2016 Project Abstract
For the Period Ending June 30, 2019

PROJECT TITLE: Assessing Neonicotinoid Insecticide Effects on Aquatic and Soil Communities
PROJECT MANAGER: William Arnold
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FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2016, Chp. 186, Sec. 2, Subd. 04e

APPROPRIATION AMOUNT: $ 400,000
AMOUNT SPENT: $ 400,000
AMOUNT REMAINING: $ 0

Sound bite of Project Outcomes and Results
The processes of hydrolysis and photolysis are relatively slow for neonicotinoid insecticides, with half-lives of years for hydrolysis and hours to days for photolysis. On surfaces, the photolysis rate is dependent on the surface the commercial formulation. The reaction products formed were non-toxic to mosquito larvae.

Overall Project Outcome and Results
Neonicotinoid insecticides are widely used and detected at varying concentrations across diverse environments, including soil, surface water, and groundwater. A key component of how persistent neonicotinoids are in the environment is their degradation rate, and the residual toxicity of the products needs evaluation. Hydrolysis is the reaction process that occurs in water, which may be affected by the pH of the water or the presence of natural trace metals and minerals. Reaction driven by sunlight (photolysis) has also been reported as an important transformation pathway for neonicotinoids. The objectives of this study were to quantify hydrolysis and photolysis rates for neonicotinoid insecticides in water and on various surfaces; understand the effects of pH and natural trace metals on hydrolysis of neonicotinoids; characterize transformation products; and assess the toxicity of hydrolysis and photolysis products to soil and aquatic species. Hydrolysis and photolysis in aqueous solutions and on surfaces were examined for various neonicotinoids, including imidacloprid, thiamethoxam, clothianidin, acetamiprid, and nitenpyram. The results showed that neonicotinoids undergo base-catalyzed hydrolysis, and the hydrolysis rates were not impacted in the presence of divalent metal cations and minerals. Direct photolysis was observed for nitenpyram, imidacloprid, thiamethoxam, and clothianidin, but not for acetamiprid. When put onto various model surfaces to simulate application to a plant leaf, the photolysis rates and mechanisms were not only dependent on the surface, but also on whether a commercial formulation or solution of pure compound (analytical standard dissolved in ultrapure water) of the pesticide was used. Photolysis of commercial products was faster than pure compounds on the tested surfaces. Product analysis indicated that the urea derivative was the most commonly detected product for neonicotinoids reacting via hydrolysis and photolysis in water, while reduction and dissociation of the nitro group led to the major photoreaction products on surfaces. Toxicity tests on mosquito (Culex pipiens) larvae were conducted with nitenpyram, imidacloprid, acetamiprid, thiamethoxam, clothianidin, and their reaction products generated via hydrolysis, photolysis in water, and photolysis on surfaces. No residual toxicity associated with reaction products was observed.

Project Results Use and Dissemination
Results from the work have been presented as oral and poster presentations at conferences (2017 Minnesota Water Resources Conference, 2017 MN Conference on the Environment, 2017 Society of Environmental Toxicology and Chemistry (SETAC) national meeting, 2019 American Chemical Society National meeting, 2019

Association of Environmental Engineering and Science Professors Conference). The paper “Neonicotinoid insecticide hydrolysis and photolysis: Rates and residual toxicity” was published in the journal *Environmental Toxicology and Chemistry.* It is open access and freely available at: https://doi.org/10.1002/etc.4256. The associated data set is archived at http://hdl.handle.net/11299/199764. Mr. Stephen Todey’s MS Thesis is available via ProQuest (https://search-proquest-com.ezp3.lib.umn.edu/docview/2268373263) and will shortly be archived in the University of Minnesota Digital Conservancy. We are preparing a manuscript that describes the photolysis and toxicity results for experiments performed on surfaces. The findings from this project will aid the development of guidelines for the management and safe use of neonicotinoids to protect the health of Minnesota’s waters.

Environment and Natural Resources Trust Fund (ENRTF)
M.L. 2016 Work Plan

Date of Report: August 9, 2019

Final Report

Date of Work Plan Approval: June 7, 2016

Project Completion Date: June 30, 2019

PROJECT TITLE: Assessing Neonicotinoid Insecticide Effects on Aquatic and Soil Communities

Project Manager: William Arnold

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Location: Statewide

Total ENRTF Project Budget:		
	ENRTF Appropriation:	**$400,000**
	Amount Spent:	**$400,000**
	Balance:	**$0**

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 04e

Appropriation Language:

$400,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to identify neonicotinoid insecticide breakdown components produced in water and plant leaves and assess their toxicity to soil and aquatic species and related biotic communities. This appropriation is available until June 30, 2019, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Assessing Neonicotinoid Insecticide Effects on Aquatic and Soil Communities

II. PROJECT STATEMENT: Neonicotinoid insecticides were introduced in the 1990s and now represent 25% of global insecticide use. Current estimates for the U.S. are that neonicotinoids are used on 95% of corn and half of sugar beets and soybeans, all important Minnesota crops. These insecticides are applied as seed dressings, so a portion of the insecticide is taken up by the plant, and the remainder enters the soil and water. Thus, neonicotinoid compounds have been detected in soil, surface water, and groundwater, but their persistence in the environment and potential toxic effects are not fully understood. Reactions of neonicotinoids in water or in sunlight will give rise to new chemicals of similar chemical structure and unknown toxicity. Because neonicotinoids are applied as seed dressings and taken up by plants, water/solar driven reactions within the plant itself must also be explored. While the potential toxic effects of neonicotinoids on honey bees and birds are known, toxic effects on aquatic or soil species have received less attention. Consequently, new studies regarding the environmental movement, fate, and toxicity of neonicotinoids are urgently needed to determine any potential effects in Minnesota waters and to develop guidelines for safe use of neonicotinoids. The hypothesis to be tested by this project is that the neonicotinoid breakdown products formed in water and plants will have residual toxicity. The goals of the project are to: 1) Identify reaction products from neonicotinoids in water in the presence of natural trace metals and minerals; 2) Identify reaction products in water and simulated plant leaves upon neonicotinoid exposure to sunlight; 3) Assess toxicity of neonicotinoids to soil and aquatic species before and after reaction in water and plants; and 4) Disseminate the findings to stakeholders, regulators, and the public. Neonicotinoids that are applied as insecticides are formulated from structurally related chemicals that may vary in toxicity and propensity to generate toxic byproducts. Our studies will evaluate which neonicotinoids are transformed most quickly in surface waters, if transformation in plant leaves occurs, and whether the breakdown products have residual toxic activity for soil and aquatic species. The results of this work will have direct impacts on management of neonicotinoid use and the environmental health of Minnesota's waters.

III. OVERALL PROJECT STATUS UPDATES:

Project Status as of January 1, 2017:
Efforts to date have focused on measuring the reaction of the neonicotinoids in water under various pH and metal ion levels. The stability of the reaction products has also been evaluated so that samples can be stored appropriately for toxicity tests. Hydrolysis rates of the neonicotinoids imidacloprid, acetamiprid, nitenpyram, clothianidin, and thiamethoxam have been determined at pH 8 and pH 10. Reactors for the same neonicotinoids at pH 4, 6.33, and 7 have been set-up and are being sampled regularly using high performance liquid chromatography to determine hydrolysis rates of reaction. More time is needed for these reactions due to the slow reaction rates at lower pH values. Experimental reactors have been set up for the neonicotinoids imidacloprid, acetamiprid, nitenpyram, clothianidin, and thiamethoxam at pH 4 and 6.33 with each of the metals zinc, copper, and nickel. These reactors have been sampled regularly, though reaction rates are slow enough more time is needed. Initial experiments have been conducted using the neonicotinoid imidacloprid to determine hydrolysis product stability and half-life in a solar-simulator. Work has been focused on hydrolysis products due to the significantly longer amounts of time needed for hydrolysis experiments to react. Baseline toxicities of parent compounds imidacloprid, acetamiprid and thiamethoxam have been established for mosquito larvae and collembola. These data establish approximate quantities of reaction products needed for toxicological testing of neonicotinoid derivatives.

Project Status as of July 1, 2017:

Over the past 6 months, efforts have been focused on monitoring hydrolysis reactors to determine aqueous rates of reactions of neonicotinoids. Hydrolysis rates have been determined for pH 8 and 10 reactors in water, and in the presence of natural trace metals. Hydrolysis rates of reactors at pH 4, 6.3 and 7 for water has been completed, and will be completed shortly for pH 4 and 6.33 metal reactors. Work has begun on mineral reactors

at pH 8. Photolysis rates in pure water and buffer solution have been determined for imidacloprid, nitenpyram, thiamethoxam and clothianidin. The next few months will be focused more on identifying impact of indirect photolysis on neonicotinoids, and replication of experiments in natural water. Hydrolysis samples for toxicity testing for imidacloprid, nitenpyram, thiamethoxam, and acetamiprid have been generated, along with photolysis samples of imidacloprid, nitenpyram, thiamethoxam, and clothianidin. Work is underway to generate natural trace metal samples of imidacloprid, nitenpyram, thiamethoxam, and acetamiprid for toxicity testing.

Project Status as of January 1, 2018:

Efforts have continues to define hydrolysis and photolysis rates. The effect of minerals on hydrolysis rates was quantified. Experiments exploring indirect photolysis were completed. High resolution mass spectrometry analysis is being used to identify the reaction products produced during hydrolysis and photolysis, which is important information that is needed to evaluate the toxicity results. An experimental protocol for the photolysis of the compounds on surfaces that simulate the surface of plant leaves continues to be developed, and these efforts will continue throughout the year. We will also test the "as applied" formulation of the neonicotinoids in addition to the pure compounds on the simulated leaf surfaces. Toxicity testing with mosquito larvae is continuing, and studies with tadpoles were conducted. Reaction products to not appear to be toxic to the target species in experiments thus far.

Project Status as of July 1, 2018:

Results from hydrolysis, photolysis, and mosquito toxicity studies have been submitted for publication. Efforts are now largely focused on photolysis on glass, wax film, and plant leave surfaces. The protocol to extract the applied pesticides from surfaces has been developed. Results to date show loss of the compounds on surfaces, but the means in which it is applied – in a water solution versus using a commercial product – appears to dramatically affect that stability of the neonicotinoid on surfaces. Work is continuing to determine the factors that affect photolysis reaction rates, means to apply the materials to plant leaves, and reaction product identification.

Amendment Request (11/26/18): The photolysis experiments of Activity 2 have required more effort than anticipated and the toxicity experiments in Activity 3 have required less activity than anticipated. Thus, it is requested that $52,839 in salaries/fringe benefits be moved from Activity 3 to Activity 2. This will allow the postdoctoral researcher to continue working on the project through June 30, 2019. Additionally, a total of $54,789 will be reallocated to postdoctoral research support from co-PI Fallon salary ($21,388), undergraduate students ($22,000) and graduate student #2 ($11,501). There will still be sufficient funds for graduate and undergraduate students working on the project. Because the toxicity studies have not required the initial effort anticipated, this alteration reflects the level of co-PI effort. **Amendment Approved by LCCMR 12/03/2018**

Project Status as of January 1, 2019:

Efforts have been focused on monitoring the photodegradation of the neonicotinoids on various surfaces. Photolysis rates on glass, aluminum foil, paraffin wax and leaves have been determined for imidacloprid, thiamethoxam, clothianidin and acetamiprid. Experiments investigating the differences between pure compounds prepared in water and commercial products upon photolysis have been completed. Photolysis products have been identified using high resolution mass spectrometry analysis. Work is ongoing to determine the quantum yields using 2-nitrobenzaldehyde as an actinometer. The method to extract the neonicotinoids from surfaces for toxicity tests has been developed. Work has begun to generate samples of imidacloprid, thiamethoxam and clothianidin to obtain LC50 values for materials photolyzed on surfaces.

Overall Project Outcomes and Results:

Neonicotinoid insecticides are widely used and detected at varying concentrations across diverse environments, including soil, surface water, and groundwater. A key component of how persistent neonicotinoids are in the environment is their degradation rate, and the residual toxicity of the products needs evaluation. Hydrolysis is the reaction process that occurs in water, which may be affected by the pH of the water or the presence of natural trace metals and minerals. Reaction driven by sunlight (photolysis) has also been reported as an important transformation pathway for neonicotinoids. The objectives of this study were to quantify hydrolysis and photolysis rates for neonicotinoid insecticides in water and on various surfaces; understand the effects of pH and natural trace metals on hydrolysis of neonicotinoids; characterize transformation products; and assess the toxicity of hydrolysis and photolysis products to soil and aquatic species. Hydrolysis and photolysis in aqueous solutions and on surfaces were examined for various neonicotinoids, including imidacloprid, thiamethoxam, clothianidin, acetamiprid, and nitenpyram. The results showed that neonicotinoids undergo base-catalyzed hydrolysis, and the hydrolysis rates were not impacted in the presence of divalent metal cations and minerals. Direct photolysis was observed for nitenpyram, imidacloprid, thiamethoxam, and clothianidin, but not for acetamiprid. When put onto various model surfaces to simulate application to a plant leaf, the photolysis rates and mechanisms were not only dependent on the surface, but also on whether a commercial formulation or solution of pure compound (analytical standard dissolved in ultrapure water) of the pesticide was used. Photolysis of commercial products was faster than pure compounds on the tested surfaces. Product analysis indicated that the urea derivative was the most commonly detected product for neonicotinoids reacting via hydrolysis and photolysis in water, while reduction and dissociation of the nitro group led to the major photoreaction products on surfaces. Toxicity tests on mosquito (Culex pipiens) larvae were conducted with nitenpyram, imidacloprid, acetamiprid, thiamethoxam, clothianidin, and their reaction products generated via hydrolysis, photolysis in water, and photolysis on surfaces. No residual toxicity associated with reaction products was observed.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Neonicotinoid reaction in water: role of trace metals and minerals

Description: Hydrolysis (water driven transformation) is an important pathway for pollutant degradation. The transformation of neonicotinoids in water shows that rates are slow at the pH conditions of natural waters. Other system components, however, such as natural trace metals and minerals (which are key plant nutrients), may increase transformation rates via hydrolysis and lead to previously unidentified reaction products. This activity will quantify reaction rates and characterize transformation products of reactions of neonicotinoids in the presence of natural trace metals present in soil that are critical for plant growth (copper, iron, calcium, etc.) and soil minerals (e.g., clays, iron oxides). Three neonicotinoids will be tested with variables including pH, temperature, trace metals, and minerals. Experiments will be largely performed in laboratory-prepared matrices, but once critical factors affecting neonicotinoid hydrolysis are determined, additional experiments in Mississippi River water (with added trace metals or minerals) will also be performed. Minerals will be purchased or in selected cases, synthesized in the laboratory. All minerals will be characterized via X-ray diffraction to confirm their identity and purity.

Reactors will be constructed preparing an aqueous solution at the desired pH (controlled by a buffer system) and target trace metal and/or soil mineral. In selected cases, (e.g., when a redox active metal such as ferrous iron is used), the solution will be deoxygenated. Experiments will be initiated by spiking in the desired neonicotinoid and monitoring its loss from solution over time by high pressure liquid chromatography. Samples at various time points (when a given fraction of neonicotinoid has been degraded) will be immediately used for the toxicity tests described in Task 3. We expect the kinetic studies will require approximately 200 reactors (approximately 2000 samples) to be run. At the end of the period where kinetics are monitored, gas and liquid chromatograph-mass spectrometry and nuclear magnetic resonance techniques will be used to identify reaction products. In selected cases, product identification may occur throughout the experiment to assist in identification of the relevant chemical reaction mechanism.

Summary Budget Information for Activity 1:

ENRTF Budget: $ 117,525
Amount Spent: $ 117,525
Balance: $ 0

Outcome	Completion Date
1. Rates of neonicotinoid reaction in water	12/31/16
2. Rates of neonicotinoid reaction in water with natural trace metals	6/30/17
3. Rates of neonicotinoid reaction in water with natural minerals	12/31/17
4. Identification of reaction products	12/31/18

Activity Status as of January 1, 2017:
The rates of hydrolysis for the neonicotinoids imidacloprid, acetamiprid, thiamethoxam, clothianidin, and nitenpyram have been determined at pH 8 and pH 10. Reactors have been set up for pH 4, 6.33 and 7, though due to the long half-life of hydrolysis at lower pH values, more time is needed to determine hydrolysis half-lives through sampling at regular intervals. Reactor vials for neonicotinoid reaction rates with trace metals have also been started. Currently, the neonicotinoids imidacloprid, acetamiprid, thiamethoxam, clothianidin, and nitenpyram have been mixed with trace amounts of copper, zinc, and nickel at pH values 4 and 6.33, and are being regularly sampled to determine degradation rates.

Activity Status as of July 1, 2017:

The rates of hydrolysis for the neonicotinoids imidacloprid, acetamiprid, thiamethoxam, clothianidin, and nitenpyram have been determined for pH 4, 6.33, and 7. The rates of hydrolysis for the same neonicotinoids has been determined for pH 8 and 10. Little to no effect on rate of hydrolysis was observed. Hydrolysis rates have also been preliminarily determined for pH 4, and 6.33 with the natural trace metals copper (II), nickel (II), and zinc (II). Due to the long half-lives, as observed in the reactions with water, more time is needed to confirm the hydrolysis rates at pH 4, and 6.33. Natural mineral reactors (goethite, kaolinite, titanium dioxide) have been started for pH 8. Mineral reactors must be stirred constantly to avoid the minerals settling out of solution, thus due to space limits, only pH 8 and 10 are currently being observed. Natural mineral reactions were started too recently to make any observations on rate of hydrolysis. Method development for high performance liquid chromatography high resolution mass spectrometry for product identification has begun, however, due to limited instrument availability, no product identification is expected for at least several months.

