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**