Neonicotinoid Insecticide Hydrolysis and Photolysis: Rates and Residual Toxicity

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Stephen Anthony Todey

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Abstract

Neonicotinoid insecticides are currently the most widely used class of insecticides worldwide, accounting for 25% of total insecticide use. They are registered in 120 countries for use on more than 140 crops. Concern has grown, however, over their widespread detection in global surface waters, soil, finished drinking water, and wastewater, and for their potential role in colony collapse disorder in honey bees. This work set out to examine hydrolysis and photolysis reaction rates of neonicotinoids, as well as to identify reaction products and determine the toxicity of the reaction products on mosquitoes. Hydrolysis rates were tested between pH 4 and pH 10. Reaction rates were pseudo-first order and highly pH dependent. Calculated half-lives ranged from >1000 days to 10 days. Divalent metal ions (Cu^{2+}, Ni^{2+}, Zn^{2+}) and minerals (kaolinite, goethite, TiO_2) were found to have little to no effect on neonicotinoid hydrolysis. Experiments from pH 4 to pH 10 revealed a non-elementary rate law for neonicotinoid degradation, with the hydroxide concentration being raised to a power of 0.55 ± 0.09.

Nitenpyram, imidacloprid, thiamethoxam, and clothianidin were found to undergo direct photolysis, with quantum yields of 0.025 ± 0.001, 0.0119 ± 0.0001, 0.0167 ± 0.0002, and 0.0133 ± 0.0001, respectively. Acetamiprid degraded very slowly via direct photolysis, but was found to undergo indirect photolysis due to reaction with OH· with a bimolecular rate constant of $1.7 \pm 0.2 \times 10^9$ M^{-1} s^{-1}. Reaction products were identified for all reactions, with the urea derivative as the most commonly detected product. Toxicity experiments on mosquitoes indicate no residual toxicity from hydrolysis or photolysis products, which may be expected given the removal of the pharmacophore during reactions. While abiotic reaction products were found to be non-toxic, results from experimental work indicates long environmental half-lives for the tested neonicotinoids, which may help to explain their observed persistence in environmental matrices.

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List of Abbreviations

A – acetamiprid

C – clothianidin

DOC – dissolved organic carbon

DT_{50} – dissipation time to 50% of initial concentration

DOM – dissolved organic matter

HPLC – high pressure liquid chromatography

I – imidacloprid

LC_{50} – lethal concentration for 50% survival

MOPS – 3-(N-morpholino)propanesulfonic acid

MRW – Mississippi River water

N – nitenpyram

nAChR – nicotinic acetylcholine receptors

pCBA – *para*-chlorobenzoic acid

PNA – *para*-nitroanisole

PPRI – photochemically produced reactive intermediate

SA – screening adjusted

T – thiamethoxam

UPLC-MS/MS – ultra performance liquid chromatography - tandem mass spectrometry

WWTPs – wastewater treatment plants

ZVI – zero-valent iron

Introduction

History and Usage

Neonicotinoid insecticides are a class of systemic pesticides which are widely used throughout the world. The class contains seven different pesticides (**Figure 1**): imidacloprid, thiamethoxam, clothianidin, acetamiprid, nitenpyram, dinotefuran, and thiacloprid. Neonicotinoids are characterized by three different possible pharmacophore moieties, or functional groups, N-nitroguanidines (=N-NO_2), N-cyanoamides (=N-CN), and nitromethylenes (=CH-NO_2). As a class, neonicotinoids are characterized by high water solubilities as well as octanol water partition coefficients (K_{ow}) and dissociation constants (pK_a) which allow for neonicotinoids to enter and move throughout all parts of a plant.[1] Selected chemical and physical properties are given in **Table 1**.

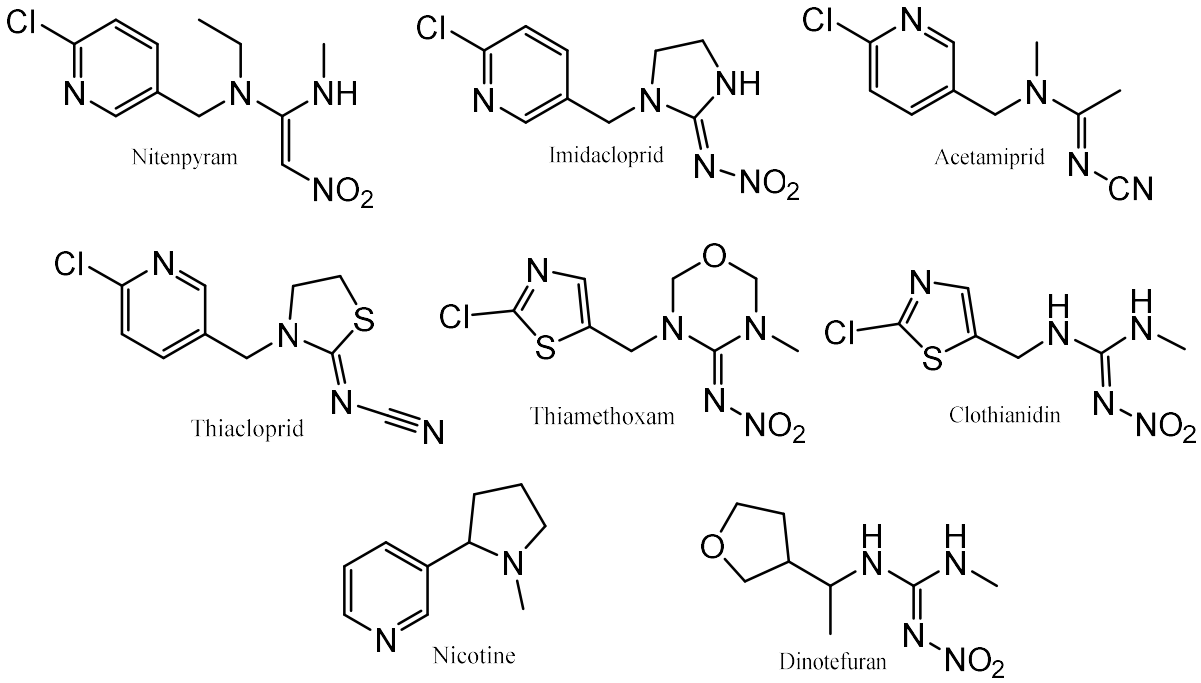

Figure 1. Commercially available neonicotinoid insecticides and the structure of nicotine. Nitenpyram, imidacloprid, acetamiprid, thiamethoxam, and clothianidin were selected for use in this study.

Table 1. Chemical and physical properties of neonicotinoid insecticides.

Neonicotinoid	Molar Mass (g/mol)	Solubility[2] (g/L)	Log K_{ow}[2]	Log K_{oc}[3]	pK_a[1]	Functional Group
Acetamiprid	222.67	4.25	0.80	2.3	0.7	=NCN
Clothianidin	249.68	0.33	0.905	2.1	11.1	$=NNO_2$
Dinotefuran	202.21	54.3	-0.549	1.4	12.6	$=NNO_2$
Imidacloprid	255.66	0.61	0.57	2.1-2.5	-	$=NNO_2$
Nitenpyram	270.72	840	-0.66	-	3.1	$=CHNO_2$
Thiacloprid	252.72	0.185	1.26	-	-	=NCN
Thiamethoxam	291.71	4.1	-0.13	1.8	-	$=NNO_2$

Imidacloprid was released globally in 1991 and was the first neonicotinoid approved for usage in the United States, with the EPA approving the registration on imidacloprid in 1994. Imidacloprid is approved for use as a seed treatment, crop insecticide, household insecticide to fight structural pests, and as flea control treatment for cats and dogs.[4] Following imidacloprid's registration, acetamiprid and nitenpyram were registered in 1995, followed by thiamethoxam in 1998, clothianidin and thiacloprid in 2001, and dinotefuran in 2002.[5,6]

Neonicotinoids are named after nicotine, a natural insecticide which was used for several hundred years, due to a shared mode of action.[7] Nicotine and neonicotinoids target the nicotinic acetylcholine receptor (nAChR).[8] Upon binding to receptors, neonicotinoids effectively keep the nAChR channel open, causing continuous nervous system stimulation which leads to paralysis and then death.[7,9] Neonicotinoids are characterized by higher selectivity to the insect nAChR over the vertebrate nAChR, leading to higher binding efficiencies of neonicotinoids to the insect nAChR.[8] This makes neonicotinoids more effective than nicotine, and results in neonicotinoids being competitive with other commonly used insecticides.[8]

Before the development of neonicotinoids, organophosphate and carbamate insecticides were widely used in agricultural and urban settings to control insects.[10] Since their original release in the 1940's and 1950's, many pests had begun to develop resistance to

organophosphates and carbamates by the 1980's.[11] Additional problems with organophosphates and carbamates included known toxicity to vertebrates and various arthropods, transport from the application site, and environmental persistence. [10,11] This led to problems for fish and aquatic invertebrate species.[10,11]

In contrast to organophosphates and carbamates, neonicotinoids were effective against species that had developed resistance to organophosphates and carbamates, because neonicotinoids were a new insecticide.[11] Another major advantage of neonicotinoids was their systemic nature, in which plant roots uptake the insecticide, allowing it to spread throughout the entire plant, including to leaves, pollen, and flowers.[6] Thus, neonicotinoids are able to protect the entire plant, no matter how or where they were applied, offering protection against any insect attempting to feed on the plant.[11] This protection was also found to be longer lasting due to the high persistence of neonicotinoids, making them very effective insecticides.[11] Neonicotinoids exhibited lower binding efficiencies to vertebrate binding sites as compared to invertebrate binding sites, resulting in higher toxicity for targeted species over non-target species.[3,7] Neonicotinoids were assumed to safer for fish and other vertebrates due to lower binding efficiencies, as well as to human workers, because neonicotinoids are often applied as seed treatments as opposed to being sprayed onto crops.[11]

Thanks to the benefits of neonicotinoids over organophosphates and carbamates, neonicotinoids became extremely popular. Their market share has increased to account for a quarter of worldwide insecticide use.[5] Neonicotinoids are now used in over 120 countries on more than 140 crops, including corn, soybeans, cotton, sorghum, sugar beets, and many fruits and vegetables.[13] In addition to seed coatings, neonicotinoids are used as foliar sprays, soil drenches, injections, and granules.[6] Use is not limited to agriculture. Garden centers, nurseries, and home gardeners use neonicotinoids to control pests.[14,15] Imidacloprid is still being used as a flea medication,[13] and neonicotinoids are currently being used to attempt to control emerald ash borer.[16]

Though there are many uses of neonicotinoids, agricultural application is still the largest use of neonicotinoids. In Minnesota, use has grown rapidly since 1996, when imidacloprid was first sold in the state.[17] Sales of neonicotinoids peaked in 2009, when nearly 67,000 kg of neonicotinoids were sold. Since the introduction of neonicotinoids to Minnesota markets, 99% of total sales have been thiamethoxam (39.4 %), imidacloprid (37.0 %), and clothianidin (22.6 %). Historical sales data are given in **Figure 2**. Use in other Midwestern agricultural states is even higher than in Minnesota. In Iowa, 335,000 kg of thiamethoxam, clothianidin, and imidacloprid were applied just to agricultural fields in 2013.[18]

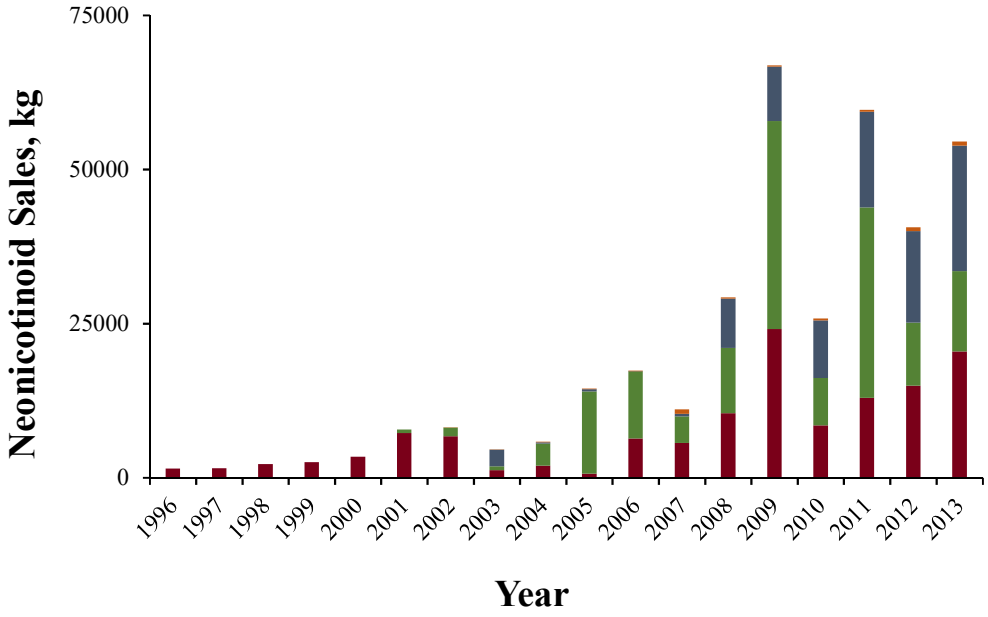

Figure 2. Historical agricultural sales of neonicotinoid insecticides in Minnesota, 1996-2013. Data compiled from Minnesota Department of Agriculture Pesticide Sales Database.[17] Legend: ■ Imidacloprid ■ Thiamethoxam ■ Clothianidin ■ Other

Environmental Detection & Fate Processes

The widespread use of neonicotinoids creates the potential for neonicotinoids to contaminate non-target areas. Runoff from agricultural fields, golf courses, lawns, and similar areas can transport neonicotinoids to water bodies.[1] Leaching potential is also

high to very high for most compounds, due in large part to their high water solubility and low hydrophobicity.[3] Drift from sprays or from dust generated by planting treated seeds can spread neonicotinoids to surface waters and other fields/plants.[19] Applications in urban areas also lead to runoff and leaching to sewer systems and surrounding water bodies.[1] Finally greenhouses and nurseries have also been shown to have the potential to contaminate urban areas.[15]

Neonicotinoids have been detected in many surface and ground water systems. In Canada's Prairie Pothole region, clothianidin and thiamethoxam were detected up to 91% of the time in a sampling of 136 wetlands, with concentrations up to 1490 ng/L for thiamethoxam and 3110 ng/L of clothianidin.[20] An additional study in southwestern Ontario, Canada found clothianidin and thiamethoxam in 76/76 and 75/76 water samples respectively near corn producing areas, with an average concentration of 2280 ng/L and a maximum concentration of 43,600 ng/L.[21] In Iowa, near high corn and soybean producing areas, clothianidin was detected in 75% of all samples taken year-round, with thiamethoxam (47%) and imidacloprid (23%) also being detected.[18] In Georgia, imidacloprid was detected 60% to 85% of the time in stream sampling, with detected concentrations ranging from 3.4 to 35.3 ng/L.[22] Detection of neonicotinoids has also been reported in Australia[23] and Puerto Rico.[24] As detection methods improve, more detections globally are expected, given the use of neonicotinoids in 120 countries.[9] Studies in the United States and Vietnam have also detected neonicotinoids in groundwater.[25,26]

Detection of neonicotinoids in soils has also been reported. In Ontario, Canada, detection in the soil ranged from 0.07 to 20.30 ng/g before planting to 0.53 to 38.98 ng/g after planting.[21] In England, total neonicotinoid concentrations in soil ranged 0.09 ng/g to 18.3 ng/g, depending on time from last application and field sampling location.[27] Wide ranges of dissipation times (DT_{50}) in soils have been reported, from 7 to 353 days for thiamethoxam, 239 to 6931 days for clothianidin, 28 – 1250 days for imidacloprid, and 388-450 for acetamiprid.[28] For thiamethoxam, one study found DT_{50} to be between 7 and

92 days and argued thiamethoxam will not accumulate in soil.[29] In a separate study, thiamethoxam could still be detected three years after its last application.[27] While DT_{50} will likely vary between soil types, accumulation is expected and has been observed, indicating DT_{50} are longer than many studies predicted.[28]

Perhaps unsurprisingly, given their widespread detection in surface water and ground water, neonicotinoids have begun to be detected in water treatment facilities and in finished drinking water. In tap water at the University of Iowa, thiamethoxam, clothianidin, and imidacloprid were detected in every sample, with concentrations ranging from 1.22 to 57.3 ng/L.[30] In rural Ontario, Canada, thiamethoxam, clothianidin, and imidacloprid were also detected in tap water with a maximum concentration of 280 ng/L.[31] During conventional drinking water treatment, thiamethoxam was found to degrade due to base-catalyzed hydrolysis in lime-soda softening, though degradation was not observed with imidacloprid and clothianidin.[30] In laboratory testing using finished drinking water, nitenpyram was found to be stable for at least four weeks while being monitored.[32] Average residence times in distribution systems range from several hours to several days,[30] suggesting stability of neonicotinoids throughout water distribution systems. Granular activated carbon has been shown to remove neonicotinoids in batch studies, indicating a potential point of use solutions for communities with high concentrations of neonicotinoids in drinking water.[30]

Neonicotinoids have also been detected in wastewater treatment plant effluents (WWTPs), and reaction rates in wastewater have been studied. In a survey of 13 WWTPs and 1 constructed wetland, imidacloprid was found year-round at every site, with a mean influent concentration of 60.5 ng/L and effluent concentration of 58.5 ng/L, indicating insignificant removal.[33] Acetamiprid (2.9 ng/L influent) was also detected, and was found to undergo an average degradation of 18% to 2.3 ng/L in the effluent.[33] In laboratory studies using wastewater from the aeration basin, thiamethoxam was found to undergo photolysis slowly, with an average half-life of 17.6 hours.[34] Biodegradation of thiamethoxam occurred in wastewater sludge with an average half-life of 25 days.[34] In

the same study, thiacloprid was found to resist photodegradation and biodegradation in wastewater sludges.[34] Due to lack of removal of neonicotinoids in WWTPs, an estimated 1000 to 3400 kg of neonicotinoids are released to surface waters every year in the United States via WWTPs.[33]

Once in water bodies, the reported hydrolysis rates for neonicotinoids vary widely.[1] While pH has been shown to affect degradation rate, its role is not well understood, and mixed results have been reported. For example, imidacloprid has been reported to be stable at pH 5 and 7 with a half-life of one year at pH 9.[35] This indicates degradation only in alkaline, or basic, conditions, which has been reported by others for imidacloprid.[36–38] Other other work has shown faster degradation of imidacloprid at pH 4 than at pH 9 (half-lives of 36.2 days vs. 41.6 days, respectively.[39] Thiacloprid and acetamiprid have also been reported to degrade under acidic conditions while remaining stable under basic conditions.[1] Thiamethoxam has been reported to degrade faster under basic conditions.[40] Based on the available literature, imidacloprid is considered to be stable at environmentally relevant pH values,[1] but the effect of pH on hydrolysis rates is not fully understood.

Photolysis of neonicotinoids has also been studied as a potential environmental degradation pathway. Direct photolysis was shown to be an important pathway for imidacloprid, clothianidin, and thiamethoxam, while thiacloprid and acetamiprid underwent photolysis slowly.[41] Indirect photolysis through photochemically produced reactive intermediates (PPRIs) may also play a part in neonicotinoid degradation with studies showing reaction of neonicotinoids with carbonate radicals,[42] singlet oxygen,[43] and hydroxyl radicals.[44] Studies showing reaction with PPRIs have not been directly compared to direct photolysis rates in experiments, which would allow for determination of environmental relevance of different photolysis pathways. Additionally, it does not appear the role of nitrate has been explored. Nitrate is known to produce hydroxyl radicals,[45] and in areas of intensive agriculture production with high levels of nitrate in

water bodies, indirect photolysis may be more likely to occur because of the potential for elevated levels of hydroxyl radicals in surface waters.

Biodegradation of neonicotinoids has also been reported. White-rot fungus (*Phanerochaere sordida*) has been shown to degrade clothianidin by 37% after a 20 day incubation at 30 ºC.[46] In a separate study, clothianidin was shown to degrade only marginally in aerobic conditions, but to degrade faster in anaerobic conditions, with DT_{50} of 28.3 days at 25 ºC and 9.7 days at 35 ºC.[47] Imidacloprid has been shown to be cometabolized by a strain of *Leifsonia*, with degradation of 37% to 58% observed over three weeks.[48] In laboratory studies, thiacloprid was hydrolyzed by *Ensifer meliloti*, an N_2 – fixing bacteria, with a half-life of 20.9 hours.[49] A separate rhizobacterium, *Ensifer adhaerens*, has been shown degrade thiamethoxam by 87% in 20 days.[50] Only one soil sample in this study, however, caused thiamethoxam degradation, indicating degradation rates will vary significantly depending on local conditions.[50]

Non-Target Organism Toxicity

While neonicotinoids were originally marketed due to their reduced toxicity to mammals and vertebrates, evidence is growing that they have impacts on non-target organisms.[51] Widespread detection of neonicotinoids in surface water, ground water, and soils is has led to impacts on non-target organisms, include many orders of aquatic arthropods, birds, and fish.[9] High acute and chronic toxicity has been observed in many different aquatic insects.[6,52,53] Sub-lethal effects have also been observed, including reproduction inhibition, immobility, delayed emergence, and feeding inhibition.[52–56] In red-legged partridges (*Alectoris rufa*), mortality was observed after birds consumed imidacloprid treated seeds.[57] Many sub-lethal effects were also observed, including: altered biochemical parameters, oxidative stress, reduced chick survival, reduced egg size, and reduced cellular immune response.[57] In a study with white-crowned sparrows (*Zonotrichia leucophrys*), decreases in body mass and fat stores were observed, as well as improper migratory orientation.[58] Neonicotinoids can also decrease food sources for

insectivorous birds in areas with highly contaminated surface waters.[51] In zebrafish (*Danio rerio*) livers, nitenpyram has been shown to damage DNA and the antioxidant enzyme system.[59]

The most widely studied non-target organism, and perhaps the species which experiences the most detrimental effects is honey bees (*Apis mellifera*).[6] Imidacloprid, thiamethoxam, and clothianidin are all considered highly toxic to honey bees with the LD_{50} values between 1 – 5 ng per bee.[6] Sub-lethal doses have also been shown to have negative impacts on honey bees, including impaired learning, decreased navigational ability, decreased success while foraging, suppressed immune response, reduce lifetimes for queens, decreased colony growth, reduced sperm viability, and reduced numbers of new queens.[60–63] Neonicotinoids are often found in beehives as well as honey, with concentrations ranging from 1-10 ng/g with concentrations over 50 ng/g in honey having been detected.[64] The impact on bees is a serious problem because an estimated 35% of world food production, spanning 87 different vegetable or seed crops, is reliant on pollination from bees and other species.[65]

An additional area of emerging concern is detection of neonicotinoids in humans, including women who were not agricultural workers[66] and children the age of 3.[67] This indicates humans are being exposed through consuming fruits, vegetables, tea, and honey with neonicotinoid residue present.[68] Levels in honey have largely been below UN recommendations,[64] and many crops do not contain high residual concentrations of neonicotinoids.[1] Residues in fruiting vegetables like tomatoes and peppers have been observed to be above the maximum residual limit, indicating a possible exposure pathway.[1] Neonicotinoids in finished drinking water are an additional potential exposure pathway. Given the relative lack of information on chronic exposure of humans to neonicotinoids, the National Institutes of Health has recommended further studying the effects of neonicotinoids on humans.[69]

It is not just the parent products of neonicotinoids which are of concern. Many metabolites have been identified in plants and animals, due to intense metabolism in plants as well as metabolism in small animals.[11] Over 110 metabolites have been identified of the seven commercially available neonicotinoids, and 27 of these have been shown to be active toward invertebrates or mammals.[11] Degradation products specific to select neonicotinoids are discussed below.

