2019 Project Abstract
For the Period Ending June 30, 2023

PROJECT TITLE: Neonicotinoid Insecticides: Occurrence And Influence on Algal Blooms
PROJECT MANAGER: William Arnold
AFFILIATION: University of Minnesota Dept of Civil, Environmental, and Geo-Engineering
MAILING ADDRESS: 500 Pillsbury Dr SE
CITY/STATE/ZIP: Minneapolis, MN 55403
PHONE: 612-625-8582
E-MAIL: arnol032@umn.edu
WEBSITE: www.williamarnold.org
FUNDING SOURCE: Environment and Natural Resources Trust Fund
LEGAL CITATION: M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04a as extended by M.L. 2022, Chp. 94, Sec. 2, Subd. 19 (c.1) [to June 30, 2023]
APPROPRIATION AMOUNT: $350,000
AMOUNT SPENT: $350,000
AMOUNT REMAINING: $0.00

Sound bite of Project Outcomes and Results
Neonicotinoid and fipronil insecticides are present in lakes, rivers, springs, and shallow groundwater across Minnesota often at concentrations exceeding chronic toxicity thresholds for aquatic invertebrates. The compounds were detected in wastewater, stormwater, and rain/snow, indicating multiple sources to Minnesota waters. No clear association with algal blooms was found.

Overall Project Outcome and Results
Neonicotinoid (acetamiprid, clothianidin, imidacloprid, thiacloprid, thiamethoxam) and fipronil insecticides are used extensively the protection of crops and animals against fleas, ticks, other destructive pests. Recent research suggests that neonicotinoids and fipronil can have lethal effects on key pollinator species including bees, butterflies, moths, and beetles. Water samples were collected from lakes , rivers, natural springs, and wastewater treatment plants from around the state over a three-year study period to determine the extent of contamination in Minnesotan groundwater and surface water systems.

Imidacloprid, thiamethoxam, and clothianidin were all extensively present in Minnesota in lakes, rivers, and in springs/shallow groundwater at concentration that often exceed U.S. EPA chronic exposure thresholds for aquatic vertebrates. Strong seasonal trends were observed in rivers in lakes with the highest concentrations and detection frequencies in the summer and fall. Sales and application rates in the previous growing season were the strongest indicator of neonicotinoid and fipronil occurrence and measured concentration, followed by watershed drainage area, imperviousness, and land use. Clothianidin and thiamethoxam were largely associated with agricultural use while fipronil, acetamiprid, and thiacloprid were associated with urbanized regions. Imidacloprid had a high prevalence in both urban and agricultural regions. Neonicotinoids appear confined to the shallowest aquifers and natural springs but there were some detections in deeper groundwater. Groundwater recharge zones and stormwater may be a substantial source of contamination in unconfined aquifers and surface waters. All of the compounds were also detected in wastewater effluents and stormwater. There were also detected in rain and snow, indicating the possibility of long range transport. Despite their widespread presence in lakes, no clear associations with specific algae grazers or formation of algal blooms was found.

Project Results Use and Dissemination
To date one journal article has been published with an associated data set, and several talks at conferences and public seminars have been presented. Additional publications will be submitted soon. This work has been included in the doctoral dissertations of two students: Matthew Berens and Grant Goedjen. The latter

dissertation and associated data will be available in the UMN digital conservancy and UMN data repository in early Fall 2023.

Environment and Natural Resources Trust Fund (ENRTF) M.L. 2019 ENRTF Work Plan (Main Document)

Today's Date: August 11, 2023

Final Report

Date of Work Plan Approval: June 5, 2019

Project Completion Date: June 30, 2023

Does this submission include an amendment request? Yes

PROJECT TITLE: Neonicotinoid Insecticides: Occurrence And Influence on Algal Blooms

Project Manager: William Arnold

Organization: University of Minnesota

College/Department/Division: Civil, Environmental, and Geo- Engineering

Mailing Address: 500 Pillsbury Dr. SE

City/State/Zip Code: Minneapolis, MN 55455

Telephone Number: 612-625-8582

Email Address: arnol032@umn.edu

Web Address: www.williamarnold.org

Location: Statewide

Total Project Budget: $350,000

Amount Spent: $350,000

Balance: $0

Legal Citation: M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04a as extended by M.L. 2022, Chp. 94, Sec. 2, Subd. 19 (c.1) [to June 30, 2023]

Appropriation Language: $350,000 the first year is from the trust fund to the Board of Regents of the University of Minnesota to quantify the occurrence of neonicotinoid insecticides in Minnesota's surface waters and groundwaters and assess if the insecticides are contributing to the formation of algal blooms.

M.L. 2022 - Sec. 2. ENVIRONMENT AND NATURAL RESOURCES TRUST FUND; EXTENSIONS. [to June 30, 2023]

I. PROJECT STATEMENT:

New studies that document the baseline occurrence of neonicotinoid insecticides *in natural and engineered waters (such as drinking water, wastewater, and storm water) are urgently needed to determine any potential effects in Minnesota waters and to develop guidelines for safe use of neonicotinoids*. Neonicotinoid insecticides, are synthetic chemicals that are widely used in urban and agricultural areas. They are highly toxic to many aquatic and terrestrial organisms. They were introduced in the 1990s and now represent a large portion of insecticides not only used in row-crop agriculture, but also in nurseries, in lawns and gardens, and for flea treatment of pets. In the few studies that have been conducted, neonicotinoid compounds have been detected in urban and agricultural streams, groundwater, and even wastewater treatment plant effluent. They have also been detected in soil and rain. Our previous ENTRF research has shown that neonicotinoid insecticides are relatively persistent and likely highly mobile in the environment. These characteristics would suggest they could move away from their point of application and into the broader aquatic environment. The neonicotinoid insecticides have been shown to produce a number of breakdown products, but little is known about the environmental behavior or environmental occurrence of these chemicals. This study would provide a baseline survey of where and at what level these chemicals occur in Minnesota and provide insight into the pathways by which they reach surface and ground waters.

The goals of the project are to:

- Quantify the occurrence of neonicotinoids and their breakdown products in Minnesota's natural and engineered waters,
- Assess any relationship between neonicotinoid levels and formation of algal blooms, and
- Disseminate the findings to stakeholders, regulators, and the public.

Data on neonicotinoid use and environmental detections in Minnesota's waters are limited. Six neonicotinoid insecticides are registered for use in Minnesota for non-agricultural (i.e., urban) and agricultural uses. The Minnesota Department of Agriculture has found four of the six in streams and three of the six in groundwater samples. *The results of this work will have direct impacts on management of neonicotinoid use and the environmental health of Minnesota's urban and out-state surface waters and ground waters.* This work will provide information to the public on the occurrence of these high profile chemicals and aid in management and regulatory decisions related to these compounds.

II. OVERALL PROJECT STATUS UPDATES:

First Update March 1, 2020

Samples have been collected and analyzed for neonicotinoids and breakdown products in natural and engineered waters. Sample sites include tap and finished drinking water (n = 25), lakes (n = 53), rivers and streams (n = 61), and treated wastewater effluents (n = 8). Samples were collected across a wide distribution of land use profiles representing agricultural (n = 45), developed (n = 99), and undeveloped (n = 16) land use regimes. The median concentration for total neonicotinoids and breakdown products ranged from 2.6 ng/L (lakes) to 23.6 ng/L (wastewater effluent). Initial results indicate different usage patterns in urban versus agricultural areas. Collaboration with the MN DNR County Geologic Atlas (CGA) and MN DNR Minnesota Spring Inventory (MSI) allowed for the collection of 38 water samples from private and municipal drinking water wells and 4 samples from shallow springs in Southeast MN. The median total neonicotinoid concentrations were 1.4 ng/L (wells) and 33 ng/L (springs). Analysis of any relationship between plankton levels and neonicotinoids is selected lakes is ongoing.

Second Update September 1, 2020

We have continued to monitor the 2019 sites with some modifications of the stream set to increase frequency but reduce the number of sites. To date, sampling locations for 2020 include lakes (n = 38), rivers and streams (n = 35), groundwater wells (n = 6), treated wastewater effluents (n = 19), passive air samplers (n = 2), springs (n = 1), and samples for quality assurance and control (n = 16). Of the samples that have been analyzed by LC-MS/MS (n = 51), median total neonicotinoid concentrations ranged from 1.3 ng/L (lakes) to 14.4 ng/L (wastewater effluent). COVID-19 has slowed groundwater sampling, with only one county sampled. Two more may be possible. No effects on algae were seen in 2019 for the lakes monitored. A manuscript has been submitted to the 2019 data.

