

2016 Project Abstract

For the Period Ending June 30, 2019

PROJECT TITLE: Game and Nongame Bird Pesticide Exposure

PROJECT MANAGER: Julia B Ponder, DVM MPH

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FUNDING SOURCE: Environment and Natural Resources Trust Fund

LEGAL CITATION: M.L. 2016, Chp. 186, Sec. 2, Subd. 03m

APPROPRIATION AMOUNT: \$349,000

AMOUNT SPENT: \$339,419

AMOUNT REMAINING: \$9,581

Sound bite of Project Outcomes and Results

We documented neurobehavioral abnormalities in chickens from neonicotinoid exposure at doses compatible with what wild birds might ingest, as well as the availability of neonicotinoid-treated seeds on the agricultural landscape. We also identified changes in gene expression associated with exposure that may be useful in developing a non-lethal test for exposure.

Overall Project Outcome and Results

Neonicotinoids are the most widely used pesticides worldwide and are commonly applied as a seed treatment to corn, soybean, and wheat seeds, which comprise the majority of Minnesota's row crops. Previous risk assessments have suggested that wild birds may be exposed to large doses of neonicotinoids through the ingestion of treated seeds. Using chickens as a model species, we evaluated the impacts of oral neonicotinoid exposure on the immune and neurological systems. We also assessed availability of treated seeds to wild birds on the agricultural landscape and analyzed grouse carcasses for residues of exposure.

Accomplishments:

- We demonstrated neurological abnormalities in chickens exposed orally to imidacloprid, a commonly used neonicotinoid in seed-treatments
- We quantified seed spills on agricultural landscapes during spring planting season that may occur during loading or refilling seed hoppers
- We documented wildlife at neonicotinoid-treated seed spills with trail cameras and documented consumption of treated seeds.
- We documented neonicotinoid residues in the tissues of hunter-harvested grouse, indicating that those birds were exposed to the pesticides
- We identified 354 genes affected by imidacloprid exposure through RNA sequencing: 37 affected genes were detected in liver and 317 affected genes were detected in blood cells (which can be non-lethally collected, which may allow future development of detection assays)

The results of this project indicate that seed-eating birds in the wild may be exposed to seeds treated with neonicotinoids in the agricultural landscape through eating at seed spills. Ingestion of neonicotinoid-treated seeds by birds can produce neurological abnormalities that may impair survivability. Exposure can be evaluated through detection of pesticide residues in carcasses, as well as fecal pellets and blood cells. The results of this study may be used by the agricultural industry to reduce impacts to wild birds through education and process

change (reduce spillage), as well as state and federal governmental agencies reviewing appropriate and safe usage of these pesticides.

Project Results Use and Dissemination

Results of this project have been communicated to a large audience of stakeholders, including directly with industry colleagues through meetings with agricultural stakeholders; with federal and state agencies through public commentary response as well as requested webinars, presentations and conversations; and with the scientific community through publications (1 paper published, 1 submitted and 4 pending), conference presentations (4) and scientific posters (2). Details of all communications are provided in the final report. The results of our work show that wild birds are at risk of exposure to agricultural seeds treated with neonicotinoids and that ingestion of field-realistic doses causes significant behavior changes in chickens that were severe at higher doses and may impair survival of free-living gallinaceous birds. The adoption of practices that would reduce seed spills on the agricultural landscape would reduce the exposure risk to wild birds.



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

Date of Report: August 1, 2019

Final Report: August 1, 2019

Date of Work Plan Approval: November 4, 2016

Project Completion Date: June 30, 2019

PROJECT TITLE: Game and Nongame Bird Pesticide Exposure

Project Manager: Julia B Ponder, DVM, MPH

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Location: Statewide

Total ENRTF Project Budget:

ENRTF Appropriation: \$349,000

Amount Spent: \$339,419

Balance: \$ 9,581

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 03m

Appropriation Language:

\$349,000 the second year is from the trust fund to the Board of Regents of the University of Minnesota to evaluate the potential risk to game and nongame birds from exposure to neonicotinoid-treated agricultural seeds. This appropriation is available until June 30, 2019, by which time the project must be completed and final products delivered.

I. PROJECT TITLE: Do neonicotinoids pose a risk to Minnesota's birds?

II. PROJECT STATEMENT: We propose to examine sub-lethal exposure of neonicotinoid pesticides in birds, using sharp-tailed grouse as a model. Neonicotinoid pesticides such as imidacloprid, thiamethoxam, thiacloprid, clothianidin are the most widely used pesticides worldwide. They are commonly applied as a seed treatment to most corn, soybean, sunflower, and wheat seeds. These crops comprise the majority of Minnesota's row crops. While their unintended impact on insect pollinators has caused the greatest amount of concern, recent studies have shown potential risk to birds. Risk assessments (American Bird Conservancy) have determined that the most likely route of exposure to large doses of neonicotinoids for birds is ingestion of treated seeds, although numerous other mechanisms exist (e.g., crops, soil, water, trophic transfer). Ingestion of a small number of treated seeds has been shown to be lethal to small birds. While larger birds are less likely to ingest a lethal dose through seed consumption, they may still be at risk for sub-lethal health impacts and may be exposed to multiple types of neonicotinoids. Sub-lethal effects found in the lab include behavioral abnormalities, declines in reproductive success, and immune suppression; but available studies have not adequately simulated field exposures nor provided tools to measure risk to wild birds.

Sharp-tailed grouse are a good model to understand risk to birds, as they utilize areas with high and low levels of agriculture in Minnesota; consume corn, wheat, and other crop types in which neonicotinoid-treated seeds would be available through spillage or after planting; and, are closely related to domestic chickens which are amenable to lab studies. Sharp-tailed grouse are also large making them less likely to consume a lethal dose, yet manifest detectable sub-lethal effects. Based on current knowledge, it is calculated that a grouse would need to eat 14 seeds for a sub-lethal dose and approximately 80 corn seeds for a lethal dose, the latter being unlikely in one feeding bout. Lastly, sharp-tailed grouse display at leks, an assembly area where multiple animals congregate for breeding displays and courtship. These leks are fairly stable in location among years, facilitating non-lethal collection of feces and blood from a large geographical area within and outside of agricultural areas, and allowing comparisons of naturally occurring low and high exposure groups.

