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

Date of Report: May 29, 2016

Date of Next Status Update Report: January 1, 2017

Date of Work Plan Approval: June 7, 2016

Project Completion Date: June 30, 2019

Does this submission include an amendment request? No

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

Project Manager: William Arnold

Organization: University of Minnesota

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Location: Statewide

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

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:

Project Status as of July 1, 2017:

Project Status as of January 1, 2018:

Project Status as of July 1, 2018:

Project Status as of January 1, 2019:

Overall Project Outcomes and Results:

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: $ 0
Balance: $ 117,525

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:

Activity Status as of July 1, 2017:

Activity Status as of January 1, 2018:

Activity Status as of July 1, 2018:

Activity Status as of January 1, 2019:

Final Report Summary:

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: $ 115,025**
Amount Spent: $ 0
Balance: $ 115,025

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:

Activity Status as of July 1, 2017:

Activity Status as of January 1, 2018:

Activity Status as of July 1, 2018:

Activity Status as of January 1, 2019:

Final Report Summary:

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: $ 167,450**
Amount Spent: $ 0
Balance: $ 167,450

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:

Activity Status as of July 1, 2017:

Activity Status as of January 1, 2018:

Activity Status as of July 1, 2018:

Activity Status as of January 1, 2019:

Final Report Summary:

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:

Activity Status as of July 1, 2017:

Activity Status as of January 1, 2018:

Activity Status as of July 1, 2018:

Activity Status as of January 1, 2019:

Final Report Summary:

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. 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	$ 0	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:	**$** 157,400	**$ 0**	

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.

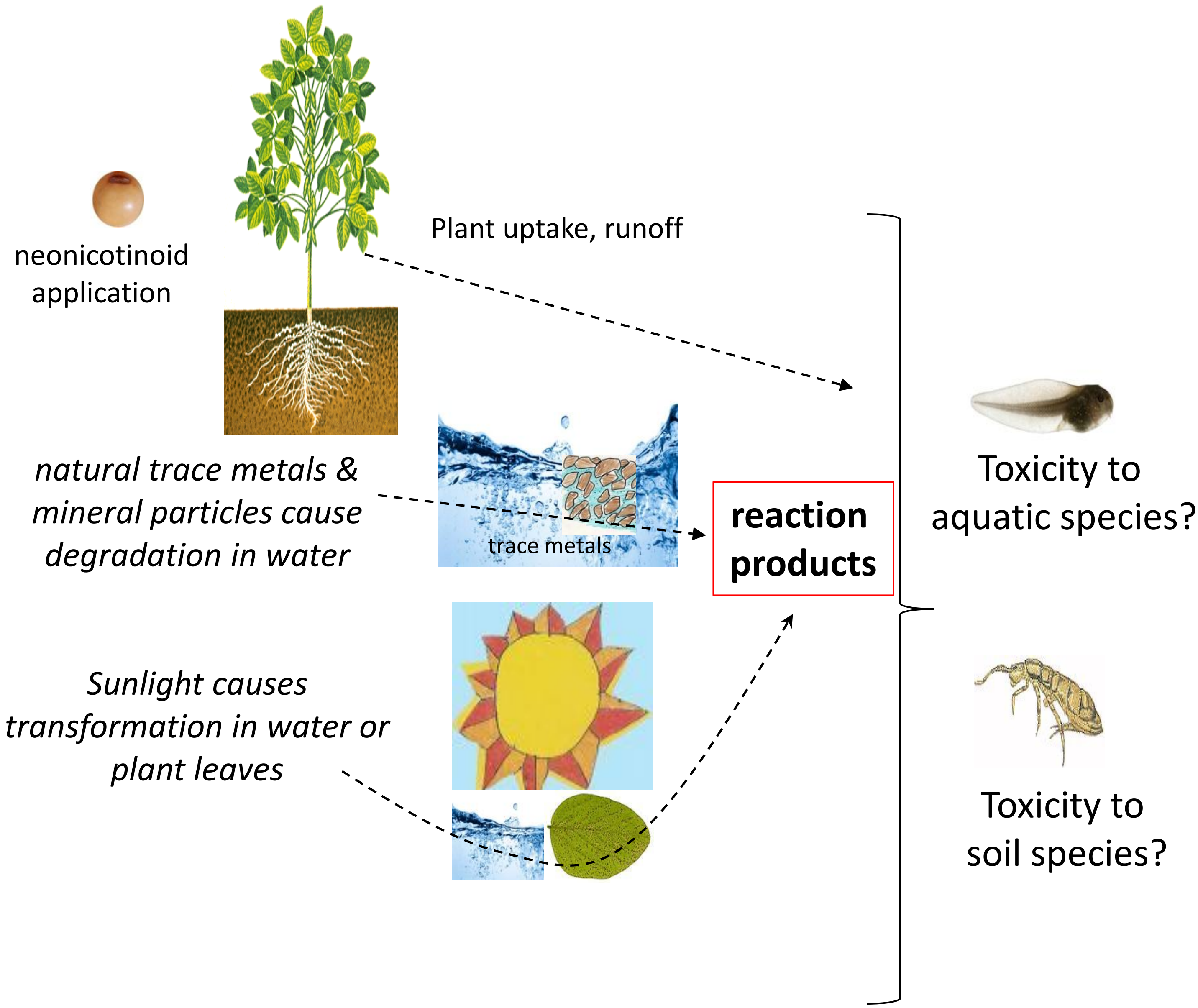
Environment and Natural Resources Trust Fund
M.L. 2016 Project Budget

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: May 29, 2016

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 trace**			**Solar effects on neonicotinoids in water and**			**Toxicity of transformation products to soil**				
Personnel (Wages and Benefits)	$100,525	$0	$100,525	$100,525	$0	$100,525	$156,950	$0	$156,950	$358,000	$358,000
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, $28,450 (74.8% salary, 25.2 % fringe benefits, 4% 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 $114,500 (25% time academic year in Y1, 50% time during academic year, 50% time in summer in Y2 and Y3; 56% salary, 33% tuition, 11% fringe benefits). Rearing organisms for toxicity studies, toxicity studies, data analysis and interpretation.											
Undergraduate students $42,000 (100% time. In Y1 and Y2, two students for 40 hr/wk in the summer (10 weeks) and 10 hours per week for one semester (15 weeks). In Y3, summer only. 100% salary). 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	$0	$8,000	$6,000	$0	$6,000	$3,000	$0	$3,000	$17,000	$17,000
Analytical time for product identification $6,000	$3,000	$0	$3,000	$3,000	$0	$3,000				$6,000	$6,000

Operating costs for laboratory instruments required for analyses and experiments; costs portioned based on usage by project $9,000	$3,000	$0	$3,000	$3,000	$0	$3,000	$3,000	$0	$3,000	$9,000	$9,000
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	$0	$1,500	$1,000	$0	$1,000	$1,500	$0	$1,500	$4,000	$4,000
Other											
Publication charges to make published journal articles (four) immediately available via open access to maximize data availability and dissemination $6000	$1,500	$0	$1,500	$1,500	$0	$1,500	$3,000	$0	$3,000	$6,000	$6,000
COLUMN TOTAL	**$117,525**	**$0**	**$117,525**	**$115,025**	**$0**	**$115,025**	**$167,450**	**$0**	**$167,450**	**$400,000**	**$400,000**

Neonicotinoid Insecticides and Their Breakdown Products May Be Toxic to Non-Target Species



Outcomes

1. Understand neonicotinoid processing
2. Identify water/plant reaction products
3. Recognize toxic effects
4. Inform use, management, regulation