Third Update March 1, 2021

We have finalized extracting and analyzing the samples for the 2020 sampling period. In 2020, two-hundred and four (204) water samples were analyzed; Sampling locations included lakes (n = 62), rivers and streams (n = 65), groundwater wells (n = 23), natural springs (n = 1), wastewater treatment plant effluent (n = 19), passive composite air samples (n = 2), and quality control replicates and recovery samples (n = 32). Median total neonicotinoid concentrations have ranged from 0.055 ng/L to 148.8 ng/L. COVID-19 has slowed groundwater and field sampling but is expected to accelerate again as COVID restrictions relax. The 2021 sampling period has begun and will focus on a subset of sampling locations with increased sampling frequency to better establish the relationship between neonicotinoid compounds presence and algae and plankton abundance and understand temporal trends of neonicotinoid loading in natural and engineered waters. Current sampling locations include lakes (n = 7), wastewater treatment plant paired influent and effluent (n = 4 plants), stormwater discharge points (n = 5), and rivers and streams (n = 3). Samples from the select locations will be sampled weekly to monthly and analyzed for neonicotinoids, neonicotinoid degradation products, fiproles, general water quality indicators, and plankton and algae abundance where applicable. The submitted article *Neonicotinoid Insecticides in Surface Water, Groundwater, and Wastewater across Land Use Gradients and Potential Effects* has been accepted by and published in the journal Environmental Chemistry and Toxicology.

Fourth Update September 1, 2021

As of July 2021, we have collected one-hundred and forty-four (n = 144) samples from eleven (11) lakes, fourteen (14) river locations, three (3) snow melt and stormwater runoff locations entering one (1) lake, nine (9) springs fed by shallow groundwater, and one (1) groundwater well. Neonicotinoid, fipronil, and their degradants have been detected in all of the classes of sites, including springs, wells, rivers, lakes, stormwater runoff, and snowmelt runoff. Median total neonicotinoids concentrations range from 0.01 ng/L in snowmelt samples and 32.2 ng/L in springs, with a maximum total neonicotinoid concentration of 82.6 ng/L in springs. The range of median total fiprole compounds range from 0.08 ng/L in snowmelt samples and 0.81 ng/L in springs, with a maximum total neonicotinoid concentration of 12.0 ng/L in springs. COVID-19 restrictions have slowed groundwater and field sampling, but sampling is expected to accelerate again as COVID restrictions relax. The remainder of 2021 will include the expansion of sampling sites to Steele County, MN groundwater wells and Olmsted County, MN based on the MN DNR sampling plan. Additional sampling sites will include Twin Cities and University of Minnesota Organics composting facilities, four (4) wastewater treatment plants, and one (1) water treatment plant. Expanded sampling will neonicotinoids, fipronil, and it degradants in composting leachate, as well as samples obtained from various points in water and wastewater treatment trains to determine fiprole and neonicotinoid removal capacity. Results from this work were presented virtually at two American Chemical Society National meetings.

Amendment Request: Because of disruptions caused by the COVID-19 pandemic, we are requesting a one year extension of the project. The restrictions on laboratory work and sampling has limited the groundwater sampling and (Activity 2) and collection of algal data both in the field and the laboratory (Activity 3). An extension will allow this work to be completed, and will also allow additional data collection for Activity 1. **Amendment pending further LCCMR and legislative action as of 10/6/21 (Approved, see below)**

Because of reduced laboratory capacity during the pandemic, we have not been able to hire as many undergraduates as planned. Thus, we are requesting to move $10,000 from undergraduate salary to laboratory supplies ($5,000) and a new category, maintenance ($5,000). We also request moving the remaining $4,420 in analytical time to maintenance. This is similar to the general operating funds category, but we want to be clear about the use of funds. These maintenance funds ($9,920) will still be used for costs related to analyzing samples, but we are able to now do this on an instrument we purchased that resides in our laboratory. Rather than pay service fees for use of an instrument in a central facility, we now need these funds to operate our own instrument. This does not change how the funds are used, but changes how they are categorized in the UMN accounting system. The maintenance costs are allocated based on instrument usage. **Amendment Approved by LCCMR 10/6/2021**.

Fifth Update March 1, 2022

We have collected two-hundred and forty-one (n = 241) samples from thirteen (13) lakes, five (5) rivers from seventeen (17) locations, three (3) snow melt and stormwater runoff collection points entering Lake Como, ten (10) natural springs fed by shallow groundwater, and twenty-three (23) groundwater wells during the 2021 sampling season. Neonicotinoids, fipronil, and their degradants were detected in all site classifications, including natural springs, groundwater wells, rivers, lakes, stormwater runoff, and snowmelt runoff, and in drinking water and wastewater plant influent. The median total fipronil was 2.23 ng/L in lakes, 5.02 ng/L in rivers, 36.30 ng/L in snow melt runoff, and 2.90 ng/L in rainfall runoff. The median total neonicotinoids concentration was 9.80 ng/L in lakes, 20.4 ng/L in rivers and streams, 9.11 ng/L in snow melt runoff, and 78.1 ng/L in rainfall runoff. Natural surface springs had significantly higher concentrations of total neonicotinoids and moderately higher concentrations of total fipronil. The median total neonicotinoids concentrations was 33.5 ng/L in surface springs and 0.45 ng/L in deep groundwater, while the median total fipronil concentration was 2.1 ng/L in surface springs and 0.75 ng/L in deep groundwater. Neonicotinoid removal in drinking water was also tested as a serendipitous portion of a pilot plant study. We also continued evaluation of the relationship between zooplankton abundance and neonicotinoid concentrations. Zooplankton samples of Lake Como were analyzed for the relationship between zooplankton and external stressors (neonicotinoid concentrations, temperature, total nitrogen, total phosphorous, etc.) using historical data collected by Capitol Region Watershed District (CRWD). Local lakes and springs will continue to be sampled regularly and an additional set of urban surface springs will be sampled to compare pesticide concentrations in springs in rural, agricultural, and urban environments. Collaboration with the MN DNR County Geologic Atlas and Minnesota Spring Inventory will continue once sampling has commenced through the spring and summer of 2022. We will also continue to evaluate any effects of neonicotinoids on zooplankton and phytoplankton communities.

Update as of June 30, 2022:

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022:

Since the start of the project, we have collected a total of 752 samples from around the midwestern territories of Minnesota and Wisconsin. Sampling locations include: 27 influent, 24 effluent, and 12 biosolids samples from 4 wastewater treatment plants, 295 samples from 28 lakes, 150 samples from 29 rivers and streams, 12 snowfall and 15 rainfall samples from one collection location, 75 stormwater samples and 13 snowmelt samples from 3 discharge points, 33 samples from 13 natural springs, and 97 groundwater samples from 87 groundwater wells. All samples have been examined for 5 primary neonicotinoids, 5 degradation products, fipronil, and three degradation products. We have expanded our suite of transformation by one compound, 6-chloronicotonic acid, terminal degradation product in the degradation of several neonicotinoids. The median total concentration of neonicotinoids in wastewater treatment plants was estimated to be 151 ng/L in influent, 164 ng/L in effluent, and 113 ng/L in biosolids. The median concentration in surface and groundwater systems was estimated to be 13.6 ng/L in lakes, 19.3 ng/L in rivers, 4.5 ng/L in natural springs, and 0.10 ng/L in groundwater. Direct capture snowfall has median total neonicotinoid concentrations of 73.1 ng/L with no detectable concentration in snow melt while rainfall had a median total concentration of 2.69 ng/L with 20.6 ng/L in the formed runoff. The median total concentration of total fiproles in wastewater treatment plants was estimated to be below the detectable limit for both influent and effluent with and 46.3 ng/L in biosolids. The median concentration in surface and groundwater systems was estimated to be 1.83 ng/L in lakes, 1.69 ng/L in rivers, 0.67 ng/L in natural springs, and 0.42 ng/L in groundwater. Direct capture snowfall has median total neonicotinoid concentrations of 1.40 ng/L with no detectable concentration in snow melt while rainfall had a median total concentration of 0.42 ng/L with 2.06 ng/L in the formed runoff. Sampling has concluded for the groundwater and spring portion of the grant with no further sampling anticipated. Sampling for the surface waters in nearing its end with samples collection continuing through the fall four urban lakes. Sampling of stormwater will continue through the fall along with direct capture of rainfall. Sampling of snowmelt and direct capture of snowfall continue in the winter of 2022 through the spring of 2023. Sampling of four wastewater treatment plants is ongoing to conduct a mass balance of the wastewater treatment plants with varying treatment technologies.