Perhaps the most prevalent degradation product of imidacloprid is imidacloprid-urea (**Figure 3**), which has been observed as a product of hydrolysis,[37,38] photolysis,[70,71] and soil metabolism.[72] Imidacloprid-urea has not been shown to exhibit residual toxicity towards invertebrates or mammals, however it has been tested only in a limited number of studies.[11] Other metabolites of imidacloprid have been shown to be much more active towards both invertebrate and mammalian receptors, namely desnitro/guanidine and nitrosoguanidine derivatives (**Figure 3**).[72] The nitrosoguanidine metabolite of metabolite imidacloprid has been shown to retain moderate to high insecticidal activity to the insect nicotinic acetylcholine receptor channels.[12] Alternatively, the desnitro/guanidine of imidacloprid has been shown to be non - active to the insect channel, but instead highly active against the mammalian nicotinic receptor channels.[8,26,27]

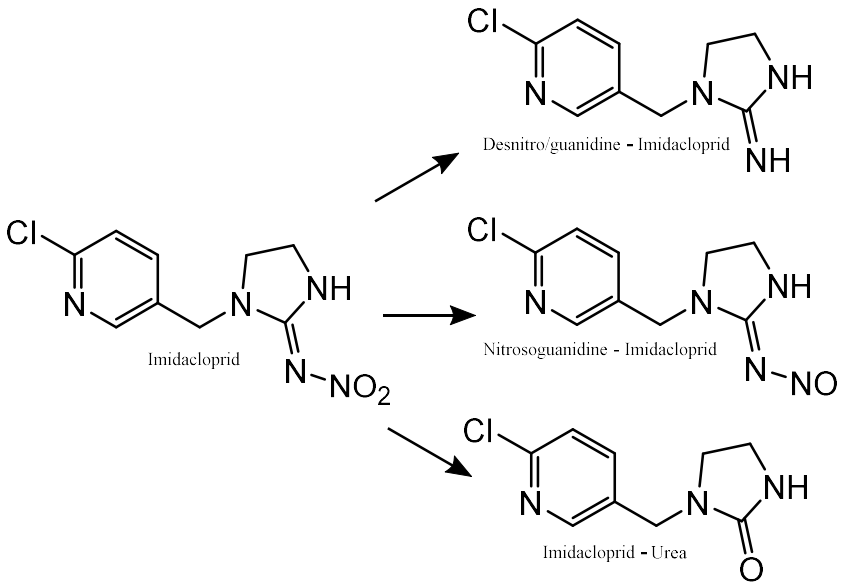

Figure 3. Structures of imidacloprid and reported degradation products.

Similar results have been observed with metabolites of thiamethoxam. Thiamethoxam-urea (**Figure 4**) has been observed as the product of hydrolysis,[40,75] photolysis,[76] and biologically in mice and soil bacteria, and similar to imidacloprid-urea, has not been shown to exhibit residual toxicity.[11,77] Formation of the desnitro/guanidine derivative (**Figure 4**) has been observed as a result of photolysis [34,71,73] and has been shown to exhibit residual toxicity.[11] Biological transformation to the neonicotinoid clothianidin via a biologically mediated ring-opening reaction has been shown to occur, indicating one of the main degradation products of thiamethoxam environmentally is clothianidin.[77,78] These results again indicate any study on degradation rates should also be focused on transformation product identification, because some products have high levels of residual toxicity while others do not appear to be toxic.

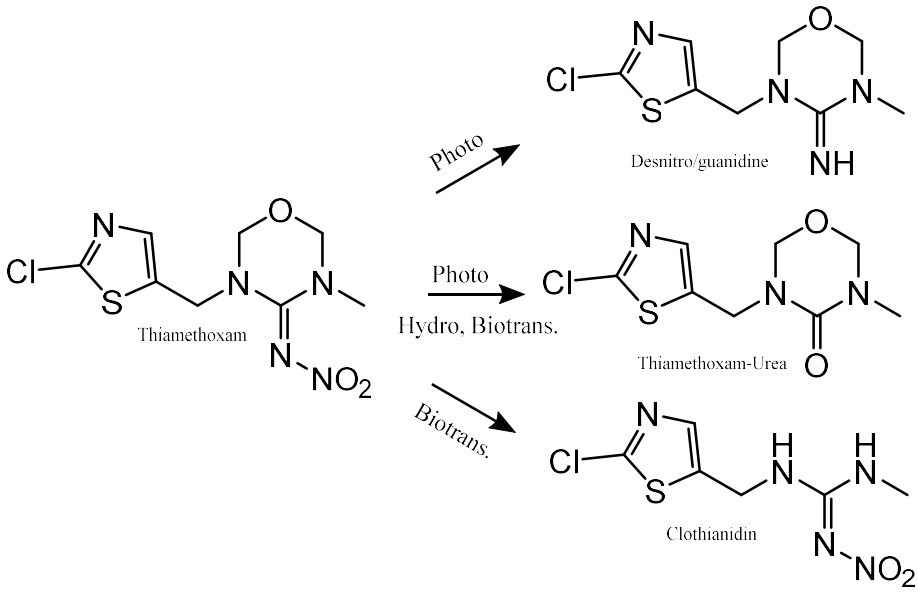

Figure 4. Observed environmental degradation products of thiamethoxam.

Similar results to thiamethoxam and imidacloprid have been observed for clothianidin, with several detected products, namely the desnitro/guanidine and nitrosoguanidine derivatives among others having been shown to retain toxicity, while the urea derivative does not.[79] Degradation products of acetamiprid and nitenpyram are not as widely studied as imidacloprid, thiamethoxam, and clothianidin, however, identified metabolites have

not been reported to be toxic.[80] These results indicate studying degradation rate may not tell the whole story in terms of environmental risk.

Rationale for Study

As previously discussed, while hydrolysis rate has been shown to change based on pH, the exact relationship of pH and hydrolysis rate has not been determined. This should be examined further to understand the likelihood of neonicotinoids to breakdown due to hydrolysis in the environment. The role of metals should also be examined in hydrolysis reactions of neonicotinoids. Previous work has shown that metals can catalyze pesticide degradation in aqueous systems.[81–83] Dissolved metal ions have also been shown to inhibit the hydrolysis of the herbicide naptalam and related amides.[84]

Similarly, the impact of minerals on neonicotinoid hydrolysis merits study. Hematite, goethite, ferrihydrite, and aluminum hydroxide have all been shown to catalyze hydrolysis of organophosphorus pesticides.[85] Similar results were observed with anatase and goethite using phenyl picolinate, a pesticide-like compound.[86] Coabsorbed species and absorbed natural organic matter were shown to inhibit picolinate hydrolysis, indicating a dependence on ecosystem for mineral hydrolysis impacts.[87,88] Neonicotinoids have been shown to adsorb to mineral surfaces, indicating the possibility of mineral catalyzed hydrolysis reactions occurring.[89,90]

To summarize, the goals of this research were to: 1) understand the effects of pH, divalent metals (Cu^{2+}, Ni^{2+}, Zn^{2+}), and minerals (kaolinite, goethite, TiO_2) on hydrolysis of neonicotinoids, 2) measure photolysis rates, 3) determine toxicity of hydrolysis and photolysis products to mosquitoes, and 4) identify reaction products. Imidacloprid, clothianidin, and thiamethoxam (all nitroguanidines) account for >99% of total neonicotinoid usage in Minnesota [17] and were thus selected for this study. Acetamiprid (a cyanoamide) and nitenpyram (a nitromethylene) were also used to allow comparison of the three pharmacologically active groups currently used in neonicotinoids.

Materials and Methods

Chemicals

Analytical grade neonicotinoids were used in all experiments. Imidacloprid (99.5%), acetamiprid (99.5%), thiamethoxam (99.5%), and clothianidin (99.5%) were purchased from Chem Service Inc. Nitenpyram (99.9%) was purchased from Fluka Analytical. HPLC-grade solvents (methanol, acetonitrile) were purchased from Sigma-Aldrich. Ultra-pure water (18.2 MΩ·cm) was obtained using a Milli-Q Academic system (Millipore). Buffers were made using ACS-Grade chemicals. Sodium acetate (99.5%) was purchased from BDH Chemicals, MOPS (3-(N-morpholino)propanesulfonic acid) (99.5%) was purchased from Sigma Aldrich, sodium tetraborate (assayed purity 102.2%) was purchased from Fisher Chemicals, and potassium phosphate monobasic (>99.0%) and sodium phosphate dibasic (>99.0%) were purchased from J.T. Baker. ACS-Grade (99.9%) acetic acid was purchased from BDH Chemicals. Zinc (II) chloride (>98%) and nickel (II) chloride (>99.9%) were purchased from Sigma Aldrich, and copper (II) chloride (99%) was purchased from Acros Organics. Titanium dioxide type P25 (>99.5%) was purchased from Acros Organics, kaolinite type KGa-1b was purchased from the Clay Mineral Society, and goethite was synthesized and characterized by Jeanette Tensfeldt of the Penn Lab at the University of Minnesota – Twin Cities. *para*-Nitroanisole (PNA; 98%) and pyridine (>99.0%) were purchased from Sigma-Aldrich. Sodium nitrate (99.2%) was purchased from Fisher Chemical, and *para*-chlorobenzoic acid (*p*CBA; 99%) was purchased from Acros Organics. Hydrochloric acid (TraceMetal^{TM} Grade) was purchased from Fisher Chemical.

Hydrolysis Experiments

Neonicotinoid Stock Solutions. Stock solutions were made for use in dosing reactors with low concentrations of neonicotinoid insecticides. Methanol was used for hydrolysis stock solutions to eliminate any hydrolytic degradation reactions from occurring in the

stock. The stocks were made by weighing ~5 mg of neonicotinoid into a 10-mL volumetric flask and recording the mass. Methanol was added, and the mass was recorded. The mass of methanol used was converted to a volume (~10.0 mL), giving the concentration of the solution. Stocks were placed in aluminum foil-wrapped 20 mL scintillation vials and stored at 4 ºC and replaced every 4-6 months. Before use, stock solutions were allowed to reach room temperature.

Buffer Solutions. To determine the hydrolysis rates over a range of pH values, buffer solutions were made at pH 4, 6.33, 7, 8, 9, and 10. pH 9 was used only for thiamethoxam reactors because hydrolysis occurred rapidly at pH 10. Acetate was used as a buffer for pH 4, MOPS was used for pH 6.33, 7, and 8 buffers, and sodium tetraborate (e.g. borate) was used for pH 9 and 10 experiments. The acetate buffer was prepared by dissolving 60 mg of sodium acetate in 500 mL of Milli-Q water, then titrating with acetic acid until pH 4 was reached. MOPS (1.046 g) was dissolved in 500 mL of Milli-Q water, and then titrated with 1 M NaOH or 1 M HCl until the desired pH was achieved. Sodium tetraborate (1.906 g) was dissolved in 500 mL of Milli-Q water and titrated with 1 M NaOH until the desired pH was reached.

Baseline Experiments. Experiments were performed at pH 4, 6.33, 7, 8, and 9/10. Reactors at each pH were dosed with a methanol stock solution to achieve an initial concentration of 1 μM. The solution containing the neonicotinoid was transferred to an aluminum foil-wrapped scintillation vial and sampled on different schedules, depending on pH. Low pH samples (4, 6.33, and 7) were sampled every two weeks, and high pH samples (8 and 10) were sampled weekly. Nitenpyram (pH 10) and thiamethoxam (pH 9) were sampled 3 times per week due to faster reaction times.

Metal Ion Experiments. To determine if trace metal ions impact the rate of neonicotinoid degradation, neonicotinoid reactors at pH 4, 6.33, 8, and 10 were dosed with either copper (II) chloride, nickel (II) chloride, or zinc (II) chloride. Reactors were started with 1.0 mM M(II) for pH 4 and 6.33, and 0.1 mM M(II) for pH 8 and 10. A lower

concentration of metal was used at high pH values to prevent precipitation of solids. Reactors at pH 4 and 6.33 were made by dissolving an appropriate mass of metal chloride salt in pH 4 or pH 6.33 buffer, and then following the same procedure for baseline reactors. Reactors at pH 8 and 10 were started by adding 1.0 mL of M(II) buffer solution to a 10 mL volumetric flask, dosing with neonicotinoid to 1 μM, and filling with pH 8 or 10 buffer. Adjustment of pH back to 8 and 10 was performed using NaOH.

Mineral Experiments. Two procedures were used to make reactors with minerals, which was dependent on the mineral stock (suspension or dry). TiO_2 (25 nm particles, BET surface area of 65 m^2/g) and kaolinite (particle size: 57.8% < 2 μm, 32.0% < 0.5 μm, BET surface area of 11.7 m^2/g) stocks were dry, while the goethite stock was a suspension with a mass loading determined to be 26.7 g/L. To create goethite (90 nm long by 15 nm wide, BET surface area of 138.4 m^2/g) reactors at 1 g/L, 0.37 mL of stock solution was added to 9.63 g of pH 8 or 10 buffer solution in 20 mL scintillation vials. Kaolinite and TiO_2 reactors were started by adding ~10 mg of dry mineral to a 20-mL scintillation vial, then adding buffer until the mass was 10 g. Exact masses were recorded to determine the mineral loading of each reactor. A stir bar was then placed in each scintillation vial and vials were stirred for 18-24 hours before neonicotinoids were added at 10 μM using methanol stock solutions. Vials were stirred constantly on a 16-channel analog stir plate (Scilogix, LLC). To eliminate all light, vials were wrapped in aluminum foil and a box was placed over the stir plate. Samples were filtered through a 0.2 μm syringe filter to remove minerals prior to analysis.

Mississippi River Water Experiments. Reactors were prepared in water taken from the Mississippi River and filter sterilized through a 0.2 μm filter. Neonicotinoids were dosed into Mississippi River water at 10 μM using methanol stock solutions. The pH was measured as 8.28, with a conductivity of 364.8 μS at 22.4 ºC, and a dissolved organic matter (DOM) concentration of 4.78 mg/L.

Data Analysis. Comparison of reaction rates between baseline reactors and metal containing reactors was done using a t-test to compare the slopes of kinetic data (ln[neonicotinoid] vs. time). Statistical analysis was performed using the Real Statistics[91] add-in for Microsoft Excel, with the 'SlopesTest' function used to compare the slope of baseline reaction rates with to reactions with metal. The slopes test function is based on analyses described by Howell.[92] Pooled variance was assumed to be applicable to each t-test. The null hypothesis tested was that the slopes are equal; if the p-value generated was greater than 0.05, the null hypothesis could not be rejected, and the slopes are assumed to be equal. If the p-value generated was less than 0.05, the null hypothesis could be rejected (the slopes are different).

Photolysis Experiments

Location. Photolysis experiments were performed both in natural sunlight as well as simulated sunlight in an Atlas Suntest CPS+ solar simulator with a xenon arc lamp fitted with a 290-nm cutoff filter. Natural sunlight experiments were conducted on the roof of the Department of Mechanical Engineering Building, University of Minnesota Twin-Cities campus (44˚58'30.6" N, 93˚14'01.1" W). A solar spectrum for this location was generated using the Natural Renewable Energy Laboratory (NREL) Simple Model of the Atmospheric Radiative Transfer of Sunshine (SMARTS) model version 2.9.5. The solar spectrum generated gave irradiance intensities on an average summer day for the latitude coordinates provided.

Neonicotinoid Stock Solutions. Photolysis experiments required stocks to be made in Milli-Q water, due to methanol being a radical quencher. Water stocks were made by weighing ~5 mg of neonicotinoid into a 10-mL volumetric flask and recording the mass, then adding 10.0 mL of water to the flask. The flasks were wrapped in foil and then shaken until a majority of the neonicotinoid in the flask was dissolved. Stocks were filtered through a 0.2 μm filter to remove undissolved compound in the solution. To determine exact concentrations, a dilution series was used, and exact concentrations of

dilutions were determined by analyzing on an HPLC and comparing to calibration standards made with methanol stocks. Stocks were stored in aluminum foil wrapped 20 mL scintillation vials and stored at 4 ºC and were remade after each set of experiments.

Experiments. Photolysis experiments were carried out in ultrapure (Milli-Q) water and Mississippi River water (MRW). MRW was collected from the University of Minnesota Boathouse dock, pre-filtered with combusted glass-fiber filters (Millipore, 0.7 µm), filter-sterilized with nitrocellulose membrane filters (Millipore, 0.22 µm), and stored at 4 ºC until used. Two separate MRW samples were collected, on July 12, 2017 and on November 3, 2017. Characterization of each sample is found in **Table 2**. Conductivity was measured using a Model 72 Engineered Systems and Design conductivity meter, and pH was measured with a WTW 340i pH meter fitted with a Sensorex S200C probe. DOC was measured on with a Shimadzu TOC-L analyzer operated in non-purgeable organic carbon mode. Photolysis experiments were run in triplicate in quartz test tubes (Ace Glass).

Table 2. Water chemistry characteristics of Mississippi River water samples.

Sample Characteristic	July	November
pH	8.28	8.40
Conductivity, µS[1]	364.8 @ 22.4 ºC	357.8 @ 20.4 ºC
DOC, mg/L	4.78	4.86

[1]Conductivity was measured at ambient laboratory temperature

To determine the pathway of neonicotinoid degradation, solutions were prepared in Milli-Q and Mississippi River water by dosing in water stocks of neonicotinoids, resulting in a 10 µM contaminant concentration. PNA – pyridine actinometers were run to allow determination of quantum yield, using 5 µM PNA and variable concentrations of pyridine, due to differences in neonicotinoid reactivity.

After initial experiments were performed, further tests were run to determine photolysis in nitrate amended waters (10 mg/L as N, added as sodium nitrate). Experiments were

performed in the solar simulator. Experiments were run in parallel in triplicate with neonicotinoid added to each of the following: Milli-Q water, Mississippi River water, and Mississippi River water amended with nitrate. A *p*CBA probe was used to determine steady state hydroxyl radical concentrations. The *p*CBA probe was prepared by dissolving 10 mg of *p*CBA in pH 12 water, stirring overnight until dissolved, neutralizing with concentrated TraceMetal™ Grade HCl, and spiking *p*CBA into nitrate amended Mississippi River water to give concentration of 5 μM.

Data Analysis. Data was analyzed using methods prescribed by Leifer[93] using the recent update to the PNA quantum yield relationship.[94] The relationship between quantum yield (ϕ) and the concentration of pyridine (*[pyr]*) is given in Eq. 1.

$$\Phi = 0.29[pyr] + 0.00029 \quad (1)$$

The reaction rate in the test tube is given by Eq. 2, where k_d is the rate constant observed in the test tube, γ is an orientation parameter with a value of 2.2 for test tubes, ϕ is quantum yield, ε_λ is the molar absorptivity at a specific wavelength, and L_λ is the irradiance at a specific wavelength. The subscripts *a* and *c* are used to distinguish between the actinometer and the contaminant (neonicotinoid).

$$k_d = \gamma * \phi_d * \sum_\lambda \epsilon_\lambda * L_\lambda \quad (2)$$

From the quantum yield calculated in Eq. 1 for the actinometer (ϕ_{da}), the molar absorptivity of PNA,[94] and the irradiance, k_{da}, the rate in the test tube for the actinometer can be calculated. Irradiance in the solar simulator was provided by the manufacturer, while irradiance in natural sunlight was estimated using the NREL SMARTS model.[95]

$$\ln\left(\frac{[C_0]}{[C_t]}\right) = k_d * t \quad (3)$$

First-order kinetic data is used to calculate k_{dc}, or the rate constant observed in the test tube for the contaminant. From Leifer,[93] Eq. 3 can also be used to described the rate in the test tube for both the contaminant and the actinometer. Photolysis experiments

involve running the actinometer and contaminant in parallel, allowing Eq. 3 for the contaminant to be divided by Eq. 3 for the actinometer. This gives Eq. 4, which relates the observed concentration data at different time points to the reaction rate in the test tube. Eq. 4 also provides a way to calculate the quantum yield of contaminants.

$$\ln\left(\frac{[C_0]}{[C_t]}\right)_c = \frac{k_{dc}}{k_{da}} * \ln\left(\frac{[C_0]}{[C_t]}\right)_a \quad (4)$$

Graphing ln([C_0]/[C_t]) of the contaminant versus ln([C_0]/[C_t]) for the actinometer gives a slope which is equal to k_{dc}/k_{da}. k_{da} is calculated from Eq. 2 for the actinometer, allowing for the k_{dc} to be calculated. Once k_{dc} is known, Eq. 2 for the contaminant can be used to find the quantum yield of the contaminant, using γ (2.2 for test tubes),[93] and the sum of molar absorptivity multiplied by irradiance over the range where the contaminant absorbs light. Molar absorptivity at different wavelengths was calculated for each neonicotinoid from collected UV-Visible spectra; see Appendix B **Table B1** and **Figure B1** for more information.

