumed by all individuals. This limited our ability to conduct studies designed to determine the effects of TM and IM on feeding. An initial feeding study conducted with amphipods exposed to IM in their water while being fed stream-conditioned maple leaves indicated a potential dose response in feeding rate (those exposed to higher concentrations ate fewer leaves), but the difference was not significant due to high variability among individuals within a treatment. Gammarus spp. may also eat the biofilm growing on the leaves before (Bottger et al., 2013), or instead of, shredding the leaves, which can be harder to quantify. Taken together, our work suggests studies to develop standardized rearing and feeding of this ecologically important species may prove worthwhile.

Results of the acute toxicity tests conducted with a mixture of TM and IM are presented in Figure 2. Since TM and IM target the same receptor and have the same predicted mode of toxicity, we would predict that mixtures of these two neonicotinoids would cause additive toxicity (the effects of each sum together, like 2+2=4) versus greater than additive toxicity (synergism; the sum of the effects is greater than when only one is present, like 2+2=6) or less than additive toxicity (antagonism; the sum of the effects is less than when only one is present, like 2+2=1). When acute tests were run for the standard duration (two days for the water fleas and four days for the amphipods), we observed no significant differences in survival among treatments (control, single insecticides and four different mixtures) for water fleas or amphipods, although again, there was significant variability in survival within treatments that may have affected these results. For example, survival was 100% for water fleas in the control, and only 55% for water fleas in one of the mixture treatments, but these were not significantly different (ANOVA, Tukeys, p<0.05). However, when we let the amphipod mixture test continue beyond the standard duration of four days to ten days (four days exposure followed by six days in clean water), we observed significant decreases in survival among some of the treatments, while the control survival remained at 100%. Delayed effects have been observed in other studies (e.g., Bottger et al., 2013). In the present study, survival was lowest where concentrations of IM were highest, so IM may, at first, appear to account for a higher proportion of the observed mortality than TM. However, actual concentrations of IM were closer to nominal, while concentrations of TM were only about half of nominal. So, using the toxic unit approach, we would expect the treatments with higher proportions of IM to be more toxic in this case.

In the <u>chronic toxicity tests conducted with a mixture of TM and IM</u>, we observed no significant effects on reproduction of water fleas or growth of amphipods. It is possible that effects on growth of amphipods



may have been observed if the test had been conducted for a longer duration of exposure, or incorporated a post-exposure observation period as observed by Cavallaro et al. (2017) where the longer the duration of exposure, the lower the effective concentration.

Overall, our findings suggest that mixtures of TM and IM cause additive toxicity in the aquatic invertebrates we tested, at the concentrations we tested. We did not observe greater-than-additive action of IM and TM mixtures in our acute or chronic mixture tests. Few studies have been conducted that have examined the effects of mixtures of two or more neonicotinoid insecticides on aquatic invertebrates. Those that have been conducted have indicated that mixtures of neonicotinoid insecticides do not always act in an additive manner. Maloney et al. studied the acute (2017) and chronic (2018b) toxicity of binary and ternary mixtures of clothianidin, IM, and TM in *C. dilutus*, and used a MIXTOX modeling approach to test the assumption of concentration additive cumulative toxicity. In their acute tests, they determined that mixtures of IM and TM exhibited a "response-additive dose-ratio-dependent synergism," with toxicity shifting from antagonism to synergism as the relative concentration of TM increased. In their chronic tests, they also determined that a mixture of IM and TM displayed a "dose-ratio dependent

synergism," in which emergence of *C. dilutus* was reduced to a higher degree than would be predicted by concentration addition alone, but only when mixtures contained higher concentrations of TM than IM. However, in a semi-controlled field setting using limnocorrals, Maloney et al. (2018a) found that emergence and biomass responses of *C. dilutus* to a mixture of IM and TM were strictly additive.

Fish Studies: Summative information regarding the toxicity of thiamethoxam and imidacloprid singly and in combination are summarized in Table 2.

Table 2: Summ imidacloprid.	ary of toxicity obs	erved in fish follow	ing chronic exposu	ire to thiamethox	am and
Species & age at exposure	Mortality	Impaired Hatching	Embryonic Motor Activity	Predator Escape	Foraging Efficiency
		Thiamet	hoxam	1	1
Fathead minnow embryo	≥ 1.6 µg/L ~20% increase	no effect obs; >155 μg/L	- reduced 155 μ g/L -25% of fish exposed to \geq 1.6 μ g/L	- delayed larvae exposed to $\geq 0.16 \ \mu g/L$ - $\sim 25-58\%$ of fish decreased burst speed	-Slight reduction in 40-50% of all exposed fish
Fathead minnow larvae	no effect obs; >155 μg/L	no effect obs; >155 μg/L	no effect obs; >155 μg/L	no effect obs; >155µg/L	no effect obs; >155µg/L
Zebrafish embryo	163 μg/L	0.21; 163 μg/L	no effect obs; >163 μg/L	-0.21 µg/L delayed response and burst speed	no effect obs; >163 µg/L
		Imidacl	oprid		
Fathead minnow embryo	≥ 1.6 µg/L 10-40% increase	≥ 1.6 µg/L	Reduced ≥ 1.6 µg/L	~42% exposed to ≥ 0.02 µg/L had delayed response -174 µg/L decreased burst speed	Not assessed
	Mixt	ures of thiamethox	am and imidaclop	rid	
Fathead minnow embryo 1:1 IM:TM	≥ 0.2 µg/L 1:1 10-30% increase	≥ 20 μg/L 1:1 Increase in hatching	Reduced ≥ 0.2 µg/L 1:1	No observed effect	Not assessed
Fathead minnow embryo 1:3, 1:4, or 1:5 IM:TM	No observed effect	No observed effect	No observed effect	1:5 ratio delayed	Not assessed

Computer modeling predicts that, as expected, neither thiamethoxam nor imidacloprid are capable of binding the nAChR with sufficient affinity to cause overt toxicity (Figure 3), which is supported by our experimental findings as described below.



nicotine for the vertebrate nAChR. Computer models indicate that both imidacloprid and thiamethoxam are capable of binding to the vertebrate nAChR; however, based upon docking and glide scores, the affinity is much lower as predicted (the more negative these scores, the stronger the molecule binds the receptor). Imidacloprid may have a slightly higher binding affinity for the nAChR than thiamethoxam.

For fish, we found that TM was not toxic to fathead minnow larvae if exposure began as larvae (posthatch), but did cause adverse health and behavioral outcomes if exposure began as embryos (just after fertilization) (Figure 4). Further, fathead minnows are more sensitive to TM compared to zebrafish larvae. We were surprised to see increased mortality for both fathead minnow and zebrafish following chronic exposure to concentrations of TM that are an order of magnitude lower than reported LC50s for other fish species. Chronic exposure to TM induced subtle alterations in behaviors (embryonic motor activities and predator escape responses) in both fathead minnows and zebrafish, indicative that TM may bind the nAChR in vertebrates with a low affinity; this is supported by our computer modeling. Further studies would be needed to clarify NOEC or LOEC values for thiamethoxam, but using a risk quotient, calculated using concentrations detected in WI, and our NOEC of 14.6 μ g TM/L for behavioral alterations in fathead minnows, chronic exposure to surface waters with concentrations below 1.5 μ g TM/L would pose low risk, concentrations between 1.5-15 μ g TM/L would pose medium risk and concentrations >15 μ g TM/L would pose high risk with respect to altering behaviors that are essential for survival. Our findings also support the need to further test the hypothesis that TM may be able to interfere with thyroid hormone or estrogen hormone regulation of early development (Zhu et al. 2019).