Seventh Update as of March 1, 2023:

Project sampling has largely been completed for the project with only 2023 spring snow melt samples remaining. Surface water, groundwater, wastewater, drinking water, and storm water samples sampling have otherwise been completed and three papers are currently in progress including (1) evaluation of neonicotinoids and fipronil in Minnesota surface and groundwater, (2) relations among watershed-level social and physical factors on the detection rate and observed concentration ranges of neonicotinoid and fipronil in surface hydrologic systems, and (3) the contributions of neonicotinoids and fiproles in stormwater runoff to stormwater-impacted urban lakes. We have identified and rectified a flaw in the fipronil analytical method causing false positives of fipronil degradants in the collected samples.

There is widespread low-grade contamination of neonicotinoids in Minnesota surface waters in agriculturally-intense areas. The observed median concentration of the individual neonicotinoids are orders of magnitude lower than the chronic and acute toxicity thresholds for humans and aquatic life. There are elevated levels of pesticide concentrations and higher frequencies of detections in lakes, rivers, and streams during the growing season and during periods of high precipitation. The periods of elevated concentrations are only for limited periods of time and can exceed the chronic toxicity threshold periodically for short periods of time, typically on the orders of days before returning to lower levels. Contamination in groundwater appears to be limited largely to shallow, unprotected aquifers and surface springs. Both neonicotinoids and fipronil appear to be isolated to surface-level contamination only with concentrations well below the chronic and acute toxicity thresholds and No contamination of sampled aquifers used for municipal drinking water have been observed.

Final Report as of June 30, 2023 (to be submitted before August 15, 2023):

Overall Project Outcomes and Results:

Neonicotinoid (acetamiprid, clothianidin, imidacloprid, thiacloprid, thiamethoxam) and fipronil insecticides are used extensively the protection of crops and animals against fleas, ticks, other destructive pests. Recent research suggests that neonicotinoids and fipronil can have lethal effects on key pollinator species including bees, butterflies, moths, and beetles. Water samples were collected from lakes , rivers, natural springs, and wastewater treatment plants from around the state over a three-year study period to determine the extent of contamination in Minnesotan groundwater and surface water systems.

Imidacloprid, thiamethoxam, and clothianidin were all extensively present in Minnesota in lakes, rivers, and in springs/shallow groundwater at concentration that often exceed U.S. EPA chronic exposure thresholds for aquatic vertebrates. Strong seasonal trends were observed in rivers in lakes with the highest concentrations and detection frequencies in the summer and fall. Sales and application rates in the previous growing season were the strongest indicator of neonicotinoid and fipronil occurrence and measured concentration, followed by watershed drainage area, imperviousness, and land use. Clothianidin and thiamethoxam were largely associated with agricultural use while fipronil, acetamiprid, and thiacloprid were associated with urbanized regions. Imidacloprid had a high prevalence in both urban and agricultural regions. Neonicotinoids appear confined to the shallowest aquifers and natural springs but there were some detections in deeper groundwater. Groundwater recharge zones and stormwater may be a substantial source of contamination in unconfined aquifers and surface waters. All of the compounds were also detected in wastewater effluents and stormwater. There were also detected in rain and snow, indicating the possibility of long range transport. Despite their widespread presence in lakes, no clear associations with specific algae grazers or formation of algal blooms was found.

Amendment Request: We are requesting minor budget modifications so that the final budget matches final expenditures. Our laboratory supplies and personnel each were overspent, and this allowed us to complete our project goals. The full amounts of maintenance/operating costs, travel, and publication were not used. Thus, we request that a total of $8,019 be moved from underspent lines to cover personnel and $4,148 be moved to cover laboratory supplies. The revised budget sheet shows these changes and a zero balance.
Amendment approved October 22, 2024

III. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1 Title: ***Frequencies and concentrations of neonicotinoids and breakdown product occurrence in natural and engineered waters***

Description: Streams (50 samples), storm water (20 samples), drinking water (10 samples), and treated wastewater effluents (20 samples) in urban and agricultural areas throughout Minnesota will be sampled to provide a baseline survey of the frequency of occurrence and concentrations of the neonicotinoid insecticides and reaction products. Locations of the sampling sites will inform and complement the work of the MN Departments of Agriculture and Public Health as well as water and wastewater treatment facilities. Water samples will be analyzed for six neonicotinoid insecticides, fipronil (a related veterinarian insecticide), and selected breakdown products.

ACTIVITY 1 ENRTF BUDGET: $128,000

Outcome	Completion Date
1. *Analytical method developed for low-level analysis neonicotinoids and degradates*	06/30/20
2. Water samples collected and analyzed for low-level neonicotinoid and degradates	09/01/22
3. Dissemination of Activity 1 findings via open access journal publication(s)	06/01/23

First Update March 1, 2020

One hundred and forty-seven (147) samples have thus far been collected and analyzed for neonicotinoids and breakdown products in natural and engineered waters. Sample sites include tap and finished drinking water (n = 25), lakes (n = 53), rivers and streams (n = 61), and treated wastewater effluents (n = 8). Samples were collected across a wide distribution of land use profiles representing agricultural (n = 45), developed (n = 99), and undeveloped (n = 16) land use regimes. Neonicotinoids and breakdown products were detected in almost all samples, however, the frequency of concentrations detected above the analytical limit of quantitation ranged from 15% (lakes) to 34% (wastewater effluent). The median concentration for total neonicotinoids and breakdown products ranged from 2.6 ng/L (lakes) to 23.6 ng/L (wastewater effluent). Additional samples have been collected from primary and secondary and final wastewater effluent (n = 19) and are pending analysis. At the time of this report, issues with the analytical method for fipronil quantitation have inhibited their analyses and future work will seek to resolve these issues such that concentrations of fipronil and its breakdown products can be measured in each of the above-mentioned samples.

Second Update September 1, 2020

One hundred and seventeen (117) samples have thus far been collected during the 2020 sampling season but restrictions imposed by COVID-19 have delayed the analysis for neonicotinoid and fiprole concentrations. To date, sampling locations for 2020 include lakes (n = 38), rivers and streams (n = 35), groundwater wells (n = 6), treated wastewater effluents (n = 19), passive air samplers (n = 2), springs (n = 1), and samples for quality assurance and control (n = 16). Of the samples that have been analyzed by LC-MS/MS (n = 51), median total neonicotinoid concentrations ranged from 1.3 ng/L (lakes) to 14.4 ng/L (wastewater effluent). Sample collection for 2020 will include fewer overall sites for rivers and streams, but measurements will be made more frequently (~2 per month). Sampling will continue through the end of the year and add up to 20 more well samples from 3 additional MN counties, stormwater samples, and winter lake samples.

Third Update March 1, 2021

Final samples for the 2020 sampling period were collected and analyzed for neonicotinoid and neonicotinoid degradation compounds. In 2020, two-hundred and four (204) water samples were analyzed; Sampling locations included lakes (n = 62), rivers and streams (n = 65), groundwater wells (n = 23), natural springs (n = 1), wastewater treatment plant effluent (n = 19), passive composite air samples (n = 2), and quality control replicates and recovery samples (n = 32). Median concentration of detected total neonicotinoids ranged from 0.055 ng/L to 148.8 ng/L during peak agricultural application. For the 2021 sampling season, a select few sites will be monitored with higher frequency along with their water quality and algae trends associated with neonicotinoid presences. In particularly, we will focus on two urban lakes (Cedar Lake in Minneapolis, Como Lake in St. Paul) and an agriculturally impacted lake (Madison Lake near Mankato, MN). Currently, sampling locations for 2021 include lakes (n = 7), wastewater treatment plant paired influent and effluent (n = 4 plants), stormwater discharge points (n = 5), and rivers and streams (n = 3). Samples from the select locations will be sampled weekly to monthly and analyzed for neonicotinoids, degradation products, fiprole compounds, and general water quality and health indicators.