Activity Status as of January 1, 2018:

The rates of hydrolysis for the neonicotinoids nitenpyram, imidacloprid, acetamiprid, thiamethoxam, and clothianidin have been determined at pH 4, 6.33, 7, 8, and 10 with natural trace metals (copper (II), nickel (II), and zinc (II)), and with natural trace minerals (goethite, kaolinite, titanium dioxide). Current analyses of hydrolysis rates show little variation between baseline reactors and reactors with natural trace metals. A statistical analysis is being completed to verify there is no statistical difference between reaction rate with natural trace metals present and no trace metals. Hydrolysis rates with natural minerals appear to be faster when titanium dioxide and goethite are present; statistical analysis is being done to verify this result. Slight variations in the pH of reactors may be the factor changing the reaction rates; if shown to be the case, this would mean pH of an abiotic environment would be the most important factor in determining degradation rate. Initial samples have been run using high performance liquid chromatography high resolution mass spectrometry for product identification. Initial results indicate only 1 main product for each neonicotinoid, with no variation in products between solutions containing no natural minerals or natural metals and solutions with natural minerals and natural metals. Additional work is needed to verify results.

Activity Status as of July 1, 2018:

After further statistical analysis, no significant variation was observed in hydrolysis reaction rates with metals or minerals present. Thus, it can be concluded pH of an abiotic environment will be the most important factor in determining degradation rate. Further statistical analysis revealed neonicotinoid insecticides do not undergo an elementary second-order reaction mechanism, which has previously been widely assumed. The updated rate law will lead to more accurate prediction of environmental hydrolysis rates and allow for more reasonable extrapolation to different environmental conditions. Reaction products have also been verified, with the major reaction product observed the urea-derivative of each compound. No variation was observed in samples containing metals or minerals.

Activity Status as of January 1, 2019:

Activity 1 has been completed.

Final Report Summary:

The hydrolysis rates for five neonicotinoid insecticides were determined, including imidacloprid, thiamethoxam, clothianidin, acetamiprid and nitenpyram. Additionally, these reactions in the presence of trace metals or minerals were studied to examine any effects on neonicotinoid hydrolysis. Hydrolysis rates were tested between pH 4 and 10, and little to no degradation was observed for all neonicotinoids in pH 4.0, 6.3, and 7.0, with half-lives calculated to be over 1000 d for most compounds. Specifically, for imidacloprid, hydrolysis was only observed to react at pH values >9. The results indicated that neonicotinoids undergo base-catalyzed hydrolysis. Experiments revealed a nonelementary rate law, with the hydroxide concentration raised to a power of 0.55 ± 0.09, which has implications for accurate prediction of environmental half-lives. Furthermore, divalent metal ions (Cu^{2+}, Ni^{2+}, Zn^{2+}) and minerals (kaolinite, goethite, TiO_2) were not observed to affect hydrolysis rates. The calculated hydrolysis rate constants do not differ between baseline and metal-containing solutions, indicating that pH may be responsible for any observed variations in reaction rates. A comparison to hydrolysis rates in a natural water was also performed. The results showed that the hydrolysis rate in natural water was slower than that predicted by experiments in buffered laboratory water.

Ultrahigh pressure liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) was used to identify neonicotinoid hydrolysis and photolysis degradation reaction products. Two hydrolysis products of nitenpyram were observed, including nitenpyram-urea, and removal of -NHCH3 and subsequent substitution with an oxygen, as either an alcohol or a ketone. For imidacloprid hydrolysis and photolysis experiments, only imidacloprid-urea was observed. For acetamiprid, product testing was performed only for hydrolysis samples, and the urea derivative of acetamiprid was the only product observed. In addition, the urea derivative of thiamethoxam was the only hydrolysis or photolysis product identified. Similarly, clothianidin-urea was the only observed hydrolysis and photolysis product. In general, the urea derivative was the most commonly detected product in both hydrolysis and photolysis experiments for all neonicotinoids.

This portion of the project demonstrated that under typical environmental conditions, hydrolysis of neonicotinoids will not be a major degradation process. The pH of the water is a critical parameter, and the proper equation (loss rate = $k[\text{neonicotinoid}][OH^-]^{0.55}$) must be used to properly predict the rate of removal.

ACTIVITY 2: Solar effects on neonicotinoids in water and plants

Description: Photolysis (solar driven transformation) is another potentially important transformation pathway for neonicotinoids in aquatic systems. Photolysis experiments will be performed in pure water solutions using artificial sunlight (which provides control and reproducibility) as an energy source. Validation of results will use natural sunlight and natural waters (e.g., Mississippi River water). The natural water experiments will also allow the potential role of indirect photolysis (i.e., reactions with hydroxyl radical, singlet oxygen, and triplet excited state natural organic matter to be explored) via use of appropriate quenchers (isopropyl alcohol for hydroxyl radicals,

histidine for singlet oxygen, and sorbic acid for triplet excited states). Experiments are performed by amending water samples with the desired neonicotinoid, exposing the solution to the light source, and monitoring concentration as a function of time with high pressure liquid chromatography. Based on the kinetic results and absorbance properties of the compounds, quantum yields for the reaction will be calculated.

Following these experiments, photolysis rates in "artificial leaves" (cuticular wax films) will be investigated. This method has been used in recent pesticide transformation studies to mimic the chemical environment of a plant leaf. The waxy leaf environment may lead to different transformation rates and products. Transformation products will be identified for reactions in water and "artificial leaves" to find any structural or comparative differences in product compositions. These analyses will be performed by the same methods as those described in Activity 1. For both the aqueous and wax film experiments, samples will also be collected at various time points throughout the reactions for use in the experiments described in Activity 3.

Summary Budget Information for Activity 2:

ENRTF Budget: $167,864
Amount Spent: $ 167,864
Balance: $ 0

Outcome	Completion Date
1. Rates of solar-driven neonicotinoid reaction in water	6/30/17
2. Rates of solar-driven neonicotinoid reaction in "artificial leaves"	6/30/18
3. Identification of products of aqueous and "artificial leaf" photolysis	12/31/18
4. Dissemination of Activity 1 & 2 findings via open access journal publication(s)	12/31/18

Activity Status as of January 1, 2017:

Initial experiments have been conducted using the neonicotinoid imidacloprid to determine reaction rates and half-life in a solar-simulator. The stability of the reaction products is also being assessed so that proper sample storage will be possible for samples to be used in Task 3.

Activity Status as of July 1, 2017:

The rate of solar degradation of neonicotinoids imidacloprid, nitenpyram, thiamethoxam, and clothianidin have been determined in simulated – sunlight and in natural light. Experiments are currently being redone in order to yield date for accurate calculation of quantum yields. Work will begin shortly to determine rates of reaction in natural Mississippi River water and to quantify the role indirect photolysis has on neonicotinoid reactions.

Activity Status as of January 1, 2018:

The rates of solar degradation of neonicotinoids nitenpyram, imidacloprid, thiamethoxam, and clothianidin in both natural Mississippi River Water and deionized water have been calculated, as well as the quantum yields for these reactions. Analysis of the data shows evidence that degradation occurs due to direct photolysis as opposed to indirect photolysis. An additional experiment with nitrate containing waters is needed to verify this, and will be completed shortly. Acetamiprid was found to not degrade significantly while exposed to natural sunlight for over a month, indicating photolysis is not an important pathway for degradation. Initial work has been done to identify a system to model artificial leaves in the laboratory. Results indicate experiments will take significantly longer than the photolysis of neonicotinoids in aqueous solutions, thus work will be done during the summer of 2018 using natural sunlight to avoid significant wear and tear on the laboratory's solar simulator. Initial samples have been run using high performance liquid chromatography mass spectrometry for product identification. Early results appear to indicate the same product for imidacloprid and slightly different products with nitenpyram, however more work is needed to verify these early findings.

Activity Status as of July 1, 2018:

Photolysis in nitrate-amended water, studied due to nitrate's ability to produce hydroxyl radicals which lead to indirect photolysis, revealed indirect photolysis, even with high levels of hydroxyl radicals does not lead to increased degradation for thiamethoxam, clothianidin, and imidacloprid. However, hydroxyl radicals were found to lead to degradation in acetamiprid, which did not undergo direct photolysis. Estimated half-lives for acetamiprid, however, are >100 days at environmentally relevant concentrations of hydroxyl radicals. Products for thiamethoxam, clothianidin, and imidacloprid were found to be the same as products for hydrolysis reactions. For nitenpyram, the same product was observed as in hydrolysis, along with an additional photolysis product. Work is on-going for degradation experiments using wax as model leaves as well as with real plant leaves. Results to date indicate that photolysis does occur on surfaces, but the type of surface and the matrix in which the neonicotinoid is applies – laboratory prepared aqueous solution versus commercial product – dramatically affect the rate of compound loss. Preliminary analyses have been performed to identify reaction products produced from photolysis of surface applied compounds. Efforts are also focused on the determining the best way to apply compounds consistently to real leaf surfaces.

Activity Status as of January 1, 2019:

Photolysis of four neonicotinoids including imidacloprid, thiamethoxam, clothianidin and acetamiprid – pure compounds prepared in water as well as commercial products – were examined on four surfaces: glass, aluminum foil, paraffin wax, and leaves of strawberry plants. Similar with results observed in water, acetamiprid on paraffin wax remained stable while exposed to simulated light for 60 hours, suggesting that photolysis is not an important degradation pathway. For imidacloprid, the degradation rates on paraffin wax and leaves were comparable but were much lower than those on glass and aluminum foil, indicating that paraffin wax best simulates the reaction environment on leaves. The loss of commercial imidacloprid was much faster than pure imidacloprid. Degradation of pure imidacloprid followed zero order kinetics on all surfaces, while commercial imidacloprid followed first order kinetics. These results imply that commercial products containing various active and inert ingredients can lead to a significant change in the photolysis process. Experiments with thiamethoxam led to similar results with imidacloprid. Commercial clothianidin disappeared fast on paraffin wax/glass, while pure clothianidin was observed to not degrade exposed to natural sunlight for over two months. Products for imidacloprid, thiamethoxam and clothianidin have been identified using LC-MS/MS. Nitro Reduction and dechlorination were found to be the major reaction processes. Products for commercial compounds were observed to be the same with pure compounds on each surface. Additional work is underway to use 2-nitrobenzaldehyde as an actinometer for the determination of quantum yields.

Final Report Summary:

Photolysis experiments for neonicotinoids in aqueous solutions, including Milli-Q water, Mississippi River water, and nitrate-amended Mississippi River water, were performed in both natural sunlight and simulated sunlight in an Atlas Suntest CPS+ solar simulator with a xenon arc lamp fitted with a 290-nm cutoff filter. The results showed that indirect photolysis does not play a part in neonicotinoid photodegradation. Direct photolysis was observed for nitenpyram, imidacloprid, thiamethoxam, and clothianidin in both ultrapure and natural waters, with average quantum yields of 0.024 ± 0.001, 0.0105 ± 0.0002, 0.0140 ± 0.0002, and 0.0101 ± 0.0001, respectively. For acetamiprid, direct photolysis was extremely show, with a half-life of >100 h. However, acetamiprid was found to undergo indirect photolysis because of reaction with hydroxyl radicals with a bimolecular rate constant of $1.7 \pm (0.2 \times 10^{9})$ M^{-1} s^{-1}. The reaction products observed from photolysis were the same as in hydrolysis experiments.

For the experiments focused on simulating the photolysis reaction on the surface of plant leaves, we measured the photochemical transformation rates of four neonicotinoid insecticides, including imidacloprid, thiamethoxam, clothianidin and acetamiprid on four surfaces: glass, aluminum foil, paraffin wax, and leaves

from strawberry plants in an Atlas Suntest CPS+ solar simulator. No disappearance was observed for acetamiprid. For imdacloprid, degradation of a commercial formulation followed first order kinetics, while the pure compound (an analytical standard dissolved in water) followed zero order kinetics. For thiamethoxam, degradation of the commercial formulation and pure compound both followed first order kinetics. For clothianidin, degradation of the commercial formulation followed zero order kinetics, while the pure compound was observed to be relatively stable. Our main observations regarding the photodegradation of neonicotinoids on surfaces were as follows:

- Photolysis rates of neonicotinoids on paraffin wax and leaves were comparable, and much slower than those on glass and aluminum foil, indicating that paraffin wax best simulates the reaction environment on leaves.
- Photodegradation of commercial products was much faster than pure compounds, suggesting that the commercial formulations contain other ingredients that affects the photolysis process.
- The rate law and perhaps the photolysis mechanism depends upon the surface used.

Transformation products were analyzed by liquid chromatography coupled to a high resolution and accurate mass – tandem mass spectrometer (LC/HRAM-MS/MS; Thermo Fisher Scientific LTQ Orbitrap Velos), and the mass spectrometer was run in both positive & negative mode. Data analysis was performed targeted and untargeted analyses of degradation product work flows. Products were identified by interpreting possible structures based on the exact mass, comparing to available literature data, or a "structure search" through molecular formula. The results showed that for imidacloprid, photodegradation products were the same for the commercial and pure compounds on various surfaces, and products were formed via the reduction and dissociation of the nitro group, addition of hydroxyl groups, the dissociation of C-N bond, and elimination reactions. Similar to imidacloprid, the photodegradation processes were consistent for commercial and pure clothianidin on different surfaces, including the reduction and dissociation of nitro group, dissociation of chlorine, and addition of hydroxyl groups. On the other hand, nitro reduction and ring rearrangement were observed to be the major reaction pathways for commercial thiamethoxam, while for pure thiamethoxam, nitro reduction was the only reaction pathway. We are finalizing work to determine the quantum yields for photodegradation of neonicotinoids on different surfaces using 2-nitrobenzaldehyde as an actinometer.

In sunlit surface waters, photolysis will be a more important loss process than hydrolysis, although the reaction products obtained are the same. The quantum yields determined will allow estimation rates in the photic zone of lakes and rivers when combined with solar intensity data. The persistence on plant leaves merits further study, but it is interesting that the commercial formulations are, in general, more reactive that the pure compounds. This result is important when considering the desired balance between environmental persistence and the time needed for effective control of insects. If a single application is all that is needed, the faster degradation is a positive. If the faster reaction of commercial formulations lead to the need for multiple applications, this may lead to additional costs and environmental loads.

ACTIVITY 3: Toxicity of transformation products to soil and aquatic species

Description: The potential impacts on soil and aquatic organisms need to be explored to fully evaluate impacts of neonicotinoids and their byproducts. The tests will use springtails (a soil arthropod commonly used in assessment of environmental contaminants), mosquito larvae, and tadpoles from three native frog species that breed in vernal pools, often impacted by agricultural runoff. Test animals will be from unexposed insects bred in the laboratory, or in the case of tadpoles, reared from eggs deposited in an artificial, converted swimming pool in which the test species have become established. The choice of organisms represents a range of species native to Minnesota. Neonicotinoid insecticides exploit the biochemical finding that insect nervous systems have proportionately more nicotinic, relative to muscarinic acetylcholine receptors, relative to vertebrates. Because vertebrates do not entirely lack neonicotinoid receptors, however, the proposed tests with both arthropods and vertebrates in an aquatic environment will provide important baseline data for future biochemical evaluation of potential insecticide targets.

Toxicity tests will be performed with the neonicotinoid insecticides, the reaction mixtures from Activity 1 and 2, and, when possible, with individual identified/isolated transformation products. While every attempt will be made to use the solutions generated at specific time points in Activity 1 and 2, it may be necessary to repeat the hydrolysis or photolysis experiments to generate the appropriate solutions depending on the experimental time scales and the capacity to perform the toxicology testing.

For each reaction condition, a minimum of seven doses are needed for each species tested (up to 2500 total experiments). The baseline experiment will be an exposure using the neonicotinoid compound at a range of concentrations. By determining the organism survival (via live/dead counts and/or protein-based estimation of biomass for collembola) after 48 hours as a function of dosage, an EC_{50} value (the concentration which kills half of the tested organism) for the compound is determined. For the reaction mixtures, the concentration of the residual parent compound must be known (and is measured in Activity 1 or 2) and tested using a similar dilution series. If the dose/response curve for a neonicotinoid byproduct is the same as the baseline case, then the reaction product does not have a toxic effect. If the effect of the hydrolyzed/photolyzed solution is greater than that seen at the equivalent neonicotinoid concentration, then the reaction products do have an effect, and the magnitude of the effect will be further assessed. When testing additive effects of neonicotinoids with trace metals or soil composition, appropriate control experiments (containing, for example, the trace metals alone) will be performed. To minimize complications, efforts will focus on reactions where the reaction product is likely to have residual activity based on its structure, and in the toxicity tests, the pH of the substrate will be adjusted to neutrality, using buffers (such as Tris-HCl) that do not precipitate trace metals. Selected experiments will also test whether there are synergistic effects of the neonicotinoid compounds with other agricultural chemicals applied to the same systems (e.g., fungicides). In the synergistic experiments, a comparison is made between the effects of the compounds at a given dose individually and together.