Screening factors were calculated for photolysis experiments run in MRW to allow for adjustment of calculated rate constants and quantum yields. Screening factors account for the absorption of light by dissolved organic matter (DOM) in Mississippi River water samples, which decreases the light absorbed by neonicotinoids in the solution. Screening factors are calculated as the ratio of light absorption in the presence and absence of DOM (i.e. the species responsible for screening). Eq. 5 describes how to calculate the rate of light absorption ($R_{a,i}$) at the wavelength λ of a neonicotinoid *(i)* without DOM present, where W_λ represents the irradiance (milli-Einstein's cm^{-2} s^{-1}), a_i is the absorbance (a.u.) at a given λ, and z is path length (1.12 cm in all calculations). Eq. 6 describes how to calculate the rate of light of absorption (R_{a,i_DOM}) of neonicotinoid in the presence of DOM at a given λ, symbols are the same as in Eq. 5. Absorbance of DOM *(a_{DOM})* is equal to the absorbance of MRW.

$$R_{a,i_\lambda} = W_\lambda \frac{(1-10^{-a_{i\lambda}z})}{z} \quad (5)$$

$$R_{a,i\_DOM_\lambda} = W_\lambda \frac{(1-1^{-(a_{i_\lambda}+a_{DOM_\lambda})z})}{z} \times \frac{a_{i_\lambda}}{a_{i_\lambda}+a_{DOM_\lambda}} \quad (6)$$

$$S_{i\_DOM} = \frac{\sum_\lambda R_{a,i\_DOM_\lambda}}{\sum_\lambda R_{a,i_\lambda}} \quad (7)$$

Eq. 7 describes the calculation of the screening factor. A summation of all rates of light absorption of neonicotinoid in the presence of DOM at all wavelengths of light absorption is divided by the summation of the rate of light absorption at over all wavelengths of light absorption of the neonicotinoid. This yields a value between 0 and 1.0, with a value of 0 representing that no light is absorbed by the neonicotinoid (i.e. DOM screens all the light), while a value of 1.0 would indicate DOM does not screen light from being absorbed by neonicotinoids. For nitenpyram, the screening factor was calculated to be 0.95, for imidacloprid 0.96, for thiamethoxam 0.96, and 0.96 for clothianidin.

To directly compare rates and quantum yields of Milli-Q and Mississippi River water studies, results from Mississippi River water experiments are divided by the screening factor to remove the effect of DOM screening on the observed quantum yield. Screening factor adjustment gives the power to determine the importance of indirect photolysis on neonicotinoid degradation. If neonicotinoids only undergo direct photolysis, screening adjusted Mississippi River water samples will not have larger rate constants/quantum yields. If indirect photolysis is important, Mississippi River water samples would have larger rate constants/quantum yields after adjusting for screening.

Results from nitrate experiments were analyzed by comparing rate constants of Milli-Q samples, Mississippi River water samples, and nitrate amended Mississippi River water samples and calculating second order rate constants using hydroxyl radical concentrations obtained from *p*CBA probes. Bimolecular rate constants of neonicotinoids with hydroxyl

$$\ln\left(\frac{[A]}{[A]_0}\right) = \frac{k_{A,HO\cdot}}{k_{pCBA,HO\cdot}} \ln\left(\frac{[pCBA]}{[pCBA]_0}\right) \quad (8)$$

radicals ($k_{A,HO\cdot}$) were derived from the linear regression of natural log normalized concentrations of neonicotinoids (A) vs. pCBA, shown in Eq. 8, where $k_{pCBA,HO\cdot}$ is the bimolecular rate constant of pCBA reaction with hydroxyl radicals. The value of $k_{pCBA,HO\cdot}$ is 5×10^9 M^{-1} s^{-1} and was obtained from literature.[96]

Analytical Methods

High Pressure Liquid Chromatography (HPLC). Neonicotinoid degradation was monitored by HPLC on an Agilent 1200 Diode-Array Detector fitted with an Ascentis Supleco RP-Amide C-16 column (15 cm × 4.6 mm, 5 µm), with an RP-Amide guard column. Nitenpyram, imidacloprid, acetamiprid, thiamethoxam, clothianidin were analyzed using methods described in in **Table 3**. PNA and pCBA required different mobile phases than the neonicotinoids, which are also provided in **Table 3**.

Analytical Standards. Analytical standards for HPLC were used to determine exact concentration of samples. Standards were made using pH 7 10 mM phosphate buffer as well as Milli-Q water. After no difference in degradation of standards was observed over time between pH 7 buffer and Milli-Q standards, Milli-Q was used for standards. Thiamethoxam and nitenpyram standards were replaced on at least a monthly basis to prevent degradation of accuracy and were stored at 4 ºC between uses. Standards were made using 10 mL volumetric flasks. Five concentrations were made from approximately 0.5 µM to 50 µM.

Table 3. HPLC methods for sample analysis.

Compound	Flow *(mL/min)*	Run Time *(min)*	Retention Time *(min)*	Method	Injection Volume	Detection Wavelength
Nitenpyram	0.8	8.0	6.65	90% **A** 10% **C**	20 µL	270 nm
Imidacloprid	1.0	5.5	3.97	70% **A** 30% **B**	20 µL	254 nm
Acetamiprid	1.0	5.5	4.21	70% **A** 30% **B**	20 µL	254 nm
Thiamethoxam	1.0	5.5	2.99	70% **A** 30% **B**	20 µL	254 nm
Clothianidin	1.0	5.5	4.01	70% **A** 30% **B**	20 µL	254 nm
PNA	1.0	7.0	5.42	50% **B** 50% **D**	50 µL	313 nm
*p*CBA	1.0	15	12.1	35% **B** 65% **D**	40 µL	238 nm

A: 10 mM pH 7 phosphate buffer w/ 10% acetonitrile, **B**: Acetonitrile, **C:** Methanol, **D:** 10 mM pH 3 phosphate buffer w/ 10% acetonitrile.

Mass Spectrometry. Ultra-pressure liquid chromatography - tandem mass spectrometry (UPLC-MS/MS) was used to identify neonicotinoid reaction products. Samples were analyzed on a Thermo Fisher Orbitrap UPLC-MS/MS system with a C18 nanoflow column at The University of Minnesota Masonic Cancer Center. The solvent gradient is given in **Table 4.** Data was analyzed using Thermo Fisher Scientific Compound

Table 4. UPLC-MS/MS mobile phase gradient for neonicotinoid analysis.

Time (min)	Flow [µL/min]	% **A**	% **B**
0.0	1.0	98.0	2.0
5.5	1.0	98.0	2.0
6.0	0.3	98.0	2.0
20.0	0.3	60.0	40.0
24.0	0.3	2.0	98.0
26.0	0.3	2.0	98.0
28.0	1.0	98.0	2.0
33.0	1.0	98.0	2.0

A: 2% acetic acid buffer, **B:** LC-MS grade acetonitrile

Discoverer 2.1 software. An untargeted environmental analysis and targeted analysis of expected neonicotinoid degradation products (from previously reported hydrolysis and photolysis products) were performed. Exact mass was used to determine molecular composition and to compare to literature results. MS2 fragmentation patterns were compared to fragmentation patterns for previously reported products to further support the observed products.

Toxicity

Samples for parent compound toxicity tests were prepared by dosing methanol stock solution to a 10 mL volumetric flask so the final concentration was 50 µM, filling with Milli-Q ultrapure water and mixing well. Concentration was verified using HPLC. Hydrolysis samples containing products were prepared by creating a 50 µM solution at pH 10 and monitoring until the neonicotinoid concentration was 10 µM. Samples were then filtered through a 0.2 µm syringe tip filter and neutralized using metals grade concentrated HCl. Photolysis samples were prepared by reacting a 50 µM solution in an Atlas CPS+ solar simulator and monitoring using HPLC until the concentration of parent compound was 10 µM. This yielded samples with an approximate 4:1 ratio of metabolites to parent compound. Samples were stored at -20 ºC before being used in experiments.

Toxicity experiments were performed using mosquito (*Culex pipiens*) 4^{th} instar larvae. Larvae were placed in distilled water and distributed into vials (5 larvae in each of three replicate vials), and volumes were adjusted to 9.0 ± 0.1 mL. Treated samples were diluted based on residual parent neonicotinoid concentrations and 1 mL was added to each vial, giving final parent neonicotinoid concentrations from 0.1 to 1.0 µM. Control vials received 1 mL of distilled water. After 20 h, larvae that exhibited movement were scored as alive. All calculated values of 0 and 100% are based on averages from three vials from two separate experiments. LC_{50} values were then calculated by plotting response (percent) vs. dose (concentration) and determining the point at which 50% of larvae died.

Results

Baseline Hydrolysis

Neonicotinoid baseline hydrolysis reactors were monitored and sampled for 50 to 150 days, with the longer sampling periods occurring for all clothianidin reactors and all low (4, 6.33, 7) pH reactors. Pseudo-first order rate constants were calculated using linear regression of the natural log of concentration vs. time for all reactors, and results are given in **Figure 5** and **Table 5**. In low pH samples for all neonicotinoids, little to no degradation was observed, with half-lives calculated to be over 1000 days for most compounds. Significant error is present in calculations for reactors below pH 8. In many cases the standard deviation of the linear regression (calculated as the sum of the 95% confidence intervals divided by the product of the number of samples times the square root of the number of samples) is the same order of magnitude as the calculated pseudo-first order rate constant.

Baseline imidacloprid results are similar to previously reported hydrolysis studies, in which imidacloprid was only observed to react at pH values greater than 9.[37,38] Thiamethoxam hydrolysis kinetics at high pH were also similar to those previously reported.[40,97] Karmakar *et al*. (2009), however, observed significantly faster degradation of thiamethoxam at pH 4 and 7 than was observed in this study. Clothianidin has been observed to be stable under acidic and neutral conditions, with slow hydrolysis observed in alkaline conditions[98] with similar results observed in this work.

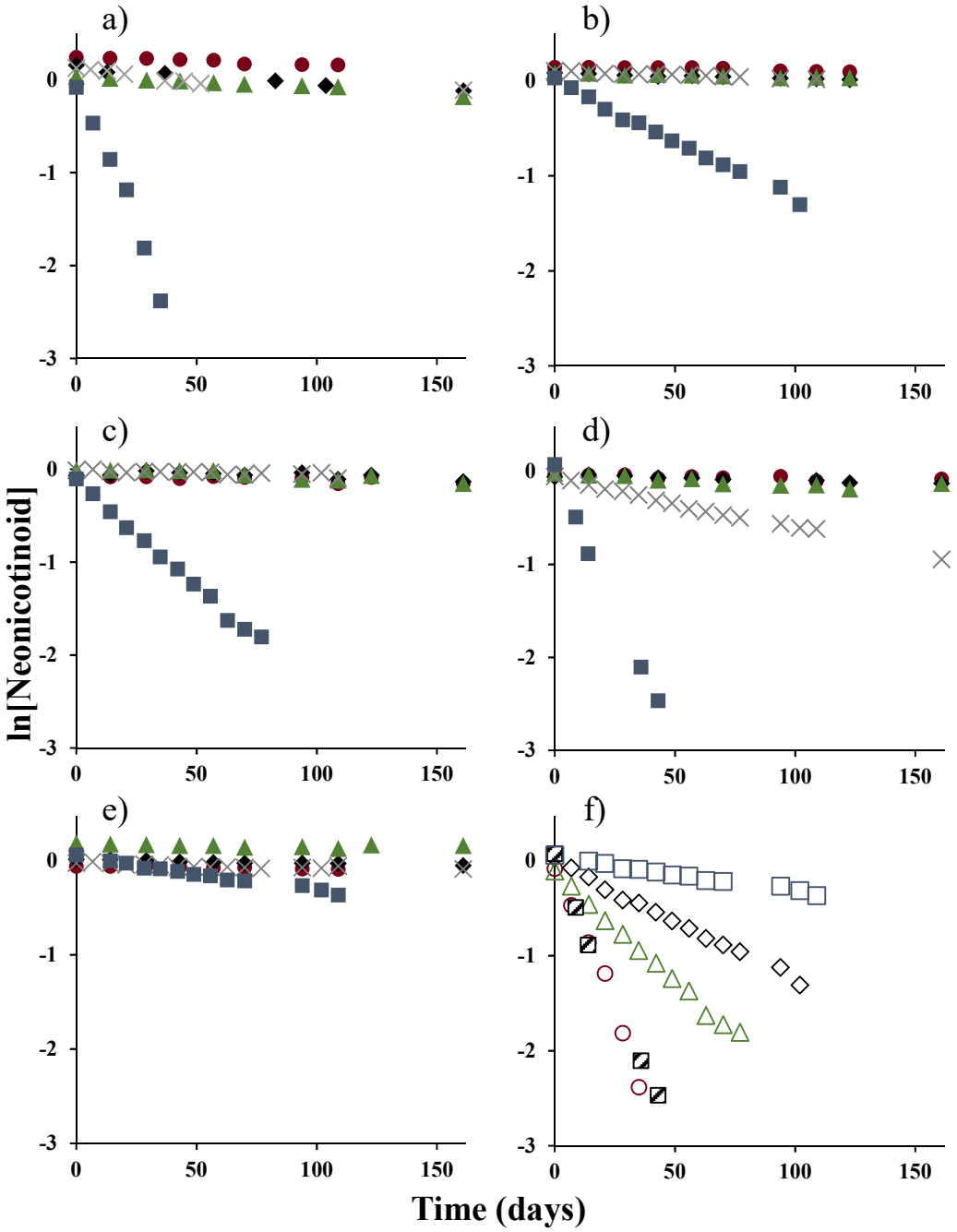

Figure 5. Baseline hydrolysis of neonicotinoid insecticides at pH 4, 6.33, 7, 8, and 10. a) Nitenpyram, b) Imidacloprid, c) acetamiprid, d) Thiamethoxam, e) Clothianidin, f) pH 10 (pH 9 for thiamethoxam) hydrolysis results. Legend graphs a)-e): ● pH 4, ♦ pH 6.33, ▲ pH 7, X pH 8, ■ pH 10. Legend graph f): ○ Nitenpyram pH 10, ◊ Imidacloprid pH 10. △ Acetamiprid pH 10, ▨Thiamethoxam pH 9, □ Clothianidin pH 10.

Table 5. Calculated pseudo-first order rate constants (k_{obs}) and half-lives ($t_{1/2}$) for hydrolysis of neonicotinoids at 21.5 ºC. Copper, nickel, and zinc columns represent observed rate constants of neonicotinoids in the presence of each metal. Error is the 95% confidence interval.

		Baseline		Copper		Nickel		Zinc	
	pH	k_{obs} (d^{-1})	$t_{1/2}$ (d)	k_{obs} (d^{-1})	$t_{1/2}$ (d)	k_{obs} (d^{-1})	$t_{1/2}$ (d)	k_{obs} (d^{-1})	$t_{1/2}$ (d)
Nitenpyram	4	$8.5 \pm 2.5\times10^{-4}$	820	$6.2 \pm 1.7\times10^{-4}$	>1000	$7.1 \pm 1.5\times10^{-4}$	970	$7.5 \pm 2.9\times10^{-4}$	920
	6.33	$1.4 \pm 0.4\times10^{-3}$	500	$1.1 \pm 0.7\times10^{-3}$	660	$8.4 \pm 3.5\times10^{-4}$	830	$1.4 \pm 0.5\times10^{-3}$	510
	7	$1.3 \pm 0.2\times10^{-3}$	550	-	-	-	-	-	-
	8	$3.4 \pm 0.4\times10^{-3}$	210	$1.5 \pm 0.3\times10^{-3}$	470	$2.5 \pm 0.6\times10^{-3}$	280	$1.7 \pm 0.4\times10^{-3}$	420
	10	$5.1 \pm 0.1\times10^{-2}$	14	$4.8 \pm 0.3\times10^{-2}$	15	$5.0 \pm 0.3\times10^{-2}$	14	$5.0 \pm 0.4\times10^{-2}$	14
Imidacloprid	4	$4.3 \pm 1.6\times10^{-4}$	>1000	$2.9 \pm 0.9\times10^{-4}$	>1000	$3.0 \pm 0.7\times10^{-4}$	>1000	$3.6 \pm 2.7\times10^{-4}$	>1000
	6.33	$5.4 \pm 1.0\times10^{-4}$	>1000	$6.9 \pm 4.3\times10^{-4}$	>1000	$5.1 \pm 3.1\times10^{-4}$	>1000	$5.4 \pm 4.4\times10^{-4}$	>1000
	7	$4.2 \pm 1.4\times10^{-4}$	>1000	-	-	-	-	-	-
	8	$7.9 \pm 0.9\times10^{-4}$	880	$9.9 \pm 5.2\times10^{-4}$	700	$1.3 \pm 0.5\times10^{-3}$	530	$9.7 \pm 5.6\times10^{-4}$	720
	10	$1.8 \pm 0.1\times10^{-2}$	39	$1.5 \pm 0.1\times10^{-2}$	47	$1.5 \pm 0.1\times10^{-2}$	46	$1.5 \pm 0.1\times10^{-2}$	46
Acetamiprid	4	$4.0 \pm 3.3\times10^{-4}$	>1000	$5.2 \pm 2.6\times10^{-4}$	>1000	$5.9 \pm 2.0\times10^{-4}$	>1000	$2.5 \pm 1.6\times10^{-4}$	>1000
	6.33	$4.2 \pm 4.2\times10^{-4}$	>1000	$7.5 \pm 2.0\times10^{-4}$	920	$5.9 \pm 2.3\times10^{-4}$	>1000	$6.6 \pm 1.6\times10^{-4}$	>1000
	7	$1.0 \pm 0.4\times10^{-3}$	670	-	-	-	-	-	-
	8	$1.5 \pm 0.2\times10^{-3}$	460	$2.0 \pm 0.5\times10^{-3}$	340	$2.2 \pm 0.6\times10^{-3}$	310	$1.9 \pm 0.5\times10^{-3}$	370
	10	$2.8 \pm 0.1\times10^{-2}$	25	$2.6 \pm 0.1\times10^{-2}$	26	$2.6 \pm 0.1\times10^{-2}$	27	$2.5 \pm 0.1\times10^{-2}$	27
Thiamethoxam	4	$2.0 \pm 1.8\times10^{-4}$	>1000	$3.4 \pm 7.4\times10^{-4}$	>1000	$2.9 \pm 1.1\times10^{-4}$	>1000	$2.8 \pm 2.1\times10^{-4}$	>1000
	6.33	$5.7 \pm 1.7\times10^{-4}$	>1000	$6.3 \pm 82\times10^{-4}$	>1000	$2.4 \pm 1.8\times10^{-3}$	290	$3.1 \pm 2.6\times10^{-3}$	230
	7	$1.0 \pm 0.3\times10^{-3}$	670	-	-	-	-	-	-
	8	$5.4 \pm 0.2\times10^{-3}$	130	$3.4 \pm 0.9\times10^{-3}$	200	$4.0 \pm 1.1\times10^{-3}$	170	$4.6 \pm 1.2\times10^{-3}$	151
	9	$5.8 \pm 0.1\times10^{-2}$	12	$5.3 \pm 0.8\times10^{-2}$	13	$5.2 \pm 0.1\times10^{-2}$	10	$5.2 \pm 0.1\times10^{-2}$	13
Clothianidin	4	$3.4 \pm 1.2\times10^{-4}$	>1000	$7.0 \pm 11\times10^{-5}$	>1000	$1.0 \pm 2.0\times10^{-4}$	>1000	$3.2 \pm 3.3\times10^{-4}$	>1000
	6.33	$4.1 \pm 0.5\times10^{-4}$	>1000	$2.8 \pm 2.6\times10^{-4}$	>1000	$2.3 \pm 2.1\times10^{-4}$	>1000	$2.9 \pm 1.4\times10^{-4}$	>1000
	7	$4.3 \pm 2.1\times10^{-4}$	>1000	-	-	-	-	-	-
	8	$4.6 \pm 1.2\times10^{-4}$	>1000	$5.3 \pm 0.1\times10^{-4}$	>1000	$8.8 \pm 0.1\times10^{-4}$	780	$8.1 \pm 11\times10^{-4}$	860
	10	$5.2 \pm 0.4\times10^{-3}$	135	$5.1 \pm 0.6\times10^{-3}$	136	$4.9 \pm 0.6\times10^{-3}$	140	$5.1 \pm 0.7\times10^{-3}$	135

Hydrolysis Experiments in the Presence of Cu^{2+}, Ni^{2+}, and Zn^{2+}

Neonicotinoid reactors containing 1 mM (pH 4, 6.33) and 0.1 mM (pH 8, 10) divalent metal ions were monitored for 50 to 150 days, depending on the rate of reaction. Pseudo-first order rate constants were calculated using linear regression of the natural log of concentration vs. time for all reactors, and results are given in **Table 5**. Kinetic data for pH 4 and pH 6.33 reactors are given in **Figure 6**, and **Figure 7** gives data for pH 8 and pH 10 reactors. Similar to baseline reactors, little to no degradation was observed at pH 4 and 6.33, with significant uncertainty in calculated results. At pH 8 and 10, these metals do not appear to have an effect on degradation rate. To allow statistical comparison of slopes, p-values for slopes comparisons were calculated, and are given in **Table 6**.

Comparison of the slopes for nitenpyram shows the null hypothesis cannot be rejected for any of the metals; all p-values are greater than 0.05. Thus, for nitenpyram, addition of the divalent metals copper, nickel, and zinc, does not appear to change the rate of hydrolysis.

Similar results were observed for imidacloprid, acetamiprid, thiamethoxam, and clothianidin. At pH 4 and pH 6.33 calculated p-values were all greater than 0.05, indicating no statistically significant difference between baseline and metal containing reactors. At pH 10, results were all statistically different between metal containing reactors and baseline reactors, while pH 8 had mixed results, with some p-values both greater and less than 0.05. This could indicate metals do change the reaction rate at higher pH values. After the pH of each sample was re-tested (see **Table A1**), however, it was clear there was some minor variation in pH between different samples that had previously been assumed to be at the same pH.