Fourth Update September 1, 2021

As of July 2021, one-hundred and forty-four (44) unique samples have been collected, processed, and analyzed for seventeen (17) neonicotinoid and neonicotinoid metabolites as well as four (4) fipronil and fiprole metabolites. One-hundred and forty-two (n = 142) archived samples from the 2020 sampling period were re-analyzed for four (4) fiprole and metabolite compounds. Samples taken in 2021 consisted of sixty-six (n = 66) lake samples obtained from eleven (11) lakes, thirty-seven (n = 37) river samples obtained from fourteen (14) river locations, nine (n = 9) snow melt runoff samples obtained from three (3) locations entering one (1) lake, nine (n = 9) spring samples obtained from (9) springs, twenty-two (n = 22) storm water runoff samples obtained from three (3) locations entering one (1) lake, and one (n = 1) groundwater sample obtained from one (1) well. Neonicotinoids and fiprole compounds have been identified in lakes, rivers, groundwater wells, natural springs, and surface runoff. The median total neonicotinoids concentration range from 0.01 ng/L in snowmelt samples and 32.2 ng/L in springs, with a maximum total neonicotinoid concentration of 82.6 ng/L in springs. The median total fipronil concentration range from 0.08 ng/L in snowmelt samples and 0.81 ng/L in springs, with a maximum total neonicotinoid concentration of 12.0 ng/L in springs. In the remainder of 2021, sampling will be expanded to include process samples from one water and four wastewater treatment plants, and leachate from up to two organics composting facilities. Sampling will also be expanded to include algae and microorganism pulls from various lakes in the Twin Cities greater metropolitan region and around the state, beginning in latke 2021.

Fifth Update March 1, 2022

Sampling for the 2021 season has been completed with two-hundred and forty-one (n = 241) unique samples collected from twenty-one (21) natural water sites around Minnesota to be analyzed for seventeen (17) neonicotinoid parent compounds and degradation products, fipronil, and three (3) fipronil degradation compounds. As of January 1st, 2022, one-hundred and thirty-nine (139) samples were collected from thirteen (13) lakes, forty-seven (47) samples were collected from five (5) rivers, forty-six (46) stormwater samples were collected from three (3) outlets entering Lake Como, and nine (9) snow melt samples were collected from three outlets entering Lake Como. The median concentrations of total fipronil is 2.23 ng/L in Lakes and 5.02 ng.L in rivers after adjusting for surrogate standard recovery. Snowmelt and stormwater runoff may be adding significant concentrations of fipronil to surface waters with concentrations of 36.20 ng/L and 2.9 ng/L total fipronil, respectively. Similarly, total neonicotinoids in lakes and rivers are 9.80 ng/L and 20.4 ng/L, respectively, with snow melt and surface runoff concentrations of 9.11 ng/L and 78.1 ng/L, respectively. We were also able to serendipitously collect fifty-nine (59) samples were during a baseline (1) and two (2) spike and removal studies from a pilot drinking water plant to determine removal efficiently of select neonicotinoids in advanced drinking water treatment oxidation processes through. In all conditions, substantial neonicotinoid removal was observed (i.e., averaging > 80% removal for both compounds in 2 spiking events) demonstrating reactivity towards both biological degradation and chemical oxidation. Future research in the spring of 2022 will focus on fate of neonicotinoids and fipronil in wastewater plants, with collection of samples planned for the spring of 2022 from four different plants including influent, effluent, and biosolids. Archived wastewater samples from previous plant collection projects will be extracted and analyzed when possible.

Update as of June 30, 2022:

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022:

We have collected 623 natural and engineered water samples since the start of the research project. Sampling locations include: 27 influent, 24 effluent, and 12 biosolids samples from 4 wastewater treatment plants, 295 samples from 28 lakes, 150 samples from 29 rivers and streams, 12 snowfall and 15 rainfall samples from one collection location, 75 stormwater samples and 13 snowmelt samples from 3 discharge points. At least one neonicotinoid was detected in 100% of wastewater samples (influent, effluent, and biosolids), 87% of lake samples, 86% or river samples, 91% of snowfall and 100% of rainfall samples, and 78% or runoff samples. At least one fiprole was detected in 15% of wastewater samples (influent, effluent, and biosolids), 80% of lake samples, 76% or river samples, 100% of snowfall and rainfall samples, and 30% or runoff samples. Concentrations of fiproles and neonicotinoids have been identified in natural waters with median concentrations of 1.8 ng/L and 13.6 ng/L in lakes, 1.7 ng/L and 19.3 ng/L in rivers and streams. Stormwater and direct precipitation samples were also collected for one sampling location. Direct precipitation has median fiproles and neonicotinoids concentrations of 0.4 ng/L and 2.7 ng/L in rainfall, 1.3 and 73.0 ng/L in snowfall. Runoff samples have median fiproles and neonicotinoid concentrations of 2.1 ng/L and 20 ng/L in runoff. Monthly sampling will continue for 4 lakes through the summer of 2022 along with stormwater and rainfall sample collection. Sampling will then be paused until the winter/spring when collection of direct snowfall and snowmelt will be collected. Following winter of 2023, no further surface water samples will be collected.

Seventh Update as of March 1, 2023:

The last round of surface water samples was obtained from the Minneapolis Parks and Recreation Board were collected as of February 2023 along with additional snow, rain, and stormwater runoff samples. Only remaining samples to be collected will be obtained following spring snow melt during the March/April. Watershed GIS analysis is being conducted to determine the relations among watershed land use, imperious area, development indicators, and crop cultivation on the likelihood and extent of pesticide contamination in surface lakes, rivers, and streams together with various socioeconomic factors. Imidacloprid and clothianidin were the two most frequently observed noenicitinoids and are the only two contaminants with median concentrations consistently above the instrument detection limit. Imidacloprid was identified in 80% of rivers and streams with a median concentration of 1.4 ng/L and a maximum observed concentration of 410 ng/L and 63% of lakes with a median concentration of 0.55 ng/L and a maximum observed concentration of 450 ng/L. Clothianidin was found in 66% of rivers with a median concentration of 0.38 ng/L and maximum observed concentration of 370 ng/L and in 31% of lakes with a median concentration below the instrumental detection limit and a maximum observed concentration of 290 ng/L.

Seasonal timing has a significant impact on surface water insecticide concentrations with elevated levels observed during the agricultural and rainy seasons. Stormwater and snow melt runoff contribute an appreciable mass of pesticides to stormwater-impacted lakes and rivers with short periods of highly elevated concentrations of pesticides lasting for several days during periods of high rainfall or runoff formation. All five neonicotinoids were found in runoff samples with total neonicotinoid concentrations up to 30 ng/L in snow melt and 120 ng/L in stormwater and fipronil concentrations up to 190 ng/L in stormwater. The land use and size of the watershed appear strongly influence the frequency of detection and concentrations of neonicotinoids. Lakes in large, agricultural watersheds having the highest likelihood of pesticide contamination, particularly imidacloprid and clothianidin. The frequency of detection of acetamiprid, thiacloprid, thiamethoxam, and fipronil appear to be less related to land use, but are still impacted by watershed size.

Final Report as of June 30, 2023 (to be submitted before August 15, 2023):
The final precipitation, stormwater, and snow melt samples were received and processed in March of 2023. No additional samples have been collected or processed.

Imidacloprid and clothianidin were the two most frequently observed noenicitinoids and are the only two contaminants with median concentrations consistently above the instrument detection limit. Imidacloprid was identified in 80% of rivers and streams with a median concentration of 1.4 ng/L and a maximum observed concentration of 410 ng/L and 63% of lakes with a median concentration of 0.55 ng/L and a maximum observed concentration of 450 ng/L. Clothianidin was found in 66% of rivers with a median concentration of 0.38 ng/L and maximum observed concentration of 370 ng/L and in 31% of lakes with a median concentration below the instrumental detection limit and a maximum observed concentration of 290 ng/L. Concentrations were often above their respective EPA chronic toxicity threshold for invertebrate organisms indicating a possible risk to local non-target organisms which reside in Minnesota surface water.