Summary Budget Information for Activity 3:

ENRTF Budget: $114,611
Amount Spent: $ 114,611
Balance: $ 0

Outcome	Completion Date
1. Quantify levels of neonicotinoids and breakdown products toxic to springtails	6/30/18
2. Quantify levels of neonicotinoids and breakdown products toxic to mosquito larvae	12/31/18
3. Quantify levels of neonicotinoids and breakdown products toxic to tadpoles (3 species)	6/30/19
4. Dissemination of findings via open access journal publication(s)	6/30/19

Activity Status as of January 1, 2017:

Work is underway to generate hydrolysis samples for use in toxicity testing. It is anticipated tests will begin in February 2017. Baseline toxicities of parent compounds imidacloprid, acetamiprid and thiamethoxam have been established for mosquito larvae and collembola. These data establish approximate quantities of reaction products that will be needed for toxicological testing of hydrolysis samples neonicotinoid derivatives.

Activity Status as of July 1, 2017:

Hydrolysis samples of nitenpyram, thiamethoxam, imidacloprid, and acetamiprid have been generated for use in toxicity testing as have photolysis sample of nitenpyram, thiamethoxam, imidacloprid, and clothianidin. Work is underway to generate samples of nitenpyram, imidacloprid, thiamethoxam, and acetamiprid in the presence of natural trace metals to be used in toxicity testing.

Activity Status as of January 1, 2018:

Hydrolysis samples have been generated for the neonicotinoids nitenpyram, imidacloprid, thiamethoxam, and acetamiprid in the presence of natural trace minerals to be used in toxicity testing. Photolysis samples in Mississippi River water and deionized water have also been generated for use in toxicity testing.

None of the parent compounds (imidacloprid, thiamethoxam, acetamiprid, and nitenpryam) were toxic to newly hatched tadpoles over a 72 h period. These results confirm 2016 data, and provide a more rigorous test with younger, presumably more sensitive, tadpoles. The LC50 values for tadpoles exceed the solubility of these compounds in water. Our findings are consistent with published data.

For imidacloprid, the LC50 was 0.15 μM for mosquito larvae. Photolysis and hydrolysis reactions that converted approximately 80% of parent imidacloprid into products had LC50 values consistent with those of residual parent compound, with no evidence for generation of toxic breakdown products. Likewise, kaolinite reaction products, and metal reactor products (with copper, nickel, and zinc) failed to generate toxic breakdown products.

For acetamiprid, the LC50 was 0.4 to 0.6 μM for mosquito larvae. Hydrolysis and metal reactive products were non-toxic, relative to residual amounts of parent compounds.

Activity Status as of July 1, 2018:

LC50 values for the parent compounds and hydrolysis and photolysis products have been finalized. When comparing solutions of parent compounds alone to parent compound + reaction products (with the parent compound at the same level in both treatments), increases in toxicity (lower LC50) was not observed, meaning the reaction products are not toxic.

Activity Status as of January 1, 2019:

Method development to extract photolysis products from surfaces (glass, aluminum foil, paraffin wax, and leaves) for toxicity tests have been finalized. Efforts are focused on generating samples of imidacloprid, thiamethoxam and clothianidin for use in toxicity testing. Work is ongoing to obtain LC50 values for both parent compounds and photolysis products on surfaces.

Final Report Summary:

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 slow degradation rate, even at pH 10.0. Similarly, photolysis products were produced in water for nitenpyram, imidacloprid, thiamethoxam, and clothianidin, but no products were produced for acetamiprid given its long half-life in simulated and natural sunlight experiments. Median lethal concentration (LC50) value, which determines the point at which 50% of mosquito larvae died, was used to quantify toxicity. Solutions with hydrolysis or photolysis products contained approximately 20% of the parent compound and 80% of the original concentration present as products. Testing was performed so that the concentration of parent neonicotinoid added to mosquito tests was consistent in all exposures. Thus, if products exhibited toxicity, the LC50 values of tests with products present would be smaller relative to values for the parent neonicotinoids, whereas if products did not exhibit toxicity, the LC50 values would remain unchanged. Calculated LC50 values are 0.15-1.0 μM for neonicotinoids under the tested conditions. Significant disparities in LC50 values were observed among different neonicotinoids, but not among a parent compound and its hydrolysis and photolysis products, indicating that the products produced are not toxic to mosquito larvae.

Samples from the photolysis of imidacloprid on glass, aluminum, and wax surfaces were prepared for toxicity testing. Either the pure compound or a commercial formulation containing imidacloprid was tested. The amount

of imidacloprid dosed on surfaces was the same for the pure compound and commercial product. Samples were taken when approximately 80% of the imidacloprid was photolyzed. Pure water was used to extract compounds from surfaces in order to minimize the interference of organic solvent on toxicity tests and to better simulate environmental scenarios (rainfall, irrigation). The extraction efficiency of imidacloprid using water varied from 13% to 19%, depending on the type of the surface. Therefore, a dark control was always included in each test to determine the extraction efficiency. For the pure imidacloprid samples photolyzed on surfaces, the LC50 values were all approximately 0.075 μM for mosquito larvae. This value was lower than the LC50 of 0.15μM for water photolysis samples. Given variations between batches of larvae, this value was considered within a reasonable range. Surface photolysis products had LC50 values that were consistent with parent imidacloprid, with no evidence for the generation of more toxic products. Noticeably, even though photolysis products were different among surfaces, the LC50 values of samples generated via surface photolysis were similar. For the commercial formulatoin containing imidacloprid, the LC50 was less than 0.038 μM for samples produced on all surfaces. The lower LC50 values of the commercial formulation could be attributed to the other constituents in the sample. Besides 0.012% (w/w) of imidacloprid, the formulation contains 0.014% of Tau-fluvalinate, 0.015% of tebuconazole, and 99.959% inert ingredients. This low LC50 value is likely a combined effect of all the ingredients. Again, for the commercial product, the LC50s of extracts containing only the parent compound and the parent plus reaction products were similar, indicating that the photolysis products of these active ingredients were not toxic to mosquito larvae.

In summary, the results indicate that there is no residual toxicity associated with products from hydrolysis or photolysis to mosquito larvae. Based on initial experiments, tests with the springtails and tadpoles were deemed unnecessary, and as stated in amendment requests, funds were re-budgeted to focus on the efforts in Activity 2, which was more complicated than originally anticipated.

V. DISSEMINATION:

Description: The results will be disseminated via peer reviewed publications in scientific journals, presentations at local/regional conferences, and via a publically available final report. Funds have been requested to pay fees for open access, so the articles will be available to the public and stakeholders without an embargo period.

Activity Status as of January 1, 2017: Nothing to report.

Activity Status as of July 1, 2017: Nothing to report.

Activity Status as of January 1, 2018:

Results have been presented at a University of Minnesota Twin Cities Civil, Environmental, and Geo-Engineering Environmental seminar. Presentations were also given at the 2017 Minnesota Water Resources Conference, 2017 MN Conference on the Environment, and 2017 Society of Environmental Toxicology and Chemistry (SETAC) national meeting. A paper on the hydrolysis and photolysis reactions is being drafted.

Activity Status as of July 1, 2018:

A journal article on the hydrolysis and photolysis results (excluded wax film/plant leaf studies) has been submitted to the journal *Environmental Toxicology and Chemistry* and is currently under review.

Activity Status as of January 1, 2019:

The paper “Neonicotinoid insecticide hydrolysis and photolysis: Rates and residual toxicity” was published in the journal *Environmental Toxicology and Chemistry.* It is open access and freely available at:

https://doi.org/10.1002/etc.4256. The associated data set is archived at http://hdl.handle.net/11299/199764. Conference presentations and additional manuscripts are in preparation.

Final Report Summary: Results from the work have been presented as oral and poster presentations at conferences (2017 Minnesota Water Resources Conference, 2017 MN Conference on the Environment, 2017 Society of Environmental Toxicology and Chemistry (SETAC) national meeting, 2019 American Chemical Society National meeting, 2019 Association of Environmental Engineering and Science Professors Conference). The paper "Neonicotinoid insecticide hydrolysis and photolysis: Rates and residual toxicity" was published in the journal *Environmental Toxicology and Chemistry.* It is open access and freely available at: https://doi.org/10.1002/etc.4256. The associated data set is archived at http://hdl.handle.net/11299/199764. Mr. Stephen Todey's MS Thesis is available via ProQuest (https://search-proquest-com.ezp3.lib.umn.edu/docview/2268373263) and will shortly be archived in the University of Minnesota Digital Conservancy. We are preparing a manuscript that describes the photolysis and toxicity results for experiments performed on surfaces. The accepted manuscript will be provided when it has undergone peer review and is published.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	$ Amount	Overview Explanation
Personnel:	$ 358,000	Arnold at 8% per year, Fallon at 4% per year. Two graduate students at 25-50% time. One postdoctoral research for two years at 100% time. Two summer undergraduate students. Costs include fringe benefits for all and tuition for the graduate students.
Equipment/Tools/Supplies:	$ 32,000	Chemical standards and reagents, instrument analytical time, laboratory consumables, supplies for toxicity assays
Travel Expenses in MN:	$ 4,000	Sample collection and presentation at local conferences to stakeholders
Other:	$ 6,000	Publication fees for open access
TOTAL ENRTF BUDGET:	**$ 400,000**	

Explanation of Use of Classified Staff: not applicable

Explanation of Capital Expenditures Greater Than $5,000: not applicable

Number of Full-time Equivalents (FTE) Directly Funded with this ENRTF Appropriation: 6.7

Number of Full-time Equivalents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: 0

B. Other Funds:

Source of Funds	$ Amount Proposed	$ Amount Spent	Use of Other Funds
Non-state			
	$ 157,400	$157,400	Because the project is overhead free, laboratory space, electricity, and other facilities/administrative costs (52% of direct costs excluding permanent equipment and graduate student

			academic year fringe benefits) are provided in-kind
State			
	$	$	
TOTAL OTHER FUNDS:	$ 157,400	$ 157,400	

VII. PROJECT STRATEGY:

A. Project Partners: The project will be led by William Arnold (U of MN, Department of Civil, Environmental, and Geo- Engineering), who will be responsible for Activities 1 and 2, and Ann Fallon (U of MN, Department of Entomology) who will be responsible for Activity 3. The team will consist of two graduate and two undergraduate student researchers. Arnold is an expert in chemical reactions of pollutants in water, and Fallon is an expert in insecticide toxicology, insecticide resistance, insect physiology and molecular biology.

B. Project Impact and Long-term Strategy: This project will provide an understanding of neonicotinoid interactions with the natural environment and their potential transformation pathways. Results of the proposed work will provide a strong basis for evaluating the persistence and toxicity of neonicotinoids thus allowing for informed use, management, and, if needed, regulatory actions. Additionally, these studies will provide the first evidence of neonicotinoid hydrolysis and photolysis beyond simple baseline experiments in pure water solutions, and will involve both arthropod and vertebrate target organisms that lie at the bottom of the food chain for fish and birds. The results will be disseminated via open-access scientific literature and publically available reports.

VIII. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS: not applicable

IX. VISUAL COMPONENT or MAP(S): See attached

X. RESEARCH ADDENDUM: to be inserted upon completion of peer review

XI. REPORTING REQUIREMENTS:
Periodic work plan status update reports will be submitted not later than January 1, 2017; July 1, 2017; January 1, 2018; July 1, 2018, and January 1, 2019. A final report and associated products will be submitted between June 30 and August 15, 2019.

Environment and Natural Resources Trust Fund
M.L. 2016 Project Budget

Project Title: Assessing Neonicotinoid Insecticide Effects on Aquatic and Soil Communities
Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 04e
Project Manager: William Arnold
Organization: University of Minnesota
M.L. 2016 ENRTF Appropriation: $ 400,000
Project Length and Completion Date: 3 Years, June 30, 2019
Date of Report: August 10, 2019

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Activity 1 Balance	Activity 2 budget	Amount Spent	Activity 2 Balance	Activity 3 budget	Amount Spent	Activity 3 Balance	TOTAL BUDGET	TOTAL BALANCE
BUDGET ITEM	**Neonicotinoid reaction in water: role of**										
Personnel (Wages and Benefits)	$100,525	$100,525	$0	$153,364	$153,364	$0	$104,111	$104,111	$0	$358,000	$0
William Arnold, Project Manager, $58,550 (74.8% salary, 25.2% fringe benefits, 8% FTE per year). Project supervision, design of experiments and data analysis of Activities 1 &2, supervision of graduate and undergraduate students and project reporting.											
Ann Fallon, co-investigator, $7112(74.8% salary, 25.2 % fringe benefits, 1% FTE per year). Project supervision, design of experiments and data analysis of Activity 3, supervision of graduate and undergraduate students and project reporting											
Graduate student #1 $114,500 (50% time during academic year, 50% time in summer in Y1 and Y2; 25% time academic year in Y3 ; 56% salary, 33% tuition, 11% fringe benefits). Hydrolysis and photolysis experiments, development of analytical methods, identification of reaction products, data analysis and interpretation.											
Graduate student #2 $59,772(56% salary, 33% tuition, 11% fringe benefits). Rearing organisms for toxicity studies, toxicity studies, data analysis and interpretation.											
Postdoctoral researcher $98,066 (75% time in Y2 and 75% time in Y3) 82% salary, 18% fringe. Photolysis experiments in water and on plant leaves. Assist with toxicity testing.											
Undergraduate students $20,000 (100% time. In Y1 and Y2, one student for 40 hr/wk in the summer (10 weeks) and 10 hours per week for one semester (15 weeks).Assist graduate students with all laboratory activities.											
Equipment/Tools/Supplies											

Supplies $17,000 (chemical standards, chemical reagents for fate experiments and toxicity assays, necessary glassware, instrument/analytical time for product identification, solvents, consumable supplies, laboratory notebooks, software licenses)	$8,000	$8,000	$0	$6,000	$6,000	$0	$3,000	$3,000	$0	$17,000	$0
Analytical time for product identification $6,000	$3,000	$3,000	$0	$3,000	$3,000	$0				$6,000	$0
Operating costs for laboratory instruments required for analyses and experiments; costs portioned based on usage by project $9,000	$3,000	$3,000	$0	$3,000	$3,000	$0	$3,000	$3,000	$0	$9,000	$0
Travel expenses in Minnesota											
Charges and univeristy vehicle rental charges for trips to water samples. Hotel/meal charges if overnight stay required. Attendence for students at local conferneces to disseminate project findings to agriculture and environmental interests $4000	$1,500	$1,500	$0	$1,000	$1,000	$0	$1,500	$1,500	$0	$4,000	$0
Other											
Publication charges to make published journal articles (four) immediately available via open access to maximize data availability and dissemination $6000	$1,500	$1,500	$0	$1,500	$1,500	$0	$3,000	$3,000	$0	$6,000	$0
COLUMN TOTAL	**$117,525**	**$117,525**	**$0**	**$167,864**	**$167,864**	**$0**	**$114,611**	**$114,611**	**$0**	**$400,000**	**$0**

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Environmental Chemistry

Neonicotinoid Insecticide Hydrolysis and Photolysis: Rates and Residual Toxicity

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Abstract: Neonicotinoid insecticides are the most widely used class of insecticides worldwide. Concern has grown over their widespread environmental presence and potential unintended adverse effects. The present study examined hydrolysis and photolysis reaction rates of neonicotinoids and assessed any residual toxicity of reaction products. Hydrolysis rates were tested between pH 4 and 10 and found to be base-catalyzed. Experiments revealed a nonelementary rate law for hydrolysis, with the hydroxide concentration raised to a power of 0.55 ± 0.09, which has implications for accurate prediction of environmental half-lives. Divalent metal ions (Cu^{2+}, Ni^{2+}, Zn^{2+}) and minerals (kaolinite, goethite, TiO_2) had no effect on hydrolysis rates. The hydrolysis rate in a natural water, however, was slower than that predicted by buffered experiments. Nitenpyram, imidacloprid, thiamethoxam, and clothianidin reacted via direct photolysis in both ultrapure and natural waters, with average quantum yields of 0.024 ± 0.001, 0.0105 ± 0.0002, 0.0140 ± 0.0002, and 0.0101 ± 0.0001, respectively. Acetamiprid primarily underwent indirect photolysis by reaction with OH· ($1.7 \pm [0.2] \times 10^9\ M^{-1}\ s^{-1}$). For all compounds, the urea derivative was the most commonly detected product in both hydrolysis and photolysis experiments. Using mosquito (*Culex pipiens*) larvae, no residual toxicity of reaction products was observed. Results indicate long environmental half-lives for the tested neonicotinoids, which may help to explain their ubiquitous presence in environmental matrices. *Environ Toxicol Chem* 2018;37:2797–2809. © 2018 The Authors. *Environmental Toxicology and Chemistry* published by Wiley Periodicals, Inc. on behalf of SETAC

Keywords: Abiotic transformation; Contaminants; Environmental fate; Insecticide; Neonicotinoids

INTRODUCTION

Neonicotinoids (Figure 1) are a class of systemic insecticides widely used worldwide, with registration in over 120 countries for usage on more than 140 crops (Jeschke et al. 2010). Since their release in the 1990s as a replacement for carbamates and organophosphates, use has increased considerably, and neonicotinoids now account for a quarter of the world's insecticide use (Bass et al. 2015). Usage has spread beyond agriculture to home garden and lawn care, garden centers, and urban forestry to combat emerald ash borer (Cowles 2009; Cloyd and Bethke 2011).