Figure 4. Effects of chronic exposure to thiamethoxam in fish. TM reduces survival by approximately 20% in both fathead minnows and zebrafish when exposure begins at early development, but TM is not toxic to fathead minnows if exposed post-hatch (A). Embryonic exposure to TM has a larger impact on behavior in fathead minnows (B) but has more general adverse health outcomes in zebrafish (C). Taken together, chronic exposure to TM poses low to medium risk in fish. Letters denote significant differences, and proportions (%) in bold are significantly different from control groups.

Imidacloprid appears to be slightly more toxic to fathead minnow larvae than thiamethoxam, which may be due to its potential to bind the nAChR at a greater affinity. Chronic exposure to IM beginning just after fertilization reduced survival by 20-40%, impaired growth by 7-24%, and altered embryonic motor activities (Figure 5). Interestingly, IM did not impair the predator escape response other than slowing the overall swimming speed following exposure to very high concentrations (174 μ g IM/L). Further studies would be needed to clarify NOEC or LOEC values for imidacloprid, using our NOEC of 0.16 μ g IM/L for mortality and reduced growth in fathead minnows to calculate a risk quotient, chronic exposure to surface waters with concentrations below 0.016 μ g TM/L would pose low risk, concentrations between 0.016-0.16 μ g IM/L would pose medium risk and concentrations 0.16 > μ g IM/L would pose high risk to wild fish.



When fish were exposed to mixtures of imidacloprid and thiamethoxam, imidacloprid appeared to drive the overall toxicity. Imidacloprid and thiamethoxam do not appear to cause additive toxicity in fish (Figure 6); however, there were some subtle differences in toxic response of IM when TM was present at equivalent nominal concentrations (confirmation of exposure concentrations are in progress). For example, when exposed to IM alone, significant mortality was observed following chronic exposure to concentrations \geq 0.16 µg IM/L, but when IM and TM were present in equivalent concentrations, significant mortality was observed following exposure to a lower concentration of IM when TM was present at an equivalent nominal concentration. Our computer modeling suggests that IM and TM may interact with slightly different residues on the nAChR, indicating that observed toxicities following exposure to mixtures of IM and TM could be explained by potential competitive interactions between the two molecules and the nAChR. Further, since it is possible that TM may cause its toxicity in fish through a different mode of action all together, that could also support the lack of any observed additive responses observed in these studies. Taken together, our data indicate that mixtures of TM and IM do not pose an

increased direct risk to wild fish populations compared to each compound together, especially at the environmentally relevant mixtures observed in the Central Sands Region of WI.



approximate mixtures of $\geq 0.2 \ \mu g \ IM/L$ and 0.2 $\ \mu g \ TM/L$ resulted in increased embryonic motor activity (C, D). Blue bars indicate data that was significantly different from control. The predator escape response was not altered.

CONCLUSIONS AND RECOMMENDATIONS

We examined the effects of two neonicotinoid insecticides (IM and TM) on three species of aquatic invertebrates and two species of fish: *Ceriodaphnia dubia* (water fleas), *Chironomus dilutus* (midges; single chemical acute tests only), *Pimephales promelas* (fathead minnow) and *Danio rerio* (zebrafish); we are the first to report acute and chronic effects of neonicotinoids on *G. pseudolimnaeus*, important in the structure (as prey) and function (organic matter processing) of local aquatic ecosystems. In some cases, acute and chronic exposure to IM and TM individually, and in combination, resulted in decreased survival, reproduction, and growth, as well as changes in ecologically-relevant behaviors in both aquatic invertebrates and fish. In addition, we used *in silico* computer modeling approaches to estimate the binding affinity of TM and IM to the vertebrate nAChR (nicotinic acetylcholine receptor) to better

understand sublethal responses in fish. We saw no evidence in any of the mixture studies that TM and IM together acted in any way other than additively for invertebrates. Mixtures likely present higher risk for aquatic invertebrates compared to fish.

In terms of survival, invertebrates (as expected) were more sensitive than fish. Invertebrate sensitivity varied with insecticide, but little is understood about the underlying reasons for species sensitivities towards neonicotinoids. Potential mechanisms could include differences in binding affinities for the nACh receptor, differences in ventilation, uptake and bioaccumulation, oxidative stress, and enzymes used in detoxification and elimination (Maloney et al., 2021; Wei et al., 2020; Chandran et al., 2018; Azevedo-Peeira et al., 2011).

In general, effect concentrations were higher than concentrations typically found in the environment. However, fish and crustaceans such as water fleas and amphipods tend to be less sensitive. Recent studies have shown mayflies are among the most sensitive (to neonicotinoids) species, and we recommend including them in future studies. Most mayflies cannot be cultured in the laboratory, but *Neocloeon triangulifer* is parthenogenetic and has been shown to be a useful model species (Chou et al., 2017).

While the present study and other studies have shown water fleas to be less sensitive to neonicotinoid insecticides than other aquatic invertebrates such as mayflies, they remain important test organisms given their role in calculation of water quality criteria and secondary values. In Wisconsin, a secondary value may be calculated for the protection of aquatic life from a toxic chemical when database requirements necessary to calculate a water quality criterion have not been fulfilled. A secondary value cannot be calculated without acute data for one of three genera of water fleas, including *Ceriodaphnia* sp. (Wis. Admin. Code Ch. NR 105). A longer-term goal might be to amend data requirements for calculation of secondary values to include more sensitive species such as mayflies. This could ultimately lead to better protection of fish and aquatic life.

Future studies designed to examine the toxicity of neonicotinoids should be run for a longer duration than required by standard protocols to allow delayed effects to be observed. Delayed toxicity is not taken into account by standard methods used for regulatory purposes, which means toxicity and risk may be underestimated (Li et al., 2021).

Future studies should also consider pulse exposures. In our study, organisms were subjected to constant exposures. In the environment, neonicotinoid insecticides often enter streams and lakes in pulses. While we may not be able to capture short-term inputs of these chemicals, such short-term, repeated exposures may be enough to exert longer-term effects on survival and health of longer-lived aquatic invertebrates and fish, especially if there is carry-over toxicity (Li et al., 2021; Raby et al., 2018b).

Our studies were all conducted in the laboratory (some with field-caught organisms). And while this allows better control of variables, it lacks environmental realism in terms of presence of other species, other chemicals, sediment, etc. Future studies should include a field biomonitoring component to determine if lab test results are predictive of effects in the field. And while measurements of insecticide concentrations in surface water and groundwater offer a snapshot in time, bioaccumulation and various aquatic invertebrate biomonitoring endpoints can integrate effects over the long-term. For example, *Gammarus pulex* amphipods have been shown to bioaccumulate neonicotinoid insecticides, including IM and TM in agricultural streams (Shahid et al., 2018). And while neonicotinoid pesticides were designed to target the nAChR, growing evidence suggests that they may have a different mode of action in fish (e.g., endocrine disruption) which warrant further study. Finally, impacts on survival, growth, reproduction, and behavior of aquatic invertebrates and fish could have cascading effects on the structure and function of aquatic ecosystem.