Seasonal timing has a significant impact on surface water insecticide concentrations with elevated levels observed during the agricultural growing and rainy seasons. Stormwater and snow melt runoff contribute an appreciable mass of pesticides to stormwater-impacted lakes and rivers with short periods of highly elevated concentrations of pesticides lasting for several days during periods of high rainfall or runoff flows. All five neonicotinoids were found in runoff samples with total neonicotinoid concentrations up to 30 ng/L in snow melt and 120 ng/L in stormwater and fipronil concentrations up to 190 ng/L in stormwater. The land use and size of the watershed appear to strongly influence the frequency of detection and concentrations of neonicotinoids. Lakes in large, agricultural watersheds have the highest likelihood of pesticide contamination, particularly for imidacloprid and clothianidin. The frequency of detection of acetamiprid, thiacloprid, thiamethoxam, and fipronil appear to be less related to land use but are still impacted by watershed size and imperviousness.

Clothianidin, imidacloprid, and thiamethoxam were all frequently identified in rain and snow samples. Individual concentration of clothianidin ranged from 0 – 2 ng/L, imidacloprid concentrations ranged from 0 – 4.4 ng/L, and thiamethoxam ranged from 0 – 1.7 ng/L. Acetamiprid and fipronil were also in snow but not observed at high frequencies in rain. Snow acetamiprid concentrations ranged from 0 – 0.58 ng/L and fipronil from 0 – 2.9 ng/L. The majority of the contamination in formed runoff appears to be picked up during runoff formation in the watershed and not a result of the insecticides being present in the precipitation itself.

ACTIVITY 2 Title: ***Quantify the occurrence of neonicotinoids and breakdown products in shallow groundwater***
Description: Neonicotinoid insecticides are persistent and mobile in the environment. They have a high potential for moving into and through shallow groundwater. Groundwater is an important source of drinking water to a large portion of the State's population, and groundwater discharge (base flow) is also an important source of water to most lakes and streams. Thus, when groundwater becomes contaminated with the neonicotinoid insecticides, the groundwater can be a vector for delivering these chemicals to both humans and aquatic biota. This portion of the study collaborates with the MN DNR County Geologic Atlas Program to investigate the occurrence of the neonicotinoid insecticides and breakdown products in 72 wells in 12 counties across the state in a mixture of urban, agricultural, and natural lands.

ACTIVITY 2 ENRTF BUDGET: $122,000

Outcome	Completion Date
1. *Groundwater samples collected and analyzed for neonicotinoid and degradates*	9/01/22
2. *Statistical summary and interpretation of the data*	02/01/23
3. *Dissemination of Activity 2 findings via open access journal publication(s)*	06/01/24

First Update March 1, 2020
Collaboration with the MN DNR County Geologic Atlas (CGA) and MN DNR Minnesota Spring Inventory (MSI) allowed for the collection of 38 water samples from private and municipal drinking water wells and 4 samples from shallow springs in Southeast MN. Samples with the CGA were collected from Hubbard, Cass, Hennepin, Dodge, and Olmstead counties. The proportion of neonicotinoids detected above the analytical limit of quantitation was 11% for lakes and 23% for springs. The median total neonicotinoid concentrations were 1.4 ng/L (wells) and 33 ng/L (springs). Future work will continue with the MN DNR to sample wells in 4 additional counties and at least 8 additional springs in 2020. The current samples are also pending analysis for fipronil and its breakdown products.

Second Update September 1, 2020
Collaboration with the MN DNR CGA and MSI has continued throughout the 2020 season. Due to COVID-19 restrictions, only 6 samples from groundwater wells have been collected, each from Kandiyohi County. Three total samples have been collected from two shallow springs in Southeast MN. To date, none of these samples have been analyzed for neonicotinoid or fipronil concentrations. Throughout the remainder of 2020, samples will potentially be collected from Rock and Nobles counties and spring sampling has not yet resumed.

Third Update March 1, 2021

Collaboration with the MN DNR CGA and MSI has continued through the 2020 season into the 2021 season. In 2020, a total of 23 groundwater samples were collected and analyzed for detectable neonicotinoid concentrations from Kandiyohi, Nobles, and Rock counties. Of the collected samples, three groundwater samples contained neonicotinoids above the limits of detection, with concentrations up to 0.02 ng/L. Additional groundwater samples will be collected as the sampling season for 2021 resumes and Covid-19 restrictions relax.

Fourth Update September 1, 2021
Collaboration with the MN DNR County Geologic Atlas (CGA) and MN DNR Minnesota Spring Inventory (MSI) has continued during the 2021 season. So far in 2021, samples have been collected from one (1) groundwater well and nine (9) springs. The total median neonicotinoid and fiprole concentrations for the spring samples collected thus far in 2021 are 32.9 ng/L and 0.81 ng/L, respectively, for samples which tested positive for neonicotinoids and fiprole compounds. The total neonicotinoid and fiprole concentrations for the groundwater sample collected in 2021 is 0.38 ng/L and 0.78 ng/L. In the remainder of 2021, up to twenty (20) additional groundwater samples are anticipated to be collected from Steele County, MN and several spring samples from Olmsted County, MN. Additional samples may be collected, dependent on the MN DNR sampling plan for the remainder of 2021. MN DNR sampling has been impacted by the continuation of the Covid-19 pandemic and may limit additional groundwater and spring sampling.

Fifth Update March 1, 2022
Collaboration with the Minnesota Dept. of Natural Resources County Geologic Atlas (CGA) and Minnesota Spring Inventory (MSI) has continued through 2021. Due to continued covid-19 restrictions, groundwater sampling through the CGA program was restricted to a single county in 2021 and several agricultural natural springs from around Minnesota. In 2021, twenty-three (23) well samples have been collected from Steele County, MN and ten (10) surface-emergent spring samples were collected from seven (7) sites around the state. Significantly higher concentrations of neonicotinoids (1-sided Wilcoxon test, $p = 1.9e-5$) and fipronil (1-sided Wilcoxon test, $p = 0.10$) have been found in near-surface natural springs compared to deeper groundwater systems. The median total neonicotinoids were 33.50 ng/L in near-surface natural springs compared to 0.45 ng/L in deeper aquifers, while median total fipronil concentrations was 2.1 ng/L in near-surface natural springs and 0.75 ng/L in deeper groundwater. Future work will include sampling of an additional five (5) urban springs will in spring of 2022 to compare neonicotinoids and fipronil concentrations in natural springs in urban, agricultural, and rural settings.

Update as of June 30, 2022:

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022:

Since the start of the project, we have collected a total of 130 samples from around the midwestern territories of Minnesota and Wisconsin: 33 samples from 13 natural springs, and 97 groundwater samples from 87 groundwater wells. At least one fiprole and neonicinoid was identified in 93% and 94% of natural springs and

80% and 70% of groundwater samples. The median concentration of total fiproles was determined to be 0.42 ng/L in groundwater and 0.67 ng/L in natural springs; The median concentration of total neonicotinoids was determined to be 4.50 ng/L in natural springs and 0.10 ng/L in groundwater wells. Summer 2022 sampling was restricted to one county. Samples were collected in close geographic grouping to compare the vertical distribution of neonicotinoids and fiproles in the hydrogeologic column and between aquifer systems; data analysis for the distribution of neonicotinoids and fiproles in the vertical water column and between hydrogeologic cycles is ongoing. Sampling has concluded with the Minnesota Department of Natural Resources County Groundwater Atlas program. At this point we do not anticipate collecting anymore samples.

Seventh Update as of March 1, 2023:

No additional groundwater samples have been collected since Update 6. The final sample total for surface and groundwater samples includes 130 samples from Minnesota and Wisconsin including 33 samples from 13 natural springs, and 97 groundwater samples from 87 groundwater wells. Clothianidin was the most commonly-detected neonicotinoid found in 41% of sampled springs with a maximum concentration of 410 ng/L and in 14% of groundwater wells a maximum concentration of 82 ng/L. Imidacloprid was the second most-commonly found neonicotinoid in 35% of natural springs and 18% of groundwater wells a maximum concentrations of 35 ng/L and 1 ng/L in springs and wells, respectively. All observed concentration are below the acute and chronic toxicity limits. Because time series samples were not taken for this project, chronic toxicity limits cannot be accurately predicted, but all observed concentrations fall below the chronic toxicity limits for their respective compounds.

Aquifer depth and the presence of confining layers appear to play an important role in the presence of pesticides in groundwater. Surface springs and shallow, unprotected groundwater appear to be at highest risk of neonicotinoid and fipronil contamination. The superficial Galena and Galena-Dubuque formations may be at the highest risk of contamination. Both the frequency of detection and concentrations of pesticides in the groundwater decline steeply with the depth of groundwater, with the deeper confined aquifers left at the lowest risk for pesticide contamination. The land use of the groundwater recharge zones also plays a role in the likelihood and extent of pesticide contamination in shallow groundwater and springs. Wells in agricultural areas appear to have the highest likelihood of neonicotinoid contamination and the highest observed concentration when the chemicals are detected. There appears to be limited risk to the deep groundwater aquifers used to source municipal drinking water in the areas sampled, but there may be elevated risk to private drinking wells extracting water from shallow, unprotected groundwater systems.