Widespread use of neonicotinoids, perhaps unsurprisingly, has led to near ubiquitous environmental detection, including in surface water and groundwater (Hladik et al. 2014; Morrissey et al. 2015; Schaafsma et al. 2015). Detection in finished drinking water has also been reported in Iowa City, Iowa, USA (Klarich et al. 2017), and in Ontario, Canada (Sultana et al. 2018), with concentrations as high as 57.3 and 280 ng/L, respectively. Wastewater effluent frequently contains neonicotinoids, and traditional activated sludge treatment does little to remove them, resulting in an estimated 1000 to 3400 kg of neonicotinoids being discharged in effluent yearly (Peña et al. 2011; Sadaria et al. 2016). In soil, neonicotinoids have been detected at concentrations up to 20 μg/g and up to 3 yr after the last application (Goulson 2013; Jones et al. 2014; Schaafsma et al. 2015). This widespread detection indicates that neonicotinoids are environmentally persistent and effectively have slow abiotic degradation rates.

Previous work has shown long half-lives in water, with reported half-lives at pH 7 of >800 and >4000 d for thiamethoxam and imidacloprid, respectively (Zheng and Liu 1999; Karmakar et al. 2009). The full effect of pH on neonicotinoid degradation, however, is not understood because some work has shown degradation occurring at pH 4, whereas other work only reported degradation at basic pH values (Zheng and Liu 1999; Liu et al.

This article includes online-only Supplemental Data.
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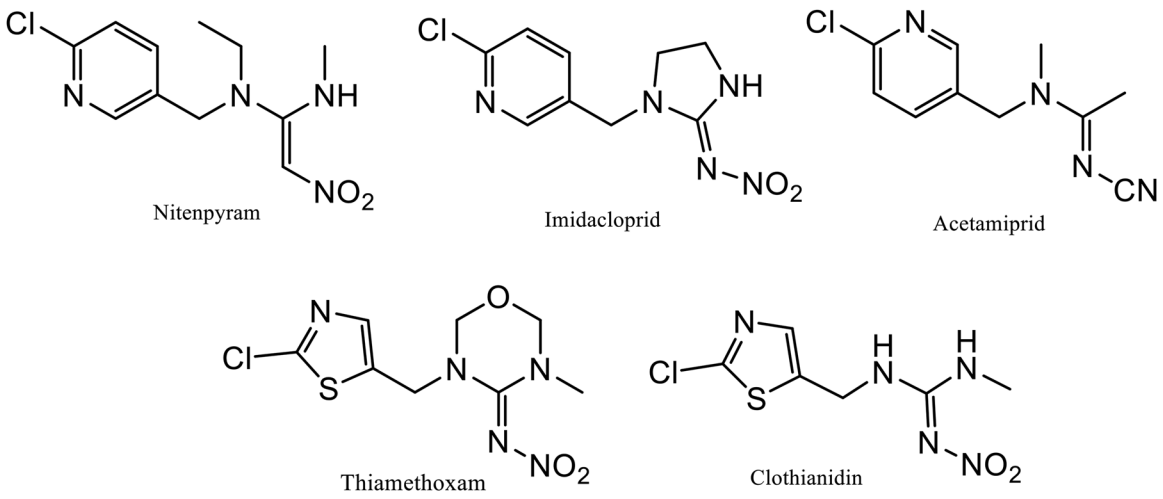

FIGURE 1: Selected neonicotinoid insecticides used in the present study.

2006; Bonmatin et al. 2015). In addition, the effect of the presence of metal ions and minerals, which have been shown to increase hydrolysis rates in other pesticides (Ketelaar et al. 1956; Smolen and Stone 1997), has not been explored for neonicotinoids. Direct photolysis has also been observed, with large variations in quantum yield between neonicotinoids and half-lives ranging from 12 min for imidacloprid to 42 h for thiacloprid (Lu et al. 2015). Indirect photolysis has been studied, with half-life estimates of 5 h to 19 d in aquatic reservoirs, indicating that hydroxyl radicals may play a role in neonicotinoid photolysis (Dell'Arciprete et al. 2009). The comparison of direct and indirect photolysis in the same study, however, has not been reported, and updated actinometry values (Laszakovits et al. 2017) require validation of previously reported quantum yields. Overall, accurate hydrolysis and photolysis rate constants would allow for increased accuracy in environmental fate modeling.

There has been growing concern over the impact of neonicotinoids on nontarget organisms. Detrimental effects have been observed at acute and subacute levels in honeybees (*Apis mellifera*), with neonicotinoids suspected of contributing to colony collapse disorder along with other problems such as decreased navigational ability and impaired learning (Henry et al. 2012; Gill et al. 2013). Although research has focused on honeybees, sublethal effects have been observed in aquatic arthropods, birds, and fish, including reproduction inhibition, delayed emergence, feeding inhabitation, and organ damage (Morrissey et al. 2015; Hladik et al. 2018). In addition, residual toxicity has been observed with several degradation products, such as the desnitro/guanidine and nitrosoguanidine derivatives of imidacloprid (Lee Chao and Casida 1997; Tomizawa and Casida 1999; Tomizawa et al. 2000).

Imidacloprid, clothianidin, and thiamethoxam (all nitroguanidines; Figure 1) account for >99% of total neonicotinoid usage in Minnesota, USA, and were thus selected for the present study. Acetamiprid (a cyanoamide) and nitenpyram (a nitromethylene) were also used, to allow for comparison of the 3 pharmacologically active groups currently used in neonicotinoids. The goals of the present study 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) identify reaction products; and 4) evaluate toxicity of hydrolysis and photolysis products.

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. Nitenpyram (99.9%) was purchased from Fluka Analytical. Solvents (methanol, acetonitrile; high-performance liquid chromatography [HPLC] grade) were purchased from Sigma-Aldrich. Ultrapure water (18.2 MΩ · cm) was obtained using a Milli-Q Academic system (Millipore). Buffers were made using American Chemical Society (ACS)–grade chemicals. Sodium acetate (99.5%) was purchased from BDH Chemicals, 3-(*N*-morpholino)propanesulfonic acid (MOPS; 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. Acetic acid (ACS-grade; 99.9%) 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 Voelz in the University of Minnesota Department of Chemistry. The compounds *p*-nitroanisole (PNA; 98%) and pyridine (>99.0%) were purchased from Sigma-Aldrich. Sodium nitrate (99.2%) was purchased from Fisher Chemical, and *p*-chlorobenzoic acid (*p*CBA; 99%) was purchased from Acros Organics.

Buffer solutions

To determine the hydrolysis rates over a range of pH values, buffer solutions were prepared at pH 4.0, 6.3, 7.0, 8.0, and 10.0, with the exception that a pH 9.0 buffer was used for thiamethoxam instead of pH 10.0, because of rapid degradation

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of thiamethoxam at pH 10.0. Acetate was used as a buffer for pH 4.0; MOPS was used for pH 6.3, 7.0, and 8.0 buffers; and sodium tetraborate (i.e., borate) was used for pH 9.0 and 10.0 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, 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.

Hydrolysis

To determine hydrolysis rates at different pH values, buffer solutions were prepared at pH 4.0, 6.3, 7.0, 8.0, and 9.0/10.0. Reactors at each pH were dosed with a methanolic stock solution of the desired neonicotinoid to achieve an initial concentration of 1 μM. Reactors were stored in foil-wrapped glass scintillation vials in cabinets to prevent photolysis. Degradation was monitored for up to 150 d.

Reactors containing metal ions and minerals were also studied. To determine if metal ions had an effect on neonicotinoid degradation, copper (II) chloride, nickel (II) chloride, and zinc (II) chloride were added to reactors at 1 mM (pH 4.0 and 6.3) or 0.1 mM (pH 8.0 and 10.0) and spiked with neonicotinoids to a concentration of 1 μM using the same buffers as baseline experiments. Although equilibrium calculations indicate that precipitation could occur for all 3 metals at pH 10 and for copper at pH 8, no formation of solids was observed during the experiments. For reactors containing minerals, kaolinite, goethite, and titanium dioxide were added (1 g/L) to the reactors and stirred for 18 to 24 h before adding neonicotinoids (10 μM). Reactors were constantly stirred on a 16-position analog stir-plate (Scilogex) using a 1/8 × 1/2″ PTFE disposable stir bar (Fisher Scientific). Regular samples were taken (250 μL) and filtered through a 13-mm PTFE syringe-tip filter (pore size 0.2 μm; Fisher Scientific) before analysis.

A comparison to hydrolysis rates in a natural water was also performed. Mississippi River water was collected from the University of Minnesota Boathouse (Minneapolis, MN, USA) dock, prefiltered with combusted glass-fiber filters (Millipore; 0.7 μm), filter-sterilized with nitrocellulose membrane filters (Millipore; 0.22 μm), and stored at 4 °C until used. Two separate Mississippi River water samples were collected, on 12 July 2017 and on 3 November 2017. Characterization of each sample is found in Supplemental Data, Table S1. 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. Dissolved organic carbon was measured with a Shimadzu TOC-L analyzer operated in nonpurgeable organic carbon mode. Samples were dosed with neonicotinoids to an initial concentration of 10 μM and monitored for 150 d.

Reactors containing metals and minerals were compared to baseline studies using a *t* test to compare slopes of kinetic regression lines, based on a method published by Howell (2011). The null hypothesis was that the slopes are equal; thus, if a *p* value ≥0.05 was calculated, the null hypothesis could not be rejected, and there was not considered to be a statistical difference between tested slopes.

Photolysis

Photolysis experiments were performed in both 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 Simple Model of the Atmospheric Radiative Transfer of Sunshine model (Ver 2.9.5). To determine the relative importance of direct and indirect photolysis, solutions were prepared in ultrapure water (Milli-Q) and Mississippi River water by dosing neonicotinoids using an aqueous stock solution, resulting in a 10 μM contaminant concentration. Pyridine–PNA actinometers were run to allow determination of quantum yield, using 5 μM PNA and variable concentrations of pyridine, because of differences in neonicotinoid reactivity. Data were analyzed using methods prescribed by Leifer (1988) with the recent update to the PNA quantum yield relationship (Laszakovits et al. 2017). Details of the equations used to calculate quantum yields and screening factors are provided in the Supplemental Data.

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 (5 μM, $k_{pCBA,HO\cdot} = 5 \times 10^9\ M^{-1}\ s^{-1}$; Westerhoff et al. 1999) was used to determine steady-state hydroxyl radical concentrations.

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 radicals ($k_{A,HO\cdot}$) were derived from the linear regression of natural log-normalized concentrations of neonicotinoids (*A*) versus *p*CBA, shown in Equation 1, where $k_{pCBA,HO\cdot}$ is the bimolecular rate constant of *p*CBA reaction with hydroxyl radicals.

$$\ln\left(\frac{[A]}{[A]_0}\right) = \frac{k_{A,HO\cdot}}{k_{p,CBA,HO\cdot}} \ln\left(\frac{[pCBA]}{[pCBA]_0}\right) \quad (1)$$

Analytical methods

Light absorbance of each neonicotinoid was measured from 200 to 800 nm using a Shimadzu UV-1601PC spectrophotometer with 1-cm quartz cuvettes. Neonicotinoid, *p*CBA, and PNA concentrations were measured using HPLC on an Agilent 1200 system equipped with a diode-array detector. All compounds were detected using an Ascentis Supelco RP-Amide C-16 column (15 cm × 4.6 mm, 5 μm); specific method information

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for each compound is provided in Supplemental Data, Table S2.

Ultrahigh pressure liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) was used to identify neonicotinoid hydrolysis and photolysis degradation reaction products. Aliquots were taken from samples generated for toxicology experiments (see *Toxicology* section) and analyzed at the University of Minnesota's Masonic Cancer Center on a Thermo Fisher UltiMate 3000 UHPLC system paired to a Thermo Fisher Linear Trap Quadrupole Orbitrap Velos UHPLC-MS/MS using a C18 nanoflow column with a gradient method (see Supplemental Data, Table S3). The mass spectrometer was run in positive mode and set to analyze for 33 min, with 6 scans, all from 80.0 to 400 (*m/z*) and using a collision energy of 35 eV. Scan 1 had a resolution of 60 000, and scan 2 was set to collect the parent neonicotinoids. Scans 3 to 6 had a resolution of 15 000 and isolated the first, second, third, and fourth most abundant peaks (not including the parent) from scan 1, with a peak exclusion area set to 500. Mass spectrometric data were analyzed using Thermo Fisher Compound Discoverer 2.1 software. Untargeted environmental analyses and targeted environmental analyses of expected degradation products were run to identify products. Products were first compared using exact mass, with MS2 data and database identification used to verify product structure by comparing to literature data when available.

Toxicology

Samples for parent compound toxicity tests were prepared by dosing methanol stock solution to a 10-mL volumetric flask so that the final concentration was 50 μM, filling with Milli-Q ultrapure water and mixing well. Concentration was verified using HPLC. Hydrolysis samples (baseline, with metals, and with minerals) containing products were prepared by creating a 50-μM parent solution at pH 10.0 and monitoring until the neonicotinoid concentration decreased to 10 μM. Samples were filtered through a 0.2-μm syringe tip filter and neutralized to pH 7 using metals-grade concentrated HCl. Photolysis samples were prepared by reacting a 50-μM aqueous solution in an Atlas CPS+ solar simulator and monitoring using HPLC until the concentration of the parent compound was 10 μM, yielding samples with an approximate 4:1 ratio of reaction products to parent compound. Samples were stored at –20 °C.

Toxicity experiments were performed using mosquito (*Culex pipiens*) fourth instar larvae. Larvae were placed in distilled water and distributed into vials (5 larvae in each of 3 replicate vials), volumes were adjusted to 9.0 ± 0.1 mL, and 1 mL of test solution 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 3 vials from 2 separate experiments. Median lethal concentration (LC50) values were then calculated by plotting response (percentage) versus dose (concentration) and determining the point at which 50% of larvae died.

RESULTS AND DISCUSSION

Hydrolysis

Baseline hydrolysis. Neonicotinoid baseline hydrolysis reactors were monitored and sampled for 50 to 150 d. Pseudo-first-order rate constants were calculated using linear regression of natural log concentration versus time for all reactors; results are given in Table 1 and Figure 2. In pH 4.0, 6.33, and 7.0 samples for all neonicotinoids, little to no degradation was observed, with half-lives calculated to be over 1000 d for most compounds. Significant error is present in calculations for reactors below pH 8.0. In many cases, the 95% confidence interval 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 >9 (Zheng and Liu 1999; Liu et al. 2006). Thiamethoxam hydrolysis kinetics at high pH were similar to previously reported work (Liqing et al. 2006; Karmakar et al. 2009; Klarich et al. 2017). Karmakar et al. (2009) observed significantly larger k_{obs} for thiamethoxam in phosphate-buffered solutions at pH 4.0 (100 times larger) and pH 7.0 (10 times larger) than was observed in the present study. Klarich et al. (2017), however, saw no hydrolysis at pH 7, consistent with the present results.

Hydrolysis in the presence of metal ions. Neonicotinoid reactors containing 1 mM (pH 4.0, 6.3) and 0.1 mM (pH 8.0, 10.0) divalent metal ions were monitored for 50 to 150 d, depending on the rate of reaction. Pseudo-first-order rate constants were calculated using linear regression of the natural log of concentration versus time; results are given in Table 1. Similar to baseline reactors, little to no degradation was observed at pH 4.0 and 6.3 (see Supplemental Data, Figure S1), with broad intervals at the 95% confidence level. At pH 8.0 and 10.0 (Supplemental Data, Figure S2), metals do not appear to have an effect on degradation rate. Calculated *p* values from slope tests are given in Supplemental Data, Table S4.

Determination of reaction order with [OH^-]. 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. Thus, second-order rate constants could be calculated by dividing the observed, pseudo-first-order rate constant by the measured values of [OH^-] in each experiment (which were ±0.05 units from the target value), giving a rate constant with units of per molar per day. Propagation of error was performed using the standard deviation of results from the pseudo-first-order linear regression. Error was calculated by dividing 95% confidence interval by [OH^-].