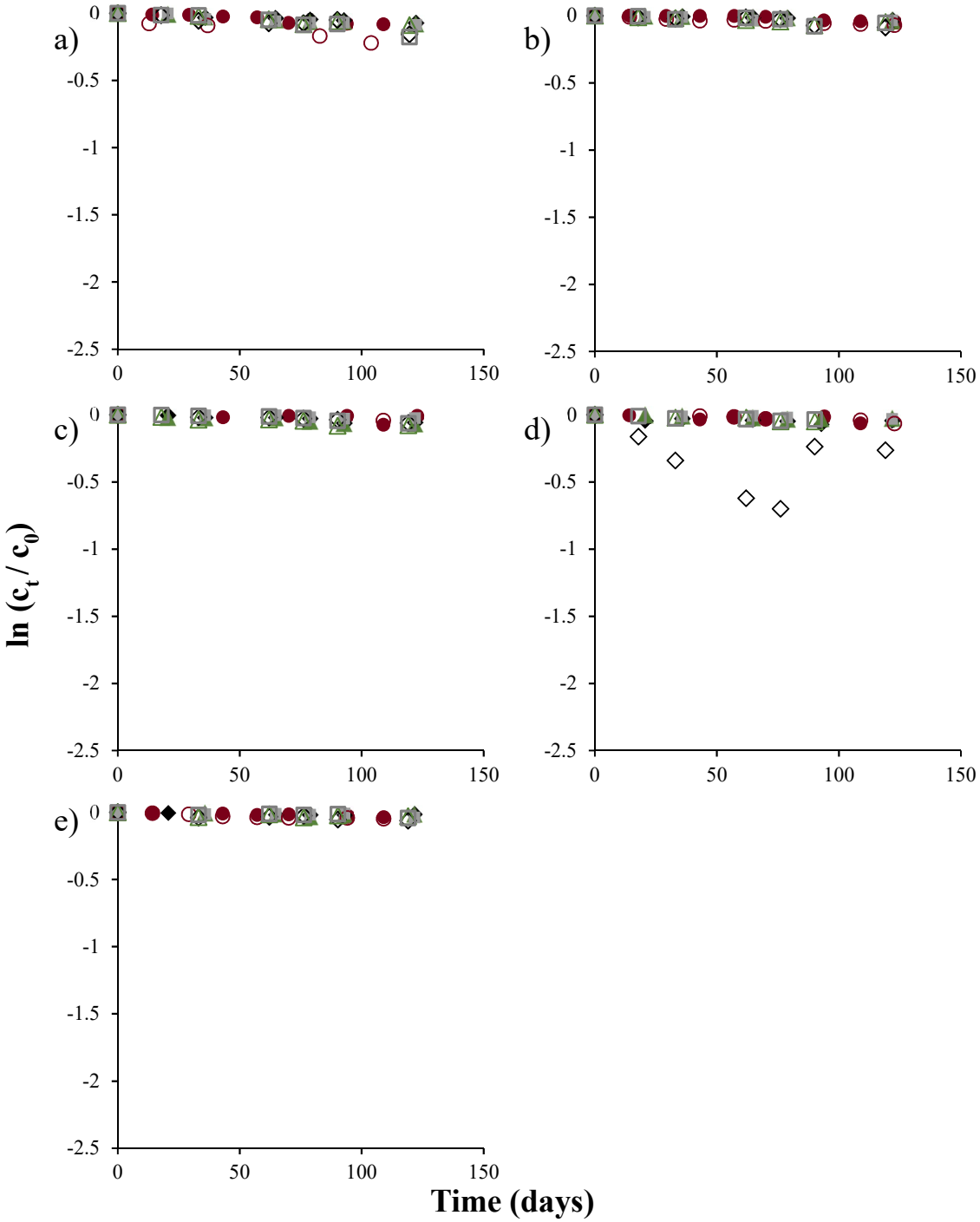

Figure 6. Hydrolysis of neonicotinoids in the presence of metals, pH 4 and pH 6.33. a) Nitenpyram, b) Imidacloprid, c) Acetamiprid, d) Thiamethoxam, e) Clothianidin. Legend: ● baseline hydrolysis, pH 4, ♦ hydrolysis in the presence of Cu^{2+}, pH 4, ▲ hydrolysis in the presence of Ni^{2+}, pH 4, ■ hydrolysis in the presence of Zn^{2+}, pH 4 ○ baseline, pH 6.33, ◊ Cu^{2+}, pH 6.33. Δ Ni^{2+}, pH 6.33, □ Zn^{2+}, pH 6.33.

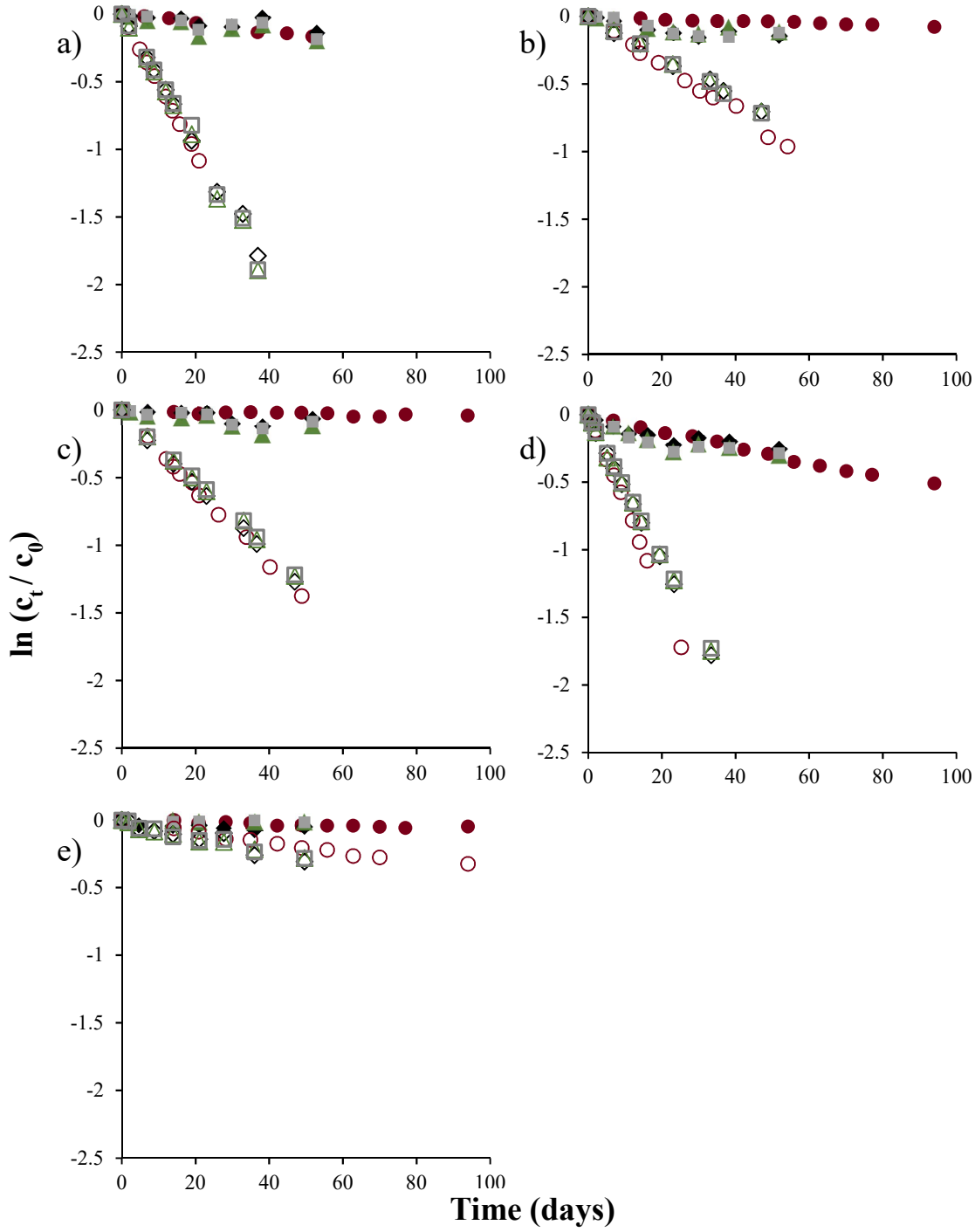

Figure 7. Hydrolysis of neonicotinoids in the presence of metals, pH 8 and pH 10. a) Nitenpyram, b) Imidacloprid, c) Acetamiprid, d) Thiamethoxam, e) Clothianidin. Legend: ● baseline hydrolysis, pH 8, ♦ hydrolysis in the presence of Cu^{2+}, pH 8, ▲ hydrolysis in the presence of Ni^{2+}, pH 8, ■ hydrolysis in the presence of Zn^{2+}, pH 8 ○ baseline, pH 10, ◊ Cu^{2+}, pH 10, Δ Ni^{2+}, pH 10, □ Zn^{2+}, pH 10.

Table 6. Calculated p-values of metal hydrolysis studies. p-values compare baseline results at a given pH with hydrolysis results of neonicotinoid hydrolysis at the same pH with a divalent metal ion present. A p-value of less than 0.05 indicates the slopes of the compared results are significantly different at the 95% confidence level.

	pH	4	6.33	8	10
Nitenpyram	Baseline + Cu	0.087	0.303	0.084	0.065
	Baseline + Ni	0.272	0.054	0.743	0.533
	Baseline + Zn	0.529	0.934	0.583	0.518
Imidacloprid	Baseline + Cu	0.160	0.341	0.480	0.0006
	Baseline + Ni	0.170	0.814	0.037	0.0003
	Baseline + Zn	0.546	0.999	0.574	0.0002
Acetamiprid	Baseline + Cu	0.186	0.228	5.22×10^{-5}	0.012
	Baseline + Ni	0.153	0.362	4.22×10^{-6}	0.00095
	Baseline + Zn	0.516	0.386	1.78×10^{-5}	0.00035
Thiamethoxam	Baseline + Cu	0.471	0.409	2.78×10^{-4}	4.0×10^{-12}
	Baseline + Ni	0.407	0.922	0.0297	1.62×10^{-13}
	Baseline + Zn	0.661	0.306	0.330	4.02×10^{-14}
Clothianidin	Baseline + Cu	0.095	0.123	0.440	0.026
	Baseline + Ni	0.746	0.279	0.302	0.0052
	Baseline + Zn	0.118	0.909	0.180	0.00489

To account for the variation in pH, hydrolysis reactions were assumed to be second order because the rate of degradation increased as the concentration of hydroxide ion increased, as described in Eq. 9. Thus, second order rate constants were calculated by dividing the observed, pseudo-first order rate constant by $[OH^-]$, shown in Eq. 10 and Eq. 11, giving a rate constant with units $M^{-1}\ d^{-1}$. Propagation of error was performed using the standard deviation results from the pseudo-first order linear regression. Error was calculated by dividing the 95% confidence interval (C.I.) by $[OH^-]$, see Eq. 12.

$$rate = k[OH^-][Neonic] \tag{9}$$

$$k_{obs} = k[OH^-] \tag{10}$$

$$k = \frac{k_{obs}}{[OH^-]} \tag{11}$$

$$Error = \frac{95\%\ C.I.}{[OH^-]} \quad (12)$$

Calculated second order rate constants are given in **Table 7**. Examination of the second order rate constants reveals there is no significant difference between hydrolysis reactions when divalent metals are present than in solutions without metals present. At a given pH, error calculated from the 95% confidence intervals shows second order rate constants are all within error of each other at a specific pH. Thus, it is likely all the variation observed in results and statistical difference is due to small differences in the pH of individual reactors.

Table 7. Second order rate constants (M^{-1} d^{-1}) for neonicotinoid insecticide hydrolysis at 21.5 ºC. Error is the 95% confidence interval.

	Reactor	pH 4	pH 6.33	pH 8	pH 10
Nitenpyram	Baseline	6600000 ± 1900000	67000 ± 19000	4200 ± 500	550 ± 8
	Copper	6000000 ± 1700000	80000 ± 51000	1900 ± 400	550 ± 31
	Nickel	6600000 ± 1400000	35000 ± 15000	3700 ± 800	560 ± 38
	Zinc	7000000 ± 2700000	63000 ± 25000	2900 ± 700	540 ± 42
Imidacloprid	Baseline	3400000 ± 1300000	24000 ± 4000	1000 ± 100	170 ± 9
	Copper	3000000 ± 900000	79000 ± 49000	3000 ± 1600	160 ± 13
	Nickel	2800000 ± 700000	30000 ± 18000	1800 ± 700	170 ± 8
	Zinc	3200000 ± 2400000	27000 ± 22000	2200 ± 1300	170 ± 9
Acetamiprid	Baseline	3300000 ± 2700000	20000 ± 20000	1800 ± 300	430 ± 15
	Copper	5400000 ± 2700000	67000 ± 18000	4600 ± 1200	300 ± 11
	Nickel	5600000 ± 1900000	26000 ± 10000	3500 ± 900	290 ± 12
	Zinc	2100000 ± 1300000	31000 ± 7000	3500 ± 900	290 ± 10
Thiamethoxam	Baseline	1700000 ± 1500000	33000 ± 10000	7400 ± 300	6790 ± 170
	Copper	3500000 ± 7600000	98000 ± 1267000	7700 ± 1900	7160 ± 110
	Nickel	2600000 ± 1000000	115000 ± 85000	7100 ± 2000	7000 ± 150
	Zinc	2600000 ± 1900000	154000 ± 132000	7800 ± 2000	7460 ± 140
Clothianidin	Baseline	3000000 ± 1100000	18000 ± 2000	600 ± 200	58 ± 4
	Copper	700000 ± 1200000	20000 ± 18000	600 ± 1200	56 ± 6
	Nickel	1000000 ± 1900000	11000 ± 10000	1100 ± 1400	54 ± 6
	Zinc	3000000 ± 3000000	13000 ± 6000	1500 ± 2100	56 ± 7

Calculated second order rate constants, however, indicate that the hydrolysis reaction that the neonicotinoids undergo, is, in fact, not a second order elementary reaction. From pH 4 to pH 10, calculated rate constants vary by 5 to 6 orders of magnitude, indicating the assumed reaction mechanism is incorrect.

Hydrolysis reactions can occur due to reaction of a compound with H^+, H_2O, or OH^-. Because the reaction at pH 4 is slow in all reactors, it was assumed there were no hydrolysis reactions occurring due to H^+, thus, the rate of reaction observed at pH 4 was assumed to be the baseline rate of hydrolysis reaction with respect to H_2O. The observed rate constant is then assumed to be a sum of the rate due to hydrolysis from water and the rate due to base catalyzed hydrolysis, as shown in Eq. 15. Because hydrolysis does increase with increasing concentration of hydroxide, the concentration of hydroxide was assumed to be part of the overall rate expression, but expressed to some unknown power of *n*. The exponent *n* is calculated by graphing the log of $k_{obs} - k_{pH\,4}$ versus the -pOH of each reactor and determining the slope of the regression line of the resulting scatterplot (**Figure 8**).

$$rate = k_{H_2O}[Neonic] + k_{OH^-}[Neonic][OH^-]^n \quad (13)$$

$$rate = k_{obs}[Neonic] \quad (14)$$

$$k_{obs} = k_{H_2O} + k_{OH^-}[OH^-]^n \quad (15)$$

$$Assume\ k_{H_2O} = k_{pH\,4}$$

$$k_{obs} - k_{pH\,4} = k_{OH^-}[OH^-]^n \quad (16)$$

$$\log\left(k_{obs} - k_{pH\,4}\right) = n * pOH + \log(k_{OH^-}) \quad (17)$$

Calculated reaction orders range from 0.50 ± 0.105 (clothianidin) to 0.67 ± 0.183 (thiamethoxam), with imidacloprid (0.52 ± 0.121), acetamiprid (0.62 ± 0.125), and

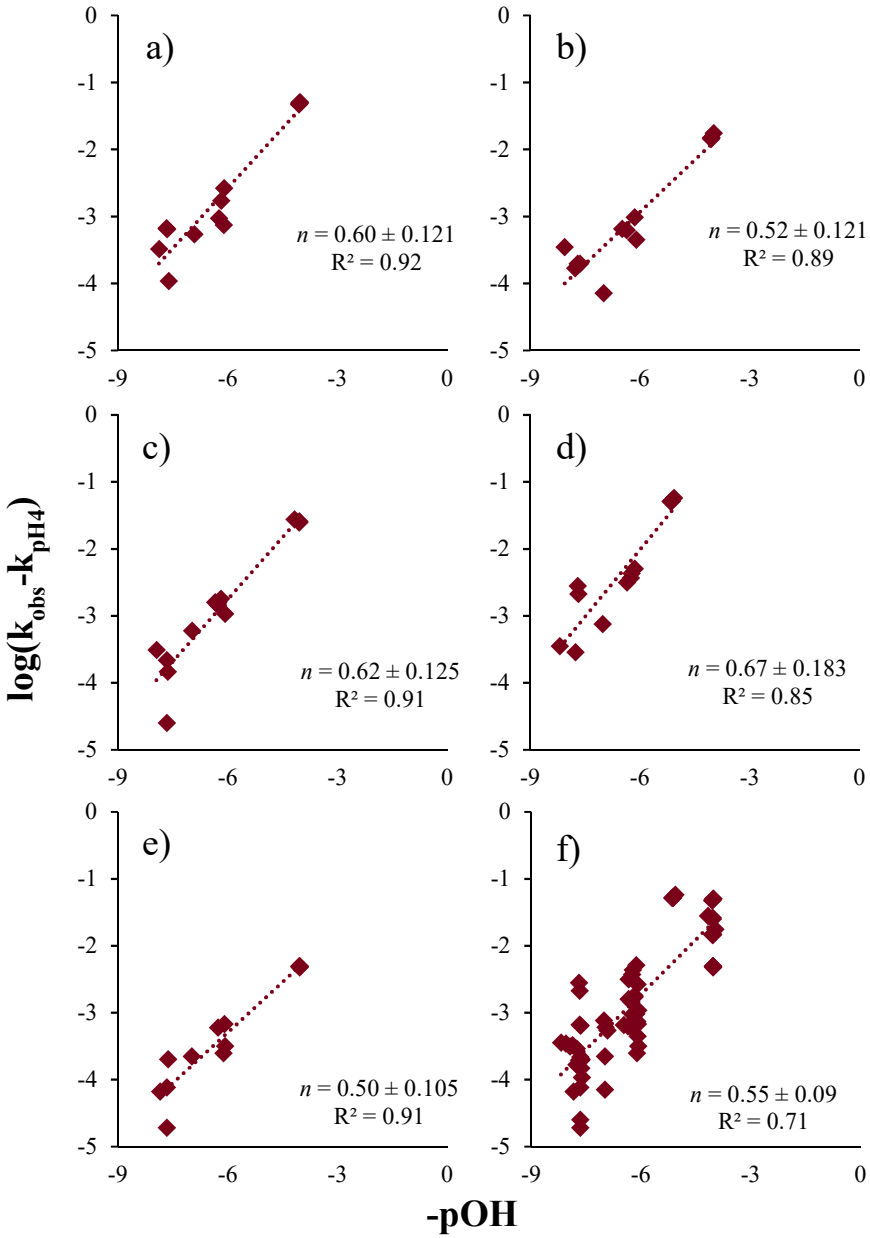

Figure 8. Log-log plot of the hydroxide concentration and the difference between k_{obs} and $k_{pH\ 4}$. The resulting slope is the approximate value of *n*, the [OH^-] in the non-elementary reaction of neonicotinoid hydrolysis. a) Nitenpyram, b) imidacloprid, c) acetamiprid, d) thiamethoxam, e) clothianidin, and f) all neonicotinoids combined. All data points were combined to estimate the value of n after slope testing revealed no statistical significance between the slopes of each of the individual neonicotinoids.

nitenpyram (0.60 ± 0.121) in the middle. Errors are 95% confidence intervals. Due to the relative similarity between the calculated reaction order, a slopes test was performed to compare each of the slopes to determine if there was a significant difference between the values of *n*. Calculated p-values (**Table 8**) show there is not a statistically significant difference between each of the calculated slopes. Thus, all data points were placed in a single plot, shown in **Figure 8f**, and linear regression yielded a value for *n* of 0.55 ± 0.09. Non-elementary rate constants were calculated for all baseline and metal reactors using a value of 0.55 for *n*. Results are given in **Table 9**.

Table 8. Calculated p-values of slopes test comparing the value of the exponent of the hydroxide concentration in non-elementary hydrolysis reactions.

	Nitenpyram	Imidacloprid	Acetamiprid	Thiamethoxam	Clothianidin
Nitenpyram	-	0.315	0.802	0.493	0.203
Imidacloprid	-	-	0.220	0.146	0.833
Acetamiprid	-	-	-	0.632	0.134
Thiamethoxam	-	-	-	-	0.090
Clothianidin	-	-	-	-	-

Generally, slopes tests had shown pH 8 and pH 10 reactions were different, when baseline and metals containing reactors were compared. When hydroxide rate constants are compared at the 95% confidence interval however, rate constants do not appear to be different at a specific pH between baseline and metals containing solutions. There is perhaps a slight difference between the acetamiprid pH 10 reactor and metal reactors, but the metals in this case appear to slightly slow the rate of reaction as opposed to increase it. Thus, these results indicate that the tested divalent metal cations in solution do not change the rate of hydrolysis of neonicotinoids.

Table 9. Hydroxide rate constants for neonicotinoid hydrolysis reactions at 21.5 ºC.

	pH	6.33	8	10
Nitenpyram	Baseline	10.3 ± 6.2	5.6 ± 0.9	8.0 ± 0.1
	Copper	6.5 ± 13.4	1.6 ± 0.7	7.7 ± 0.4
	Nickel	1.6 ± 5.1	4.1 ± 1.3	8.0 ± 0.5
	Zinc	9.7 ± 8.3	2.4 ± 1.1	7.8 ± 0.6
	Avg.[1]		6.1 ± 0.9	
Imidacloprid	Baseline	8.0 ± 1.5	1.7 ± 0.2	2.6 ± 0.1
	Copper	17.4 ± 10.9	3.4 ± 1.8	2.4 ± 0.2
	Nickel	9.0 ± 5.5	2.9 ± 1.1	2.5 ± 0.1
	Zinc	8.6 ± 7.0	2.9 ± 1.7	2.4 ± 0.1
	Avg.[1]		4.2 ± 0.5	
Acetamiprid	Baseline	0.4 ± 5.8	2.3 ± 0.5	5.3 ± 0.2
	Copper	6.8 ± 4.4	4.7 ± 1.5	4.2 ± 0.2
	Nickel	2.2 ± 3.4	4.3 ± 1.4	4.2 ± 0.2
	Zinc	3.4 ± 2.4	3.8 ± 1.4	4.1 ± 0.2
	Avg.[1]		3.8 ± 0.5	
Thiamethoxam	Baseline	5.0 ± 2.9	11.5 ± 0.5	33.8 ± 0.8
	Copper	10.5 ± 242.6	9.3 ± 2.5	33.4 ± 0.5
	Nickel	33.2 ± 27.9	9.6 ± 2.9	32.7 ± 0.7
	Zinc	44.8 ± 42.0	10.9 ± 2.9	33.7 ± 0.6
	Avg.[1]		23.4 ± 2.3	
Clothianidin	Baseline	3.0 ± 0.7	0.5 ± 0.3	0.8 ± 0.1
	Copper	1.3 ± 5.0	0.7 ± 2.1	0.8 ± 0.1
	Nickel	0.3 ± 3.2	1.4 ± 2.4	0.8 ± 0.1
	Zinc	1.2 ± 2.1	1.6 ± 3.0	0.8 ± 0.1
	Avg.[1]		1.1 ± 0.5	

Rate constants are in units of $M^{-0.55}$ d^{-1}. Error is the 95% confidence interval. Thiamethoxam samples were studied at pH 8 and 9. [1] Average rate constants were calculated by averaging rate constants from baseline and metal experiments at pH 6.33, 8, and 10. Error was calculated by divided the sum of the 95% confidence interval by the product of the number of data points times the square root of the number of points. Thiamethoxam pH 6.33 copper was excluded as an outlier due to the large error associated with the value. Imidacloprid pH 6.33 copper was excluded as an outlier as it had an outsized effect on mean.