The overall goal of this project is to assess whether birds are at risk from exposure to neonicotinoid-treated seeds in agriculture landscapes using sharp-tailed grouse as a model species. Our specific objectives are to:

- Assess exposure in wild grouse
 - Identify birds consuming neonicotinoid-treated seeds, quantify consumption per foraging bout, and measure neonicotinoid concentrations of seeds
 - Quantify grouse neonicotinoid residues in feces of breeding birds and tissues from hunter-harvested birds
 - Quantify the rate of seed spillage along roads and edges of agricultural fields (transect study)
- Establish exposure-response relationships in the lab
 - Assess impacts of exposure to neonicotinoid mixtures on the immune system in the lab using chickens as a surrogate
- Provide a means to link exposure to effect in field studies
 - Quantitatively link exposure to neonicotinoid mixtures, tissue residue concentrations (dose), and immune suppression in the lab to interpret tissue residue concentrations in wild birds

This study will provide preliminary data to evaluate the risk to Minnesota's birds from neonicotinoids by documenting access to neonicotinoid-treated seeds, comparing tissue residue in wild birds from agricultural areas and non-agricultural areas, establishing non-lethal methods of assessing exposure, demonstrating sub-lethal impacts of exposure, and assessing whether exposure to multiple neonicotinoids worsens their impact.

III. OVERALL PROJECT STATUS UPDATES:

Amendment request (10/18/2016):

Due to the inability to hire a post-doctoral candidate to oversee the laboratory studies, data collection and data analysis in the first year of this project, we are requesting to move one year of the budgeted salary for this

position to a graduate student classification. The only impact on the budget is to add a line for graduate student salary and adjust effort of post-doc, all within the original budget for personnel.

Amendment approved: 11/4/2016

Project Status as of: 30 January 2017

After an unsuccessful attempt to identify a qualified post-doctoral candidate, an alternate plan to utilize a graduate student for implementation of Activity 1/year 1 was developed and an amendment request submitted. A graduate student was successfully approved and funded for this project. Subawards have been put in place for Minnesota Department of Natural Resources (field work – Activity 2) and Southern Illinois University – Carbondale (sample analysis). Collection of field samples started in Fall 2016 and preparatory work for Activity 1 has been done. All activities are on schedule

Project Status as of: 30 July 2017

Activity 1 exposure and sample collection has been completed for four dosage groups and initial immune assays run. Initial analysis has been started of immune assay results. Tissue samples collected for residue analysis have been frozen pending shipment to SIUC.

For Activity 2, video was captured from 40 trail cameras placed on simulated seed spills to document animals that consumed treated seeds. Video analysis is pending. To quantify seed spills, a balanced sample of 50 townships with >50% of area planted in soybean, corn or wheat in 2014 were surveyed. Planting status was documented along with approximate size (e.g., area or count) of seed spills on roads, field edge or in field and crop type (where possible). Finally, fresh fecal pellets were collected from 46 sharp-tailed grouse leks and 27 prairie-chicken leks, and sent to SIUC for analysis.

A post-doctoral candidate has been hired for Activity 3 and is in the process of reviewing the protocols and research methodology.

Amendment request (9/08/2017):

Preliminary data analysis for Activity 1 indicates that there is not a statistically significant difference in immune function between the current treatment groups, although there is substantial individual variability. Based on this preliminary information and a power analysis of the current data, we are requesting the following changes to Activity 1:

- Reduction of dosing levels from six to five: based on preliminary findings, there is no value in assessing dosages lower than 3.3% LD₅₀
- Increase group numbers from 10 to 20: based on a power analysis of the preliminary results, this increase in sample size at each dose level is needed to detect a significant difference in immune function, if it does exist.
- Eliminate the clothianidin exposure groups: Clothianidin has the same mechanism of action of imidacloprid, so while the exposure dose needed to cause a clinical or subclinical effect may be different, the type of effect would likely be similar. By eliminating these groups, we would have the budget and resource capacity to increase the imidacloprid group sizes to 20, allowing us to fully evaluate if there is a statistical difference on immune function.

We are also proposing to amend Activity 3 based on the results of Activity 1. The use of genomics (RNA sequencing) to evaluate immune function is a more sensitive assay of immunotoxicity than the assays used in Activity 1 and is the basis for development of a biomarker for assessing exposure. We propose to reduce our exposure groups from four to three (0.25-20% LD₅₀) and add a second phase of RNA sequencing sampling to expand the potential for development of a biomarker.

In order to complete these efforts, we are requesting the following budget adjustments:

- Increase in budget for supplies from \$4,250 to \$15,900: the costs of supplies for immune assays and RNA sequencing was originally underestimated
- Increase in budget for research animal housing from \$13,944 to \$17,078 to reflect current pricing
- Increase budget for RNA sequencing from \$12,600 to \$38,600 to accommodate analysis of additional tissues
- Decrease in budget for analysis of neonicotinoid residues (SIUC) from \$98,445 to \$76,615 – we will evaluate residues in fewer tissues in Activity 3 as results from Activity 1 will provide adequate information on tissue distribution.

Amendment Approved by LCCMR 10/9/17

Project Status as of: 30 January 2018

Activity 1 data collection and analysis of immune assay results completed with final assessment of tissue residue analysis pending. Results are being written up for publication and have been presented at two scientific conferences.

All field work is complete for Activity 2 and final assessment of tissue residue analysis pending. Results have been presented at a scientific conference and discussed with both regulatory agency personnel (EPA) and industry representatives (Bayer).

Data collection for Activity 3 has been completed and samples processed for analysis (in process).

Amendment request (6/12/2018):

We are requesting a one year extension to our workplan (as allowed by appropriations language) in order to complete the previously approved activities, which behind schedule due to delays in receiving results from the Genomics Center. This will allow us to run our second phase of genomics sequencing, which has been postponed as it is dependent on the results of the first phase. As the genomics analysis and research animal costs were below budget projections, we are also requesting that those funds be moved to personnel to allow us to extend the post-doctoral fellow's appointment to facilitate completion of the genomics work and reporting. This requires the following budget changes:

- Activity 1: Reduce by \$7,768, reflecting reduced research animal costs
- Activity 3: Reduce research animal costs (\$9,850) and RNA sequencings costs (\$5,339)
- Activity 3: Increase personnel costs by \$22,957 (sum of above reductions)

Amendment Approved: [07/06/2018]

Project Status as of: 30 June 2018

All laboratory and field work has been completed, as has most data analysis. The final data analysis is pending completion of the second phase of genomic sequencing. Currently, three scientific manuscripts are in development with additional ones to come.