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APPENDIX A

PUBLICATIONS (*undergraduate student; [†]Master of Science graduate student)

S Victoria[†], M Hein^{*}, E Harrahy and TC King-Heiden. 2022. Potency matters: Impacts of embryonic exposure to nAChR agonists thiamethoxam and nicotine on hatching success, growth, and neurobehavior in larval zebrafish. J Toxicol Env Health, A. 85(18):767-782. https://pubmed.ncbi.nlm.nih.gov/35650526/

S Victoria[†], S Duffy^{*}, E Harrahy and TC King-Heiden. 2022. Embryonic exposure to thiamethoxam reduces survival and alters neurobehavior of fathead minnows. Env. Tox. and Chem. 41(5):1276-1285. https://setac.onlinelibrary.wiley.com/doi/abs/10.1002/etc.5301

PRESENTATIONS AND AWARDS (Presenting author underlined; *undergraduate student; [†]Master of Science graduate student)

International/Global Research Conferences

<u>A Jeninga</u>[†], S Victoria[†], S Duffy^{*}, Z Wallace^{*}, E Harrahy, and TC King-Heiden. 2022. You act differently around your friends: The developmental and neurobehavioral toxicities of imidacloprid and thiamethoxam change during binary exposures. SETAC-NA² Annual meeting, Pittsburg, PA ~2000 attendees

<u>TC King-Heiden</u>, S Victoria[†], S Duffy^{*}, M Hein^{*}, and E Harrahy. 2022. Incorporating ecologically relevant behaviors, age at exposure, and two species in assessing the toxicity of thiamethoxam in fish. SETAC-NA Annual meeting, Pittsburg, PA ~2000 attendees

<u>S (Michel) Victoria[†]</u>, M. Hein*, E Harrahy, and TC King-Heiden. 2021. Effects of chronic thiamethoxam exposure on fathead minnow larvae. SETAC- NA virtual meeting. ~2000 attendees

Midwest Regional Research Conferences

<u>A Jeninga</u>[†], S Duffy^{*}, E Harrahy, and TC King-Heiden. 2022. Toxicity of single and binary exposures of the neonicotinoids thiamethoxam and imidacloprid on *Pimephales promelas*. Mississippi River Research Consortium Annual Meeting ~300 attendees *Awarded Best Student Poster Presentation*

<u>A Jeninga</u>[†], S Duffy*, E Harrahy, and TC King-Heiden. 2022. Do the neonicotinoids thiamethoxam and imidacloprid exhibit additive toxicity in *Pimephales promelas* larvae? MRC-SETAC³ annual meeting ~100 attendees. *Awarded Best Student Poster Presentation*

<u>S (Michel) Victoria</u>[†], M Hein*, A Helgeson*, S Duffy*, E Harrahy, and TC King-Heiden. 2021. Adverse effects of chronic exposure to thiamethoxam in zebrafish and fathead minnow embryos. Joint meeting between MRC-SETAC and Northland Society of Toxicology. ~100 attendees

² SETAC-NA: Society of Environmental Toxicology and Chemistry-North America

³ MRC-SETAC: Midwest Regional Chapter of SETAC

<u>J Lacki</u>*, A Draper*, A Jeninga*, TC King-Heiden and E Harrahy. 2021. Acute and chronic toxicity of the neonicotinoid insecticides thiamethoxam and imidacloprid to select aquatic invertebrates. Platform presentation. MRC-SETAC annual meeting with Northland Society of Toxicology, April 29. Virtual. ~100 attendees

<u>S (Michel) Victoria</u>[†], S Duffy^{*}, T deDianous^{*}, E Harrahy, and TC King-Heiden. 2021. Local Fish Species exposed to the pesticide, thiamethoxam, exhibits changes to survival, embryonic motor activity, and predator escape in early life stages. Mississippi River Research Consortium. Virtual \sim 300 attendees

<u>T King-Heiden</u>. 2021. It's a matter of time: Delayed toxic responses in fathead minnow larvae following chronic exposure to environmentally relevant concentrations of thiamethoxam. Puget Sound Toxics Workshop, WA. Virtual ~50 attendees

<u>E Harrahy</u>, A Draper*, J Lacki*, A Jeninga*, TC King-Heiden. 2021. Acute and chronic toxicity of the neonicotinoid insecticides thiamethoxam and imidacloprid to select aquatic invertebrates. Platform presentation. Wisconsin Chapter of the American Water Resources Association Annual Meeting, March 3-4. Virtual. ~100 attendees

Presentations to Community Members/Outreach Presentations

Note: Due to the COVID-19 pandemic, several planned outreach presentations were cancelled

<u>S Duffy</u>*, S Victoria[†], E Harrahy, and TC King-Heiden. 2022. Secret life of fish: bee-harming pesticide makes fish larvae susceptible to predators. Research on the Rotunda, WI State Capitol, Madison, March 9. Poster presentation to various UW system administrators, state legislators, and the public. ~200 attendees

<u>F Fisher</u>*, A Draper*, J. Lacki*, A Herrmann*, A. Jeninga*, TC King-Heiden, and E Harrahy. 2022. Effects of a mixture of two neonicotinoid insecticides on reproduction of water fleas and growth of amphipods. Research in the Rotunda, WI State Capitol, Madison, March 9. Poster presentation to various UW system administrators, state legislators, and the public. ~200 attendees

Presentations at Undergraduate Research Conferences

<u>F Fisher</u>*, J Lacki*, A Herrmann*, TC King-Heiden, and E Harrahy. 2022. Effects of a mixture of two neonicotinoid insecticides on reproduction of water fleas and growth of amphipods. Poster presentation. UW System Symposium for Undergraduate Research, UW-Whitewater, April 22. ~400 attendees

<u>J Lacki</u>*, A Draper*, A Jeninga*, TC King-Heiden, and E Harrahy. 2021. Acute and chronic toxicity of the neonicotinoid insecticides thiamethoxam and imidacloprid to select aquatic invertebrates. Poster presentation. UW System Symposium for Undergraduate Research, UW- Whitewater, April 28. Virtual. ~400 attendees

<u>A Draper</u>*, J Lacki*, TC King-Heiden, and E Harrahy. 2020. Effects of two neonicotinoid insecticides on survival and growth of *Gammarus pseudolimnaeus* amphipods. Poster presentation. Fall Undergraduate Research Day, UW-Whitewater, November 11. Virtual. ~200 attendees

<u>J Lacki</u>*, A Draper*, TC King-Heiden, and E Harrahy. 2020. Acute and chronic toxicity of neonicotinoid insecticides thiamethoxam and imidacloprid to the water flea, *Ceriodaphnia dubia*. Poster presentation. Fall Undergraduate Research Day, UW-Whitewater, November 11. Virtual. ~200 attendees