Hydrolysis Experiments in the Presence of the Minerals Kaolinite, Goethite, and Titanium Dioxide

Reactors containing minerals (kaolinite, goethite, or titanium dioxide) as well as new baseline reactors were monitored for up to 100 days, depending on the speed of the reaction. Placement of a box to reduce the possibility of photolysis over the stir plate created the possibility of a slightly increased rate of reaction, for other work has shown neonicotinoid hydrolysis reaction rate increases with temperature.[37,40] To account for the potential effect of temperature, and the potential effect of stirring mineral reactors constantly while previous reactors had not been stirred, new baseline hydrolysis reactors were run along with mineral reactors. Pseudo-first-order rate constants were calculated for all reactions and are given in **Table 10**. Reaction kinetics are shown in **Figure 9**.

Table 10. Pseudo-first order rate constants (d^{-1}) for neonicotinoid insecticide mineral reactors at 28 ºC. Error is the 95% confidence interval.

	pH	Baseline	Kaolinite	Goethite	Titanium Dioxide
Nitenpyram	8	$1.8 \pm 0.4\times10^{-3}$	$3.0 \pm 0.3\times10^{-3}$	$2.1 \pm 0.4\times10^{-3}$	$5.0 \pm 0.5\times10^{-3}$
	10	$1.0 \pm 0.1\times10^{-1}$	$9.8 \pm 1.0\times10^{-2}$	$1.2 \pm 0.2\times10^{-1}$	$1.2 \pm 0.2\times10^{-1}$
Imidacloprid	8	$1.6 \pm 1.6\times10^{-4}$	$9.9 \pm 5.6\times10^{-4}$	$4.8 \pm 1.9\times10^{-4}$	$1.6 \pm 0.5\times10^{-3}$
	10	$3.6 \pm 0.1\times10^{-2}$	$3.9 \pm 0.2\times10^{-2}$	$4.0 \pm 0.1\times10^{-2}$	$4.0 \pm 0.1\times10^{-2}$
Acetamiprid	8	$2.1 \pm 1.6\times10^{-4}$	$7.1 \pm 0.5\times10^{-4}$	$5.5 \pm 1.6\times10^{-4}$	$1.8 \pm 0.2\times10^{-3}$
	10	$4.6 \pm 0.1\times10^{-2}$	$5.1 \pm 0.1\times10^{-2}$	$6.4 \pm 0.2\times10^{-2}$	$6.3 \pm 0.2\times10^{-2}$
Thiamethoxam	8	$6.8 \pm 0.7\times10^{-3}$	$8.5 \pm 0.5\times10^{-3}$	$1.0 \pm 0.1\times10^{-2}$	$1.1 \pm 0.1\times10^{-2}$
	9	$9.5 \pm 0.1\times10^{-2}$	$1.0 \pm 0.1\times10^{-1}$	$1.7 \pm 0.1\times10^{-1}$	$1.6 \pm 0.1\times10^{-1}$
Clothianidin	8	$6.0 \pm 20\times10^{-6}$	$1.1 \pm 0.5\times10^{-3}$	$7.0 \pm 5.0\times10^{-4}$	$2.0 \pm 48\times10^{-5}$
	10	$1.0 \pm 0.1\times10^{-2}$	$1.2 \pm 0.2\times10^{-2}$	$1.2 \pm 0.1\times10^{-2}$	$1.2 \pm 0.1\times10^{-2}$

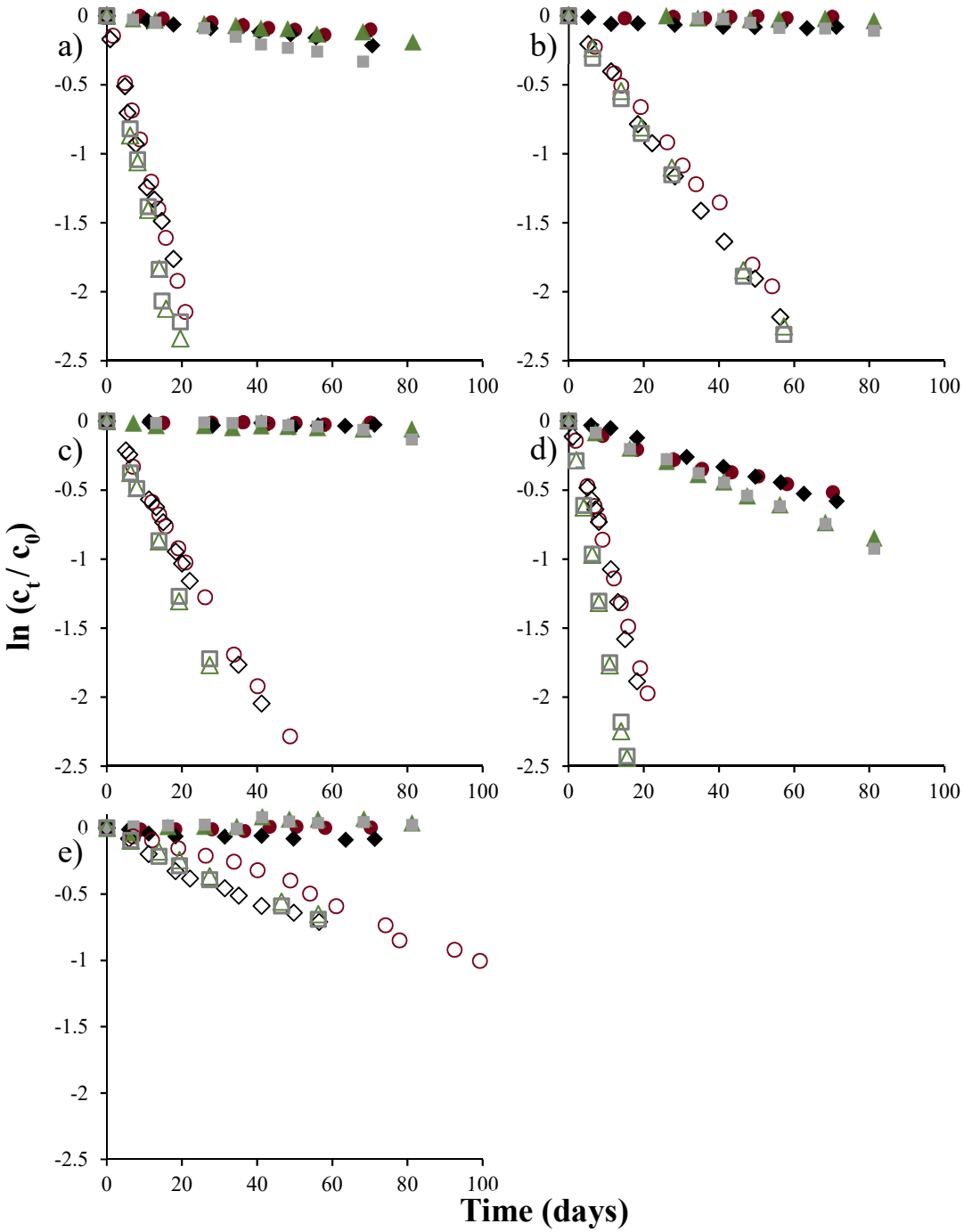

Figure 9. Hydrolysis of neonicotinoids in the presence of minerals. a) Nitenpyram, b) Imidacloprid, c) Acetamiprid, d) Thiamethoxam, e) Clothianidin. Legend: ● baseline hydrolysis, pH 8, ♦ hydrolysis in the presence of kaolinite, pH 8, ▲ hydrolysis in the presence of goethite, pH 8, ■ hydrolysis in the presence of TiO_2, pH 8, ○ baseline, pH 10, ◊ kaolinite, pH 10. Δ goethite, pH 10, □ TiO_2, pH 10. Note: thiamethoxam reactors were run at pH 9.

Reaction rates were varied between reactors containing minerals and stir plate baseline reactors. At pH 8, long half-lives were still observed, with many imidacloprid, acetamiprid, and clothianidin reactors having calculated half-lives of over 1000 days. With the temperature increase of 7 ºC compared to previous reactors, pH 10 reactors showed faster reaction rates. Goethite and titanium dioxide reactors appeared to increase reaction rate slightly, most notably in pH 10 reactors. The same slopes test as used for mineral reactors was run to compare all experiments, to determine if a difference between trials did exist. Resulting p-values are given in **Table 11**.

Table 11. Calculated p-values from slopes-test results for hydrolysis reactions in the presence of minerals.

	pH	8	10
Nitenpyram	Baseline + kaolinite	0.0004	0.260
	Baseline + goethite	0.2568	0.0006
	Baseline + TiO_2	1.28×10^{-8}	0.0279
Imidacloprid	Baseline + kaolinite	0.002	0.016
	Baseline + goethite	0.012	0.002
	Baseline + TiO_2	1.35×10^{-6}	0.001
Acetamiprid	Baseline + kaolinite	0.020	0.001
	Baseline + goethite	0.005	4.40×10^{-10}
	Baseline + TiO_2	5.19×10^{-6}	8.30×10^{-10}
Thiamethoxam	Baseline + kaolinite	7.08×10^{-4}	1.62×10^{-4}
	Baseline + goethite	2.79×10^{-9}	1.89×10^{-15}
	Baseline + TiO_2	1.34×10^{-9}	4.76×10^{-12}
Clothianidin	Baseline + kaolinite	3.21×10^{-4}	0.026
	Baseline + goethite	0.021	0.201
	Baseline + TiO_2	0.948	0.061

Results from the slopes test are varied. For nitenpyram, results indicate no statistical difference between baseline results and kaolinite results at pH 10, and between baseline results and goethite results at pH 8. All imidacloprid, acetamiprid, and thiamethoxam p-values are less than 0.05 for all conditions, indicating no slopes can be said to be the same. For clothianidin, calculated p-values indicate no difference between baseline and

TiO_2 results at pH 8 and pH 10, nor a difference between the baseline and goethite reactors at pH 10. As with neonicotinoid reactors containing metals, slight variations in pH (see **Table A1**) were observed in reactors containing minerals. To account to the variation in pH, bimolecular rate constants for reaction with OH^- were calculated for all reactors. Results from the baseline pH 4 reactors were used in calculating mineral hydroxide rate constants, because no pH 4 mineral reactors were made.

Table 12. Hydroxide rate constants ($M^{-0.55}$ d^{-1}) for neonicotinoid insecticide reactions in the presence of minerals at 28 ºC. Error is the 95% confidence interval.

	pH	8	10
Nitenpyram	Baseline	3.0 ± 1.1	16.8 ± 0.2
	Kaolinite	5.9 ± 0.9	17.4 ± 1.8
	Goethite	2.8 ± 0.8	20.2 ± 2.4
	TiO_2	8.9 ± 1.0	19.5 ± 3.9
	Avg.	11.8 ± 0.5	
Imidacloprid	Baseline	0*	5.5 ± 0.2
	Kaolinite	1.7 ± 1.5	6.1 ± 0.3
	Goethite	0.3 ± 0.4	6.4 ± 0.2
	TiO_2	2.6 ± 1.0	6.2 ± 0.2
	Avg.	4.3 ± 0.2	
Acetamiprid	Baseline	0*	7.2 ± 0.1
	Kaolinite	0.8 ± 1.3	8.1 ± 0.2
	Goethite	0.2 ± 0.3	10.5 ± 0.4
	TiO_2	2.8 ± 0.4	10.2 ± 0.3
	Avg.	5.0 ± 0.2	
Thiamethoxam	Baseline	18.7 ± 2.1	58.4 ± 0.6
	Kaolinite	23.8 ± 1.3	61.6 ± 3.6
	Goethite	20.1 ± 0.6	89.0 ± 3.8
	TiO_2	22.1 ± 0.8	85.8 ± 2.7
	Avg.	47.4 ± 0.7	
Clothianidin	Baseline	0*	1.8 ± 0.1
	Kaolinite	2.2 ± 1.3	1.8 ± 0.3
	Goethite	1.0 ± 1.0	2.0 ± 0.1
	TiO_2	0*	1.9 ± 0.2
	Avg.	1.3 ± 0.2	

Thiamethoxam samples were studied at pH 8 and 9. Averages are calculated as a summation of pH 8 and pH 10 results of a neonicotinoid; 95% confidence interval is calculated as the sum of errors divided by $n\times\sqrt{n}$. *indicates calculated value was negative (i.e. rate constant was slower than pH 4 rate constant) and a value of 0 was used instead.

Calculated mineral hydroxide rate constants are given in **Table 12**. Several results were negative (pH 8 baseline imidacloprid, acetamiprid, and clothianidin, and pH 8 clothianidin with TiO_2), which indicates reaction rates are slower than the observed pH 4 reaction rates. Negative values are listed as zero in **Table 12**. Of particular note is the hydroxide reaction rates are higher for pH 9 thiamethoxam reactors in the presence of goethite and TiO_2. Rates increased from the baseline rate of 58.4 $M^{-0.55}$ d^{-1} to 89.0 $M^{-0.55}$ d^{-1} for goethite and 85.8 $M^{-0.55}$ d^{-1} for TiO_2, while kaolinite is within error of the baseline. This trend is not observed at pH 8 for thiamethoxam, for goethite and TiO_2 reactors are within error of the baseline while the kaolinite reactor is not. Thus, it is inconclusive as to the effect of selected minerals on neonicotinoid hydrolysis, however, they do not appear to have a significant effect across the two pH values tested.

Hydrolysis in Mississippi River Water

Mississippi River water samples were monitored for 150 days. Pseudo-first order rate constants were calculated as ln[Neonicotinoid] vs. time for experiments in MRW and are given in **Table 13**. Kinetic data is given in **Figure 10**. The pH of the MRW was 8.28, thus, pseudo-first order rate constants were expected to be faster than hydrolysis rates at pH 8. This was observed for nitenpyram, where the pseudo-first order rate constant is slightly larger than the average pseudo-first order at pH 8 (3.4×10^{-3} d^{-1} vs. 2.3×10^{-3} d^{-1}).

Table 13. Calculated pseudo-first order and hydroxide rate constants for hydrolysis reactions in MRW at 21.5 ºC. Error is the 95% confidence interval.

	$k_{obs,\ MRW}$ d^{-1}	$k_{avg,\ pH\ 8}$ d^{-1}	$k_{OH^-,\ MRW}$ $M^{-0.55}$ d^{-1}	$k_{OH^-,\ avg}$ $M^{-0.55}$ d^{-1}
Nitenpyram	$3.4 \pm 1.2\times10^{-3}$	$2.3 \pm 0.2\times10^{-3}$	3.5 ± 1.6	6.1 ± 0.9
Imidacloprid	$6.5 \pm 2.7\times10^{-4}$	$1.0 \pm 0.2\times10^{-3}$	0.4 ± 0.4	5.3 ± 0.7
Acetamiprid	$3.5 \pm 2.2\times10^{-4}$	$1.9 \pm 0.2\times10^{-3}$	0.1 ± 0.3	3.8 ± 0.5
Thiamethoxam	$4.4 \pm 0.5\times10^{-3}$	$4.4 \pm 0.4\times10^{-3}$	5.5 ± 0.7	22 ± 8
Clothianidin	$6.4 \pm 4.4\times10^{-4}$	$6.7 \pm 4.2\times10^{-4}$	0.6 ± 0.6	1.1 ± 0.5

$k_{obs,MRW}$: pseudo-first order rate constant for hydrolysis reactions in Mississippi River water (pH 8.28), $k_{avg,\ pH\ 8}$: Averaged pseudo-first order rate constants at pH 8, $k_{OH^-,\ MRW}$: hydroxide rate constant for Mississippi River water hydrolysis experiments, $k_{OH^-,\ avg}$: Average hydroxide rate constant across all pH values.

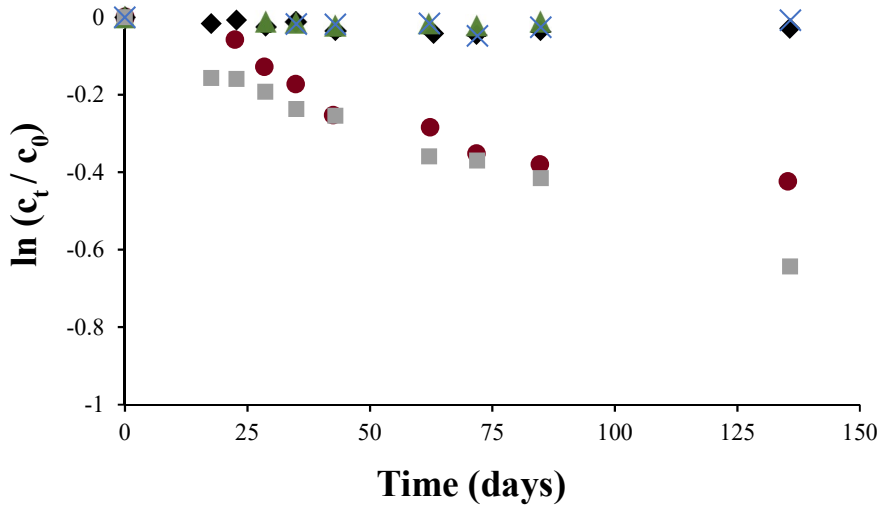

Figure 10. Hydrolysis of neonicotinoids in Mississippi River water (pH 8.28). Legend: ● Nitenpyram, ♦ Imidacloprid, ▲ Acetamiprid, ■ Thiamethoxam, × Clothianidin.

Thiamethoxam pseudo-first order rate constants were the same (4.4×10^{-4} d^{-1}) even with the slight pH variation. Clothianidin was marginally slower (6.4×10^{-4} d^{-1} in Mississippi River water vs. 6.7×10^{-4} d^{-1} pH 8 average), though results are well within error. Acetamiprid and imidacloprid, however, were significantly slower in Mississippi River water experiments.

Comparison of hydroxide rate constants, which accounts for comparison across several pH values, indicates every neonicotinoid reacts slower in Mississippi River water, once accounting for the pH of Mississippi River water. No explanation is currently available to account for changes in reaction rates, though these results would indicate hydrolysis degradation rates may be even slower in natural water bodies, as MRW used in these experiments was filter-sterilized.

Photolysis

Kinetic data for experiments run on the roof the UMN Mechanical Engineering building and in an Atlas solar simulator are given in **Figure 11** and **Figure 12**, respectively. Quantum yields (ϕ) were calculated used the described method and are shown in **Table 14**. Screening factors were applied to Mississippi River water samples to allow for direct comparison of quantum yields between experiments, also given in **Table 14**. Calculated first-order rate constants are available in Appendix B (see **Table B2**).

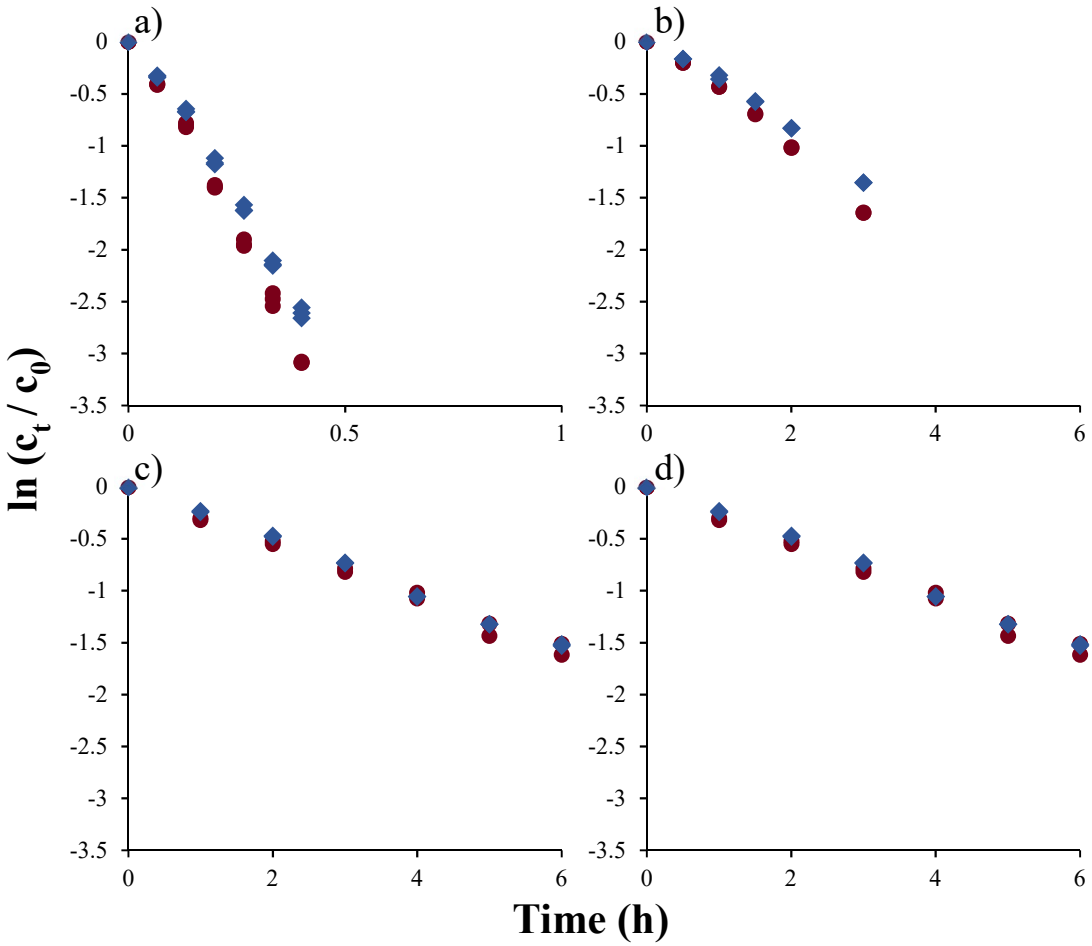

Figure 11. Photolysis kinetics of neonicotinoid insecticides in Milli-Q water and Mississippi River water in natural sunlight. a) Nitenpyram, b) Imidacloprid, c) Thiamethoxam, d) Clothianidin. Legend: ● represents experiments run in Milli-Q water; ♦ represents experiments run in Mississippi River water.