Information from this work was used in providing public comment on the technical merits of the EPA's recently released draft neonicotinoid ecological risk assessment. Additional communications with scientists, agencies and industry representatives have occurred.

Project Status as of: 30 January 2019

Statistical analysis of the clinical neurological signs has been completed for Activity 1 and a manuscript is in process with expected submission to peer reviewed literature in April 2019. The final data analysis for two

phases of genomic sequencing has been completed for Activity 3. Analyses and results are being written up for publication. Sequences and analytical codes have been uploaded to public domains.

Project Status as of: 11 April 2019

First manuscript has been submitted for publication for work done under Activity 2, a final draft is substantially complete for submission for work done under Activity 1 and a publication draft has been started for work done under Activity 3.

Amendment request as of 4/9/2019:

With our project substantially complete, we are requesting to use unused funds from personnel, travel and research lab services to do one additional round of laboratory analysis, looking for residues of neonicotinoid metabolites (products made by the liver from the original neonicotinoid exposure) to identify both possible presence of additional active ingredients in the body as well as potential new markers for exposure. In addition, we had an unexpected budget overrun on lab supplies and would like to adjust the budget to cover this.

- \$5,567 moved from personnel salary and benefits
- \$4,621 staying within laboratory and service contracts (moving from genomics analysis to metabolite analysis)
- Activity 3 supplies increased by \$1,200 (\$912 from Activity 1 supplies, \$1,188 from personnel salary/benefits) to cover overage
- \$10,000 line added under service contracts for metabolite analysis of samples

Amendment Approved by LCCMR 4/30/2019

Overall Project Outcomes and Results:

Neonicotinoids are the most widely used pesticides worldwide and are commonly applied as a seed treatment to corn, soybean, and wheat seeds, which comprise the majority of Minnesota's row crops. Previous risk assessments have suggested that wild birds may be exposed to large doses of neonicotinoids through the ingestion of treated seeds. Using chickens as a model species, we evaluated the impacts of oral neonicotinoid exposure on the immune and neurological systems. We also assessed availability of treated seeds to wild birds on the agricultural landscape and analyzed grouse carcasses for residues of exposure.

Accomplishments:

- We demonstrated neurological abnormalities in chickens exposed orally to imidacloprid, a commonly used neonicotinoid in seed-treatments.
- We quantified seed spills on agricultural landscapes during spring planting season that may occur during loading or refilling seed hoppers.
- We documented wildlife at neonicotinoid-treated seed spills with trail cameras and documented consumption of treated seeds.
- We documented neonicotinoid residues in the tissues of hunter-harvested grouse, indicating that those birds were exposed to the pesticides.
- We identified 354 genes affected by imidacloprid exposure through RNA sequencing: 37 affected genes were detected in liver and 317 affected genes were detected in blood cells (which can be non-lethally collected, which may allow future development of detection assays).

The results of this project indicate that seed-eating birds in the wild may be exposed to seeds treated with neonicotinoids in the agricultural landscape through eating at seed spills. Ingestion of neonicotinoid-treated seeds by birds can produce neurological abnormalities that may impair survivability. Exposure can be evaluated through detection of pesticide residues in carcasses, as well as fecal pellets and blood cells. The results of this study may be used by the agricultural industry to reduce impacts to wild birds through education and process

change (reduce spillage), as well as state and federal governmental agencies reviewing appropriate and safe usage of these pesticides.

IV. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1: Development of tools to assess neonicotinoid exposure and impacts in birds

Description:

Immune function is known to be altered by many factors, of which contaminants may be one. Immunology is increasingly being used to study toxicology in wild birds and immune function can be a sensitive indicator of contaminant exposure (Smits et al, 1999). A laboratory exposure study will be conducted at the University of Minnesota College of Veterinary Medicine (UM) to establish the neonicotinoid exposure concentration that impacts immunity. Using domestic chickens as a model species, we will determine what concentrations of imidacloprid (the most common neonicotinoid seed treatments used in Minnesota) effect immunity and what component of the immune system is most impacted by these exposures, providing specific data for Activity 3.

Domestic chickens will be used as our model species given their suitability to captivity and close taxonomic relationship with wild grouse. Using sub-lethal doses of imidacloprid, we will expose chickens at five different dosages (plus controls) and run a panel of assays on each chicken to assess immune function. We will utilize assays that measure antigen-independent cell and humoral-mediated immune responses (Tier I assays), as well antigen specific responses (Tier II assays). Each of these assays is easily adapted to wild bird species and well-documented in the avian literature.

Summary Budget Information for Activity 1:

ENRTF Budget: \$ 88,893
Amount Spent: \$ 79,431
Balance: \$ 9,462

Outcome	Completion Date
1. Lab exposure study and sample collection	30 JUN 2017
2. Laboratory analysis of samples for neonicotinoid concentrations	30 NOV 2017
3. Validate novel sensitive immune assay	30 NOV 2017

Activity Status as of: 30 January 2017

A graduate student has been funded to implement this activity. Study methodology has been refined and a detailed timeline established for the laboratory exposure study and sample collection. Supplies have been purchased in preparation for running the data collection part of the study. A proposal for animal care and use has been developed, submitted and is under review. Data collection for this activity is expected winter/spring 2017 (on schedule).

The subaward terms, conditions and deliverables have been finalized for Southern Illinois University – Carbondale (sample analysis) and the agreement completed.

Activity Status as of: 30 July 2017

Working in study groups of ten chickens, we have completed exposure and sample collection on four dosage groups (dosages 0.039mg/kg, 0.34mg/kg, 3.43mg/kg, and 10.41mg/kg of imidacloprid). On the samples collected, the following assays have been completed: Hemagglutination and hemolysis assay, phytohemagglutinin test, delayed hypersensitivity test. Preliminary data analysis indicates that there is not a statistically significant difference in immune function between the current treatment groups. There has been, however, a documentation of neurological effects in sublethally dosed birds ranging from mild depression to profound sedation.