Calculated second-order rate constants (see Supplemental Data, Table S5) indicate that the hydrolysis reaction that the neonicotinoids undergo is, in fact, not a second-order elementary reaction. From pH 4.0 to 10.0, calculated second-order rate constants vary by 5 to 6 orders of magnitude (e.g., for clothianidin, the calculated rate constants range from 3.0 $[\pm 1.1] \times 10^{-6}\ M^{-1}$

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TABLE 1: Calculated pseudo-first-order rate constants for hydrolysis of neonicotinoids (per day)[a]

		Baseline	Copper[b]	Nickel[b]	Zinc[b]	Baseline (stir-plate)[c]	Kaolinite[d]	Goethite[d]	TiO_2[d]
Compound	pH	21.5 °C				28.0 °C			
Nitenpyram	4.0	$8.5(\pm 2.5)\times 10^{-4}$	$6.2(\pm 1.7)\times 10^{-4}$	$7.1(\pm 1.5)\times 10^{-4}$	$7.5(\pm 2.9)\times 10^{-4}$	—	—	—	—
	6.3	$1.4(\pm 0.4)\times 10^{-3}$	$1.1(\pm 0.7)\times 10^{-3}$	$8.4(\pm 3.5)\times 10^{-4}$	$1.4(\pm 0.5)\times 10^{-3}$	—	—	—	—
	7.0	$1.3(\pm 0.2)\times 10^{-3}$	—	—	—	—	—	—	—
	8.0	$3.4(\pm 0.4)\times 10^{-3}$	$1.5(\pm 0.3)\times 10^{-3}$	$2.5(\pm 0.6)\times 10^{-3}$	$1.7(\pm 0.4)\times 10^{-3}$	$1.8(\pm 0.4)\times 10^{-3}$	$3.0(\pm 0.3)\times 10^{-3}$	$2.1(\pm 0.4)\times 10^{-3}$	$5.0(\pm 0.5)\times 10^{-3}$
	10.0	$5.1(\pm 0.1)\times 10^{-2}$	$4.8(\pm 0.3)\times 10^{-2}$	$5.0(\pm 0.3)\times 10^{-2}$	$5.0(\pm 0.4)\times 10^{-2}$	$10(\pm 0.1)\times 10^{-2}$	$9.8(\pm 1.0)\times 10^{-2}$	$12(\pm 1.5)\times 10^{-2}$	$12\pm 2.4)\times 10^{-2}$
Imidacloprid	4.0	$4.3(\pm 1.6)\times 10^{-4}$	$2.9(\pm 0.9)\times 10^{-4}$	$3.0(\pm 0.7)\times 10^{-4}$	$3.6(\pm 2.7)\times 10^{-4}$	—	—	—	—
	6.3	$5.4(\pm 1.0)\times 10^{-4}$	$6.9(\pm 4.3)\times 10^{-4}$	$5.1(\pm 3.1)\times 10^{-4}$	$5.4(\pm 4.4)\times 10^{-4}$	—	—	—	—
	7.0	$4.2(\pm 1.4)\times 10^{-4}$	—	—	—	—	—	—	—
	8.0	$7.9(\pm 0.9)\times 10^{-4}$	$9.9(\pm 5.2)\times 10^{-4}$	$1.3(\pm 0.5)\times 10^{-3}$	$9.7(\pm 5.6)\times 10^{-4}$	$1.6(\pm 1.6)\times 10^{-4}$	$9.9(\pm 5.6)\times 10^{-4}$	$4.8(\pm 1.9)\times 10^{-4}$	$1.6(\pm(0.5)\times 10^{-3}$
	10.0	$1.8(\pm 0.1)\times 10^{-2}$	$1.5(\pm 0.1)\times 10^{-2}$	$1.5(\pm\times 10^{-2}$	$1.5(\pm 0.1)\times 10^{-2}$	$3.6(\pm 0.1)\times 10^{-2}$	$3.9(\pm 0.2)\times 10^{-2}$	$4.0(\pm 0.1)\times 10^{-2}$	$4.0(\pm(0.1)\times 10^{-2}$
Acetamiprid	4.0	$4.0(\pm 3.3)\times 10^{-4}$	$5.2(\pm 2.6)\times 10^{-4}$	$5.9(\pm 2.0)\times 10^{-4}$	$2.5(\pm 1.6)\times 10^{-4}$	—	—	—	—
	6.3	$4.2(\pm 4.2)\times 10^{-4}$	$7.5(\pm 2.0)\times 10^{-4}$	$5.9(\pm 2.3)\times 10^{-4}$	$6.6(\pm 1.6)\times 10^{-4}$	—	—	—	—
	7.0	$1.0(\pm 0.4)\times 10^{-3}$	—	—	—	—	—	—	—
	8.0	$1.5(\pm 0.2)\times 10^{-3}$	$2.0(\pm 0.5)\times 10^{-3}$	$2.2(\pm 0.6)\times 10^{-3}$	$1.9(\pm 0.5)\times 10^{-3}$	$2.1(\pm 1.6)\times 10^{-4}$	$7.1(\pm 4.6)\times 10^{-4}$	$5.5(\pm 1.6)\times 10^{-4}$	$1.8(\pm 0.2)\times 10^{-3}$
	10.0	$2.8(\pm 0.1)\times 10^{-2}$	$2.6(\pm 0.1)\times 10^{-2}$	$2.6(\pm 0.1)\times 10^{-2}$	$2.5(\pm 0.1)\times 10^{-2}$	$4.6(\pm 0.1)\times 10^{-2}$	$5.1(\pm 0.1)\times 10^{-2}$	$6.4(\pm 0.2)\times 10^{-2}$	$6.3(\pm 0.2)\times 10^{-2}$
Thiamethoxam	4.0	$2.0(\pm 1.8)\times 10^{-4}$	$3.4(\pm 7.4)\times 10^{-4}$	$2.9(\pm 1.1)\times 10^{-4}$	$2.8(\pm 2.1)\times 10^{-4}$	—	—	—	—
	6.3	$5.7(\pm 1.7)\times 10^{-4}$	$6.3(\pm 82)\times 10^{-4}$	$2.4(\pm 1.8)\times 10^{-3}$	$3.1(\pm 2.6)\times 10^{-3}$	—	—	—	—
	7.0	$1.0(\pm 0.3)\times 10^{-3}$	—	—	—	—	—	—	—
	8.0	$5.4(\pm 0.2)\times 10^{-3}$	$3.4(\pm 0.9)\times 10^{-3}$	$4.0(\pm 1.1)\times 10^{-3}$	$4.6(\pm 1.2)\times 10^{-3}$	$6.8(\pm 0.7)\times 10^{-3}$	$8.5(\pm 0.5)\times 10^{-3}$	$10(\pm 0.3)\times 10^{-3}$	$11(\pm 0.4)\times 10^{-3}$
	9.0	$5.8(\pm 0.1)\times 10^{-2}$	$5.3(\pm 0.8)\times 10^{-2}$	$5.2(\pm 0.1)\times 10^{-2}$	$5.2(\pm 0.1)\times 10^{-2}$	$9.5(\pm 0.1)\times 10^{-2}$	$10(\pm 0.6)\times 10^{-2}$	$17(\pm 0.7)\times 10^{-2}$	$16(\pm 0.5)\times 10^{-2}$
Clothianidin	4.0	$3.4(\pm 1.2)\times 10^{-4}$	$7.0(\pm 11)\times 10^{-5}$	$1.0(\pm 2.0)\times 10^{-4}$	$3.2(\pm 3.3)\times 10^{-4}$	—	—	—	—
	6.3	$4.1(\pm 0.5)\times 10^{-4}$	$2.8(\pm 2.6)\times 10^{-4}$	$2.3(\pm 2.1)\times 10^{-4}$	$2.9(\pm 1.4)\times 10^{-4}$	—	—	—	—
	7.0	$4.3(\pm 2.1)\times 10^{-4}$	—	—	—	—	—	—	—
	8.0	$4.6(\pm 1.2)\times 10^{-4}$	$5.3(\pm 0.1)\times 10^{-4}$	$8.8(\pm 0.1)\times 10^{-4}$	$8.1(\pm 11)\times 10^{-4}$	$6.0(\pm 20)\times 10^{-6}$	$1.1(\pm 0.5)\times 10^{-3}$	$7.0(\pm 5.0)\times 10^{-4}$	$2.0(\pm 48)\times 10^{-5}$
	10.0	$5.2(\pm 0.4)\times 10^{-3}$	$5.1(\pm 0.6)\times 10^{-3}$	$4.9(\pm 0.6)\times 10^{-3}$	$5.1(\pm 0.7)\times 10^{-3}$	$1.0(\pm 0.1)\times 10^{-2}$	$1.2(\pm 0.2)\times 10^{-2}$	$1.2(\pm 0.1)\times 10^{-2}$	$1.2(\pm 0.1)\times 10^{-2}$

[a] Errors are the 95% confidence intervals.
[b] Copper, nickel, and zinc columns represent observed rate constants of neonicotinoids in the presence of each metal cation at 21.5 °C.
[c] Baseline (stir-plate) gives observed rate constant of baseline reactors run on the stir-plate used for mineral reactions.
[d] Kaolinite, goethite, and TiO_2 columns give observed rate constants in the presence of each mineral at 28 °C.

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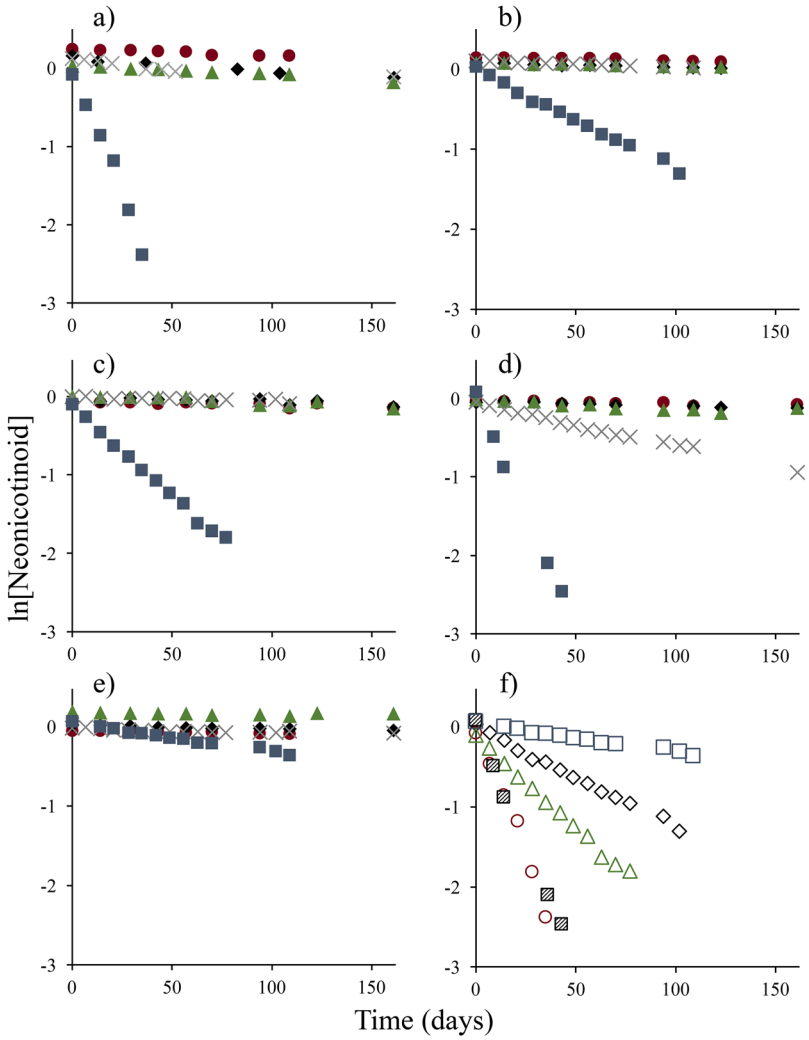

FIGURE 2: Baseline hydrolysis of neonicotinoid insecticides at pH 4, 6.33, 7, 8, and 10: (**a**) nitenpyram, (**b**) imidacloprid, (**c**) acetamiprid, (**d**) thiamethoxam, (**e**) clothianidin, (**f**) pH 10 (pH 9 for thiamethoxam) hydrolysis results. Legend graphs: (**a–e**) ● pH 4, ♦ pH 6.33, ▲ pH 7, X pH 8, ■ pH 10; (**f**) ○ nitenpyram pH 10, ◊ imidacloprid pH 10, △ acetamiprid pH 10, ▨ thiamethoxam pH 9, □ clothianidin pH 10.

d^{-1} at pH 4 to 58 M^{-1} d^{-1} at pH 10), indicating that the assumed reaction order is incorrect and the reaction with OH^- is not elementary.

Hydrolysis reactions can occur because of the reaction of a compound with H^+, H_2O, or OH^-. Because the reaction at pH 4 for all neonicotinoids is slower than all higher pH reactors, it was assumed that there were no hydrolysis reactions occurring attributable to catalysis by 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 attributable to hydrolysis from water and the rate attributable to base-catalyzed hydrolysis. 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 run at higher than pH 4.0 and calculating the regression line of the resulting scatterplot. Plots are given in Figure 3.

$$\text{rate} = k_{H_2O}[\text{Neonic}] + k_{OH^-}[\text{Neonic}][OH^-]^n = k_{obs}[\text{Neonic}] \quad (2)$$

$$k_{obs} = k_{H_2O} + k_{OH^-}[OH^-]^n \quad (3)$$

$$\text{Assume } k_{H_2O} = k_{pH\,4} \quad (4)$$

$$k_{obs} = k_{pH\,4} = k_{OH^-}[OH^-]^n \quad (5)$$

$$\log(k_{obs} - k_{pH\,4}) = n \times -pOH + \log(k_{OH^-}) \quad (6)$$

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 nitenpyram (0.60 ± 0.121) in the middle. Errors are 95% confidence intervals. Because of the relative similarity between the calculated reaction orders, a slope test was performed to compare each of the log–log regression lines to determine if there was a significant

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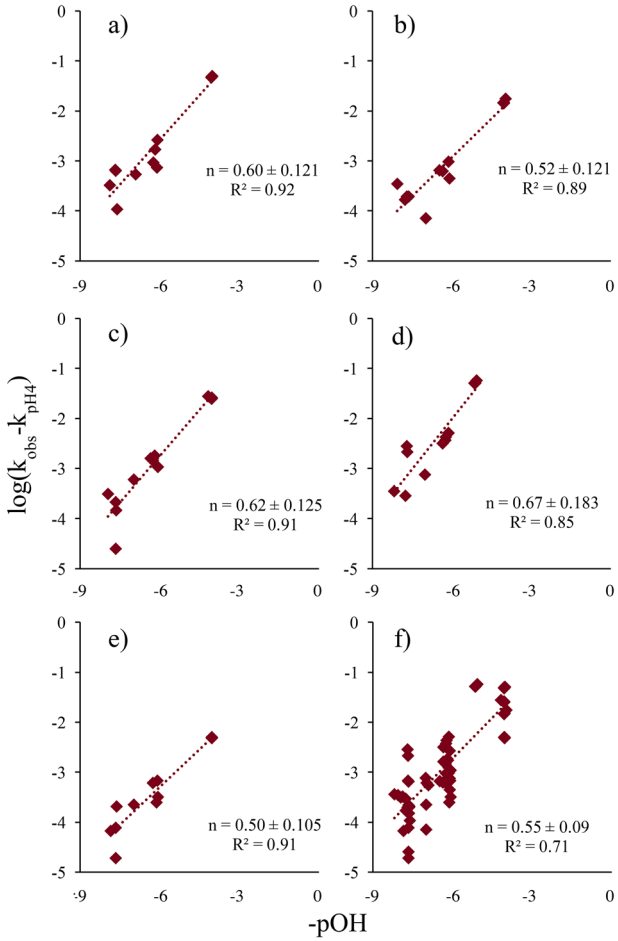

FIGURE 3: Log–log plot of hydroxide concentration and the difference between k_{obs} and $k_{pH\,4}$. The resulting slope is the approximate value of *n*, the exponent for [OH^-] in the nonelementary reaction of neonicotinoid hydrolysis: (**a**) nitenpyram, (**b**) imidacloprid, (**c**) acetamiprid, (**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.

difference between individual neonicotinoid reaction orders. Calculated *p* values (see Supplemental Data, Table S6) show that there is not a statistically significant difference between each of the calculated slopes, with *p* values ranging from 0.09 to 0.83, indicating highly correlated slopes. All data points were placed in a single plot to provide a comprehensive estimate of the value of *n*. Linear regression of the resulting plot returned a slope of 0.55 ± 0.09. Hydroxide rate constants were then calculated for all experiments and are given in Table 2. The nonelementary rate expression indicates that the hydrolysis mechanism is likely not the straightforward process previously depicted (e.g., Zheng and Liu 1999; Karmakar et al. 2009) but rather one where reversible, preequilibrium steps occur and where OH^- is involved in multiple steps. Further work would be necessary to determine the elementary reaction steps that occur leading to the observed approximately 0.5 power dependence on [OH^-].

When hydroxide rate constants are compared at the 95% confidence interval, rate constants do not differ between baseline and metal-containing solutions, with the exception of the acetamiprid pH 10.0 baseline reactor and metal reactors, in which the metals slightly decrease the rate of reaction. Thus, these results indicate that divalent metal cations in solution do not change the rate of hydrolysis of neonicotinoids.

Hydrolysis in the presence of minerals. Reactors containing minerals (kaolinite, goethite, or titanium dioxide) were monitored for up to 100 d, depending on the speed of the reaction. Placement of a box over the stir plate to reduce the possibility of light contamination created the possibility of a slightly increased rate of reaction because other work has shown that the neonicotinoid hydrolysis reaction rate increases with temperature (Zheng and Liu 1999; Liqing et al. 2006). To account for the potential effect of temperature and the potential effect of stirring mineral reactors constantly whereas previous reactors had not been stirred, new baseline reactors were run along with mineral reactors. Pseudo-first-order rate constants were calculated for all reactions and are given in Table 1. Reaction kinetics are shown in Supplemental Data, Figure S3, and the slopes tests comparisons are given in Supplemental Data, Table S7. At pH 10, the faster reaction rates (2.1–2.5 times increase) compared to original baseline and metals experiments is attributed to the increased temperature. When accounting for the actual [OH^-] in each experiment (which again varied ±0.05 units from the target

TABLE 2: Hydroxide rate constants (k_{OH} in $M^{-0.55}\,d^{-1}$) for neonicotinoid hydrolysis reactions at 21.5 °C[a]

		pH		
Compound	Experiment	6.3	8.0	10.0
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
	Average[b]		6.1 ± 0.9	
Imidacloprid	Baseline	8.0 ± 1.5	1.7 ± 0.2	2.6 ± 0.1
	Copper	17.4 ± 10.9[c]	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
	Average[b]		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
	Average[b]		3.8 ± 0.5	
Thiamethoxam	Baseline	5.0 ± 2.9	11.5 ± 0.5	33.8 ± 0.8
	Copper	10.5 ± 242.6[d]	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
	Average[b]		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
	Average[b]		1.1 ± 0.5	

[a] Errors are the 95% confidence intervals.
[b] Average rate constants were calculated using rate constants from baseline and metal experiments at pH 6.33, 8, and 10.
[c] Imidacloprid pH 6.33 copper was excluded as an outlier because it had an outsized effect on the mean.
[d] Thiamethoxam pH 6.33 copper was excluded as an outlier because of the large error associated with the value.