<u>A Jeninga</u>*, A Draper*, TC King-Heiden, and E Harrahy. 2020. Acute and chronic toxicity of the insecticide thiamethoxam to select aquatic macroinvertebrates. Poster presentation. Spring Undergraduate Research Day, UW-Whitewater, May 1. Virtual. ~200 attendees

A Jeninga*, A Draper*, TC King-Heiden, and E Harrahy. 2019. Acute and chronic toxicity of the insecticide thiamethoxam to select aquatic macroinvertebrates. Poster presentation. Fall Undergraduate Research Day, UW- Whitewater, November 13. ~200 attendees

STUDENTS FUNDED OR SUPPORTED BY GRANT AND CURRENT AFFILIATIONS

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- Megan Hein (UWL), meganhein98@gmail.com, graduated May 2021; Marquette PA program
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Impact of the Work

Insecticides, both natural and synthetic, have become an integral component of our agricultural industry allowing food growers to increase crop yields while keeping the costs of our food down. It is important that we balance the need for using insecticides with their potential risks to wildlife and human health. Neonicotinoid insecticides are uniquely designed to reduce risks to some non-target species by specifically targeting the nicotinic acetylcholine receptors (nAChR) in insects, which has been shown to reduce their toxicity to vertebrate species such as fish. In addition, they are frequently applied as a seed coating instead of as a spray to reduce environmental contamination. Unfortunately, their widespread use has resulted in their presence in aquatic ecosystems in Wisconsin, posing a potential risk for aquatic invertebrates and fish living in our streams, rivers, and lakes, especially in the Central Sands Region. For this reason, we sought to better understand their effects on aquatic invertebrates and fish. Our work shows that the two most prevalent neonicotinoids found in Wisconsin's waters, thiamethoxam and imidacloprid,

do cause toxicity in aquatic invertebrates and fish, impacting survival, growth, and various ecologically relevant behaviors. Further, these insecticides are typically found as mixtures in the environment, and our work indicates that for the invertebrate species we tested, their combined toxicity is additive, while for the fish species we tested, we saw no evidence for additive toxicity. Our data can be used to help develop use restrictions where necessary to maintain water quality. Future avenues of research, including determining longer-term effects of cumulative short-term exposures to important, local species will allow a better understanding of potential cascading effects of neonicotinoids on Wisconsin's aquatic ecosystems.

Potential use for management:

- Since the toxicity of a mixture of thiamethoxam and imidacloprid appears to be additive for invertebrates, it may be important to consider total concentrations of neonicotinoid pesticides in deriving criteria, much the same as has been done for atrazine and its metabolites in groundwater (Chapter NR 140, Wisconsin Administrative Code).
- Relative sensitivities of exposure to thiamethoxam and imidacloprid varied among species. In general, water flea species are less sensitive to neonicotinoids than other aquatic invertebrates such as mayflies (based on data from the literature). However, in Wisconsin, a secondary value cannot be calculated for the protection of aquatic life without acute data for one of three genera of water fleas, including *Ceriodaphnia* sp. (Wis. Admin. Code Ch. NR 105). A longer-term goal might be to amend data requirements for calculation of secondary values to include more sensitive species such as mayflies. This could ultimately lead to better protection of fish and aquatic life.
- While concentrations shown to be lethal to invertebrates in this study were orders of magnitude higher than concentrations detected in surface waters in WI, it should be noted that these were shorter-term exposures. Future studies and management decisions should take into account potential longer-term cumulative effects of repeated pulse exposures.
- Incorporating earlier age-at exposure (beginning exposure pre-hatch) for toxicity tests in standardized fish toxicity assays may better predict the potential risks that neonicotinoids pose to wild fish populations.
- Incorporating behavioral endpoints that are essential for survival and recruitment of wild fish populations could also enhance our understanding of the risks that environmental contaminants pose to wild fish populations, particularly if the target mode of action for a contaminant is part of the nervous system.

APPENDIX B

Table 1. Acute and chronic toxicity estimates for imidacloprid and thiamethoxam, for water fleas, amphipods, chironomids and mayflies. Note: This table is not meant to be exhaustive.

Neonic	Species	Endpoint	LC/EC50/	Estimate	Citation
	-		NOEC/NOEC/	μg/L	
			Duration	unless noted	
IMIDA Water I	CLOPRID: ACU	UTE			
water f	leas	C 1	1.050	0.420	D -1
IMD	Ceriodaphnia dubia	Survival	6 d	8,420 (5360-11480)	Raby et al. 2018c
IMD	Ceriodaphnia	Survival	EC50	571.62	Hayasaka
	dubia		48 h	(289.63- 841.19)	et al. 2013
IMD	Ceriodaphnia	Survival	LC50	72,124.9	Raby et al.
	dubia		48 h	(51,000.0, 102,000.0)	2018a
IMD	Daphnia	Survival	LC50	35,440	Raby et al.
	magna		21 d	(22,780-	2018c
				48,090)	
IMD	Daphnia	Survival	LC50	8,470	Li et al.
	magna		96 h	(6,070-	2021
				11,800)	
IMD	Daphnia	Survival	LC50	85,000	In Jamec et
	magna		48 h		al. 2007
IMD	Daphnia	Survival	LC50	10,400	In Jamec et
	magna	~	48 h		al. 2007
IMD	Daphnia	Survival	EC50	56,600	In Jamec et
	magna	~	48 h	100.000	al. 2007
IMD	Daphnia	Surv1val	LC50	>102,000	Raby et al.
A 11	magna		48 h		2018a
Amphip	ods		1.050	50.6	7 T
IMD	Hyalella	Survival		526	In Jamec et
	azteca		96 h		al. 2007
IMD	II	Courseis of 1	Juveniles	51.000	La Taura a at
IMD	Hyalella	Survival	LC30	51,800	Ih Jamec et
	aziecu		14-21 d old		al. 2007
IMD	Hyalolla	Survival		9/ 800	In Ismec et
	azteca	Survivar	1C30 96 h	94,000	$\frac{1}{2}$ $\frac{1}{2}$ $\frac{1}{2}$ $\frac{1}{2}$
	u2iecu		7-21 d old		al. 2007
IMD	Hyalella	Survival	LOLC	43,800	In Jamec et
	azteca		96 h 14-21 d old		al. 2007
IMD	Hyalella	Survival	LC50	230	Bartlett et
	azteca		7 d		al. 2019
IMD	Hyalella	Survival	LC50	90	Bartlett et
	azteca		28 d		al.