Amendment request (9/08/2017):

Preliminary data analysis for Activity 1 indicates that there is not a statistically significant difference in immune function between the current treatment groups, although there is substantial individual variability. Based on this preliminary information and a power analysis of the current data, we are requesting the following changes to Activity 1:

- Reduction of dosing levels from six to five: based on preliminary findings, there is no value in assessing dosages lower than 3.3% LD₅₀
- Increase group numbers from 10 to 20: based on a power analysis of the preliminary results, this increase in sample size at each dose level is needed to detect a significant difference in immune function, if it does exist.
- Eliminate the clothianidin exposure groups: Clothianidin has the same mechanism of action of imidacloprid, so while the exposure dose needed to cause a clinical or subclinical effect may be different, the type of effect would likely be similar. By eliminating these groups, we would have the budget and resource capacity to increase the imidacloprid group sizes to 20, allowing us to fully evaluate if there is a statistical difference on immune function.

Activity Status as of: 30 January 2018

Data collection from live animals as well as the majority of laboratory analyses are complete. Groups of 20 domestic chickens were exposed to five doses of imidacloprid (0.04 mg/kg, 0.34 mg/kg, 3.44 mg/kg, 10.41 mg/kg and 15.62 mg/kg). One group of 20 chickens was used as a vehicle control group to represent normal immune function. Data collection and statistical analysis is complete for the following immune function assays: phytohemagglutinin-A (PHA) response test, delayed type hypersensitivity (DTH) test, antibody response to sheep red blood cells, and the microbicidal assay for *Staphylococcus aureus* and *Candida albicans*. There was no detectable immune suppression or stimulation in the five imidacloprid groups. Statistical analysis is currently being done on the complete white blood cell counts. The microbicidal assay using *Escherichia coli* is underway.

The chickens did exhibit significant, dose-dependent neurologic signs after oral imidacloprid exposure. Neurologic signs ranged from mild sedation to complete inability to stand and lack of response to external stimulation in the most severe cases. This data was used to calculate an estimated median effective dose (ED₅₀) that can be used in ecological risk assessments. Moderate clinical signs included moderate sedation, increased respiratory effort, ataxia and whole-body tremors. Additional statistical analysis methods are underway in order to gather more information regarding the potential risk imidacloprid treated seeds may pose to wild granivorous birds.

This portion of the study provides evidence that field realistic doses of imidacloprid may impair avian survival due to neurologic signs, but may not be immunotoxic. These results were presented in poster format at the Society of Environmental Toxicology and Chemistry (SETAC) conference in Minneapolis, MN in November 2017. Additional conference presentation opportunities are being pursued. A manuscript is currently in preparation for publication in the peer reviewed scientific literature.

Activity Status as of: 30 June 2018

Statistical analysis has been completed on the immunotoxicity and an estimated ED₅₀ value has been calculated. As none of the immune assays showed detectable immune suppression, we did not identify a sensitive test (and therefore did not validate).

Activity Status as of: 30 January 2019

Additional statistical analysis has been performed and refined to thoroughly explain the clinical neurologic abnormalities observed in the study. Manuscript writing is well underway with anticipated submission to the peer reviewed literature in April 2019.

Project Status as of: 11 April 2019

Manuscript is in final stages of preparation for submission for publication.

Amendment request as of 4/9/2019:

We request to use leftover funds to further analyze our previously collected samples, looking for neonicotinoid metabolites residues.

Final Report Summary:

This activity demonstrated health impacts of neonicotinoid exposure to chickens, which were used as sentinels for wild, granivorous birds (seed/grain eating). Four groups of chickens were exposed to imidacloprid, a neonicotinoid pesticide commonly used in seed treatments for agricultural crops. Each group received a different dose of imidacloprid, given with food; chickens were treated daily for seven days. Assays were done to evaluate impacts on the immune system functioning and neurological/behavioral abnormalities post-exposure were observed, quantified and recorded. There were changes seen in immune system function assays evaluated in this study between the exposed chickens and the controls. There were significant neurological abnormalities seen in the chickens after ingestion of imidacloprid. The chickens presented dose-dependent abnormalities ranging from no responses through mild sedation to comatose. Additional clinical signs included increased respiratory effort, loss of neurological coordination and tremors. Neurological changes were temporary with chickens recovering within minutes to a few hours.

ACTIVITY 2: Establish risk to wild birds from neonicotinoid-treated agricultural seeds

Description: Using trail cameras, we will document any bird species that forage on spilled or recently planted seeds and the amount consumed. Trail cameras will be placed at the corners of recently planted fields to capture images of birds eating spilled or submerged seeds on tilled land in public ownership at twelve sites in highly agricultural areas. In addition, cameras will be put on simulated seed spills from these natural foraging areas to document the time it takes for birds to discover the spills and the number of seeds consumed in each foraging bout (per bird). Cameras will be placed in locations where risk of theft will be minimized by restricted access or opportunity for concealment.

Field observations of seed spills in recently planted fields will be used to quantify rate of seed spillage by field type (e.g., corn, soybean, wheat) from road-based transects in agricultural areas in the southern and western portions of the state. We will record locations and approximate number of seeds in spills near recently planted fields. To determine the proportion of seed spills that contain neonicotinoid-treated seeds, we will collect seeds from accessible spills and quantitatively assess for seven neonicotinoids.

Finally, feces and/or blood will be collected from grouse at leks in agricultural and non-agricultural areas and analyzed for neonicotinoid residues. Additional samples (ingesta and/or tissue) will be collected from 40-60 hunter-harvested grouse in the fall for analysis. Winter wheat is planted in September and October in Minnesota, so grouse might be newly exposed to treated seeds in the fall.

Summary Budget Information for Activity 2:

ENRTF Budget: \$ 118,837
Amount Spent: \$ 118,837
Balance: \$ 0

Outcome	Completion Date
1. Camera study to document which species of birds consume spilled seeds	03 June 2017

2. Transect study to estimate seed spillage rates in Minnesota	30 June 2017
3. Analysis of grouse tissues for neonicotinoid residues	30 March 2018

Activity Status as of: 30 January 2017

The subaward terms, conditions and deliverables have been finalized for the Minnesota Department of Natural Resources and the agreement completed. Thirty-one samples have been collected from hunter-harvested sharp-tailed grouse with an additional 19 samples from hunter-harvested prairie-chickens and pheasants. These latter samples will be considered for inclusion in this study (via amendment) if needed to complete deliverables for hunter-harvested samples to demonstrate exposure of wild birds to neonicotinoids (pending final numbers of grouse samples acquired). All samples have been sent to Southern Illinois University – Carbondale where they are pending analysis.

Position advertisements have been posted for seasonal technicians to collect field samples in spring 2017. These postings will close on January 30th and candidate selection finalized in February/March.

