



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

**Date of Report:** May 29, 2016

**Date of Next Status Update Report:** January 30, 2017

**Date of Work Plan Approval:** June 7, 2016

**Project Completion Date:** 30 June 2018

**Does this submission include an amendment request?** No

**PROJECT TITLE:** Game and Nongame Bird Pesticide Exposure

**Project Manager:** Julia B Ponder, DVM, MPH

**Organization:** University of Minnesota dba The Raptor Center

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**Location:** Statewide

**Total ENRTF Project Budget:**

**ENRTF Appropriation:** \$349,000

**Amount Spent:** \$0

**Balance:** \$349,000

**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:**

**Project Status as of:** 30 January 2017

**Project Status as of:** 30 July 2017

**Project Status as of:** 30 January 2018

**Overall Project Outcomes and Results:**

**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 chickens as a model species, we will determine what concentrations of imidacloprid and clothianidin (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.

Chickens will be used as our model species given their suitability to captivity and close taxonomic relationship with grouse. Using sub-lethal doses of two neonicotinoids, imidacloprid and clothianidin, we will expose chickens at six 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:** \$ 76,223  
**Amount Spent:** \$ 0  
**Balance:** \$ 76,223

<b>Outcome</b>	<b>Completion Date</b>
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

**Activity Status as of:** 30 July 2017

**Activity Status as of:** 30 January 2018

**Final Report Summary:**

**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 discovery 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 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: \$ 137,591**  
**Amount Spent: \$ 0**  
**Balance: \$ 137,591**

<b>Outcome</b>	<b>Completion Date</b>
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

**Activity Status as of:** 30 July 2017

**Activity Status as of:** 30 January 2018

**Final Report Summary:**

**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 single and combined neonicotinoids (imidacloprid and clothianidin), and immunity will be measured using the assay determined in Activity 1 to be the most sensitive to imidacloprid and clothianidin. 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 blood, excreta, and organ tissues. In addition, we will measure immunity using the most sensitive assay determined in Activity 1 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: \$ 135,187**  
**Amount Spent: \$ 0**  
**Balance: \$ 135,187**

<b>Outcome</b>	<b>Completion Date</b>
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 2018

**Activity Status as of:** 30 January 2017

**Activity Status as of:** 30 July 2017

**Activity Status as of:** 30 January 2018

**Final Report Summary:**

**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

**Status as of:** 30 July 2017

**Status as of:** 30 January 2018

**Final Report Summary:**

**VI. PROJECT BUDGET SUMMARY:**

**A. ENRTF Budget Overview:**

<b>Budget Category</b>	<b>\$ Amount</b>	<b>Overview Explanation</b>
Personnel:	\$ 121,583	Post-doc (2 years, 1 FTE) responsible for project management, laboratory studies and data collection/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): \$97,978 Subcontract to Southern Illinois University, Carbondale (SIUC) for laboratory analysis of neonicotinoid residues (350 samples ), production of stock supplies and graduate student 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): \$12,600

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)
<b>TOTAL ENRTF BUDGET:</b>	<b>\$ 349,000</b>	

**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 Equivalents (FTE) Directly Funded with this ENRTF Appropriation:** 2.2 FTE

**Number of Full-time Equivalents (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
<b>Non-state</b>			
	\$	\$	
<b>State</b>			
University of Minnesota	\$ 182,552	\$	52% indirect rate
<b>TOTAL OTHER FUNDS:</b>	<b>\$ 182,552</b>	<b>\$</b>	

**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, USFWS, 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  
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