					2019	
IMD	Hvalella	Survival	LC50	65.43	Stoughton	
	azteca		96 h	(39.78,	et al. 2008	
				107.62)		
IMD	Hvalella	Survival	LC50	363.2	Raby et al.	
	azteca		96 h	(301.3, 425.1)	2018a	
IMD	Gammarus	Survival	EC50	18	Van Den	
	nulex		96 h	(8.8. 38)	Brink	
	1			()	et al. 2016	
IMD	Gammarus	Survival	EC50	49	Van Den	
	nulex		96 h	(29, 81)	Brink	
	<i>r</i>				et al. 2016	
IMD	Gammarus	Survival	LC50	263	Roessink et	
11112	pulex	2	96 h	(155-446)	al. 2013	
IMD	Gammarus	Survival	LC50	33.8	Roessink et	
11112	nulex	2	28 d	(20.9, 54.6)	al. 2013	
IMD	Gammarus	Survival	EC50	14.2	Bottger et	
min	roeseli	Survivui	96 h	1 112	al. 2012	
Chiron	omids				un 2012	
IMD	Chironomus	Survival	LC50	>1 43	Raby et al	
IIIID	dilutus	Survivui	14 d	1115	2018b	
IMD	Chironomus	Survival	LC50	11.8	Raby et al	
IIIID	dilutus	Survivui	96 h	(8.3, 15.4)	2018a	
IMD	Chironomus	Survival	LC50	4 63	Malonev et	
mil	dilutus	Survivui	96 h	1.05	al. 2017	
IMD	Chironomus	Survival	LC50	1.52	Cavallaro	
	dilutus	2	14 d	(0.99, 1.82)	et al. 2017	
IMD	Chironomus	Survival	LC50	31.5	Chandran	
	riparius		24 h	(15.1, 75.9)	et al. 2018	
IMD	Chironomus	Survival	LC50	2.33	Chandran	
	riparius		10 d	(1.30, 4.41)	et al.	
					2018	
IMD	Chironomus	Survival	LC50	10.5	In Jamec et	
	tentans		96 h		al. 2007	
			2 nd instar			
IMD	Chironomus	Survival	LOLC	3.39	In Jamec et	
	tentans		96 h		al. 2007	
			2 nd instar			
IMD	Chironomus	Survival	LC50	5.75	Stoughton	
	tentans		96 h	(4.10, 8.08)	et al. 2008	
Mayflies						
IMD	Cloeon sp.	Survival	LC50	1,152.0	Raby et al.	
	1		96 h	(5,13.1,	2018a	
				1,790.8)		
IMD	Cloeon	Survival	LC50	26.3	Roessink et	
	dipterum		96 h	(17.7, 39.1)	al. 2013	
IMD	Cloeon	Survival	LC50	0.195	Roessink et	
	dipterum		28 d	(0.113, 0.338)	al. 2013	
IMD	Neocloeon	Survival	LC50	5.2	Raby et al.	
	triangulifer		96 h	(4.2, 6.2)	2018a	

IMD	Isonychia	Survival	LC50	18.77	Camp and
	bicolor		96 h (@15oC)		Buchwalter 2016
IMD	Deleatidium	Survival	LC50	0.28	Macaulay
	spp.		28 d	(0.21, 0.36)	et al. 2019
IMD	<i>Hexagenia</i> sp.	Survival	LC50	900	Bartlett et
			96 h	(290, 2800)	al. 2018
	CLODDID, CIU			NOMINAL	
INIIDA Watar H	LUPKID: CHI	KUNIC			
	Covio danhuia	Denneduction	EC50	2 080	Daby at al
	dubia	Reproduction	6 d	(2,980)	2018c
IMD	Danhnia	Reproduction	EC50	4 590	Raby et al
INID	тарппа	reproduction	21 d	(4,230-5,050)	2018c
IMD	Daphnia	Reproduction	LOEC	7,300	In Jamec et
	magna	1	21 d	,	al. 2007
IMD	Daphnia	Reproduction	LOEC	2,500	In Jamec et
	magna	-	21 d		al. 2007
IMD	Daphnia	Body residue	96 h	10,200	Li et al.
	magna		LR50	(7,300-	2021
			Internal	14,200)	
			exposure		
			threshold (body		
A	a da		residue)		
Априр	Unalolla	Immobility	LOEC	0.07	In Iamaa at
	azteca	minoomty	101C 96 h	0.97	$\frac{1}{2}$ Jamee et
	uziecu		iuveniles		al. 2007
IMD	Hvalella	Immobility	NOEC	94,800	In Jamec et
	azteca	5	96 h	,	al. 2007
			7-21 d old		
IMD	Hyalella	Growth	EC50	4.3	Bartlett et
	azteca		28 d		al.
					2019
IMD	Gammarus	Immobility	EC50	18.3	Roessink et
	pulex	T	96 h	(8.84, 37.8)	al. 2013
IMD	Gammarus nulex	Immobility	28 d	(9.80, 24.1)	al 2013
IMD	Gammarus	Feeding rate	EC50	5 34	Agatz et al
	nulex	(inhibition)	96 h	5.54	2013
Chirono	omids	(initional)			2015
IMD	Chironomids	Emergence	Sig. diff.	≥2.0	Williams
	(mult. spp;	U	3 weeks?		and
	community)				Sweetman
					2019
IMD	Chironomus	Emergence	EC50	0.39	Cavallaro
	dilutus		40 d	(0.31, 0.42)	et al.
	Chinanar	Emorrowss	EC50	0.5	201/ Malanassat
IND	dilutur	Emergence	28 d	0.3	al 2017