Activity Status as of: 30 July 2017

In spring 2017, we placed 40 trail cameras to capture video at simulated spills at each of 24 privately-owned fields and 16 WMAs. WMAs were selected to have food plots or Cooperative Farming Agreements (CFAs) and a land cover composition similar to that of the surrounding landscape based on the 2014 National Cropland Data Layer (USDA-NASS 2015). Spills were simulated with 1,000 wheat, corn, or soybean seeds. We checked cameras once weekly to replace batteries and data cards and deployed cameras in each location for 2 weeks. Videos will be examined in the upcoming months. Videos from our 2016 DNR-funded pilot study documented brown-headed cowbirds (*Molothrus ater*), red-winged blackbirds (*Agelaius phoeniceus*), Harris’s Sparrow (*Zonotrichia querula*), American crows (*Corvus brachyrhynchos*), blue jays (*Cyanocitta cristata*), and brown thrashers (*Toxostoma rufum*) consuming treated seeds at spills.

To quantify seed spills, we drew a spatially balanced sample of 50 townships with at least 50% of the area planted in soybean, corn, or wheat during 2014 (USDA NASS 2015) and at least 50 miles of roads (DOT 2008). We surveyed the 38 most western townships selected due to a later start to planting during the spring of 2017. We began in the southern counties in late April and worked north as crops were planted. We recorded locations and approximate spill size near recently planted fields with the DNRSurvey mobile computer application. We recorded each quarter quarter-section in agricultural production, whether any part of it was recently planted (i.e., \leq early seedling stage), documented the size of seed spills on the road, field edge, or visible in the field, and crop type (when possible). Data will be analyzed for upcoming reports.

We collected fresh fecal pellets from prairie grouse leks during 2017, based on findings from our pilot study which indicated that feces were more reliable indicators of recent exposure to neonicotinoids than blood samples. We collected fresh fecal pellets from 46 sharp-tailed grouse leks and 27 prairie-chicken leks in 2017. Samples will be sent for analysis at SIUC.

Activity Status as of: 30 January 2018

In July 2017, we sent 182 samples to Southern Illinois University Carbondale for neonicotinoid analysis. These samples included 27 greater prairie-chicken fecal pellets, 47 sharp-tailed grouse fecal pellets, 7 sharp-tailed grouse livers, and 101 seed samples collected from seed spills or used in seed exposure experiments.

In December 2017, we sent 52 samples to SIUC for analysis of neonicotinoid concentrations. These samples included 17 greater prairie-chicken livers, 27 sharp-tailed grouse livers, 1 liver from an unidentified prairie grouse, 4 sharp-tailed grouse fecal pellet samples, and 3 gizzards with contents- 2 from sharp-tailed grouse and 1 from a greater prairie-chicken. The livers and gizzards were from hunter-harvested submissions in fall 2017. This was the last shipment of samples for analysis.

Videos of seed spills from the 2017 field season are still being reviewed. GIS Analysis is underway to quantify seed spill rates in 2017. Laboratory results have not yet been received for 2017 samples. Progress will continue and be included in the next report.

Activity Status as of: 30 June 2018

Final laboratory results were received from SIUC on June 29, 2018. Data analysis is underway on these results. Statistical analyses of all previous results are underway in preparation for manuscripts.

Activity Status as of: 30 January 2019

We plan to complete review of videos at seed spills in the next few weeks and are in process of preparing manuscripts for submission to peer-reviewed journals.

Project Status as of: 11 April 2019

All work completed and first manuscript submitted for publication.

Final Report Summary:

Activity 2 demonstrated the availability of pesticide-treated seeds to wildlife on the agricultural landscape, documented wild birds and mammals consuming these seeds and also documented neonicotinoid exposure in wild birds. Transect surveys were conducted throughout Minnesota's agricultural townships and both locations and approximate size of seed spills were recorded. All work was done from public roads. Seeds and seed spills were quantified on the soil surface after spring planting. Follow-up surveys were down to document what crops were planted at each survey point.

Forty trail cameras were used to capture video at simulated seed spills on privately-owned fields and wildlife management areas, and to document wildlife species eating the seeds. Over a dozen species of birds and mammals consumed seeds at these spills. Bird species included pheasants, geese, wild turkeys, doves, blue jays, brown thrasher, rose-breasted grosbeak, various sparrow species and blackbirds.

In order to document neonicotinoid exposure in wild birds, over 80 fecal pellets from grouse were collected from leks and analyzed for neonicotinoid residues. Pilot work for this project had previously demonstrated persistence of neonicotinoid residues in feces for up to two weeks after exposure. In addition to finding residues in fecal pellets from wild grouse, tissues from over 80 hunter-harvested birds (sharp-tailed grouse, prairie chickens and pheasant) were also found to have residues, documenting exposure in free-ranging game birds in two different ways.

ACTIVITY 3: Quantify impacts of sub-lethal exposure to neonicotinoid mixtures on the immune system

Description: Using the results of Activity 1, we will determine the quantitative relationship between neonicotinoid residues in tissues and immune function to provide direct information for field-based residue studies in wild grouse. The surrogate species, chicken, will be exposed to a single neonicotinoids (imidacloprid), and dose-dependent immune suppression will be measured using RNA sequencing and gene expression framework to evaluate immune function, which will be correlated with neonicotinoid residues in tissues. Our study will provide the necessary link between effects information ascertained via controlled laboratory experiments with field studies aimed at assessing exposure in wild grouse. Residues will be measured in liver tissue and excreta. In addition, we will measure immunity using the most sensitive assay determined in Activity 1 (if a statistically significant impact is found) and gene expression in white blood cells acquired from a blood sample. Gene expression will allow us to identify biomarkers of exposure and effect in neonicotinoid exposed birds and will be assessed against immune function and residue concentrations to provide managers with non-lethal assays to understand exposure and effect in wild birds.

Summary Budget Information for Activity 3:

ENRTF Budget: \$ 141,270

Amount Spent: \$ 141,151

Balance: \$ 119

Outcome	Completion Date
1. Measurement of immune toxicity in exposed chickens	31 JAN 2018
2. Analysis of chicken tissue residues for neonicotinoids	31 JAN 2018
3. Complete data analysis of relationship between exposure and immune effects	30 JUN 2019

Activity Status as of: 30 January 2017

Other than finalization of subaward (SIUC), this activity is pending completion of Activity 1.