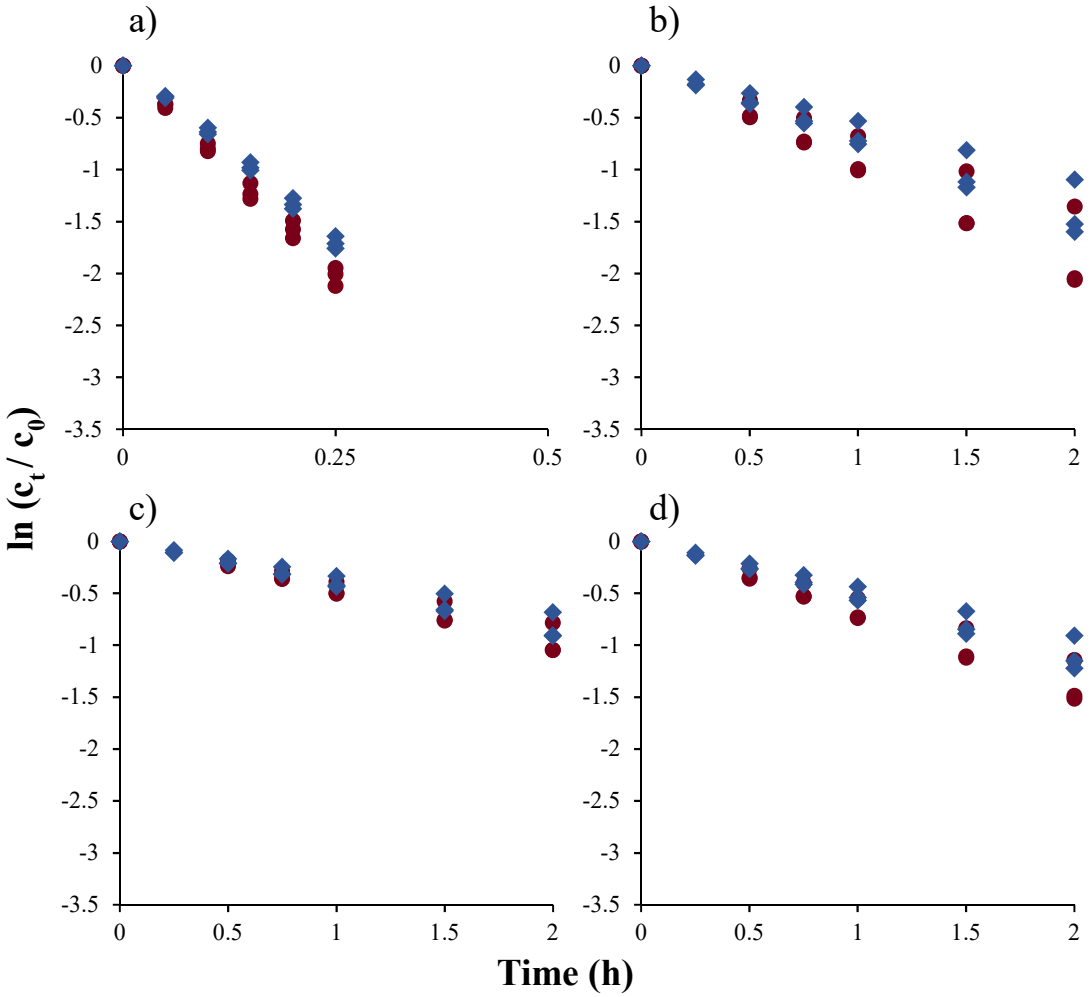

Figure 12. Photolysis kinetics of neonicotinoid insecticides in Milli-Q water and Mississippi River water in an Atlas Solar Simulator. a) Nitenpyram, b) Imidacloprid, c) Thiamethoxam, d) Clothianidin. Legend: ● represents experiments run in Milli-Q water; ♦ represents experiments run in Mississippi River water.

In the solar simulator, calculated quantum yields for nitenpyram, imidacloprid, thiamethoxam, and clothianidin in Milli-Q water are all larger than quantum yields in Mississippi River water after adjusting for screening, indicating indirect photolysis does not play a part neonicotinoid photodegradation. Results were similar in natural sunlight experiments, with similar quantum yields calculated between natural sunlight and solar simulator experiments, though calculated quantum yields were lower for thiamethoxam (25%) and clothianidin (32%). Additionally, in natural sunlight, once Mississippi River water samples were adjusted for screening, thiamethoxam had a lower quantum yield in

Table 14. Calculated average quantum yields for neonicotinoid insecticides in natural and simulated sunlight.

		Nitenpyram	Imidacloprid	Thiamethoxam	Clothianidin
Solar Sim	Milli-Q	0.025 ± 0.001	0.0119 ± 0.0001	0.0167 ± 0.0002	0.0133 ± 0.0001
	MRW[1]	0.022 ± 0.001	0.0086 ± 0.0001	0.0131 ± 0.0001	0.0095 ± 0.0001
	MRW S.A.[2]	0.023 ± 0.001	0.0089 ± 0.0001	0.0136 ± 0.0001	0.0099 ± 0.0001
Natural Sunlight	Milli-Q	0.025 ± 0.001	0.0115 ± 0.0005	0.0127 ± 0.0003	0.0091 ± 0.0002
	MRW[1]	0.023 ± 0.001	0.0097 ± 0.0005	0.0126 ± 0.0003	0.0078 ± 0.0001
	MRW S.A.[2]	0.024 ± 0.001	0.0100 ± 0.0005	0.0130 ± 0.0003	0.0080 ± 0.0001
Literature	Lu et al[41]	-	0.0092 ± 0.0005	0.019 ± 0.001	0.013 ± 0.001
	Other Work	-	0.0055[99]	0.013[100]	0.0073[99]

[1]Mississippi River water, [2]Screening Adjusted Mississippi River water samples. Error is the 95% confidence interval of average quantum yield, calculated as the summation of 95% confidence intervals divided by the product of the number of samples times the square root of the number of samples) (∑ 95% C.I.) / n*√n.

Milli-Q (0.0127 ± 0.0003) compared to screening adjusted Mississippi River water (0.0130 ± 0.0003). A one-tailed paired t-test comparing the two means, however, gives a value of 0.12, indicating at the 95% confidence interval, the two quantum yields cannot be distinguished. Thus, thiamethoxam is likely to follow the same behavior as imidacloprid, nitenpyram, and clothianidin, which photolyze only due to direct photolysis.

Calculated quantum yields in this study are similar to previously reported values. Quantum yields of imidacloprid calculated in this study (0.0089 to 0.119) are similar to previously reported quantum yields of 0.0092[41] and 0.0055.[99] Quantum yields of thiamethoxam calculated in this study (0.0130 to 0.0167) are between previously reported quantum yields of 0.019[41] and 0.013.[100] Similarly with clothianidin, quantum yields of 0.0073[99] and 0.013[41] have been reported, which are in the range of those calculated in this study (0.0080 to 0.0133).

Quantum yields of nitenpyram have not been published and few studies having examined photolysis of nitenpyram. While comparison of kinetic data of photolysis is certainly not

the most accurate method of comparison, the kinetic rate constant of nitenpyram for photolysis has been reported as 0.26 to 1.24 d^{-1},[101] which is similar to results found in this study of 3.1 to 3.3 d^{-1}.

Acetamiprid samples were originally studied in the solar simulator, where results after 3 hours of exposure gave an estimated half-life of >100 hours. While experiments were conducted on the rooftop of the University of Minnesota Mechanical Engineering building, exposure to sunlight for >1 month yielded little to no degradation of acetamiprid in Mississippi River water samples or Milli-Q samples, indicating direct photolysis was not an important environmental degradation pathway. These indicate a much longer half-life than observed in literature, where Lu *et al.* (2015) found acetamiprid to have a half-life of 26 hours with a quantum yield of 0.0022.[41]

Based on similar rates and quantum yields observed in Milli-Q and MRW, indirect photolysis does not initially appear to be important. Mississippi River water, however, does not represent the highest levels of nitrate that can be observed environmentally. Agricultural runoff may contain high levels of nitrate (4-20 mg/L as N),[102] which can generate higher concentrations of hydroxyl radicals. Further experiments were conducted using imidacloprid, acetamiprid, thiamethoxam, and clothianidin to study if high concentrations of nitrate, and thus hydroxyl radicals would increase degradation rates. Nitenpyram was not used in nitrate experiments because direct photolysis is rapid.

Pseudo-first order rate constants were calculated using linear regression of ln[C] vs. time, and rate constants shown represent averages of experiments run in triplicate. Kinetic data for photolysis in nitrate photolysis is given in **Figure 13**, and calculated rate constants are given in **Table 15**. Error was calculated as the 95% confidence interval; average error was calculated as the summation of 95% confidence intervals, divided by the product of $n \cdot \sqrt{n}$, where n is the number of rate constants used in the calculation (3 for all experiments). Screening constants were calculated for Mississippi River water samples

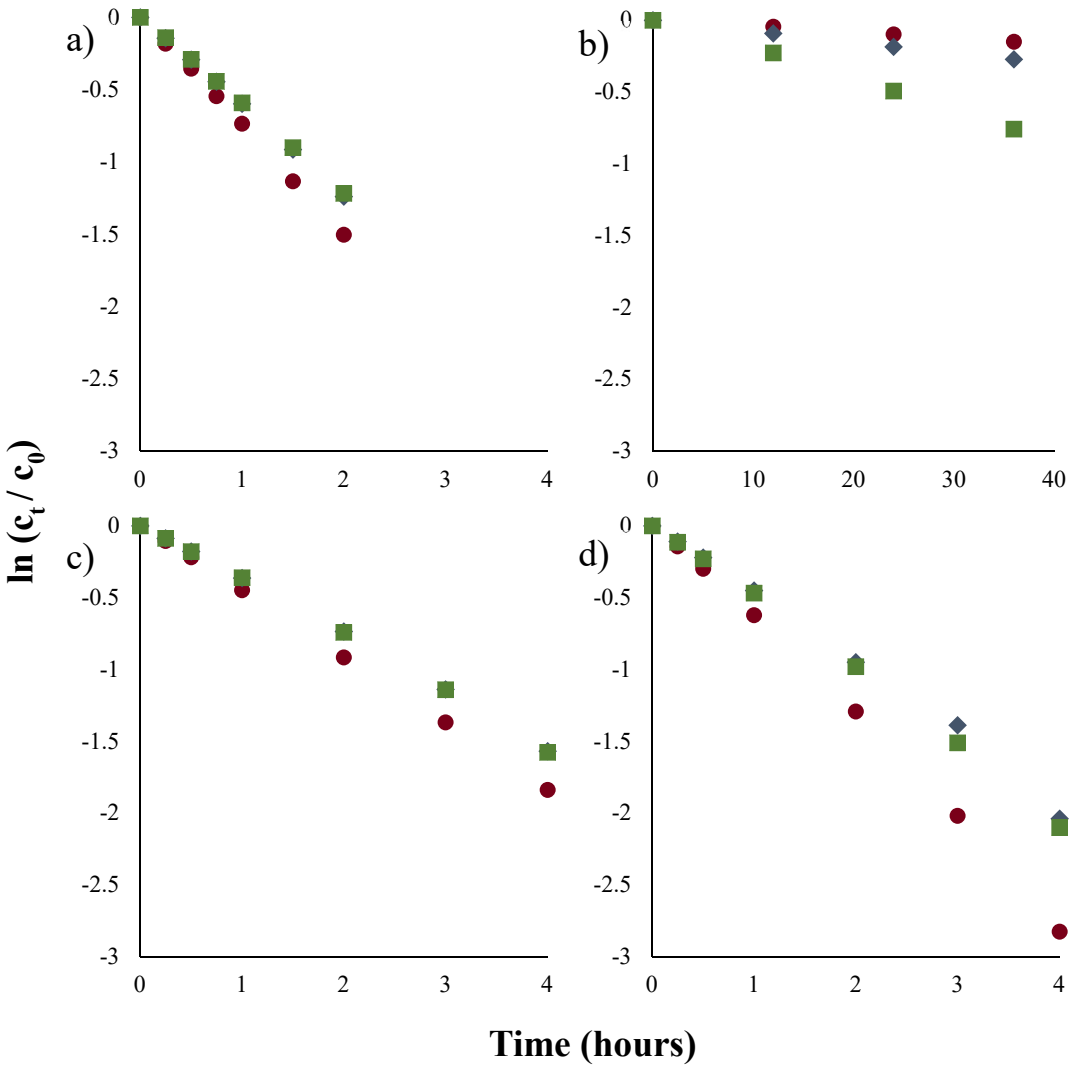

Figure 13. Averaged kinetic data of photolysis of neonicotinoid insecticides in nitrate (10 mg/L as N) amended water in an Atlas Suntest CPS+ solar simulator. a) Imidacloprid, b) Acetamiprid, c) Thiamethoxam, d) Clothianidin. Legend: ● experiments in Milli-Q water; ♦ experiments in Mississippi River water, ■ experiments in nitrate amended Mississippi River water.

and nitrate amended Mississippi River water samples and applied to calculated rate constants.

From calculated pseudo-first order rate constants in **Table 15**, it is clear for imidacloprid, thiamethoxam, and clothianidin that indirect photolysis, even in nitrate-amended waters

Table 15. Calculated rate constants (h^{-1}) for photolysis experiments in nitrate (10 mg/L as N) amended water.

	Imidacloprid	Acetamiprid	Thiamethoxam	Clothianidin
Milli-Q	0.76 ± 0.01	$5 \pm 3\times10^{-3}$	0.46 ± 0.01	0.70 ± 0.02
MRW	0.62 ± 0.01	$7.6 \pm 0.3\times10^{-3}$	0.39 ± 0.01	0.50 ± 0.02
MRW*	0.68 ± 0.01	$8.0 \pm 0.3\times10^{-3}$	0.41 ± 0.01	0.53 ± 0.02
MRW – NO_3	0.61 ± 0.01	$2.1 \pm 0.2\times10^{-2}$	0.39 ± 0.01	0.52 ± 0.01
MRW – NO_3*	0.67 ± 0.01	$2.2 \pm 0.2\times10^{-2}$	0.41 ± 0.01	0.55 ± 0.01

MRW: Mississippi River water samples, MRW – NO_3: nitrate amended Mississippi River water samples. * represents rate constants with screening factors applied. Calculated screening factors: I MRW (0.91), I MRW-NO_3 (0.91), A MRW (0.95), A MRW-NO_3 (0.96), T MRW (0.95), T MRW-NO_3 (0.95), C MRW (0.94), C MRW-NO_3 (0.94). Error is the 95% confidence interval, calculated as (∑ 95% C.I.) / n*√n.

with high levels of hydroxyl radicals, is not an important degradation pathway. For imidacloprid in Milli-Q, k_{obs} was 0.76 while k_{obs} for both Mississippi River water samples and nitrate-amended Mississippi River water samples both were lower, 0.68 and 0.67 respectively, even after adjusting for screening. The steady state concentration of hydroxyl radicals determined from use of a pCBA probe was calculated to be $2.0 \pm 0.1\times10^{-15}$ M^{-1} s^{-1} in imidacloprid experiments. Thiamethoxam samples showed a similar effect, with a k_{obs} of 0.46 in Milli-Q water and k_{obs} of 0.41 for both the Mississippi River water samples and nitrate amended Mississippi River water samples. Clothianidin (k_{obs} of 0.70 in Milli-Q), again gave a similar result, with k_{obs} of 0.53 and 0.55 for Mississippi River water and nitrate-amended Mississippi River water samples. Steady-state hydroxyl radical concentration was calculated to be $2.26 \pm 0.02 \times10^{-15}$ M^{-1} s^{-1} for thiamethoxam and clothianidin experiments using a pCBA probe. These results indicate indirect photolysis is not an important mechanism for imidacloprid, thiamethoxam, and clothianidin degradation.

Hydroxyl radicals do play a part in acetamiprid degradation. As shown in **Figure 13**, direct photolysis over 36 hours resulted in only an average degradation of 14% in Milli-Q water. In Mississippi River water an average degradation of 24% was observed over 36 hours, indicating indirect photolysis has a role in acetamiprid degradation. This effect

was not observed in natural sunlight, where over 1 month of exposure, little degradation was observed in Mississippi River water and Milli-Q samples. In nitrate-amended Mississippi River water, average degradation over 36 hours increased to 53% in the solar simulator, indicating hydroxyl radicals are an important mechanism in indirect photolysis degradation of acetamiprid. Calculation of bimolecular rate constants gave a value of 1.7 $\pm$ 0.2$\times10^{9}$ M^{-1} s^{-1} for acetamiprid, with a steady-state hydroxyl radical concentration of 2.8 $\pm$ 0.1$\times10^{-15}$ M^{-1} s^{-1}. A literature value of 5.5$\times10^{10}$ M^{-1} s^{-1} has been reported, though in solutions with hydroxyl radicals generated from H_2O_2.[44]

Toxicity

Hydrolysis reaction products for toxicity tests were generated for nitenpyram, imidacloprid, acetamiprid, and thiamethoxam, including samples amended with metal ions and minerals. No hydrolysis products were generated for clothianidin because of the long hydrolysis degradation rate, even at pH 10. Similarly, photolysis products were produced for nitenpyram, imidacloprid, thiamethoxam, and clothianidin, but no products were produced for acetamiprid given its long half-life in simulated and natural sunlight experiments.

Solutions with reaction products contained ~20% parent compound and ~80% products. Testing was performed so that the concentration of parent neonicotinoid added to mosquito tests was the same as tests with only parent present, along with ~4 times that concentration of product. Thus, if products exhibited toxicity, the LC_{50} values of tests with product present would be smaller, while if products did not exhibit toxicity, LC_{50} values would remained unchanged or increase. Example LC_{50} curves are given in **Figure 14** for imidacloprid and acetamiprid. Calculated LC_{50} values are given in **Table 16**. Results indicate there is no residual toxicity associated with products from hydrolysis or photolysis reactions, because no decrease is observed in LC_{50} values.

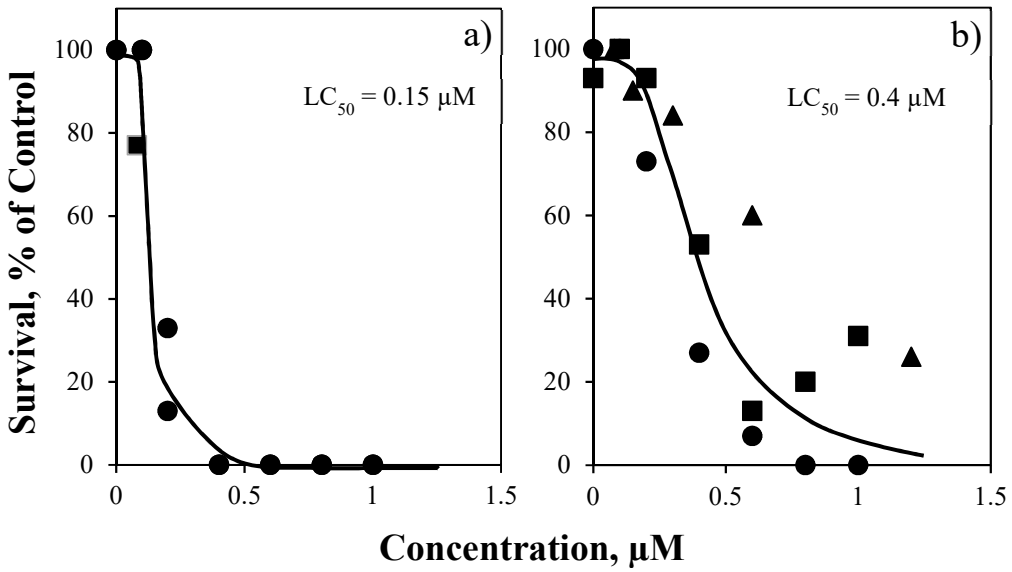

Figure 14. LC_{50} curves for a) imidacloprid and b) acetamiprid. Survival is calculated as the percentage that survived compared to the control. Legend: a) ● data from imidacloprid dilution series toxicity experiments ■ data point from independent toxicity experiment. b) ● replicate 1, ■ replicate 2, ▲replicate 3.

Table 16. LC_{50} (µM) values for tested neonicotinoid insecticides. Reaction products were tested by exposing mosquitoes to a 20% parent 80% product solution. LC_{50} values were normalized to parent concentrations and not total concentration of products + parent. MRW samples were photolysis samples exposed to light in MRW. Metal samples contained 0.1 mM of metal ions, while minerals were filtered out of samples.

LC_{50} (µM)	Nitenpyram	Imidacloprid	Acetamiprid	Thiamethoxam	Clothianidin
Parent	**0.3**	**0.15**	**0.4**	**0.6**	**0.15**
Photolysis Rep 1	0.3	0.15	-	0.7	0.15
Photolysis Rep 2	0.4	0.15	-	0.7	0.15
MRW	0.4	0.2	-	0.6	0.15
Base Hydrolysis	0.4	0.2	0.5	1.0	-
Ni^{2+}	0.5	0.2	0.4	0.9	-
Cu^{2+}	0.4	0.3	0.4	0.8	-
Zn^{2+}	0.5	0.2	0.6	0.8	-
Kaolinite	0.5	0.2	0.6	0.8	-
Goethite	0.3	0.2	0.3	0.9	-
TiO_2	0.3	0.2	0.4	0.8	-

Reaction Product Identification

Two hydrolysis products of nitenpyram were identified, with substitution of the $=CHNO_2$ functional group for =O with an exact mass of 227.0825 (nitenpyram – urea), and removal of $-NHCH_3$ and subsequent substitution with an oxygen, either as an alcohol or a ketone giving an exact mass of 257.0567. Exact mass and MS/MS data were used to identify products. As there was not enough product generated to use NMR to determine which structural isomer of the nitenpyram degradation product (257) was produced, it is assumed both structural isomers were generated via keto-enol tautomerization. The nitenpyram product with exact mass 257.0567 has previously been identified in literature[32] as has nitenpyram – urea.[32] Photolysis samples also generated two reaction products, the urea-derivative as well as a product with exact mass 211.0876 where the pharmacological moiety is removed entirely and replaced with a double bond from the carbon to methyl substituted nitrogen. The structure of the product with mass 211 was obtained by comparing MS/MS data with available literature.[32] Detected compounds are given in **Figure 15**, and MS2 fragmentation patterns are given in Appendix C (**Figures C1 – C4**).