IMD	Chironomus	Emergence	EC50	0.24	Raby et al.
	dilutus	Comment's a	14 d	(0.22, 0.27)	2018b
IMD	Chironomus	Sex ratios	40 d	No sig diff	Cavallaro
	allulus				2017
IMD	Chironomus	Ventilation	Sig diff		2017
IIVID	ringrius	v chimation	06 h	0.55	Azevedo- Dereiro et
	riparius		90 II 48 h post	0.33	relena et
			40 II post	0.5	al. 2011
IMD	Chironomus	Locomotion	Sig Diff		Azevedo
	ringrius	Locomotion	06 h	1.2	Azevedo- Dereira et
	riparias		48 h post	1.2	al 2011
			exposure	1.2	al. 2011
IMD	Chironomus	AChE	Sig diff		Azevedo-
INID	rinarius	decline	96 h	12	Pereira et
	riparias	deenne	48 h post	0.3	al 2011
			exposure	0.5	ul. 2011
IMD	Chironomus	Growth	EC50	5.03	Chandran
	riparius			(4.23, 6.00)	et al. 2018
	<i>T</i> · · · · · ·		NOEC	0.625	
			LOEC	1.25	
			10 d		
Mayflie	S	•		•	•
IMD	Cloeon	Immobility	EC50	1.02	Roessink et
	dipterum		96 h	(0.460, 2.28)	al. 2013
IMD	Cloeon	Immobility	EC50	0.123	Roessink et
	dipterum		28 d	(0.075, 0.201)	al. 2013
IMD	Neocloeon	Imago	EC50	1.75	Raby et al.
min				(1 42 2 09)	20101
	triangulifer	Emergence	14 d	(1.72, 2.07)	20180
IMD	triangulifer Deleatidium	Emergence Immobility	I4 d IC50	0.26	Macaulay
IMD	triangulifer Deleatidium spp.	Emergence Immobility	14 d IC50 28 d	$\begin{array}{c} (1.42, 2.09) \\ 0.26 \\ (0.2, 0.33) \end{array}$	Macaulay et al. 2019
IMD IMD IMD	triangulifer Deleatidium spp. Deleatidium	Emergence Immobility Impairment	I4 d IC50 28 d EC50	$\begin{array}{c} (1.42, 2.09) \\ 0.26 \\ (0.2, 0.33) \\ 0.19 \\ (0.14, 0.25) \end{array}$	Macaulay et al. 2019 Macaulay
IMD IMD	triangulifer Deleatidium spp. Deleatidium spp.	Emergence Immobility Impairment	14 d IC50 28 d EC50 28 d	$\begin{array}{c} (1.42, 2.09) \\ \hline 0.26 \\ (0.2, 0.33) \\ \hline 0.19 \\ (0.14, 0.25) \end{array}$	Macaulay et al. 2019 Macaulay et al. 2019
IMD IMD IMD	triangulifer Deleatidium spp. Deleatidium spp. Isonychia	Emergence Immobility Impairment Immobility	14 d IC50 28 d EC50 28 d EC50	$\begin{array}{c} (1.42, 2.09) \\ \hline 0.26 \\ (0.2, 0.33) \\ \hline 0.19 \\ (0.14, 0.25) \\ \hline 5.88 \end{array}$	Macaulay et al. 2019 Macaulay et al. 2019 Camp and
IMD IMD IMD	triangulifer Deleatidium spp. Deleatidium spp. Isonychia bicolor	Emergence Immobility Impairment Immobility	I4 d IC50 28 d EC50 28 d EC50 96 h	$\begin{array}{c} (1.42, 2.09) \\ \hline 0.26 \\ (0.2, 0.33) \\ \hline 0.19 \\ (0.14, 0.25) \\ \hline 5.88 \end{array}$	Macaulay et al. 2019 Macaulay et al. 2019 Camp and Buchwalter
IMD IMD IMD	triangulifer Deleatidium spp. Deleatidium spp. Isonychia bicolor	Emergence Immobility Impairment Immobility	14 d IC50 28 d EC50 28 d EC50 96 h	$\begin{array}{c} (1.42, 2.09) \\ \hline 0.26 \\ (0.2, 0.33) \\ \hline 0.19 \\ (0.14, 0.25) \\ \hline 5.88 \end{array}$	Macaulay et al. 2019 Macaulay et al. 2019 Camp and Buchwalter 2016
IMD IMD IMD IMD	triangulifer Deleatidium spp. Deleatidium spp. Isonychia bicolor Hexagenia sp.	Emergence Immobility Impairment Immobility Behavior	I4 d IC50 28 d EC50 28 d EC50 96 h EC50	$\begin{array}{c} (1.42, 2.09) \\ \hline 0.26 \\ (0.2, 0.33) \\ \hline 0.19 \\ (0.14, 0.25) \\ \hline 5.88 \\ \hline 10 \\ (2.5, 42) \end{array}$	Macaulay et al. 2019 Macaulay et al. 2019 Camp and Buchwalter 2016 Bartlett et
IMD IMD IMD IMD	triangulifer Deleatidium spp. Deleatidium spp. Isonychia bicolor Hexagenia sp.	Emergence Immobility Impairment Immobility Behavior (remaining in	14 d IC50 28 d EC50 28 d EC50 96 h EC50 96 h	$\begin{array}{c} (1.42, 2.09) \\ \hline 0.26 \\ (0.2, 0.33) \\ \hline 0.19 \\ (0.14, 0.25) \\ \hline 5.88 \\ \hline 10 \\ (2.5, 42) \\ \hline 10 $	Macaulay et al. 2019 Macaulay et al. 2019 Camp and Buchwalter 2016 Bartlett et al. 2018
IMD IMD IMD IMD	triangulifer Deleatidium spp. Deleatidium spp. Isonychia bicolor Hexagenia sp.	Emergence Immobility Impairment Immobility Behavior (remaining in burrows)	14 d IC50 28 d EC50 28 d EC50 96 h EC50 96 h	0.26 (0.2, 0.33) 0.19 (0.14, 0.25) 5.88 10 (2.5, 42) NOMINAL	Macaulay et al. 2019 Macaulay et al. 2019 Camp and Buchwalter 2016 Bartlett et al. 2018
IMD IMD IMD IMD	triangulifer Deleatidium spp. Deleatidium spp. Isonychia bicolor Hexagenia sp. Hexagenia sp.	Emergence Immobility Impairment Immobility Behavior (remaining in burrows) Behavior	14 d IC50 28 d EC50 28 d EC50 96 h EC50 96 h IC50 IC50	0.26 (0.2, 0.33) 0.19 (0.14, 0.25) 5.88 10 (2.5, 42) NOMINAL 1	Macaulay et al. 2019 Macaulay et al. 2019 Camp and Buchwalter 2016 Bartlett et al. 2018 Bartlett et
IMD IMD IMD IMD	triangulifer Deleatidium spp. Deleatidium spp. Isonychia bicolor Hexagenia sp. Hexagenia sp.	Emergence Immobility Impairment Immobility Behavior (remaining in burrows) Behavior (remaining in burrows)	14 d IC50 28 d EC50 28 d EC50 96 h EC50 96 h NOEC LOEC 96 h	0.26 (0.2, 0.33) 0.19 (0.14, 0.25) 5.88 10 (2.5, 42) NOMINAL 1 10 NOMINAL	Macaulay et al. 2019 Macaulay et al. 2019 Camp and Buchwalter 2016 Bartlett et al. 2018 Bartlett et al. 2018
IMD IMD IMD IMD IMD	triangulifer Deleatidium spp. Deleatidium spp. Isonychia bicolor Hexagenia sp. Hexagenia sp.	Emergence Immobility Impairment Immobility Behavior (remaining in burrows) Behavior (remaining in burrows)	14 d IC50 28 d EC50 28 d EC50 96 h EC50 96 h NOEC LOEC 96 h	0.26 (0.2, 0.33) 0.19 (0.14, 0.25) 5.88 10 (2.5, 42) NOMINAL 1 10 NOMINAL	Macaulay et al. 2019 Macaulay et al. 2019 Camp and Buchwalter 2016 Bartlett et al. 2018 Bartlett et al. 2018
IMD IMD IMD IMD IMD THIAM Water F	triangulifer Deleatidium spp. Deleatidium spp. Isonychia bicolor Hexagenia sp. Hexagenia sp. ETHOXAM: A	Emergence Immobility Impairment Immobility Behavior (remaining in burrows) Behavior (remaining in burrows) CUTE	14 d IC50 28 d EC50 28 d EC50 96 h EC50 96 h NOEC LOEC 96 h	0.26 (0.2, 0.33) 0.19 (0.14, 0.25) 5.88 10 (2.5, 42) NOMINAL 1 10 NOMINAL	Macaulay et al. 2019 Macaulay et al. 2019 Camp and Buchwalter 2016 Bartlett et al. 2018 Bartlett et al. 2018
IMD IMD IMD IMD IMD THIAM Water F	triangulifer Deleatidium spp. Deleatidium spp. Isonychia bicolor Hexagenia sp. Hexagenia sp. ETHOXAM: A Fleas	Emergence Immobility Impairment Immobility Behavior (remaining in burrows) Behavior (remaining in burrows) CUTE	14 d IC50 28 d EC50 28 d EC50 96 h EC50 96 h NOEC LOEC 96 h	0.26 (0.2, 0.33) 0.19 (0.14, 0.25) 5.88 10 (2.5, 42) NOMINAL 1 10 NOMINAL	Macaulay et al. 2019 Macaulay et al. 2019 Camp and Buchwalter 2016 Bartlett et al. 2018 Bartlett et al. 2018
IMD IMD IMD IMD IMD IMD THIAM Water F TMX	triangulifer Deleatidium spp. Deleatidium spp. Isonychia bicolor Hexagenia sp. Hexagenia sp. Hexagenia sp. ETHOXAM: A Tleas Ceriodaphnia dubia	Emergence Immobility Impairment Immobility Behavior (remaining in burrows) Behavior (remaining in burrows) CUTE Survival	14 d IC50 28 d EC50 28 d EC50 96 h EC50 96 h NOEC LOEC 96 h	0.26 (0.2, 0.33) 0.19 (0.14, 0.25) 5.88 10 (2.5, 42) NOMINAL 1 10 NOMINAL Not calculable > 80,000	Acaulay et al. 2019 Macaulay et al. 2019 Camp and Buchwalter 2016 Bartlett et al. 2018 Bartlett et al. 2018 Raby et al. 2018c
IMD IMD IMD IMD IMD IMD THIAM Water I TMX	triangulifer Deleatidium spp. Deleatidium spp. Isonychia bicolor Hexagenia sp. Hexagenia sp. ETHOXAM: A Fleas Ceriodaphnia dubia Ceriodaphnia	Emergence Immobility Impairment Immobility Behavior (remaining in burrows) Behavior (remaining in burrows) CUTE Survival	14 d IC50 28 d EC50 28 d EC50 96 h NOEC LOEC 96 h	0.26 (0.2, 0.33) 0.19 (0.14, 0.25) 5.88 10 (2.5, 42) NOMINAL 1 10 NOMINAL Not calculable > 80,000 >80,000	Macaulay et al. 2019 Macaulay et al. 2019 Camp and Buchwalter 2016 Bartlett et al. 2018 Bartlett et al. 2018 Raby et al. 2018c Raby et al.
IMD IMD IMD IMD IMD IMD THIAM Water F TMX TMX	triangulifer Deleatidium spp. Deleatidium spp. Isonychia bicolor Hexagenia sp. Hexagenia sp. ETHOXAM: A Fleas Ceriodaphnia dubia Ceriodaphnia dubia	Emergence Immobility Impairment Immobility Behavior (remaining in burrows) Behavior (remaining in burrows) CUTE Survival Survival	14 d IC50 28 d EC50 28 d EC50 96 h EC50 96 h NOEC LOEC 96 h LC50 6 d LC50 6 d LC50 48 h	0.26 (0.2, 0.33) 0.19 (0.14, 0.25) 5.88 10 (2.5, 42) NOMINAL 1 10 NOMINAL Not calculable > 80,000 >80,000	Macaulay et al. 2019 Macaulay et al. 2019 Camp and Buchwalter 2016 Bartlett et al. 2018 Bartlett et al. 2018 Raby et al. 2018c Raby et al. 2018a
IMD IMD IMD IMD IMD IMD IMD THIAM Water F TMX TMX	triangulifer Deleatidium spp. Deleatidium spp. Isonychia bicolor Hexagenia sp. Hexagenia sp. Hexagenia sp. ETHOXAM: A Tleas Ceriodaphnia dubia Ceriodaphnia dubia Daphnia	Emergence Immobility Impairment Immobility Behavior (remaining in burrows) Behavior (remaining in burrows) CUTE Survival Survival	14 d IC50 28 d EC50 28 d EC50 96 h EC50 96 h NOEC LOEC 96 h LC50 6 d LC50 48 h LC50	0.26 (0.2, 0.33) 0.19 (0.14, 0.25) 5.88 10 (2.5, 42) NOMINAL 1 10 NOMINAL Not calculable > 80,000 >80,000	Acaulay et al. 2019 Macaulay et al. 2019 Camp and Buchwalter 2016 Bartlett et al. 2018 Bartlett et al. 2018 Raby et al. 2018c Raby et al. 2018a Raby et al.