Activity Status as of: 30 July 2017

A post-doctoral candidate with expertise in ecotoxicogenomics and wild birds has recently been hired for Activity 3. He has reviewed the proposed protocols and methodologies, as well as the preliminary data from Activity 1 and suggested a revised protocol for Activity 3 (see amendment request).

Amendment request (9/08/2017):

We are proposing to amend Activity 3 based on the results of Activity 1. The use of genomics (RNA sequencing) is a more sensitive assay of toxicity than the assays used in Activity 1. We propose to add a second phase of RNA sequencing sampling (cryo-preserved organ tissues in addition to peripheral blood mononuclear cells). We are also proposing to remove the combined exposure groups as we will have no results for clothianidin exposure from Activity 1. The number of tissues evaluated for neonicotinoid residues will be reduced to two (liver and excreta) to allow budget space for the second phase of RNA sequencing as our residue results from Activity 1 are expected to be adequate for our analysis.

Activity Status as of: 30 January 2018

We completed our 23-day exposure experiments and collected RNA-quality blood and tissue samples. In total, we had 40 chickens divided into three treatments of imidacloprid exposures (2.72, 5.43, and 10.86 mg/kg - determined based on immune and behavioral functions in Activity 1) and one control group. We followed our time-series sampling strategy with blood samples were collected through 4 sampling occasions (day 7, 9, 16, and 23) and tissues were collected through 3 different end-points (12 chickens on day 9, 12 on day 16, and 16 on day 23). Laboratory work, including the isolation of peripheral blood mononuclear cells from whole blood samples and for the preservation of tissues was completed. RNA extraction and quantification from peripheral blood mononuclear cells and from preserved tissue samples is currently being performed. Because a statistically significant immune impact was not found in Activity 1, that assessment was not done for Activity 3.

Activity Status as of: 30 June 2018

RNA sequencing data has been generated from tissues collected during the exposure trials. Bioinformatics analysis was done to prepare sequences, which were then aligned with the Chicken Reference Genome and expressed genes in our samples recorded. A total of 24,881 expressed genes were detected in our 48 samples and preliminary pairwise differential analyses identified 499 genes having significant expressions. More than one third of the significant expressed genes belong to peripheral blood cells, which may be the basis for future development of a non-lethal exposure detection method.

Activity Status as of: 30 January 2019

Laboratory analysis of imidacloprid residues was completed. We found detectable levels of imidacloprid residues in livers that corresponded to dose group: none detected in the liver of the control group; 3 ng/g in liver of the 2.72 mg/kg treatment group (low dose); 8.8 ng/g in liver of the 5.43 mg/kg treatment group (medium dose), and 13.9 ng/g in the liver of the 10.86 mg/kg treatment group (high dose). Two phases of RNA sequencing data from 85 samples – including 58 peripheral blood mononuclear cell (PBMC—an assemblage of specific circulating

immune cells) samples, 16 brain tissue samples, and 16 liver tissue samples – was also completed. Bioinformatics analyses and gene expression analyses are completed. There were 24,881 genes observed in our samples. Based on a rigorous analysis of the current data, we detected:

- A total of 354 genes were affected by imidacloprid exposure.
- Specifically, the 58 blood samples had 317 significantly expressed genes which were distinctly different between groups and the number of affected genes increased with dose: 33 genes were changed in the control group; 55 genes in the low dose group; 84 genes in the medium dose group; and 145 genes in the high dose group.
- The 16 liver samples had only 37 affected genes detected. There was no gene which was significantly different in treated birds compared to control birds among all 16 tissue samples.
- The vast majority of the affected genes in PMBCs (259 of 317 genes, or 82%) were down-regulated, an affect that correlated with dose level. In contrast, 20 out of 28 expressed genes (71%) in livers were up-regulated.
- Statistical analysis showed that the 317 significantly expressed genes in PMBCs are different than the 37 affected genes in livers, in terms of their associated physiological functions, and in their correspondence to dose level (significance between treatment groups in PMBC genes, but not in brains or livers).

This portion of the study provides strong preliminary evidence that non-lethally acquired blood cells (i.e., PMBCs) constitute a sampling medium from which a molecular assay (a potential biomarker) may be developed for the detection of a bird's response to imidacloprid exposure. We have developed novel evidence indicating that the approach is *sensitive* to imidacloprid exposure, but do require further laboratory and field evidence for the *specificity* of the approach to imidacloprid/neonicotinoid exposure before field applications are warranted. In sum, the identification of a dose-dependent change in gene expression in non-lethally acquired immune cells is a significant contribution toward the goal of equipping scientists and managers with a means to detect a bird that has responded to imidacloprid exposure. Further work within the scope of this project will include the linking of the detected changes in gene expression to biological function as well as to doses known to alter bird behavior as reported in Activity 2 and that we detected through our field work in Activity 1.

Project Status as of: 11 April 2019

Manuscript in preparation for publication.

Final Report Summary:

In Activity 3, genetic analysis was used to identify potential biomarkers in chickens exposed to imidacloprid orally. RNA-sequencing was used to evaluate samples collected from exposed chickens to identify impacts on gene expression (the effect a gene has).

Four groups of chickens were exposed to imidacloprid doses identified in the earlier work (Activity 1). Two types of tissue samples (liver, brain) as well as peripheral blood cells were genetically sequenced. Liver and feces were also analyzed for residue analysis. Imidacloprid was detected in livers and feces in a dose-dependent manner. A total of 354 genes were affected by imidacloprid; 37 of these were found in liver samples and 317 were identified in blood samples, with the number of affected genes increasing with dose. The identification of significant gene expression alteration in blood cells may be the basis for future development of a non-lethal exposure detection method.

- Samples from chickens exposed to oral imidacloprid were evaluated for impacts on the expression of genes through RNA sequencing
- Imidacloprid residues were detected in livers in a dose-dependent manner. The liver samples had 37 genes affected by the imidacloprid exposure while peripheral blood cells (which can be collected non-lethally) had 317 affected genes.
- This information may be used in the future to develop an assay to identify non-lethal methods of detecting imidacloprid exposure in birds.

V. DISSEMINATION:

Description:

This study will help ensure that food plots and crops on state managed lands are planted with seed safe for wildlife. We will use outreach to inform stakeholders and partners managing for wildlife. This study would be among the first to examine exposure and consumption of these pesticides in wild birds, with broader impacts extending to population and pesticide management.