Final Report as of June 30, 2023 (to be submitted before August 15, 2023):

No additional groundwater samples have been collected since Update 7.

The final sample total for surface and groundwater samples includes 130 samples from Minnesota and Wisconsin including 33 samples from 13 natural springs, and 97 groundwater samples from 87 groundwater wells. Clothianidin was the most commonly-detected neonicotinoid found in 41% of sampled springs with a maximum concentration of 410 ng/L and in 14% of groundwater wells a maximum concentration of 82 ng/L. Imidacloprid was the second most-commonly found neonicotinoid in 35% of natural springs and 18% of groundwater wells a maximum concentrations of 35 ng/L and 1 ng/L in springs and wells, respectively. All observed concentration are below the acute and chronic toxicity limits. Because time series samples were not taken for this project, chronic toxicity limits cannot be accurately predicted, but all observed concentrations fall below the EPA chronic toxicity threshold for all plants and organisms for their respective compounds.

Aquifer depth and the presence of confining layers drive the presence of pesticides in groundwater. Surface springs and shallow, unprotected groundwater appear to be at highest risk of neonicotinoid and fipronil contamination. The superficial Galena and Galena-Dubuque formations may be at the highest risk of contamination. Both the frequency of detection and concentrations of pesticides in the groundwater decline steeply with the depth of groundwater, with the deeper confined aquifers left at the lowest risk for pesticide contamination. The land use of the groundwater recharge zones also plays a role in the likelihood and extent of pesticide contamination in shallow groundwater and springs. Wells in agricultural areas appear to have the highest likelihood of neonicotinoid contamination and the highest observed concentration when the chemicals are detected. There appears to be limited risk to the deep groundwater aquifers used to source municipal drinking water in the areas sampled, but there may be elevated risk to private drinking wells extracting water from shallow, unprotected groundwater systems.

Groundwater infiltration by stormwater runoff infiltration ponds may be a contributor to shallow groundwater contamination. A small survey of an groundwater infiltration ponds fed by stormwater at a organics composting facility showed the presence of acetamiprid (0 – 0.78 ng/L), clothianidin (0 – 0.16 ng/L), imidacloprid (0 – 1.6 ng/L), and thiamethoxam (0 – 0.46 ng/L).
following rainfall and stormwater runoff.

ACTIVITY 3 Title: ***Assess any relationship between neonicotinoid levels and formation of algal blooms***
Description: Neonicotinoid insecticides present in surface waters are toxic to many aquatic fauna including aquatic insects and zooplankton, which eat algae, are important in controlling the algal populations in surface waters. If the neonicotinoid insecticides are present at concentrations that affect the *fauna*, then their control on the algal population may be comprised, and the insecticides may indirectly facilitate in the development of an algal bloom. This Activity examines this possible relationship by quantifying neonicotinoid insecticides concentrations and relative abundance of *Daphnia* populations during periods with and without algal blooms in selected lakes (4 lakes, sampled weekly during one spring to fall season).

ACTIVITY 3 ENRTF BUDGET: $100,000

Outcome	**Completion Date**
1. *Sampling and analysis of neonicotinoids and Daphnia during and without algal blooms*	09/01/22
2. Data analyzed and statistical relationships established	02/01/23
3. Dissemination of Activity 3 findings via open access journal publication(s)	06/01/23

First Update March 1, 2020
The richness and abundance of several zooplankton (n = 9) and phytoplankton (n = 133) communities were measured in 6 lakes within Ramsey County by Ramsey County staff as part of their routine monitoring. Additionally, the physical size distribution of *Daphnia* was determined in each of these samples. Approximately 6 samples were collected for plankton analyses from each of the sites over the sampling season (May – October 2019). These data have been collected and tentatively reviewed but

are pending a proper statistical analysis and comparison to our measured neonicotinoid data. It should be noted that none of our sampling locations met the regulatory criteria for being at risk of a toxic algal blooms during 2019.

Second Update September 1, 2020
For the 2019 data in Ramsey County lakes, no relationship between neonicotinoids was found. Samples for plankton analysis will continue to be collected throughout 2020 and the data will become available at the end of the year.

Third Update March 1, 2021
To date, 2020 samples are currently be analyzed for causal relationships between algae and plankton blooms and neonicotinoid concentrations using historical data collected as part of the Minnesota Pollution Control Agency (MPCA) Sentinel Lakes program and the Minneapolis Board of Parks and Recreation (MPRC). During the 2021 season, we are increasing sampling frequency and data collection of both neonicotinoid concentrations and algae information in collaboration with the MPRB and MPCA. During 2021, we hope to assess the relationship between plankton, algae, and neonicotinoid presence using a subset of select lakes (n = 6) with well-studied trends in algae and plankton abundance.

Fourth Update September 1, 2021
Currently, the Minnesota Pollution Control Agency and Minneapolis Parks and Recreation Board (MPRB) are collecting seasonal algae and plankton counts as a part of the Minnesota Sentinel Lakes monitoring program and seasonal MPRB water quality monitoring program, respectively. Once completed, the data provided by the partner agencies will be obtained and compiled with these analytical results for of the monitored lakes selected for closer study during the 2021 sampling period. The compiled algae and pesticide data will be used to identify any correlations between algae and microorganism population trends, and neonicotinoid and fiprole concentrations relative to algae blooms in the Minnesota lakes.

Fifth Update March 1, 2022
For the recent data of Lake Como, no relationship between zooplankton and neonicotinoids was found. Neonicotinoid impacts may be modified by the presence of other external stressors. The historic data of Lake Como have been used to identify correlations between zooplankton and external stressors. The density of Zooplankton (1984-2020) in Lake Como has been analyzed, results show that the density trends of zooplanktons were periodic with a 7 to 8 year cycle. This work will be expanded to phytoplankton and other lakes.

Update as of June 30, 2022:

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022:

The periodic trend of zooplankton density in Lake Como does not correspond to the years of El Nino or La Nina. At this moment, the reason for this trend is unknown. No further work has been performed pertaining to statistical correlation of algal blooms and neonicotinoid concentrations in Lake Como. Sampling has continued for Lake Madison and the four urban lakes: Cedar, Loring, Nokomis, and

Powderhorn. Following completion of summer sampling, we will work with project partners to examine any correlations between lake pesticide concentrations and the occurrence or severity of algal blooms in the five aforementioned lakes. The analysis is anticipated to be completed during the winter 2022 period after the final data sets for algae are available.

Seventh Update as of March 1, 2023:
We are currently waiting for the final data sets for algae to be available from the Minnesota Pollution Control Agency and Minneapolis Parks and Recreation Board (MPRB). After that, we will start to analyze potential relations among pesticide concentrations and algal bloom occurrences. At this moment, no obvious ecological relation is apparent between lake pesticides level and algal bloom occurrences. However, risk assessment models will be applied to quantify potential risks of lake pesticides to aquatic organisms. The relations among the results of risk assessment models, trends in populations of aquatic organisms, and lake pesticide concentrations and the occurrence of algal blooms will be explored.

Final Report as of June 30, 2023 (to be submitted before August 15, 2023):
The density of zooplankton and phytoplankton (1984-2020), neonicotinoid concentrations, and related environmental conditions in Lake Como were analyzed. The periodic population trend of zooplankton was observed. No direct evidence showed that this trend was related to the neonicotinoid levels. Neither was the relationship between neonicotinoid levels and formation of algal blooms found in Lake Como. This work was expanded to other lakes. Some water quality and phytoplankton data on Cedar, Nokomis, Loring, and Powderhorn over the past 4 years were obtained from the Minnesota Pollution Control Agency and Minneapolis Parks and Recreation Board (MPRB). Those data were analyzed with the neonicotinoid concentrations. Neonicotinoid was not a significant factor that impacted the biomass of phytoplankton and zooplankton when compared to other environmental conditions. No clear relationship between neonicotinoids and algal blooms was apparent.