TMX	Daphnia	Survival	EC50		Finnegan
	magna		24 h	>100,000	et al. 2017
	-		static		(Syngenta)
TMX	Daphnia	Survival	EC50	>100,000	Finnegan
	magna		48 h		et al. 2017
	U U		static		(Syngenta)
TMX	Daphnia	Survival	EC50	>100,000	Finnegan
	pulex		24 h		et al. 2017
	Î		static		(Syngenta)
Amphip	ods			•	
TMX	Hyalella	Survival	LC50	290	Bartlett et
	azteca		7d		al.
					2019
TMX	Hyalella	Survival	LC50	220	Bartlett et
	azteca		28 d		al.
					2019
TMX	Hyalella	Survival	LC50	801.0	Raby et al.
	azteca		96 h	(518.7,	2018a
				1,083.3)	
TMX	Gammarus	Survival	LC50	3,751	Ugurlu et
	kischineffensis		96 h		al. 2015
TMX	Gammarus sp.	Survival	EC50	15,000	Finnegan
			24 h	(10,000-	et al. 2017
			static	23,000)	(Syngenta)
TMX	Gammarus sp.	Survival	EC50	2,800	Finnegan
			48 h	(1,700-4,100)	et al. 2017
			static		(Syngenta)
Chirono	omids	1		1	
TMX	Chironomus	Survival	LC50	54.3	Phillips et
	dilutus		96 h	(49.1, 60.1)	al. 2021
TMX	Chironomus	Survival	LC50	58.5	Phillips et
	dilutus		96 h	(49.3, 69.4)	al. 2021
TMX	Chironomus	Survival	LC50	31.8	Phillips et
	dilutus		10 d	(28.2, 35.9)	al. 2021
TMX	Chironomus	Survival	LC50	30.3	Phillips et
	dilutus		10 d	(29.1, 31.7)	al. 2021
TMX	Chironomus	Survival	LC50	61.9	Raby et al.
	dilutus		96 h	(45.4, 78.4)	2018a
ТМХ	Chironomus	Survival	LC50	45.88	Raby et al.
	dilutus	~	14 d		2018b
TMX	Chironomus	Surv1val	LC50	55.34	Maloney et
	dilutus	~	96 h	•••	al. 2017
TMX	Chironomus	Surv1val	LC50	23.60	Cavallaro
	dilutus	~	14 d	(20.36, 26.89)	et al. 2017
TMX	Chironomus	Surv1val	NOEC	1,300 µg/g	Finnegan
	dilutus		LOEC	2,600 µg/g	et al. 2017
			10 d	Sediment dry	(Syngenta)
			Static renewal	wt	
			Sediment		
			application		