Our findings will be communicated with state (e.g. DNR) and federal (e.g. USFWS) land managers, as well as agencies tasked with agricultural regulation and environmental protection (MDA, USDA, EPA). Findings will be presented at state, regional, and national meetings (e. SETAC, TWS) as appropriate given the results. Publications will be produced for peer-reviewed journals, outreach newsletters, and annually for the DNR's Summaries of Wildlife Research Findings. Media outreach will also be pursued.

Status as of: 30 January 2017

No activity to date.

Status as of: 30 July 2017

Findings to date have been compiled as part of the Annual DNR Wildlife Research Summaries and will be posted online following internal review. In addition, an abstract for a scientific poster presentation has been submitted and accepted by SETAC (Society of Environmental Toxicology and Chemistry) for their annual conference.

Status as of: 30 January 2018

In November 2017, Drs. Roy and Franzen-Klein attended the meeting of the Society for Environmental Toxicology and Chemistry in Minneapolis. Dr. Roy gave a presentation on the results of Activity 2 in this study and Dr. Franzen-Klein submitted a poster on Activity 1 results. Feedback was good. A representative from the Environmental Protection Agency (EPA) Headquarters Office of Pesticide Programs, Environmental Fate and Effects Division asked Dr. Roy to provide a webinar this winter for EPA staff and to provide public comments on draft risk assessment documents as appropriate. Representatives from Bayer Crop Science asked for recommendations to reduce seed spill rates. We suggested that they survey farmers to learn more about the problem and possible solutions.

In November 2017, Dr. Ponder attended the Raptor Research Foundation annual meeting in Salt Lake City, UT and presented on Exploring the Risk of Neonicotinoids in Wild Birds based on results from this study.

The results have also been presented at two internal seminar presentations at the University of Minnesota's College of Veterinary Medicine.

Status as of: 30 June 2018

Results to date have been summarized and submitted to the Environmental Protection Agency in response to their public commentary period for re-registration. In addition, Drs. Roy, Jankowski and Ponder participated in a webinar to share our results directly with employees in the EPA Office of Pesticide Programs in April 2018 and Dr. Jankowski led a presentation to the EPA Office of Environmental Review and Assessment in July.

In February 2018, Dr. Roy met with a group of Agricultural stakeholders representing the MN Farm Bureau and MN Crop Production Retailers. Dr. Ponder attended remotely to discuss options to clean up spills and reduce exposed seed in Minnesota. Internal communications include several presentations to Department of Natural Resource staff by Dr. Roy and a graduate student research seminar at the University of Minnesota by Dr. Franzen-Klein.

A scientific poster sharing the results of Activity 1 was presented at the International Conference on One Medicine, One Science in April 2018.

Status as of: 30 January 2019

The results of this study were presented to the clinical veterinary community at the ExoticsCon conference in September 2018. Other presentations completed in 2018 and planned in 2019 include:

- 1) Crop Production Retailers (stakeholder group), Dec 11 2018, Minneapolis
- 2) DNR Wildlife Managers on 4 dates at locations statewide (state managers), 2018
- 3) University of Minnesota- Mankato, Department of Biological Sciences, Nov 2 2018, Mankato
- 4) Minnesota Prairie Chicken Society (stakeholder group), Apr 21 2018, Glyndon
- 5) Minnesota Chapter of the Wildlife Society (state, federal and non-profit natural resource managers), Feb 20 2019, Duluth
- 6) Crop Production Retailers (stakeholder group), Mar 29 2019, Owatonna

In addition, the final report for Activity 2 is available online at:

https://files.dnr.state.mn.us/wildlife/research/summaries/forest/2016_neonictoids.pdf

Project Status as of: 11 April 2019

Manuscript submitted. Additionally, the work has been presented at The Wildlife Society (MN Chapter) and to seed distributors at UMN Extension Services training.

Final Report Summary:

Scientific papers: 1 published, 1 submitted, 4 pending:

- The first manuscript, *Multi-scale availability of neonicotinoid-treated seed to wildlife in an agricultural landscape during spring planting*, has been accepted for publication in Science of the Total Environment and is available on-line: <https://www.sciencedirect.com/science/article/pii/S0048969719320212>
- The second manuscript, *Evaluation of Neurobehavioral Abnormalities and Immunotoxicity in Response to Oral Imidacloprid Exposure in Domestic Chickens (Gallus gallus domesticus)*, has been submitted for publication.
- A third manuscript is in process of being finalized for submission

Oral presentations:

- Activity 2 results presented in November 2017 at the Society for Environmental Toxicology and Chemistry: *Neonicotinoids on the Landscape: Evaluating Avian Exposure to Treated Seeds in an Agricultural Region*.
- Initial results presented in *Effects of oral neonicotinoid exposure on domestic chickens (Gallus gallus domesticus)*, at the Raptor Research Foundation annual conference, November 2017
- Results presented at two internal seminars at the University of Minnesota
- Crop Production Retailers (stakeholder group), Dec 11 2018, Minneapolis
- DNR Wildlife Managers on 4 dates at locations statewide (state managers), 2018
- University of Minnesota- Mankato, Department of Biological Sciences, Nov 2 2018, Mankato
- Minnesota Prairie Chicken Society (stakeholder group), Apr 21 2018, Glyndon
- Minnesota Chapter of the Wildlife Society (state, federal and non-profit natural resource managers), Feb 20 2019, Duluth
- Crop Production Retailers (stakeholder group), Mar 29 2019, Owatonna
- ExoticsCon/Association of Avian Veterinarians Annual Conference 2018, Wildlife Track: *Potential Risks to Wild Birds from Neonicotinoid Pesticides*

- Prairie Grouse Technical Council (Bartlesville, OK), Nov 2019: *Neonicotinoids on the Landscape: Evaluating Avian Exposure to Treated Seeds in an Agricultural Region* (pending)

Other communications:

- Activity 1 results presented in poster format at the Society for Environmental Toxicology and Chemistry in November 2017: *Effects of oral neonicotinoid exposure on immune function in domestic chickens*
- Results submitted and comments shared in response to the Environmental Protection Agency public commentary period for re-registration process for neonicotinoids
- In Feb 2018, results were shared with various farming stakeholder groups
- The interim report for Activity 2 is available on-line on the DNR web site at: https://files.dnr.state.mn.us/wildlife/research/summaries/forest/2016_neonictoids.pdf
- A poster, *Effects of oral neonicotinoid exposure on immune function in domestic chickens*, was presented at the International Conference on One Medicine, One Science (April 2018).
- A poster, *Tracking the transcriptome: a non-lethal indicator of exposure to neonicotinoids in birds*, has been accepted for a poster in November, 2019 at Society for Environmental Toxicology and Chemistry annual conference.