IV. DISSEMINATION:

Description: Results will be disseminated by publication in peer-reviewed journals, presentations and conferences, and via a final report. Funds are requested to make journal articles open access. Data sets will be archived in the Data Repository for the University of Minnesota (https://conservancy.umn.edu/handle/11299/166578), a publically available collection of research data. ENTRF support will be acknowledged on all disseminated materials.

The Minnesota Environment and Natural Resources Trust Fund (ENRTF) will be acknowledged through use of the trust fund logo or attribution language on project print and electronic media, publications, signage, and other communications per the ENRTF Acknowledgement Guidelines.

First Update March 1, 2020
Nothing to report for this activity.

Second Update September 1, 2020
Planned conference presentations were cancelled due to the COVID-19 pandemic. A manuscript on the 2019 occurrence data in lakes, streams, and groundwater was submitted and is being revised after peer-review.

Third Update March 1, 2021

The article Neonicotinoid Insecticides in Surface Water, Groundwater, and Wastewater across Land Use Gradients and Potential Effects has been submitted to and accepted by the journal of Environmental Chemistry and Toxicology outlining the findings from the research during the 2019 sampling season. This article is open access.

Berens, M.J., Capel, P.D., and Arnold, W.A., 2020, Neonicotinoid Insecticides in Surface Water, Groundwater, and Wastewater across Land Use Gradients and Potential Effects, Environmental Chemistry and Toxicology Journal, Accepted, online at: https://doi.org/10.1002/etc.4959

Fourth Update September 1, 2021

Final details are available for the publication above

Berens, M.J., Capel, P.D., and Arnold, W.A., 2020, Neonicotinoid Insecticides in Surface Water, Groundwater, and Wastewater across Land Use Gradients and Potential Effects, Environmental Chemistry and Toxicology Journal, 40(4), 1017-1033 online at: https://doi.org/10.1002/etc.4959. The data set is available in Data Repository for the U of MN: https://doi.org/10.13020/760y-wc14. The article was highlighted by Minnesota Public Radio and other media outlets.

Two presentations have been given at American Chemical Society Meetings

Berens, Matthew. Neonicotinoid insecticides in surface water, groundwater, and wastewater across land use gradients and potential effects. Oral Presentation. *American Chemical Society Spring 2021 Virtual Meeting*, **2021.**

Goedjen, Grant. (2021, August 22-26). *Neonicotinoid insecticides in Minnesota surface and groundwater: Occurrence, trends, and future work. American Chemical Society Fall 2021 Convention. Atlanta, GA, United States.*

Fifth Update March 1, 2022

Goedjen, Grant. (2022, March 23). *Contributing Factors of Neonicotinoid and Fipronil Insecticides Detection Across Urban and Agricultural Land Use.* American Chemical Society Spring 2022 Convention. San Diego, CA, United States.

Update as of June 30, 2022:

Project extended to June 30, 2023 by LCCMR 6/30/22 as a result of M.L. 2022, Chp.94, Sec. 2, Subd. 19, legislative extension criteria being met.

Sixth Update as of September 1, 2022:

Poster Presentation, "Evaluation of Neonicotinoid and Fipronil Insecticides in Minnesota's Drinking and Wastewater Treatment Plants", *Gordon Research Conference: Environmental Science: Waters*, Holderness, NH. June 19-22

Poster Presentation, "Evaluation of Neonicotinoid and Fipronil Insecticides in Minnesota's Drinking and Wastewater Treatment Plants", *Gordon Research Series: Environmental Science: Waters*, Holderness, NH. June 17-18.

Seventh Update as of March 1, 2023:

A presentation abstract was submitted for the 2023 Spring AEESP in Boston, MA was submitted as of 24 February 2023.

A paper that includes analysis of neonicotinoids in drinking water has been submitted. We expect to submit papers about the surface and groundwater samples in the coming months.

Final Report as of June 30, 2023 (to be submitted before August 15, 2023):

To date one journal article has been published with an associated data set, and several talks at conferences and public seminars have been presented. Additional publications will be submitted soon. This work has been included in the doctoral dissertations of two students: Matthew Berens and Grant Goedjen. The latter dissertation and associated data will be available in the UMN digital conservancy and UMN data repository in early Fall 2023.

The final list of presentations is listed below:

Publications:

Berens, M.B., Capel, P.D., Arnold, W.A. 2021. Neonicotinoid Insecticides in Surface Water, Groundwater, and Wastewater across Land Use Gradients and Potential Effects. *Environ. Tox. Chem.* 40(4)1017-1033. Open Access. Associated data set.

Three additional publications are will be provided following submission and publication in 2023 and 2024 following the defense of Grant Goedjen (Sept 2023).

Dissertations:

Berens, Matthew. (2020). Exploring the Reactions and Presence of Munitions Compounds and Insecticides in Aquatic Systems. Retrieved from the University of Minnesota Digital Conservancy, https://hdl.handle.net/11299/220617.

Goedjen, Grant. (2023). Trends in Systemic Insecticides in Minnesota Hydrology: An Investigation into the Presence and Fate of Neonicotinoids and Fipronil Compounds in Minnesota's Hydrologic Systems and Treatment Processes. (Expected September 2023).

Oral Presentations

Berens MJ, Capel PD. Arnold WA. (2020). Occurrence of Neonicotinoid Insecticides in Minnesota Waters and Their Effects on Aquatic Ecosystems. 2020 Minnesota Water Resources Conference. (Virtual, Oral).

Berens MJ, Capel PD, Arnold WA. (2021). Neonicotinoid insecticides in Minnesota surface and groundwater: Occurrence, trends, and future work. 2021 ACS Spring Meeting. (Virtual, Oral).

Goedjen GJ, Berens MJ, Capel PD, Arnold WA. (2021). *Neonicotinoid insecticides in Minnesota surface and groundwater: Occurrence, trends, and future work. American Chemical Society Fall 2021 Convention. virtual.* August 22-26

Goedjen GJ, Berens MJ, Capel PD, Arnold WA (2022). *Contributing Factors of Neonicotinoid and Fipronil Insecticides Detection Across Urban and Agricultural Land Use.* American Chemical Society Spring 2022 Convention. San Diego, CA, United States. March 20 – 24.

Poster Presentation:

Goedjen GJ, Berg S, Capel PD, Arnold WA. (2022). "Evaluation of Neonicotinoid and Fipronil Insecticides in Minnesota's Drinking and Wastewater Treatment Plants", *Gordon Research Conference: Environmental Science: Water*, Holderness, NH. June 19-22.

Goedjen GJ, Berg S, Capel PD, Arnold WA. (2022). "Evaluation of Neonicotinoid and Fipronil Insecticides in Minnesota's Drinking and Wastewater Treatment Plants", *Gordon Research Seminar: Environmental Science: Water*, Holderness, NH. June 17-18.

Goedjen GJ, Berens MJ, Capel PD, Arnold WA. (2022). "Neonicotinoid Contamination in Hydrogeologic Systems: The Depth Distribution of Neonicotinoid Insecticides Within the Midwestern Region", *Association of Environmental Engineering and Science Professors*, Boston, MA. June 20 – 23.

V. ADDITIONAL BUDGET INFORMATION:

A. Personnel and Capital Expenditures

Explanation of Capital Expenditures Greater Than $5,000: N/A

Explanation of Use of Classified Staff: N/A

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

Enter Total Estimated Personnel Hours for entire duration of project: 4770	Divide total personnel hours by 2,080 hours in 1 yr = TOTAL FTE: 2.3

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

Enter Total Estimated Contract Personnel Hours for entire duration of project: N/A	Divide total contract hours by 2,080 hours in 1 yr = TOTAL FTE: N/A

VI. PROJECT PARTNERS:

Name	Title	Affiliation	Role
Paul Capel	Adjunct Assoc. Prof.	U of MN	Co-investigator

A. Partners outside of project manager's organization receiving ENRTF funding: N/A

B. Partners outside of project manager's organization NOT receiving ENRTF funding

Name	Title	Affiliation	Role
John Barry	Hydrogeologist	MN DNR	Collect groundwater samples
Shawn Schottler	Environmental scientist	Science Museum of Minnesota	Algal bloom sampling

VII. LONG-TERM- IMPLEMENTATION AND FUNDING: This project will provide a baseline assessment of the occurrence of the neonicotinoid and selected reaction products in various waters impacted by humans and if they play any role in algal blooms. This work will provide methods to the Minnesota State Agencies for continued studies of these chemicals if needed. 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. The results will be disseminated via open-access scientific literature and reports.