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value) and using 0.55 for n and pH 4 baseline results for k_{H_2O} (Supplemental Data, Table S8), the calculated hydroxide rate constants indicate that pH may be responsible for any observed variations in reaction rates between mineral and baseline experiments as determined by the slope test (Supplemental Data, Table S7). Thus, minerals likely do not have an impact on neonicotinoid hydrolysis rates.

Hydrolysis in Mississippi River water. Samples of Mississippi River water were monitored for 150 d. Pseudo-first-order rate constants were calculated as ln[Neonicotinoid] versus time for experiments in Mississippi River water and are given in Table 3. Kinetic data are given in Supplemental Data, Figure S4. The pH of the Mississippi River water was 8.3; thus, pseudo-first-order rate constants were expected to be faster than hydrolysis rates at pH 8.0. This was observed for nitenpyram, where the pseudo-first-order rate constant is marginally larger than the average pseudo-first order at pH 8.0. Thiamethoxam pseudo-first-order rate constants were the same, whereas clothianidin, imidacloprid, and acetamiprid pseudo-first-order rate constants were slower.

Comparison of hydroxide rate constants, which accounts for comparison across several pH values, indicates that every neonicotinoid reacts 45 to 90% slower in Mississippi River water, accounting for the pH of Mississippi River water. No explanation is currently available to account for the changes in reaction rates, although a buffer effect from carbonate is one possibility. These results do indicate that hydrolysis degradation rates may be slower in natural water bodies than predicted by laboratory tests performed in less complicated matrices.

Photolysis

Kinetic data for photolysis experiments in natural sunlight and in a solar simulator are given in Figure 4. Calculated quantum yields are given in Table 4. In the solar simulator, calculated quantum yields for nitenpyram, imidacloprid, thiamethoxam, and clothianidin in Milli-Q water are all larger by 8 to 25% than quantum yields in Mississippi River water after adjusting for screening, indicating that 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 and clothianidin. In natural sunlight, once adjusted for screening, thiamethoxam Milli-Q results were lower than Mississippi River water quantum yields. A one-tailed paired t test comparing the 2 means gave a value of 0.12, indicating that at the 95% confidence interval the 2 quantum yields cannot be distinguished. Thus, thiamethoxam is likely to follow the same behavior as imidacloprid, nitenpyram, and clothianidin, which photolyze only because of direct photolysis.

Calculated quantum yields in the present study are similar to previously reported values. For imidacloprid, quantum yields of 0.0092 (Lu et al. 2015; medium-pressure mercury lamp) and 0.0055 (von Gunten 2012; natural sunlight, 47°N latitude, Zurich, Switzerland) have previously been reported, as compared with the quantum yields calculated in the present study, which ranged from 0.0089 to 0.0119. Quantum yields of thiamethoxam (0.0130–0.0167) are between previously reported quantum yields of 0.019 (Lu et al. 2015) and 0.013 (European Comission 2006). Similarly, with clothianidin, quantum yields of 0.0073 (von Gunten 2012) and 0.013 (Lu et al. 2015) have been reported, which are in the range of those calculated in the present study (0.0080–0.0133). The differences between the present values and those previously reported could be attributable to differences in the light sources (i.e., there is wavelength dependence of quantum yield) or because we have used the updated values for the PNA actinometer (Laszakovits et al. 2017). The updated actinometer values should give 29% lower quantum yields, which is the effect seen for thiamethoxam and clothianidin in the natural sunlight experiments and in the solar simulator with Mississippi River water when comparing with values of Lu et al. (2015). It is not clear why the same effect is not observed for imidacloprid.

Acetamiprid samples were originally studied in the solar simulator, where results after 3 h of exposure gave an estimated half-life of >100 h. Although experiments were conducted on the rooftop of the University of Minnesota Mechanical Engineering building, exposure to sunlight for >1 mo yielded little to no degradation of acetamiprid in Mississippi River water samples or Milli-Q samples, indicating that direct photolysis was not an important environmental degradation pathway. These indicate a much longer half-life than reports in the literature, where Lu et al. (2015) found acetamiprid to have a half-life of 26 h with a quantum yield of 0.0022 ± 0.0003.

TABLE 3: Calculated pseudo-first-order and hydroxide rate constants for hydrolysis reactions in Mississippi River water (MRW) hydrolysis experiments at 21.5 °C[a]

Compound	$k_{obs,\ MRW}$[b] (d^{-1})	$k_{avg,\ pH\ 8}$[c] (d^{-1})	$k_{OH-,\ MRW}$[d] ($M^{-0.55}\ d^{-1}$)	$k_{OH-,\ avg}$[e] ($M^{-0.55}\ d^{-1}$)
Nitenpyram	$3.4 \pm (1.2) \times 10^{-3}$	$2.3 \pm (0.2) \times 10^{-3}$	3.5 ± 1.6	6.1 ± 0.9
Imidacloprid	$6.5 \pm (2.7) \times 10^{-4}$	$1.0 \pm (0.2) \times 10^{-3}$	0.4 ± 0.4	5.3 ± 0.7
Acetamiprid	$3.5 \pm (2.2) \times 10^{-4}$	$1.9 \pm (0.2) \times 10^{-3}$	0.1 ± 0.3	3.8 ± 0.5
Thiamethoxam	$4.4 \pm (0.5) \times 10^{-3}$	$4.4 \pm (0.4) \times 10^{-3}$	5.5 ± 0.7	22 ± 8
Clothianidin	$6.4 \pm (4.4) \times 10^{-4}$	$6.7 \pm (4.2) \times 10^{-4}$	0.6 ± 0.6	1.1 ± 0.5

[a] Errors are the 95% confidence intervals.
[b] Pseudo-first-order rate constant for hydrolysis reactions in Mississippi River water (pH 8.3).
[c] Averaged pseudo-first-order rate constants at pH 8.0.
[d] Hydroxide rate constant for Mississippi River water hydrolysis experiments.
[e] Average hydroxide rate constant across all pH values.

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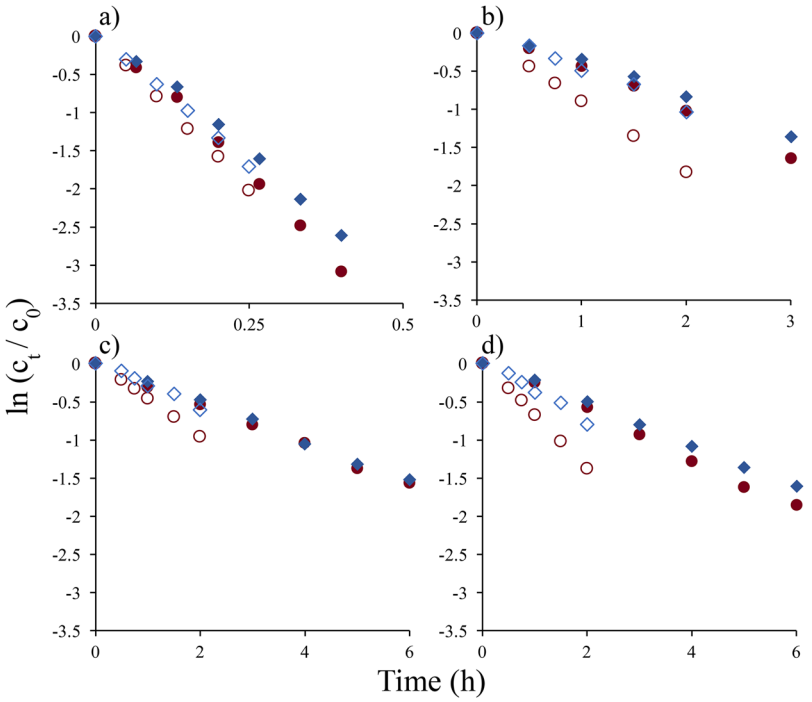

FIGURE 4: Photolysis of neonicotinoid insecticides in Milli-Q and Mississippi River water in natural and simulated sunlight: (**a**) nitenpyram, (**b**) imidacloprid, (**c**) thiamethoxam, (**d**) clothianidin. ● Milli-Q, natural sunlight; ♦ MRW, natural sunlight; ◊ MRW, solar simulator; ○ Milli-Q, solar simulator.

From the quantum yields calculated, indirect photolysis does not initially appear to be important. However, Mississippi River water generally contains lower levels of nitrate, a hydroxyl radical sensitizer, than could potentially be present in other waters, such as agricultural runoff. Further experiments were conducted using imidacloprid, acetamiprid, thiamethoxam, and clothianidin to study the effect of high concentrations of hydroxyl radicals using nitrate-amended Mississippi River water (10 mg/L as N). Nitenpyram was not used in nitrate experiments because direct photolysis is rapid.

First-order rate constants were calculated using linear regression of ln[C] versus time (see Supplemental Data, Figure S5). At a hydroxyl radical concentration of 2×10^{-15} M, as determined by the *p*CBA, imidacloprid, thiamethoxam, and clothianidin showed no increased degradation, indicating that hydroxyl radicals do not play a part in their photolysis. In acetamiprid experiments, with a hydroxyl radical concentration of $2.8 \pm (0.1) \times 10^{-15}$ M, hydroxyl radicals approximately doubled photolysis rates over 36 h in the solar simulator. A bimolecular rate constant of $1.7 (\pm 0.2) \times 10^{9} = M^{-1} s^{-1}$ was calculated for acetamiprid degradation by hydroxyl radicals.

Toxicity studies

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 degradation rate, even at pH 10.0. 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.

TABLE 4: Calculated average quantum yields for neonicotinoid insecticides in natural and simulated sunlight[a]

Light source		Nitenpyram	Imidacloprid	Thiamethoxam	Clothianidin
Solar simulator	Milli-Q	0.025 ± 0.001	0.0119 ± 0.0001	0.0167 ± 0.0002	0.0133 ± 0.0001
	MRW[b]	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[b]	0.024 ± 0.001	0.0100 ± 0.0005	0.0130 ± 0.0003	0.0080 ± 0.0001
Average		0.024 ± 0.001	0.0105 ± 0.0002	0.0140 ± 0.0002	0.0101 ± 0.0001
Literature	Lu et al. (2015)	—	0.0092 ± 0.0005	0.019 ± 0.001	0.013 ± 0.001
	Other Work	—	0.0055[c]	0.013[d]	0.0073[c]

[a] Errors are the 95% confidence interval.
[b] Mississippi River water samples were adjusted for screening by dividing by calculated screening factors, leading to an increase of 4 to 5%.
[c] von Gunten (2012).
[d] European Comission (2006).
MRW = Mississippi River water.

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Solutions with reaction products contained approximately 20% parent compound and approximately 80% products. Testing was performed so that the concentration of parent neonicotinoid added to mosquito tests was the same in all exposures. Thus, if products exhibited toxicity, the LC50 values of tests with product present would be smaller relative to values for the parent neonicotinoids, whereas if products did not exhibit toxicity, the LC50 values would remain unchanged or increase. Calculated LC50 values are given in Table 5. The results indicate that there is no residual toxicity associated with products from hydrolysis or photolysis reactions to mosquito larvae. Although other studies have also shown lower toxicity of the urea derivatives (Simon-Delso et al. 2015) to insects, structural modifications of neonicotinoids are known to lead to binding to other receptors (Lee Chao and Casida 1997; Tomizawa and Casida 1999; Tomizawa et al. 2000). Thus, other relevant endpoints and organisms would need to be tested to confirm that no undesired toxic effects remain.

Product identification

All structures of identified compounds and MS/MS data are available in the Supplemental Data; UHPLC-MS/MS studies were only run in positive mode. It is possible there are reaction products which could be detected in negative mode. In addition, products were not preconcentrated prior to analysis, so it is possible that additional compounds could have been detected if this procedure was performed. 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, as either an alcohol or a ketone, giving an exact mass of 257.0567. Exact mass and MS/MS data were used to identify products. Because there was not enough product generated to use nuclear magnetic resonance spectroscopy to determine which structural isomer of the nitenpyram degradation product (257) was produced, it is assumed that both structural isomers were generated. The nitenpyram product with exact mass 257.0567 has previously been identified in the literature (Noestheden et al. 2016), as has nitenpyram-urea. Photolysis samples also generated 2 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 the exterior nitrogen. The structure of the product with mass 211 was obtained by comparing MS/MS data with the available literature (Noestheden et al. 2016).

For imidacloprid hydrolysis and photolysis experiments, only imidacloprid-urea was observed, with exact mass 211.05124. Fragmentation patterns of imidacloprid-urea were collected from MS/MS results, yielding the same fragmentation pattern as previous work (Zheng and Liu 1999). Compound Discoverer matched MS2 fragmentation patterns to databases, resulting in positive identification of imidacloprid-urea. No variation was observed with hydrolysis products from metal ion or mineral experiments.

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 could be generated for toxicity studies or reaction product identification. The urea derivative of acetamiprid was the only product observed. Exact mass was used to initially identify the product, and MS2 results were compared for all baseline, metal, and mineral studies, yielding the same fragmentation pattern. The observed product matches the expected hydrolysis product (Si et al. 2016).

The urea derivative of thiamethoxam was the only hydrolysis or photolysis product identified through UHPLC-MS/MS. Identification was performed using exact mass. 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.

Clothianidin-urea was the only observed hydrolysis and photolysis product by UHPLC-MS/MS. Initial identification was performed using exact mass; additional identification was performed by comparing MS2 data to the literature. The MS2 fragmentation gave peaks at 132 and 113, matching literature MS2 fragmentation data (Žabar et al. 2012). Results did not vary

TABLE 5: Median lethal concentration values for tested neonicotinoid insecticides[a]

LC50 (μM)	Nitenpyram	Imidacloprid	Acetamiprid	Thiamethoxam	Clothianidin
Parent	0.3	0.15	0.4	0.6	0.15
Photolysis 1	0.3	0.15	—	0.7	0.15
Photolysis 2	0.4	0.15	—	0.7	0.15
MRW[b]	0.4	0.2	—	0.6	0.15
Baseline Hydrolysis	0.4	0.2	0.5	1.0	—
Ni^{2+}[c]	0.5	0.2	0.4	0.9	—
Cu^{2+}[c]	0.4	0.3	0.4	0.8	—
Zn^{2+}[c]	0.5	0.2	0.6	0.8	—
Kaolinite[d]	0.5	0.2	0.6	0.8	—
Goethite[d]	0.3	0.3	0.3	0.9	—
TiO_2[d]	0.3	0.3	0.4	0.8	—

[a] Reaction products were tested by exposing mosquitoes to a 20% parent, 80% product solution. LC50 values were normalized to parent concentrations and not total concentration of products + parent.
[b] The Mississippi River water samples were photolysis samples exposed to light in Mississippi River water.
[c] Metal samples contained 0.1 mM of metal ions.
[d] Minerals were filtered out of samples prior to testing.
LC50 = median lethal concentration; MRW = Mississippi River water.

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between baseline hydrolysis, metal, and mineral experiments, as with photolysis experiments.

Implications for environmental fate of neonicotinoids

Previous work had shown that neonicotinoid hydrolysis rates increased with increasing pH, indicating pH dependence; however, some results had indicated faster hydrolysis at acidic pH values (Zheng and Liu 1999; Liqing et al. 2006; Karmakar et al. 2009). Results of the present study indicate that neonicotinoids hydrolyze only under base-catalyzed conditions. Furthermore, these results indicate that in an environmentally relevant pH range (5–8.5) hydrolysis is unlikely to contribute meaningfully to degradation in the environment. This is backed by results from Mississippi River water experiments. At pH 8.3, in Mississippi River water, observed half-lives ranged widely, with significant error present. Expected environmental hydrolysis half-lives are 140 to 180 d for thiamethoxam, 150 to 320 d for nitenpyram, 800 to 1800 d for imidacloprid, 600 to 3500 d for acetamiprid, and 1200 to 5300 d for clothianidin. These half-lives will be longer at lower temperatures. This helps to explain the widespread detection of neonicotinoids in surface waters globally.

It is also of critical importance that the reaction order of OH^- is approximately 0.5 and not the 1.0 expected for an elementary reaction. Thus, second-order rate constants for base-catalyzed hydrolysis measured at a single pH value and assuming a reaction order of 1.0 will lead to incorrect values of half-life if extrapolated to other pH values. For example, the baseline imidacloprid pH 10 k_{obs} was 0.018 d^{-1}, which gave a second-order rate constant of 550 M^{-1} d^{-1}. Using this value to calculate a pseudo-first-order rate constant at pH 8 gives a value of 0.00055 d^{-1}, with a predicted half-life of 790 d. Using a reaction order of 0.5 and the same k_{obs} of 0.018 d^{-1}, however, gives a hydroxide rate constant of 2.6 $M^{-0.55}$ d^{-1} (see Table 2). The predicted pseudo-first-order rate constant at pH 8.0 is then 0.0013 d^{-1}, which gives a half-life of 530 d. Assuming a second-order elementary reaction will yield inaccurate estimates for extrapolated rate constants and half-lives.