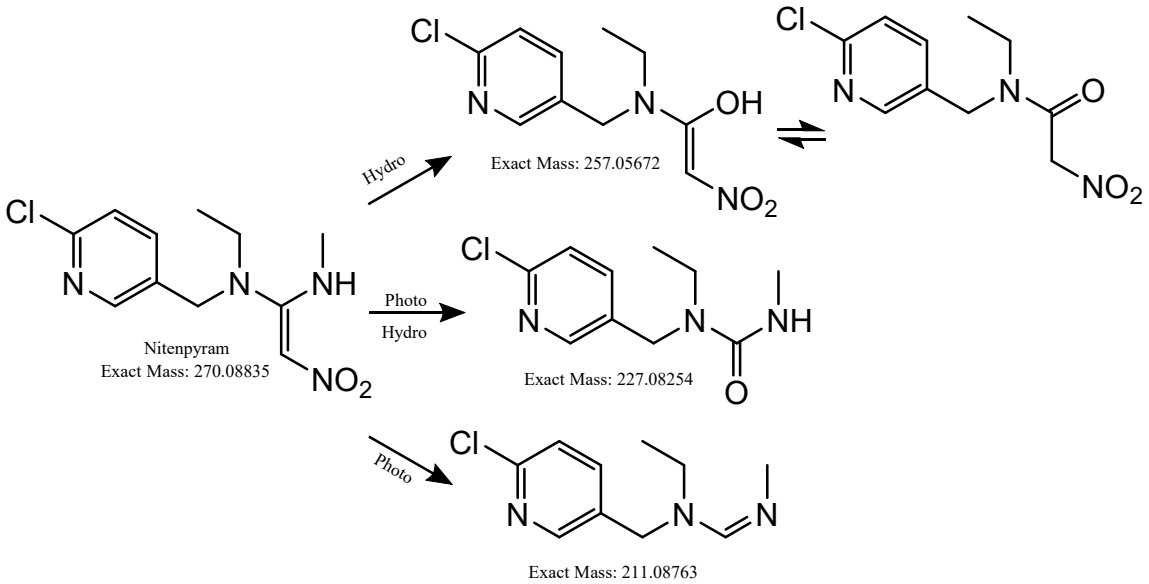

Figure 15. Observed photolysis and hydrolysis reaction products of nitenpyram.

In samples used for imidacloprid toxicity experiments, imidacloprid-urea (**Figure 16**) was the only observed product, with substitution of an oxygen for the -NNO_2 functional group, resulting in the formation of a ketone. Fragmentation patterns of imidacloprid-urea were collected from MS2 results, yielding the same fragmentation pattern as previous work.[37] Thermo Fisher Scientific Compound Discoverer 2.1 software gave database match results for imidacloprid-urea as well. The same MS2 fragmentation pattern (Appendix C, **Figure C4**) was observed for all baseline, metal, and mineral studies. Similarly, imidacloprid-urea was the only product observed in photolysis reactions. Desnitro/guanidine and nitrosoguanidine reaction products, which have been shown to be retain nAChR activity,[8,12,72–74] were not observed.

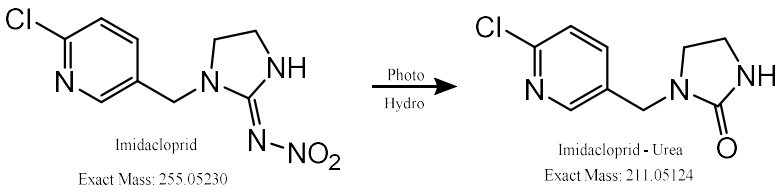

Figure 16. Observed hydrolysis and photolysis product of imidacloprid.

For acetamiprid, product testing was performed only for hydrolysis samples. As previously discussed, acetamiprid did not undergo any photolysis in an environmentally relevant time frame, and no samples were generated for toxicity studies or reaction product identification. The urea-derivative of acetamiprid was the only product observed, shown in **Figure 17**. The same MS2 fragmentation pattern (Appendix C, **Figure C5**) was observed for all baseline, metal, and mineral studies. The observed product matches the expected hydrolysis product[103] as well as an observed biodegradation product.[104]

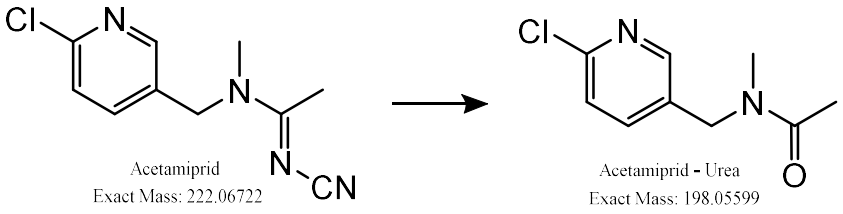

Figure 17. Observed hydrolysis product of acetamiprid.

The urea-derivative of thiamethoxam was the only hydrolysis or photolysis product identified, shown in **Figure 17**. Identification was performed using exact mass, and MS2 fragmentation patterns. Results did not vary between baseline hydrolysis, metal, and mineral experiments, and MS2 fragmentation patterns for the urea-derivative of thiamethoxam matched each other, as did the MS2 for the photolysis sample. MS2 fragmentation is shown in Appendix C (**Figure C6**).

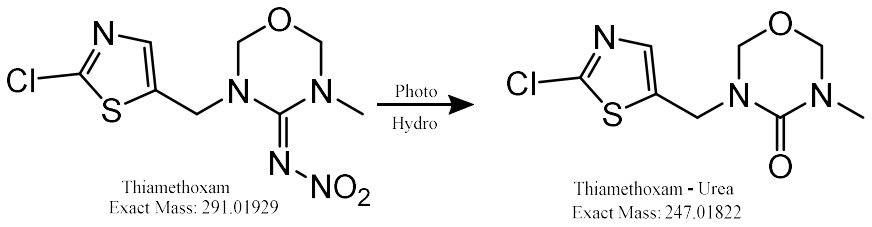

Figure 18. Observed hydrolysis and photolysis reaction product of thiamethoxam.

Clothianidin – urea (**Figure 19**) was the only observed hydrolysis and photolysis product by UPLC-MS/MS. Initial identification was performed using exact mass. Additional identification verification was performed by comparing MS2 data to literature.[105] MS2 fragmentation gave peaks at 132 and 113, matching literature MS2 fragmentation data.[106] The same urea-derivative has also been reported as a photolysis transformation.[107] Results did not vary between baseline hydrolysis, metal, and mineral experiments, as with photolysis experiments. MS2 fragmentation data is given in Appendix C (**Figure C7**).

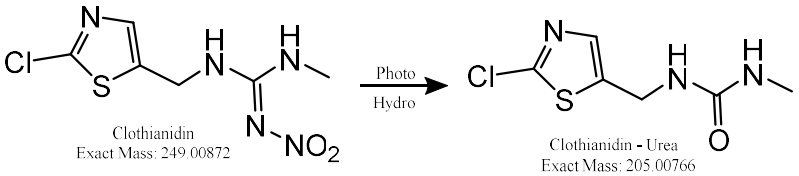

Figure 19. Observed hydrolysis and photolysis reaction product of clothianidin.

Discussion

Previous work had shown neonicotinoid hydrolysis rates increased with increasing pH, indicating pH dependence, however some results had indicated faster hydrolysis at acidic pH values.[38,40,75,98] Results of this work indicate neonicotinoids hydrolyze under base-catalyzed conditions. Furthermore, these results indicate that in an environmentally relevant pH range (5 – 8.5), hydrolysis is likely to be very slow.

This is backed by results from Mississippi River water experiments. At pH 8.28, in Mississippi River water, observed half-lives ranged widely, with significant error present. Expected environmental hydrolysis half-lives are 140 – 180 days for thiamethoxam, 150 – 320 days for nitenpyram, 800 – 1800 days for imidacloprid, 600 to 3500 days for acetamiprid, and 1200 – 5300 days for clothianidin. Hydrolysis is not expected to be a major environmental degradation pathway, given these long reaction times. In Minnesota, and in other states with intensive agriculture production in the Upper-Midwest, where many lakes and small streams are frozen from November/December until March/April, hydrolysis times are expected to be even longer. This helps to explain the widespread detection of neonicotinoids in surface waters globally.[9,18,21]

As shown in this study and in previous work,[41] several neonicotinoids do undergo direct photolysis, with nitenpyram reacting very quickly in sunlight. These experiments, however, do not necessarily take into account the change in solar intensity throughout the day or seasonally. To estimate photolysis half-lives in environment, integrated solar irradiances (L_λ) for 40º N at Midsummer obtained from Leifer (1988), quantum yields calculated from natural sunlight Mississippi River water samples, and molar absorptivity values calculated in this study were used to estimate photolysis rate constants (k_{dcE}) using Eq. 18. Estimated near surface environmental direct photolysis half-lives are 9 minutes for nitenpyram, 45 minutes for imidacloprid, 90 minutes for clothianidin, and 120

$$k_{dcE} = \emptyset_{dc} \sum_\lambda \epsilon_\lambda L_\lambda \quad (18)$$

minutes for thiamethoxam. These values may be overestimates. In experiments in natural sunlight, using averaged rate constants, half-lives were calculated to be 14 minutes for nitenpyram, 140 minutes for imidacloprid, 260 minutes for thiamethoxam, and 250 minutes for clothianidin. Imidacloprid, clothianidin, and thiamethoxam were all exposed to sunlight from 9am to 6pm on clear summer days in July and August, giving them exposure to changing irradiance intensities throughout the day. This indicates actual photolysis times are likely longer than those predicted using Eq. 18, given the variability of solar irradiance.

The indirect photolysis half-life of acetamiprid is calculated by assuming a hydroxyl radical concentration of 1×10^{-16} M, assuming 7 hours of sunlight per day, and using the bimolecular rate constant calculated in this study. Calculations were based on indirect photolysis half-life calculations from literature.[108] Direct photolysis for acetamiprid was not considered, given the negligible degradation observed due to direct photolysis. For acetamiprid, the estimated environmental half-life is 131 days for these conditions. Environmental half-lives are likely to change somewhat significantly due to changes in hydroxyl radical concentrations from differences in water chemistry and water depth. Overall, photolysis is not expected to contribute significantly to environmental degradation of acetamiprid.

Furthermore, these values are only relevant in near surface conditions. Neonicotinoids have been shown to only breakdown in the top 8 cm of a water body.[41] In any lake or larger river, such as the Mississippi River, environmental half-lives will be much longer. For example, if near-surface photolysis is expected to occur in the top 10 cm of a water body such as the Mississippi River, which is approximately 3 m deep, assuming a well-mixed system, only neonicotinoids in the top 10cm / 300 cm would degrade. Accounting for this would increase estimates of environmental half-life 30×, to 2.9 days for imidacloprid, 5.5 days for thiamethoxam, and 5.2 days for clothianidin. Additionally, experiments were conducted in filter-sterilized (0.2 μM) water. While there are some

lakes which are quite pristine in Minnesota (e.g., Green Lake), many lakes and rivers particularly in agricultural areas are much more sediment impaired and have higher turbidity than observed in laboratory experiments. This would lead to more light screening and likely longer degradation times, which helps to explain the persistence of neonicotinoids in the natural water bodies.

The observed reaction product of most reactions results in the removal of the pharmacologically active moiety (-NO_2/-CN), with formation of the urea-derivative of each compound. UPLC-MS/MS studies were only run in positive mode. It is possible there are reaction products which could be detected in negative mode. Additionally, products were not pre-concentrated prior to analysis, so it is possible additional compounds could have been detected if this procedure was performed. Examples of other imidacloprid photolysis products previously detected are given in **Figure 20**. From the information collected for this work, however, it appears the urea-derivative of each neonicotinoid is the major hydrolysis and photolysis reaction product. The formation of the same products also implies a photohydration reaction occurs during photolysis.

Results from toxicity tests further confirm literature results, which have generally concluded urea-derivatives do not have residual toxicity to the nicotinic receptor channels.[11] The exception is clothianidin-urea, which has shown to retain residual toxicity.[11] This result was not observed in toxicity experiments in this study.

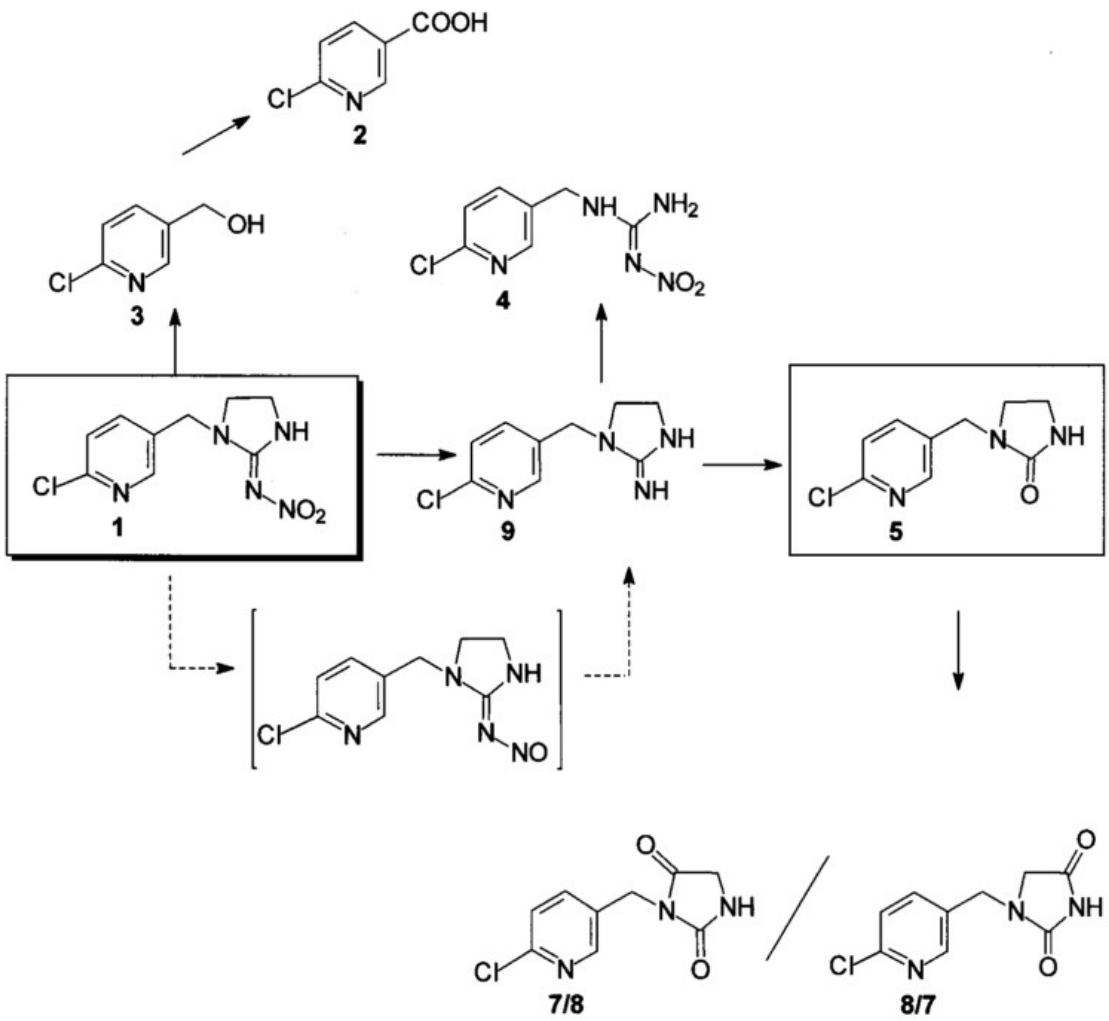

Figure 20. Previously observed photodegradation products of imidacloprid.[70] Only imidacloprid-urea was observed in this study. Figure reproduced from Wamhoff and Schneider.

Future Work

There are several areas where more research is appropriate and could give more insight on the environmental degradation of neonicotinoids. First, it would be appropriate to further study the photolysis of neonicotinoids on waxy plant surfaces (leaves). While a major use of neonicotinoids is via seed treatments in intensive agriculture, uses also include home treatments, such as tree and shrub insect drenches. While there are few studies that have focused on thin-film degradation of neonicotinoids, there is some indication from available literature that neonicotinoid photolysis is decreased substantially on thin-film/waxy surfaces.[109,110] Initial laboratory tests also indicate significantly longer photolysis degradation times. If photolysis degradation times are substantially increased after neonicotinoids are sprayed on plant leaves, this could help to explain long environmental residence times. Studies on simulated leaves made of carnauba wax and on harvested organic (neonicotinoid free) plant leaves would be recommended.

Secondly, high bisulfide ion (HS^-, or sulfide) concentrations can result from geologic formations, sulfate reduction, or mining activities.[111] Sulfide is a stronger nucleophile than hydroxide, which indicates sulfide may result in faster degradation times. Experiments could be performed in an anaerobic glove bag, using sulfide amended pH 6.33 and pH 8 buffers in foil-wrapped glass syringes. Use of glass syringes would prevent pressurization of reaction vials if sulfide is converted to a gas (H_2S). This is particularly a concern at pH 6.33, which is below the pK_a of 7.0. Verification of sulfide concentration using methylene blue tests would be important, as initial concentrations of sulfide may decrease due to speciation. There may also be new products generated, as changing the nucleophile may result in formation of a thioketone. This could allow for further product identification and toxicity experiments.

Study of hydrolysis and photolysis degradation rates of observed products with residual toxicity is an additional area in which more work should be done. No study to date has

studied or estimated the expected environmental half-lives of metabolites that have been shown to retain toxicity to invertebrates and mammals. While none of the metabolites detected in this studied exhibited any toxicity, nor were any metabolites detected which are known to retain activity, this study focused on abiotic reaction pathways. Metabolites of imidacloprid, clothianidin, and thiamethoxam are also generated due to microbial activity in soil, wetlands/prairie potholes, or other environmental matrices. These metabolites will be present in water bodies. Thus, it would be relevant to study to hydrolysis and photolysis of active metabolites, for little to nothing is known about their persistence in the environment.

An additional area of study to pursue should be simulation of neonicotinoid degradation in model groundwater environments. Nitroaromatic pesticides have been shown to be reduced by zero-valent iron (ZVI).[112] It would be valuable to determine if neonicotinoids could also be reduced by ZVI or Fe^{2+}, which is also a reactive species. This could be accomplished via batch reactors for abiotic reduction transformation experiments using iron as a reducing agent. An additional condition to consider could be reactivity in the presence of sand using batch reactors. Sand is widely present in aquifers used for drinking water production, and the behavior of neonicotinoids in the presence of sand could yield important information on subsurface fate of neonicotinoids.

Finally, further photolysis studies in Mississippi River water, and perhaps other lake waters could provide information on expected environmental degradation. Screening from DOM slows down photolysis rates, as does depth, because light cannot penetrate to the bottom of many lakes and rivers. This has previously been shown in mesocosm studies, which indicated neonicotinoids were unlikely to break down below the top 8 cm of a water body.[41] Further studies to better understand the effect of depth on neonicotinoid degradation could provide information on expected environmental half-lives of neonicotinoids in water bodies. If unfiltered water were to be used, it would provide more accurate results and could lead to observation of biodegradation reactions.

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Appendix A: Supplemental Information, Hydrolysis Experiments

Table A1. Measured pH values of neonicotinoid insecticide reactors.

Desired pH	Sample	N	I	A	T	C
4	Baseline	4.11	4.1	4.08	4.07	4.05
4	Copper	4.01	3.99	3.99	3.99	3.99
4	Nickel	4.03	4.03	4.02	4.04	4.03
4	Zinc	4.03	4.05	4.09	4.03	4.04
6.33	Baseline	6.39	6.36	6.33	6.24	6.37
6.33	Copper	6.12	5.94	6.05	5.81	6.15
6.33	Nickel	6.38	6.23	6.36	6.32	6.33
6.33	Zinc	6.34	6.3	6.33	6.3	6.33
7	Baseline	7.08	7.01	7.02	6.98	7.01
8	Baseline	7.75	7.9	7.92	7.86	7.88
8	Copper	7.89	7.52	7.65	7.65	7.92
8	Nickel	7.82	7.86	7.81	7.75	7.91
8	Zinc	7.76	7.64	7.73	7.77	7.73
8	Baseline - Stirred	7.67	7.81	7.71	7.67	7.79
8	Kaolinite	7.73	7.74	7.66	7.66	7.77
8	Goethite	7.94	7.93	7.91	7.93	7.93
8	TiO_2	7.91	7.92	7.93	7.93	7.94
10	Baseline	9.93	9.91	9.83	8.93	9.87
10	Copper	9.94	9.95	9.95	8.87	9.96
10	Nickel	9.95	9.94	9.94	8.87	9.96
10	Zinc	9.96	9.96	9.95	8.84	9.96
10	Baseline - Stirred	9.94	9.99	9.97	8.89	9.9
10	Kaolinite	9.88	9.97	9.96	8.92	9.98
10	Goethite	9.94	9.95	9.94	9.00	9.94
10	TiO_2	9.95	9.98	9.95	9.00	9.96

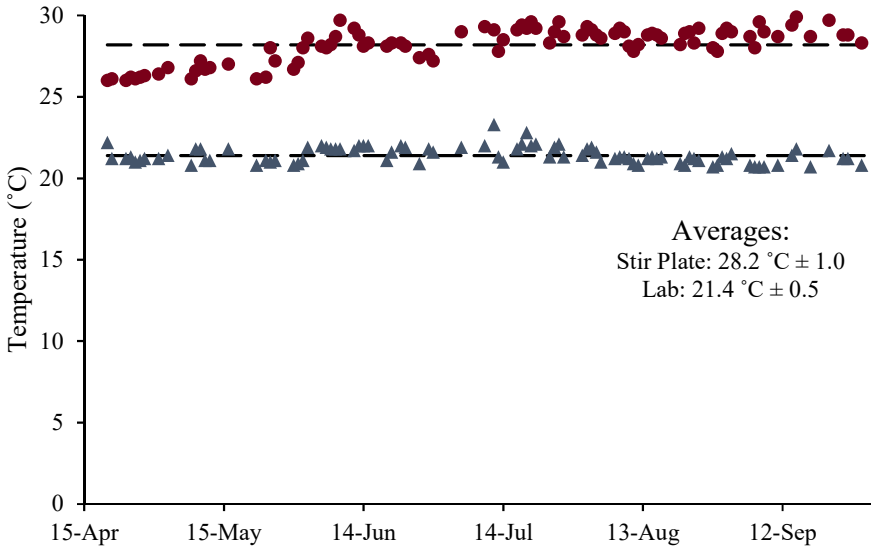

Figure A1. Stir plate temperature (●) and ambient laboratory temperature (▲) for mineral reactors. Average ambient laboratory was 21.4 ºC with a standard deviation of 0.5 ºC, while the average temperature of reactors on the stir plate was 28.2 ºC with a standard deviation of 1.0 ºC.

Appendix B: Supplemental Information, Photolysis Experiments

Molar absorptivity values were calculated for each neonicotinoid using standards which had been made for use with HPLC. For each neonicotinoid, five standards were used to calculate molar absorptivity at each wavelength. To calculate molar absorptivity, absorbance was measured on a Shimadzu UV-1601 PC UV Visible Spectrophotometer. Concentrations used are given in **Table B1**.

Table B1. Concentrations of standards used to determine molar absorptivity of neonicotinoid insecticides.