VIII. REPORTING REQUIREMENTS:

- Project status update reports will be submitted March 1 and September 1 each year of the project
- A final report and associated products will be submitted between June 30 and August 15, 2023

IX. SEE ADDITIONAL WORK PLAN COMPONENTS:

A. Budget Spreadsheet
B. Visual Component or Map
C. Parcel List Spreadsheet: N/A
D. Acquisition, Easements, and Restoration Requirements: N/A
E. Research Addendum

Attachment A:
Environment and Natural Resources Trust Fund
M.L. 2019 Budget Spreadsheet - Final
Legal Citation: M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 04a
Project Manager: William Arnold
Project Title: Neonicotinoid Insecticides: Occurrence And Influence on Algal Blooms
Organization: University of Minnesota
Project Budget: $350,000
Project Length and Completion Date: 4 years, June 30, 2023
Today's Date: August 8, 2023

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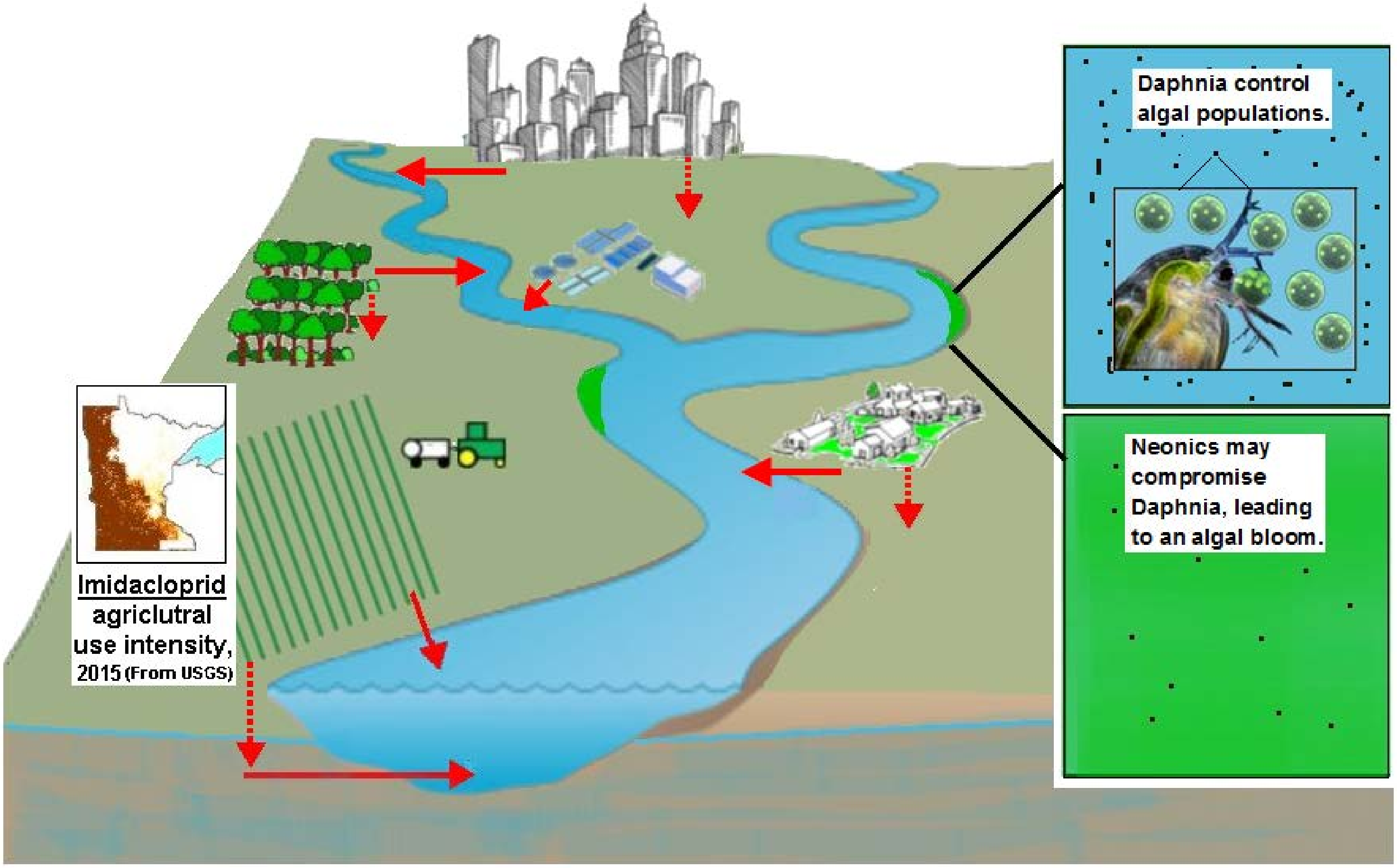
ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	**Budget [10/06/2021]**	**Revised Budget [08/08/2023]**	**Amount Spent**	**Balance**
BUDGET ITEM				
Personnel (Wages and Benefits)	$ 287,500	$ 295,519	$ 295,519	$ -
William Arnold, Project Manager (75% salary, 25% fringe benefits). 6% FTE for years 1-3. Project coordination, development of analytical protocols. supervision of graduate and undergraduate researchers. $51,000				
Paul Capel, co-Project Manager (75% salary, 25% fringe benefits). 6% FTE for years 1-3. Field sampling study design, supervision of graduate and undergraduate researchers. $51,000				
Graduate student Research assistant, sample collection, processing, measurement of concentrations (57% salary, 43% fringe benefits) 50% FTE for years 1-3. $149,000				
Undergraduate researcher #1. Assist with field sampling, processing of samples, data analysis. 10 hrs per week during academic year, 40 hours per week in summer. (100% salary) $23,350				
Undergraduate researcher #2. Assist with field sampling, processing of samples, data analysis. 10 hrs per week during academic year, 40 hours per week in summer. (100% salary) ~~$23,350~~ $13,250				
Equipment/Tools/Supplies				
Laboratory Supplies (chemical and isotopically labelled standards, chemical reagents, necessary glassware, solvents, consumable supplies, laboratory notebooks, software licenses	$ 21,000	$ 25,148	$ 25,148	$ -
Analytical time for compound quantification (210 samples including surface waters, groundwaters, and samples during algal blooms	$ 16,580	$ 16,580	$ 16,580	$ -
Operating costs for laboratory instruments required for analyses and experiments; costs portioned based on usage by project	$ 3,000	$ 1,128	$ 1,128	$ -
Maintenance for mass spectrometry equipment system components	$ 9,420	$ 3,191	$ 3,191	$ -
Travel expenses in Minnesota				
Charges and university vehicle rental charges for trips to collect water samples. Hotel/meal charges if overnight stay required. Attendence for students at local conferences to disseminate project findings to agriculture and environmental interests. Reimbursement will be according to University of Minnesota guidlines.	$ 7,500	$ 5,557	$ 5,557	$ -
Other				
Publication charges to make to make published journal articles (2-3) immediately available via open access to maximize data availability and dissemination	$ 5,000	$ 2,877	$ 2,877	$ -
COLUMN TOTAL	$ 350,000		$ 350,000	$ -

OTHER FUNDS CONTRIBUTED TO THE PROJECT	**Status (secured or pending)**			**Spent**	**Balance**
Non-State:				$ -	$ -
State:				$ -	$ -
In kind: Because the project is overhead free, laboratory space, electricty, and other facilities/adminstrative costs (54% of direct costs excluding permanent equipment and graduate student tuition benefits) are provided in-kind. Additionally, in kind sample collection will be performed by MN DNR and St. Croix Watershed Research Station.	secured	$ 164,000		$ 164,000	$ -

PAST AND CURRENT ENRTF APPROPRIATIONS	**Amount legally obligated but not yet spent**			**Spent**	**Balance**
Current appropriation:				$ -	$ -
Past appropriations: M.L.2016,Chp186,Sec.2,Subd.04e,Assessing Neonicotinoid Insecticide Effects on Aquatic and Soil Communities $400,000		$ 400,000		$ 400,000	$ -

Neonicotinoids have a variety of sources and may lead to algal blooms



Key Outcomes:

1. Assess urban, suburban, and agricultural sources of neonicotinoids to surface waters
2. Quantify neonicotinoids in groundwater
3. Identify reaction products
4. Assess role of neonicotinoids on formation of algal blooms