As shown in the present study and in previous work (Lu et al. 2015), 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 the environment, integrated solar irradiances (L_λ) for 40°N at midsummer obtained from Leifer (1988), quantum yields calculated from natural sunlight Mississippi River water samples, and calculated molar absorptivity values were used to estimate photolysis rate constants (k_{dcE}) using Equation 7, where ϕ_{dc} is the calculated quantum yield, ε_λ is the molar absorptivity, and L_λ is the irradiance.

$$k_{dcE} = \phi_{dc} \Sigma_\lambda \epsilon_\lambda L_\lambda \quad (7)$$

Estimated near-surface environmental direct photolysis half-lives are 9 mins for nitenpyram, 45 min for imidacloprid, 90 min for clothianidin, and 120 min for thiamethoxam. These values are likely overestimates, given that midsummer clear days would give maximum rates. At 45°N, where the quantum yields were calculated, exposure on a midsummer day gave half-lives of 14 min for nitenpyram, 140 min for imidacloprid, 250 min for clothianidin, and 260 min for thiamethoxam.

The indirect photolysis half-life of acetamiprid is calculated by assuming a hydroxyl radical concentration of 1×10^{-16} M, assuming 7 h of sunlight per day and using the bimolecular rate constant calculated in the present study, leading to an estimated environmental half-life of 131 d. 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 break down in the top 8 cm of a water body (Lu et al. 2015). 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, the observed half-life would be 30 times the experimental half-life. At 45°N, environmental half-lives would increase to 2.9 d for imidacloprid, 5.5 d for thiamethoxam, and 5.2 d for clothianidin. In addition, experiments were conducted in filter-sterilized water. Although some lakes are pristine, 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 scattering and thus longer degradation half-lives, which helps to explain the widespread detection 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. It appears the urea derivative of each neonicotinoid is the major hydrolysis and photolysis reaction product, but the limitations in our detection method need to be taken into account. The formation of the same products also implies that a photo-hydration reaction occurs during photolysis.

Results from toxicity tests further confirm literature results, which have generally concluded that urea derivatives do not have residual toxicity to the nicotinic receptor channels but that some may target other receptors (Simon-Delso et al. 2015). *Culex pipiens* larvae have previously been studied when exposed to thiacloprid, with a 14-d LC50 at 0.02 μM and a 5-d LC50 at 0.04 μM observed (Larissa et al. 2017). Experiments with *Aedes* sp., which is in the same family (Culicidae) as *Culex pipiens*, found 48-h LC50 values of 0.23 μM for thiamethoxam, 0.16 μM for imidacloprid, 0.11 μM for clothianidin, and 0.71 μM for acetamiprid (Raby et al. 2018), which are similar to results found in the present study. Assessment of other potential toxicological endpoints may still be needed.

CONCLUSIONS

Neonicotinoids, although widely used, have come under more scrutiny because of their observed environmental persistence,

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near ubiquitous environmental presence, and impact on nontarget organisms (namely *Apis mellifera*). The present study has shown that neonicotinoids undergo base-catalyzed hydrolysis and that the reaction is nonelementary, with the hydroxide concentration raised to a power of 0.55 in the rate law. Furthermore, divalent metal cations and minerals were not observed to change hydrolysis rates. Direct photolysis was observed for nitenpyram, imidacloprid, thiamethoxam, and clothianidin, 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 because of reaction with OH· with a bimolecular rate constant of $1.7 \pm (0.2 \times 10^9)\ M^{-1}\ s^{-1}$. The urea derivative was the most commonly detected product, but in experiments using mosquitoes (*Culex pipiens*), no residual toxicity was observed. Results from experimental work indicate long environmental half-lives for the tested neonicotinoids, which may help to explain their observed persistence in environmental matrices.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI:10.1002/etc.4256

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Data Accessibility—Data are available in the Supplemental Data section, in the Data Repository for the University of Minnesota, (https://doi.org/10.13020/D6XQ2S) and on request to the authors (arnol032@umn.edu).

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Photolysis of Neonicotinoid Insecticide in systems simulating leaf surfaces: Rates and Toxicity Assessments

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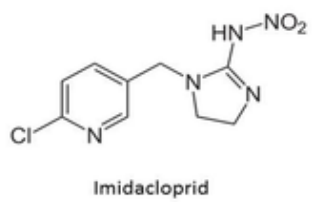
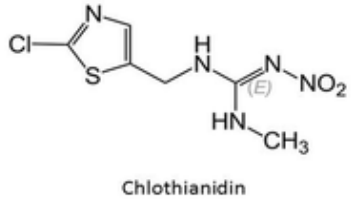
- Widely used

  --- introduced in 1990s

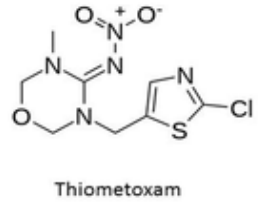
  --- represented 24% of the global market for insecticides in 2008



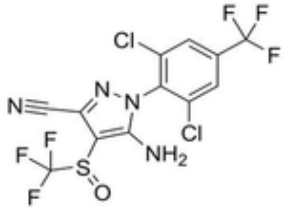
Imidacloprid



Chlothianidin



Thiometoxam



Fipronil

- Frequently detected

  --- in surface water and groundwater

  --- in drinking water

  --- in soil

- Stephen A. Todey, Ann M. Fallon, and William A. Arnold. *Environmental Toxicology and Chemistry*, **2018.**

Why Neonicotinoids?

- Break down slowly in the environment

  --- taken up by the plant

  --- long half-lives in water

  --- degrade slowly in the absence of sunlight and micro-organisms

- Affect the insect central nervous system

  --- nervous stimulation, death and paralysis

- Peter Jeschke, Ralf Nauen, Michael Schindler, and Alfred Elbert. *J. Agric. Food Chem.*, **2011**, *59* (7), pp 2897–2908.

Why Neonicotinoids?

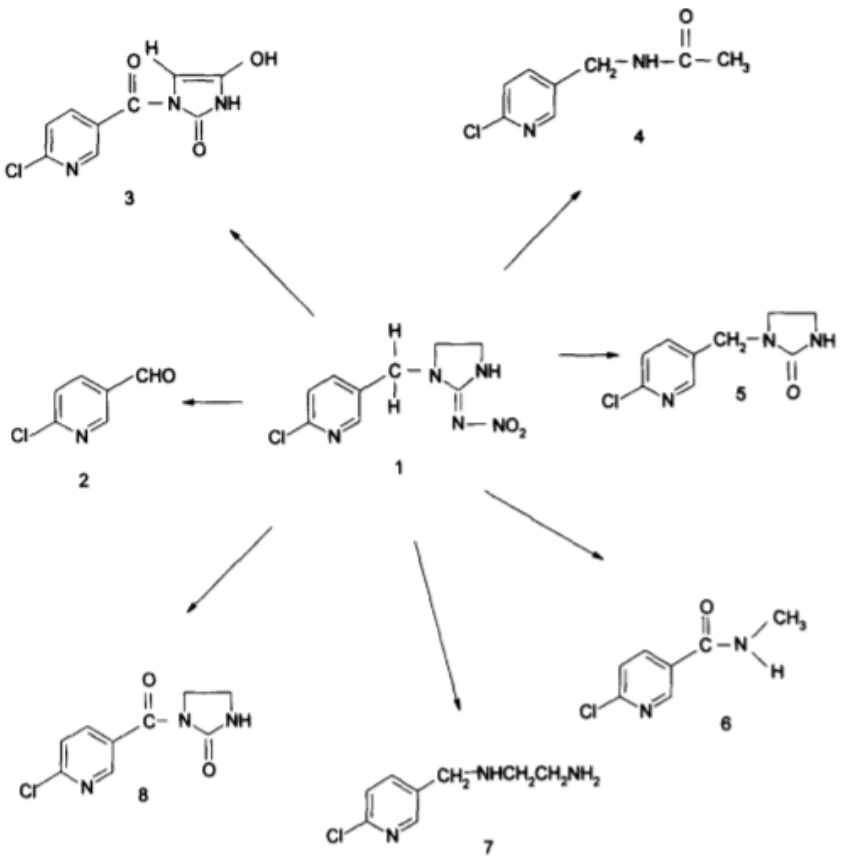
- Susceptible to photolysis

  --- half-lives of 5-36 hours in near surface waters

  --- restricted at depths greater that 8 cm

  --- can also occur on plant surfaces

The persistence of neonicotinoids in the environment and their potential toxic effects are not fully understood.

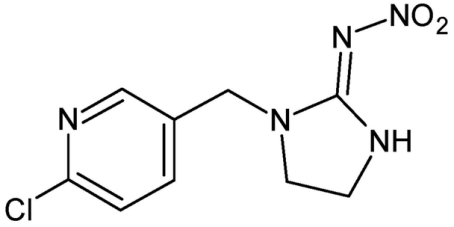


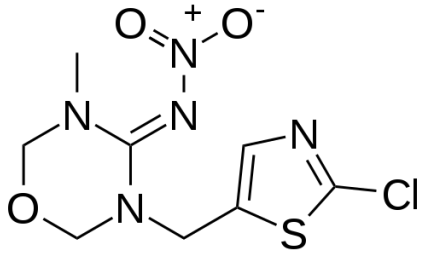
Photoproducts of imidacloprid in water

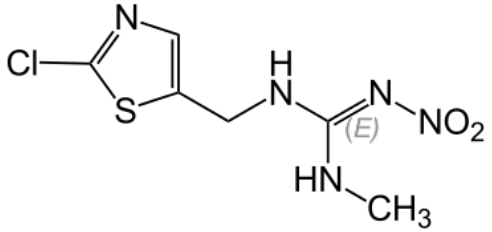
- Moza, P.N., Hustert, K .Feicht, E. Kettrup, A.. *Chemosphere*, **1998**, *36* (3), pp 497–502.

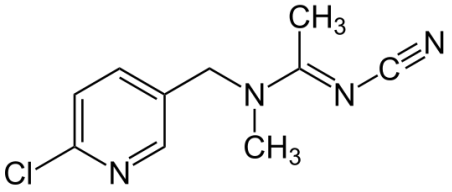
Objectives

- Identify reaction kinetics and products on various surface upon exposure to sunlight.
- Assess toxicity of neonicotinoids to soil and aquatic species before and after photolysis.
- Disseminate the findings to stakeholders, regulators, and the public.









Imidacloprid **thiamethoxam** **clothianidin** **acetamiprid**

- **commercial product containing other active ingredients:**

tebuconazole;	difenoconazole;	piperonyl butoxide;	N/A
tau-fluvalinate	lambda-cyhalothrin	metofluthrin	

- **pure compound prepared in DI water**

- Reaction kinetics

  --- real product & pure compound in H_2O

  --- various surfaces: wax, glass, alum foil, leaf

- Product identification

  --- Analysis by Orbitrap Velos LC-MSn

- Actinometry; Assessment of toxicity (in process)

Monitor the photodegradation on glass & Al foil surface

--- 1 mL of neonics deposited onto the surface

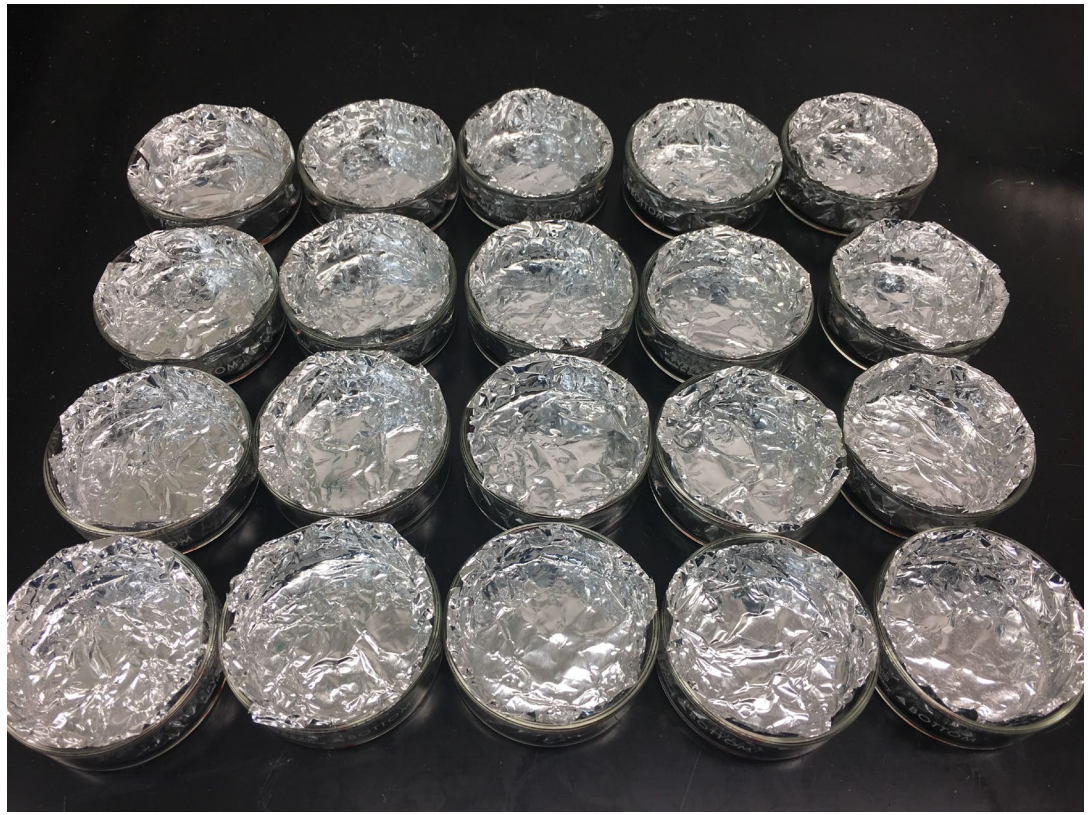
--- allow to evaporate

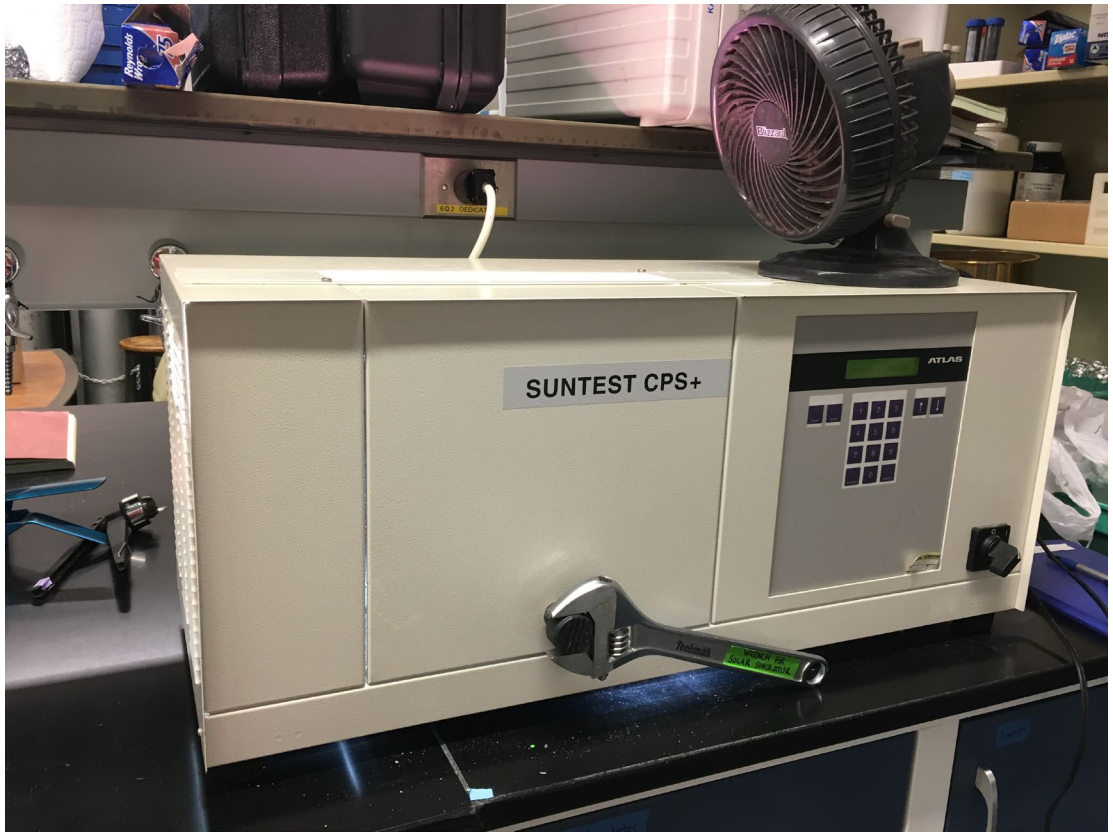
--- reactors exposed to artificial sunlight (765 w/m^2) (5 replicates)