Conc.	Nitenpyram	Imidacloprid	Acetamiprid	Thiamethoxam	Clothianidin
1	1.76 µM	0.97 µM	1.00 µM	1.02 µM	1.41 µM
2	3.52 µM	2.92 µM	3.01 µM	3.06 µM	2.83 µM
3	5.28 µM	4.87 µM	5.02 µM	5.09 µM	4.24 µM
4	10.56 µM	9.73 µM	10.03 µM	10.19 µM	8.49 µM
5	24.65 µM	24.33 µM	25.08 µM	25.47 µM	19.80 µM

Linear regression of the Beer-Lambert law was used to determine molar absorptivity at each wavelength, using the 'linest' function in excel. Molar absorptivity results are shown in **Figure B1**, along with PNA results from literature.[94]

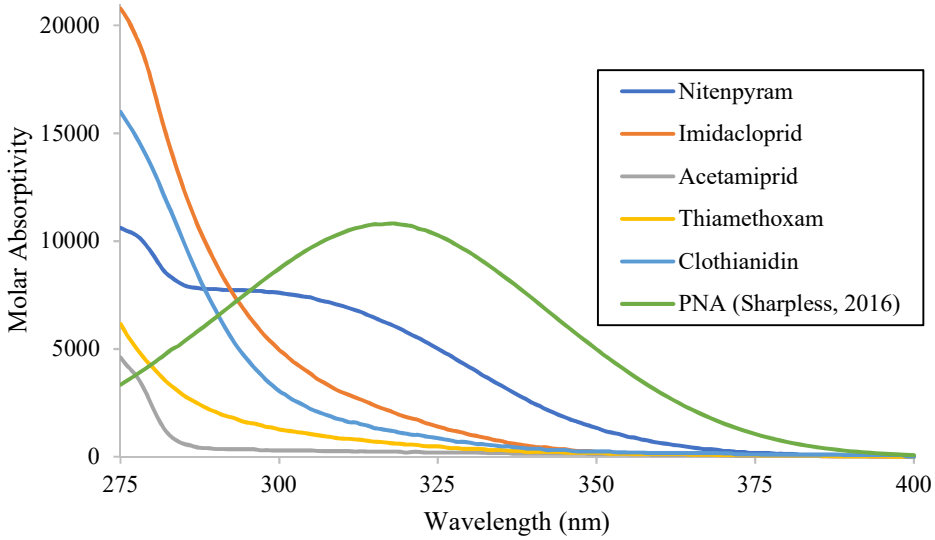

Figure B1. Molar absorptivities (ε) of neonicotinoid insecticides. Units of ε are L mol^{-1} cm^{-1}. PNA molar absorptivity data was downloaded from ES&T online supporting information.[94]

Table B2. Calculated first order photolysis rate constants (h^{-1}) of neonicotinoid insecticides. Error is the 95% confidence interval of each rate constant, calculated as the sum of all errors divided by the number of samples times the square root of the number of samples. S.A. stands for screening adjusted rate constants, calculated by dividing the Mississippi River water rate constant by the calculated screening factor.

		Nitenpyram	Imidacloprid	Thiamethoxam	Clothianidin
Solar Sim.	Milli-Q	3.1 ± 0.1	0.396 ± 0.005	0.206 ± 0.006	0.296 ± 0.005
	MRW	2.6 ± 0.1	0.285 ± 0.002	0.161 ± 0.002	0.212 ± 0.002
	MRW S.A.	2.7 ± 0.1	0.297 ± 0.002	0.168 ± 0.002	0.221 ± 0.002
Natural Sunlight	Milli-Q	3.3 ± 0.1	0.36 ± 0.02	0.160 ± 0.004	0.197 ± 0.005
	MRW	3.0 ± 0.1	0.30 ± 0.02	0.159 ± 0.004	0.168 ± 0.004
	MRW S.A.	3.2 ± 0.1	0.31 ± 0.02	0.166 ± 0.004	0.175 ± 0.004

Appendix C: Supplemental Information, UPLC – MS/MS

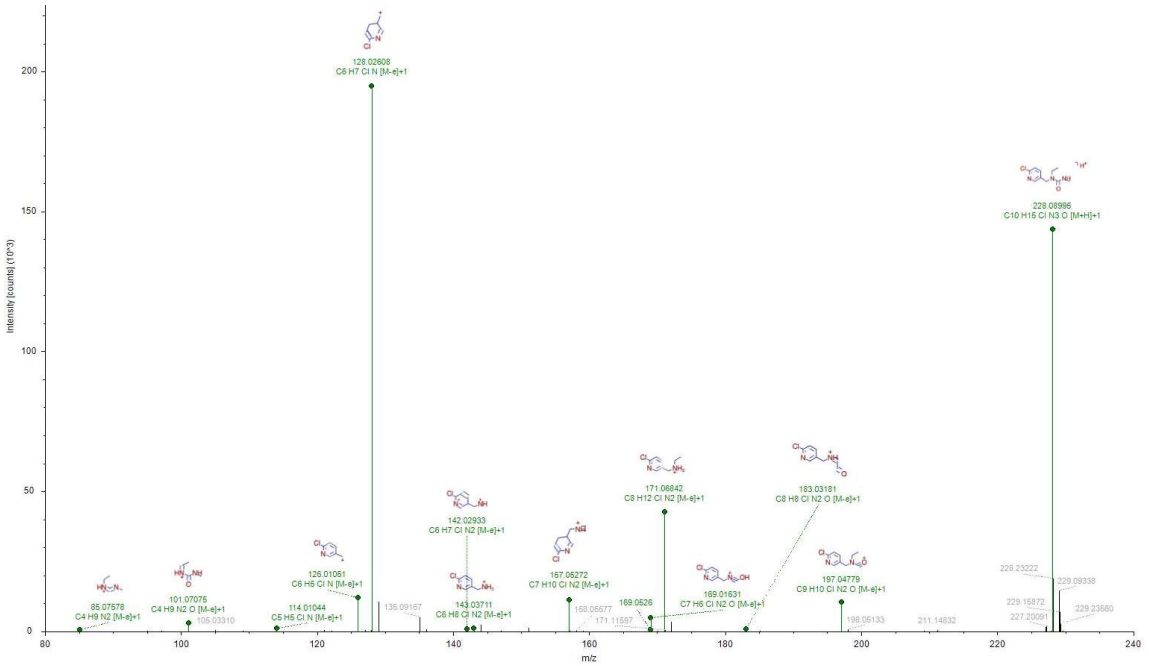

Figure C1. Nitenpyram – urea hydrolysis and photolysis MS2 data (exact mass 227.08254).

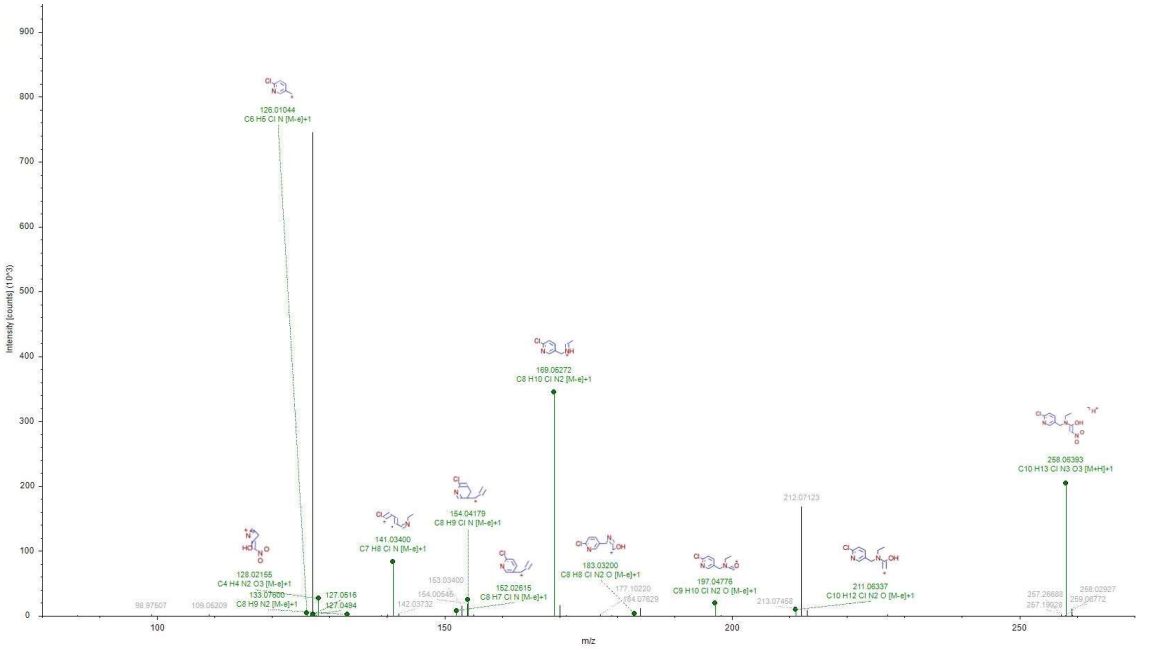

Figure C2. Nitenpyram hydrolysis products MS2 data with exact mass 257.05672.

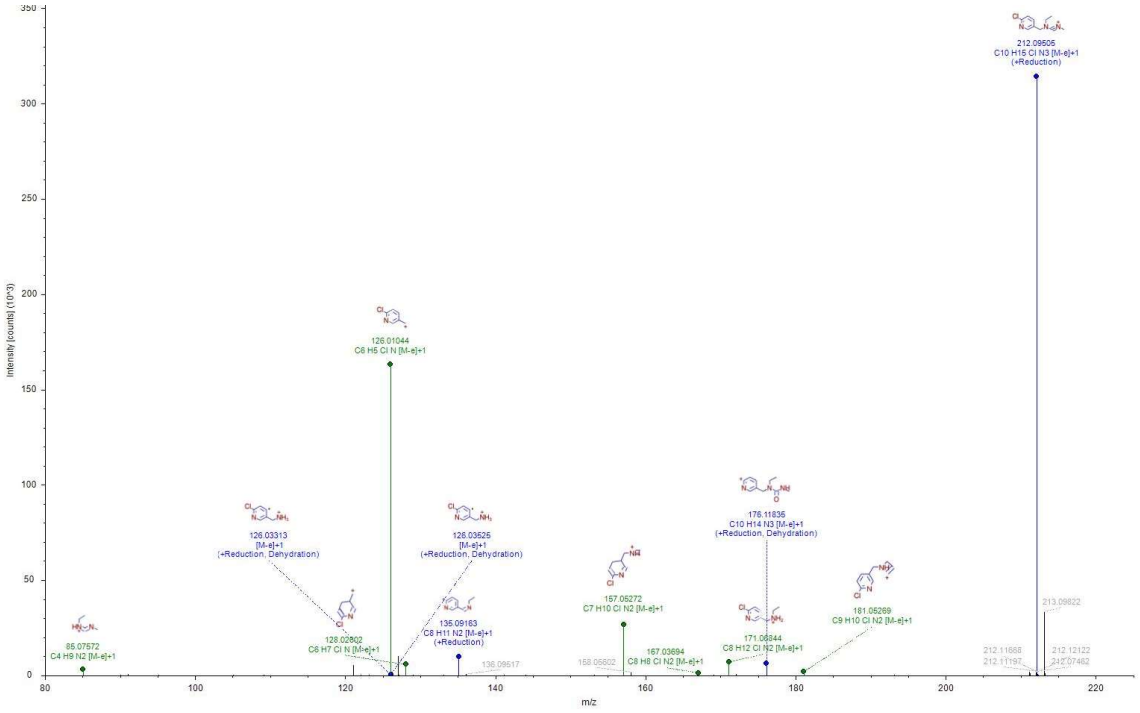

Figure C3. Nitenpyram photolysis product MS2 data with exact mass 211.08763.

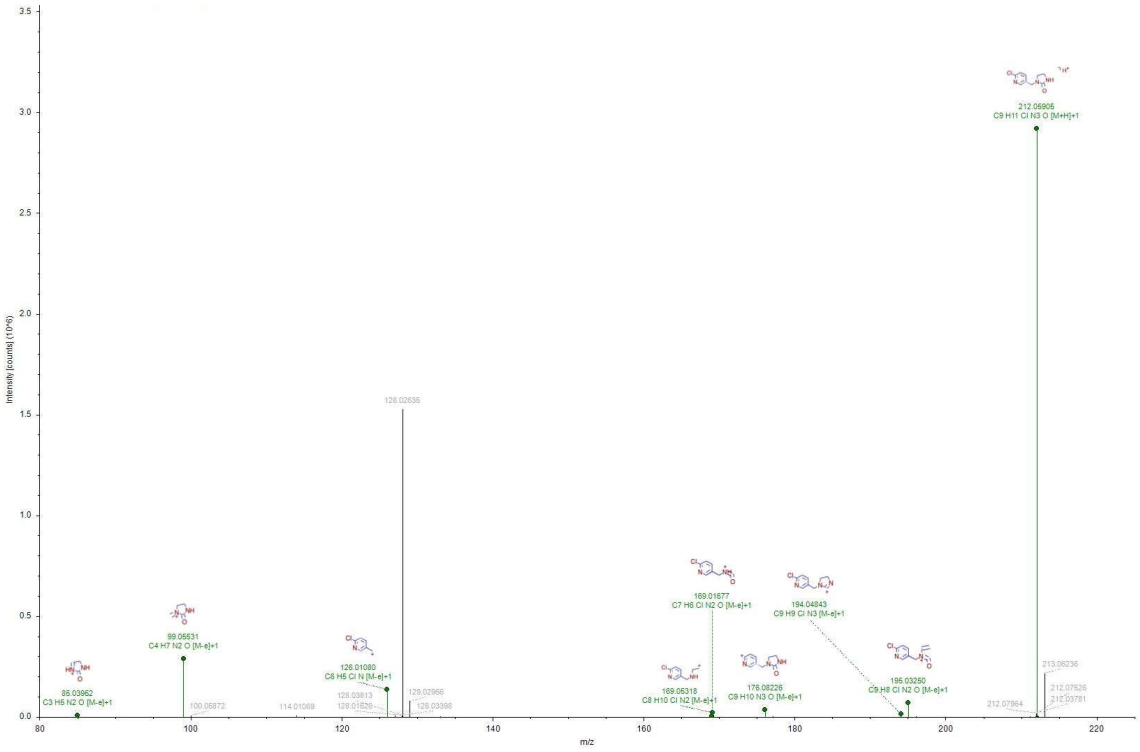

Figure C4. Imidacloprid – urea MS2 data (exact mass 211.05124). MS2 was identical for photolysis and hydrolysis degradation experiments.

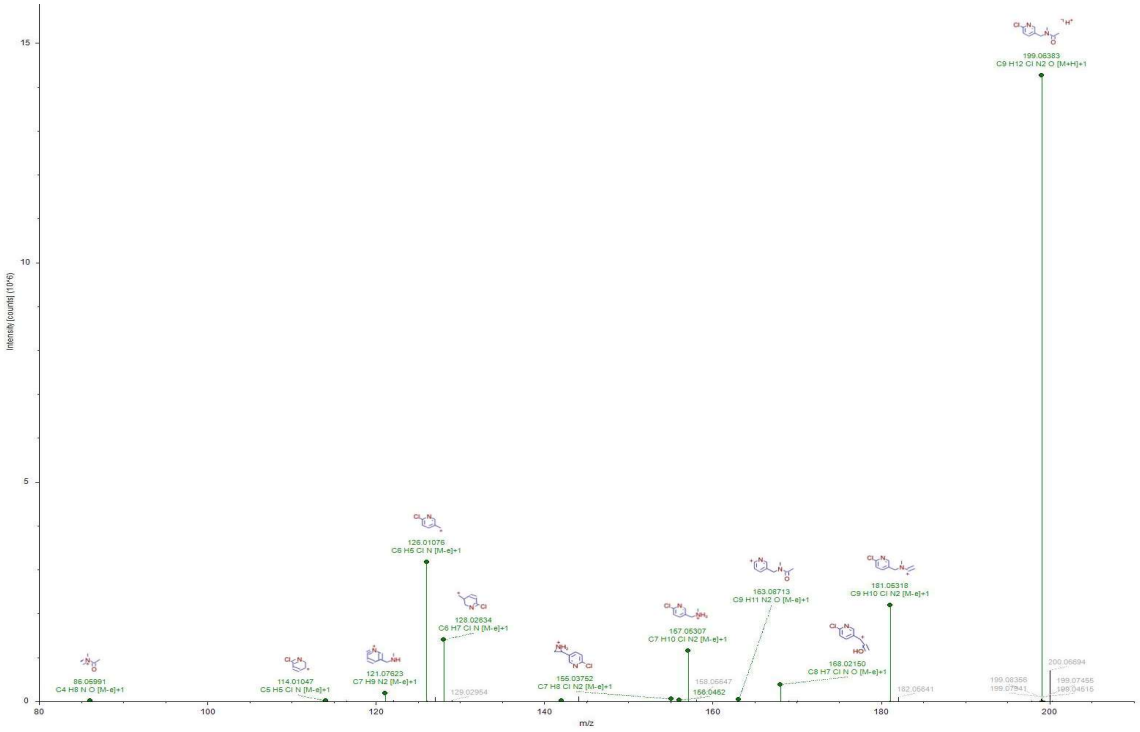

Figure C5. Acetamiprid – urea hydrolysis product MS2 data (exact mass 198.05599).

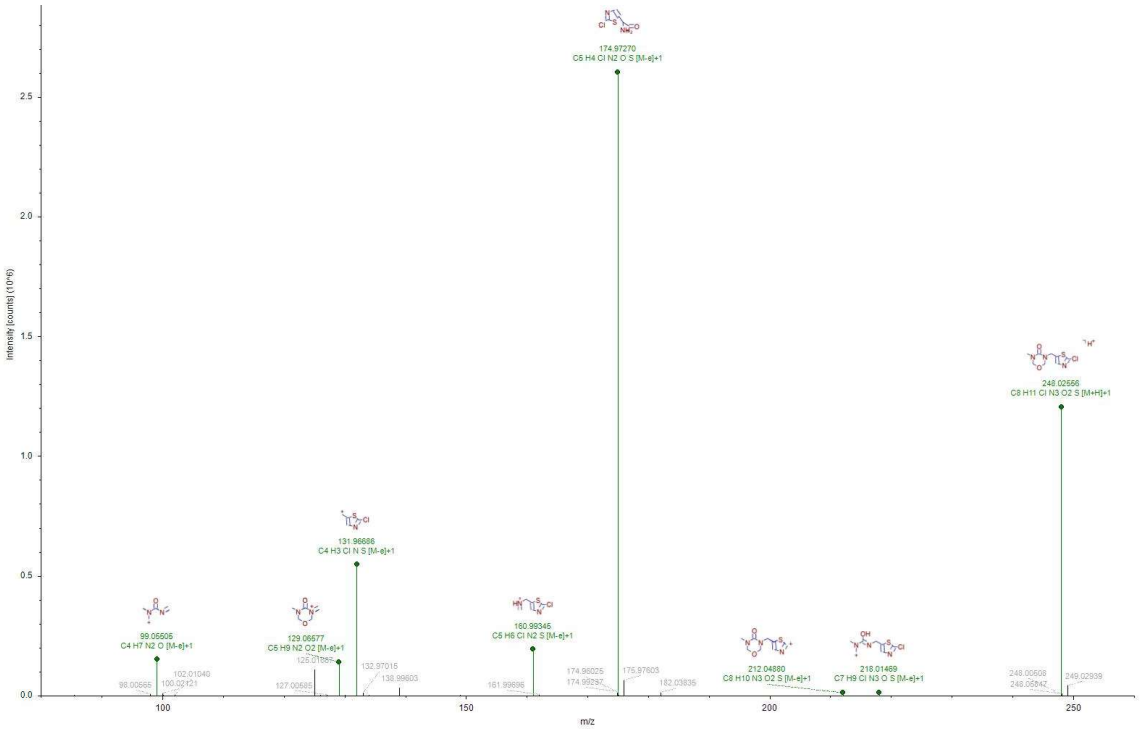

Figure C6. Thiamethoxam - urea hydrolysis and photolysis product MS2 data (exact mass 247.01822).

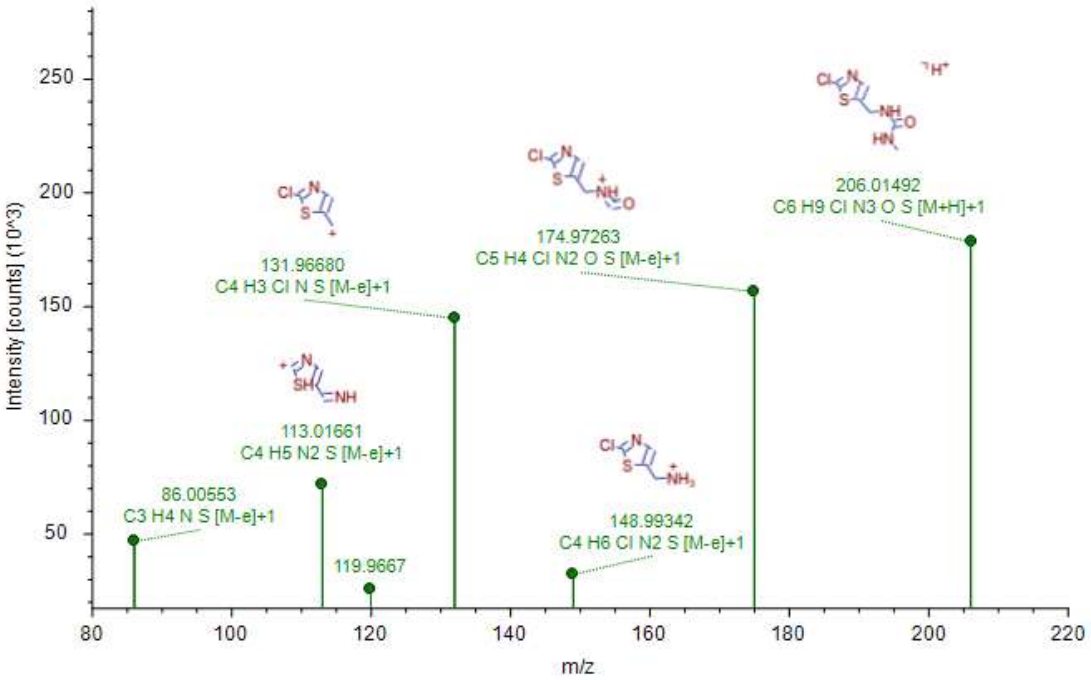

Figure C7. Clothianidin - urea hydrolysis and photolysis product MS2 data (exact mass 205.00766).