VI. PROJECT BUDGET SUMMARY:

A. ENRTF Budget Overview:

Budget Category	\$ Amount	Overview Explanation
Personnel:	\$ 121,583	Post-doc (1 year, 1 FTE) responsible for project management, laboratory studies and data collection/analysis Graduate student (1 year, 1 FTE) responsible for laboratory studies, data collection and analysis. Lab technicians (1 year, .2 FTE) to run perform immune assays
Professional/Technical/Service Contracts:	\$ 222,967	Subcontract to DNR for field collection of samples (200 samples), field observations around state and camera study (12 sites): \$98,978 Subcontract to Southern Illinois University, Carbondale (SIUC) for laboratory analysis of neonicotinoid residues (350 samples), production of stock supplies for analysis: \$98,978 Research animal housing for lab studies (Activity 1 – 28 days/130 chickens; Activity 3 – 28 days/48 chickens): \$13,944 Research laboratory (UMN) for RNA sequencing (36 samples @\$350): \$12,067
Equipment/Tools/Supplies:	\$ 4,450	Consumables for laboratory studies and immune assays (sample collection supplies, antigen for immune studies, plates for immune assays, chicken acquisition - 178)
TOTAL ENRTF BUDGET: \$ 349,000		

Explanation of Use of Classified Staff: This is not classified staff, but we need to contract with SIUC for sample analysis because there are not labs in Minnesota that will quantify residues in animal tissues. SIUC lab has established analytical methods and applied the methods to various projects in the past. Minnesota Department of Agriculture does to neonicotinoid assays, but their minimum level of detection is not sensitive enough and they have not established methods for detection in tissues.

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

Number of Full-time Equivalentents (FTE) Directly Funded with this ENRTF Appropriation: 2.2 FTE

Number of Full-time Equivalentents (FTE) Estimated to Be Funded through Contracts with this ENRTF Appropriation: 1.67

B. Other Funds:

Source of Funds	\$ Amount Proposed	\$ Amount Spent	Use of Other Funds
Non-state			
	\$	\$	
State			
University of Minnesota	\$ 182,552	\$ 90,947	53% indirect rate
TOTAL OTHER FUNDS:	\$ 182,552	\$	

VII. PROJECT STRATEGY:

A. Project Partners:

Dr. Julia Ponder, University of Minnesota, Avian and Conservation Medicine – PI, oversight of lab studies
 Dr. Charlotte Roy, MN DNR, Research Scientist – co-PI, oversight of field studies
 Dr. Da Chen, SIUC, Assistant Professor of Environmental Chemistry– co-PI, laboratory analysis of samples
 Dr. Mark Jankowski, USEPA, Ecotoxicologist – consultant for lab study design and interpretation

B. Project Impact and Long-term Strategy:

This study will provide information about the safety of neonicotinoid seed treatments to birds, using sharp-tailed grouse as a model. It will provide information to assess the risk of consumption of seeds and evaluate whether other bird species are potentially at risk for exposure. This study would be the first to holistically examine exposure to mixtures of these pesticides in wild birds. We know insects are at risk from neonicotinoids, but the information gained will be important for more informed management of risk to vertebrates.

C. Funding History:

Funding Source and Use of Funds	Funding Timeframe	\$ Amount
DNR pilot funding for camera work and small numbers of grouse samples for residue analysis to inform LCCMR study	July 2015 – June 2016	\$ 96,500

VIII. FEE TITLE ACQUISITION/CONSERVATION EASEMENT/RESTORATION REQUIREMENTS: N/A

IX. VISUAL COMPONENT or MAP(S): Attached

X. RESEARCH ADDENDUM: Submitted

XI. REPORTING REQUIREMENTS:

Periodic work plan status update reports will be submitted no later than 30 January 2017, 30 July 2017, and 30 January 2018. A final report and associated products will be submitted between June 30 and August 15, 2018.

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



Project Title: Game and Nongame Bird Pesticide Exposure

Legal Citation: M.L. 2016, Chp. 186, Sec. 2, Subd. 03m

Project Manager: Julia B. Ponder, DVM, MPH

Organization: University of Minnesota

M.L. 2016 ENRTF Appropriation: \$ 349,000

Project Length and Completion Date: 2 Years, June 30, 2018

Date of Report: August 1, 2019

**ENVIRONMENT AND NATURAL RESOURCES TRUST
 FUND BUDGET**

BUDGET ITEM	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
Personnel (Wages and Benefits)	\$60,073	\$60,073	\$0				\$78,900	\$78,900	\$0	\$138,973	\$0
TBD: Post-doc - 1 FTE, \$56,010 (80%- salary, 20% benefits), 1 year											
TBD: Graduate student - 1 FTE, (55% salary, 45% benefits) 1 year = \$56,010											
TBD: Technician - 0.2 FTE \$9,563 (77.6% salary, 22.4% benefits), 1 year											
Professional/Technical/Service Contracts											
MN DNR: field collection of grouse samples over 2 seasons plus camera study and seed spillage documentation				\$79,224	\$79,224	\$0				\$79,224	\$0
Southern Illinois University, Carbondale (SIUC): laboratory analysis of neonicotinoid residues	\$2,980	\$2,980	\$0	\$39,613	\$39,613	\$0	\$34,022	\$33,987	\$35	\$76,615	\$35
UMN Research laboratory: RNA sequencings							\$24,129	\$24,129		\$24,129	\$0
D Chen Lab services - metabolite analysis	\$10,000	\$538	\$9,462							\$10,000	\$9,462
University of Minnesota Research Animal Resources: research subject housing and oversight	\$3,152	\$3,152	\$0				\$819	\$819	\$0	\$3,971	\$0
Equipment/Tools/Supplies	\$12,688	\$12,688	\$0				\$3,300	\$3,300	\$0	\$15,988	\$0
Laboratory consumables (\$4,000)											
Acquisition of research subjects (chickens) (\$250)											
Travel expenses in Minnesota											
Mileage to pick up chickens	\$0		\$0				\$100	\$16	\$84	\$100	\$84
COLUMN TOTAL	\$88,893	\$79,431	\$9,462	\$118,837	\$118,837	\$0	\$141,270	\$141,151	\$119	\$349,000	\$9,581