TMX	Chironomus	Survival	LC50	86.41	Saraiva et
	riparius		48 h	(74.35,	al. 2017
	1			100.04)	
TMX	Chironomus	Survival	EC50	61	Finnegan
	riparius		24 h	(50, 75)	et al. 2017
			static	()	(Syngenta)
TMX	Chironomus	Survival	EC50	35	Finnegan
	riparius		48 h	(30, 41)	et al. 2017
	T		static		(Syngenta)
ТМХ	Chironomus	Survival	EC50	45	Finnegan
	rinarius		48 h	(not	et al. 2017
	ripartas		static	calculable)	(Syngenta)
тмх	Chironomus	Survival	EC50	71	Finnegan
110121	rinarius	Survivar	48 h	(34 194)	et al 2017
	ripartas		static	(31, 191)	(Syngenta)
ТМХ	Chironomus	Survival	LC50	260	Finnegan
1 1012 1	rinarius	Survivar	48 h	(130, 520)	et al 2017
	riparias		static	(150, 520)	(Syngenta)
Mavflie	s		Statie		(Syngenau)
TMY	Closon sn	Survival	1.050	4 633 6	Raby et al
IWIA	Cibeon sp.	Survivar	06 h	(1 835 8	2018_{2}
			90 II	(1,035.0, 7.431.3)	2018a
TMV	Nacalogon	Survivol	I C 50	5 5	Doby at al
IWIA	triangulifor	Survival	96 h	(3978)	2018
TMV	Cleaser	Surviva1	FC50	(3.9, 7.0)	2016a
IWA	diretormum	Survival	EC30 48 h	21 (not	Finnegan
	aipierum		40 II	(IIOt	(Syncente $)$
TMV	Classer	Courseiron 1			(Syngenta)
IWA	diptomum	Survival	10 LC 30	(38, 72)	rinnegan at al. 2017
	uipierum		40 II	(38, 75)	(Syngenta $)$
TMV	Clear	Survivo1		4.4	(Syngenta)
IWA	Cloeon	Survival	EC30	44	rinnegan
	aipierum		48 n	(42, 43)	et al. 2017
TMV	Classic	. C1		10	(Syngenia)
IMX	Cloeon sp.	Survival	EC50	19	Finnegan
			24 h	(16, 23)	et al. 2017
	01	Q : 1	static	1.4	(Syngenta)
IMX	Cloeon sp.	Survival	EC50	14	Finnegan
			48 h	(11, 17)	et al. 2017
	D 1 1.	~	static		(Syngenta)
TMX	Deleatidium	Survival	LC50	>4	Macaulay
	spp.		28 d		et al. 2019
TMX	Hexagenia sp.	Survival	LC50	>10,000	Bartlett et
			96 h	NOMINAL	al. 2018
THIAMETHOXAM: CHRONIC					
Water	leas	1			
TMX	Ceriodaphnia	Reproduction	EC50	Not calculable	Raby et al.
	dubia		6 d	>80,000	2018c
TMX	Daphnia	Reproduction	NOEC	100,000	Finnegan
	magna		LOEC	>100,000	et al. 2017
			14 d		(Syngenta)

			semi-static						
TMX	Daphnia magna	Reproduction	NOEC LOEC 21 d semi-static	100,000 >100,000	Finnegan et al. 2017 (Syngenta)				
Amphipods									
TMX	Hyalella azteca	Growth	EC50 28 d	200	Bartlett et al. 2019				
Chironomids									
TMX	Chironomus dilutus	Sex ratios	40 d	No sig diff	Cavallaro et al. 2017				
TMX	Chironomus dilutus	Emergence	EC50 40 d	4.13 (3.53, 4.76)	Cavallaro et al. 2017				
TMX	Chironomus dilutus	Emergence	EC50 28 d	8.91 (5.79, 12.37)	Maloney et al. 2017				
TMX	Chironomus dilutus	Emergence	EC50 14 d	12.95 (8.54, 17.35)	Raby et al. 2018b				
TMX	Chironomus dilutus	Growth	IC25 10 d	17.8 (12.2, 20.1)	Phillips et al. 2021				
TMX	Chironomus dilutus	Growth	IC25 10 d	17.3 (4.2, 29.6)	Phillips et al. 2021				
TMX	Chironomus dilutus	Growth	NOEC LOEC MATC 10 d	21 42 29.7	Phillips et al. 2021				
TMX	Chironomus dilutus	Growth	NOEC LOEC MATC 10 d	16 32 22.6	Phillips et al. 2021				
TMX	Chironomus dilutus	Growth ash-free dw	NOEC LOEC 10 d Static renewal Sediment application	600 μg/g 1,300 μg/g Sediment dry wt	Finnegan et al. 2017 (Syngenta)				
TMX	Chironomus riparius	Growth	NOEC LOEC 10 d	10.5 18	Saraiva et al. 2017				
TMX	Chironomus riparius	Emergence	NOEC LOEC 10 d	6.5 10.5	Saraiva et al. 2017				
TMX	Chironomus riparius	Emergence	NOEC LOEC 30 d Static Sediment application	20 μg/g 100 μg/g Sediment dry wt	Finnegan et al. 2017 (Syngenta)				

TMX	Chironomus riparius	Development rate	NOEC LOEC 30 d	>10 µg/g >10 µg/g Sediment dry	Finnegan et al. 2017 (Syngenta)					
			Sediment application	wt						
Mayflies										
TMX	Cloeon dipterum	Larval abundance	NOEC 34 d	3.0	Pickford et al. 2018 (Syngenta)					
TMX	Cloeon dipterum	Larval abundance	LOEC 34 d	10	Pickford et al. 2018 (Syngenta)					
TMX	Cloeon dipterum	Emergence	NOEC 35 d	0.3	Pickford et al. 2018 (Syngenta)					
TMX	Cloeon dipterum	Emergence	LOEC 35 d	1.0	Pickford et al. 2018 (Syngenta)					
TMX	Deleatidium spp.	Immobility	IC50 28 d	>4	Macaulay et al. 2019					
TMX	Deleatidium spp.	Impairment	EC50 28 d	>4	Macaulay et al. 2019					
TMX	Neocloeon triangulifer	Imago Emergence	EC50 14 d	2.18 (1.60, 3.20)	Raby et al. 2018b					
TMX	Heptageniidae (Stenacron, Stenonema, Maccaffertiu m)	Survival	EC50 96 h Trials with diff pops of each genus	Ranged from 19.8 to 86.5 µg/L	Rackliffe and Hoverman 2020					
TMX	<i>Hexagenia</i> sp.	Behavior (remaining in burrows)	EC50 96 h	630 (140, 2900) NOMINAL	Bartlett et al. 2018					
TMX	Hexagenia sp.	Behavior (remaining in burrows)	NOEC LOEC 96 h	100 1,000 NOMINAL	Bartlett et al. 2018					

NOTES:

LC50 = median lethal concentration (50%)

EC50 = median effective concentration (50%)

LOLC = lowest observed lethal level

NOEC = no observed effect concentration = highest concentration tested that was not significantly different from the control

LOEC = lowest observed effect concentration = lowest concentration tested that was significantly different from the control

MATC = maximum allowable toxicant concentration = geometric mean of the NOEC and LOEC concentrations

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PUBLICATIONS CURRENTLY IN PREPARATION:

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A Jeninga[†], Z Wallace^{*}, S Victoria[†], E Harrahy, and TC King-Heiden. Chronic exposure to environmentally relevant concentrations of imidacloprid impact survival and ecologically relevant behaviors of fathead minnow larvae.

E Harrahy, A Jeninga, and TC King-Heiden. Acute and chronic toxicity of imidacloprid and thiamethoxam to select aquatic invertebrates.

E Harrahy, A Jeninga[†], S Duffy^{*}, and TC King-Heiden. Exposure to binary mixtures of thiamethoxam and imidacloprid causes additive toxicity in aquatic invertebrates, but not fish.