--- extract back into 50% ACN, 3 mL x 3 times

--- 0.2 µm filter

--- HPLC



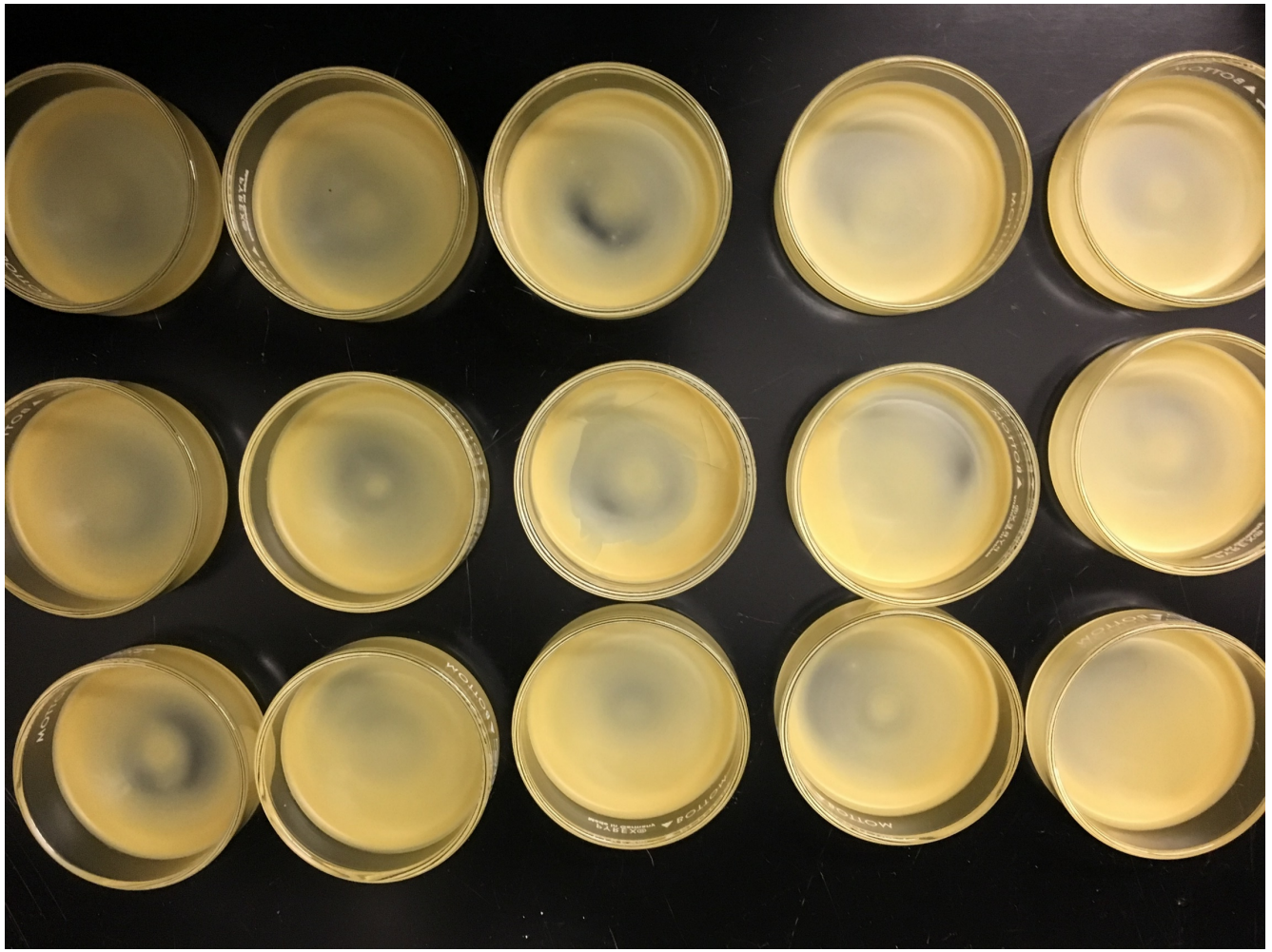


Atlas Suntest CPS+ solar simulator, using a xenon arc lamp with a 290 nm cutoff filter.

Monitor the photodegradation on wax surface

--- melt ~ 1 gm wax

--- 1 mL of neonics deposited onto wax surface



Monitor the photodegradation of imidacloprid on strawberry leaf in solar sim

--- soak 0.25 g of strawberry leaf into imidacloprid solution for ~10 s

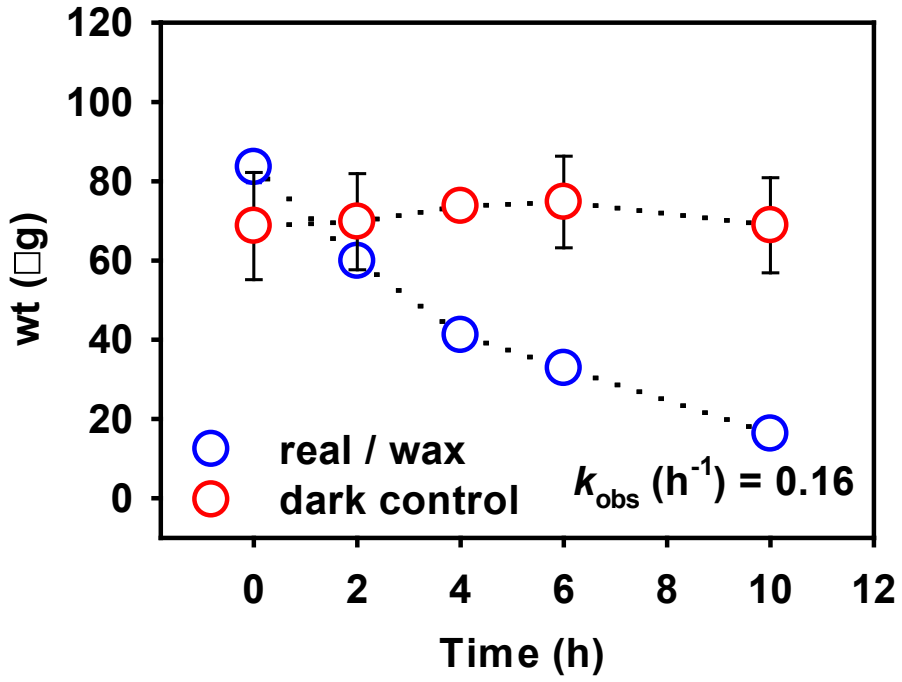
--- allow to dry in hood for 30 min

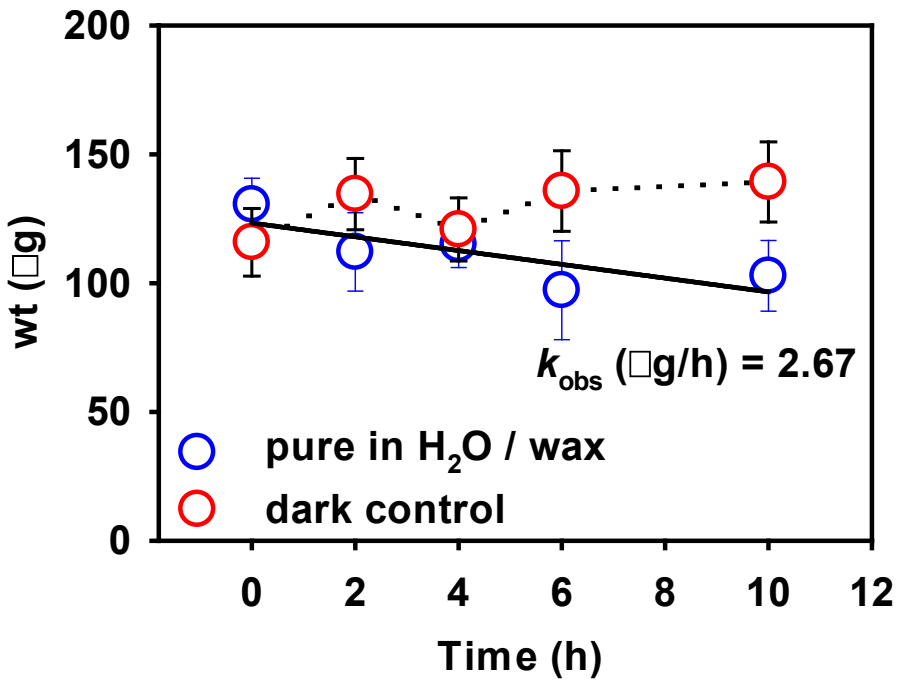
--- 4 replicates

--- extract back into 50% ACN, 2 mL x 3 times

- imidacloprid degradation on wax

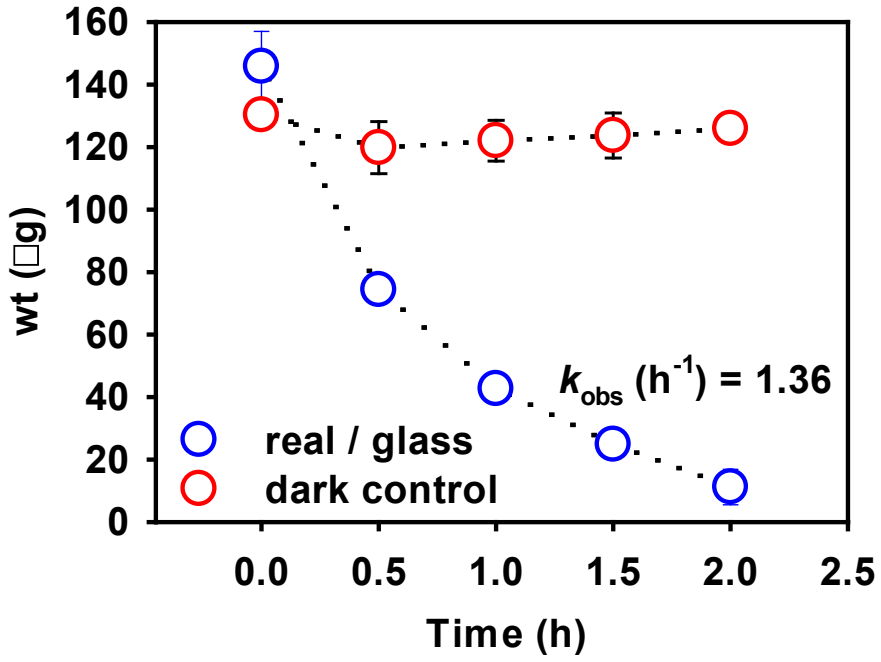
  --- initial concentration: 550 µM

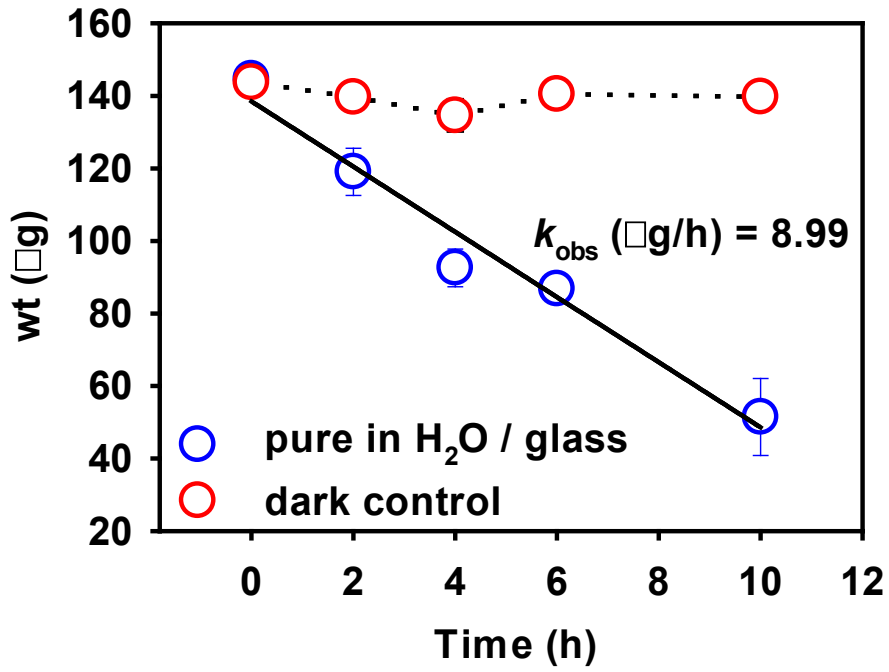




- imidacloprid degradation on glass

  --- initial concentration: 550 µM





Results

Summary of kinetics

- Photolysis rates on glass and aluminum foil were much faster than those on paraffin wax and leaves.
- For imidacloprid, degradation of real product followed first order kinetics, while pure compound followed zero order kinetics.
- For thiamethoxam, degradation of real product and pure compound both followed first order kinetics.
- For clothianidin, degradation of real product followed zero order kinetics, while pure compound was observed to be relatively stable.
- No disappearance observed for acetamiprid.

Conclusions: Kinetics

- Photodegradation of commercial products were much more reactive than pure compounds.
- Various neonics on different surfaces follow different photodegradation rate laws and mechanisms.
- Paraffin wax best simulates the reaction environment on leaves.

- Reaction kinetics

  --- real product & pure compound in H_2O

  --- various surfaces: wax, glass, alum foil, leaf

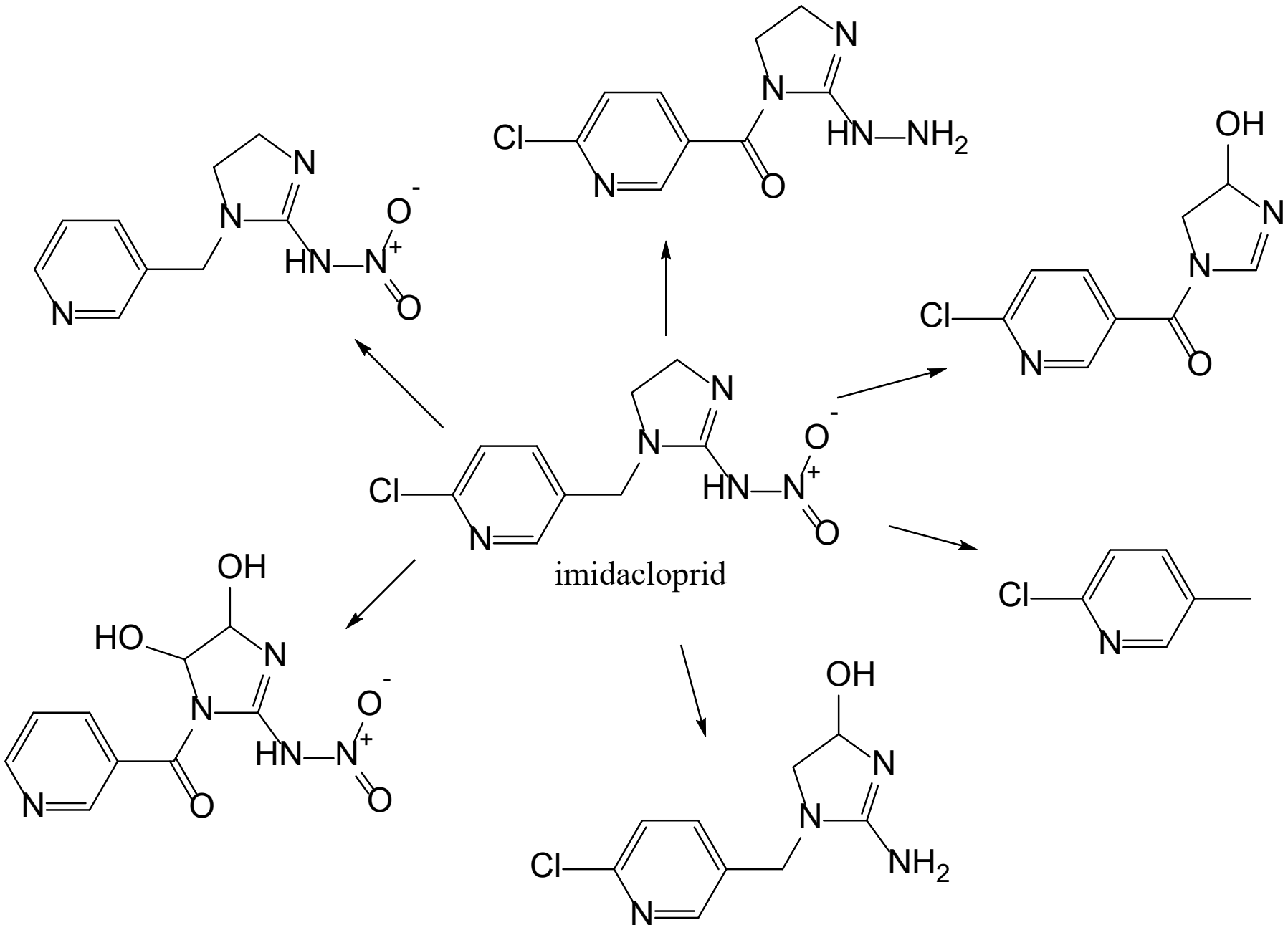
- Product identification

  --- Analysis by Orbitrap Velos LC-MSn

- Actinometry; Assessment of toxicity (in process)

- Liquid chromatography coupled to a high resolution and accurate mass – tandem mass spectrometer (LC/HRAM-MS/MS; Thermo Fisher Scientific LTQ Orbitrap Velos)
- Positive & negative mode
- Compound Discoverer 3.0 (Thermo Fisher Scientific)
- work-flows: targeted and untargeted
- Products identification in various approaches.

Summary of proposed transformation products for imidacloprid



“Conclusions”: Products

- Products were observed to vary on different surfaces.
- Products for commercial and pure compounds were different on each surface.
- Nitro Reduction and dichlorination were the major reaction processes.

- Reaction kinetics

--- real product & pure compound in H_2O

--- various surfaces: wax, glass, alum foil, leaf

- Product identification

--- Analysis by Orbitrap Velos LC-MSn

- Actinometry; Assessment of toxicity (in process)

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