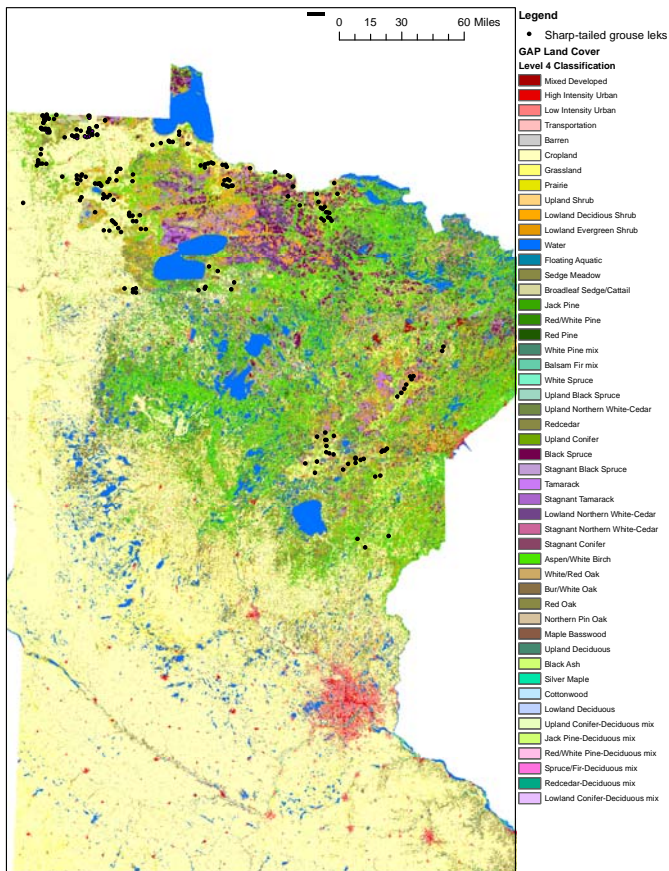
**Project Length and Completion Date:** 2 Years, June 30, 2018

**Date of Report:** May 29, 2016

ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET	Activity 1 Budget	Amount Spent	Activity 1 Balance	Activity 2 Budget	Amount Spent	Activity 2 Balance	Activity 3 Budget	Amount Spent	Activity 3 Balance	TOTAL BUDGET	TOTAL BALANCE
<b>BUDGET ITEM</b>	Development of tools to assess neonicotinoid exposure and impacts in birds			Establish risk to wild birds from NN-treated seeds			Quantify impacts of sub-lethal exposure to neonicotinoid mixtures on the immune system				
<b>Personnel (Wages and Benefits)</b>	\$60,073		\$60,073				\$61,510		\$61,510	\$121,583	\$121,583
TBD: Post-doc - 1 FTE, \$112,020 (80%- salary, 20% benefits), 2 years											
TBD: Technician - 0.2 FTE \$9,563 (77.6% salary, 22.4% benefits), 1 year											
<b>Professional/Technical/Service Contracts</b>											
MN DNR: field collection of grouse samples over 2 seasons plus camera study and seed spillage documentation				\$97,978		\$97,978				\$97,978	\$97,978
Southern Illinois University, Carbondale (SIUC): laboratory analysis of neonicotinoid residues	\$2,980		\$2,980	\$39,613		\$39,613	\$55,852		\$55,852	\$98,445	\$98,445
UMN Research laboratory: RNA sequencings							\$12,600		\$12,600	\$12,600	\$12,600
University of Minnesota Research Animal Resources: research subject housing and oversight	\$10,920		\$10,920				\$3,024		\$3,024	\$13,944	\$13,944
<b>Equipment/Tools/Supplies</b>	\$2,150		\$2,150				\$2,100		\$2,100	\$4,250	\$4,250
Laboratory consumables (\$4,000)											
Acquisition of research subjects (chickens) (\$250)											
<b>Travel expenses in Minnesota</b>											
Mileage to pick up chickens	\$100		\$100				\$100		\$100	\$200	\$200
<b>COLUMN TOTAL</b>	<b>\$76,223</b>	<b>\$0</b>	<b>\$76,223</b>	<b>\$137,591</b>	<b>\$0</b>	<b>\$137,591</b>	<b>\$135,186</b>	<b>\$0</b>	<b>\$135,186</b>	<b>\$349,000</b>	<b>\$349,000</b>



# Do neonicotinoids pose a threat to Minnesota's birds?



Wild birds may consume neonicotinoid treated seeds spilled during seed loading into farm machinery or available in recently planted crop fields at a rate sufficient to cause health impacts.

Sharp-tailed grouse lek locations and landcover data illustrating range overlap with croplands in the northwest and areas with less agriculture in the east-central part of their range



A treated seed spill (photo by G. Hoch)



Neonicotinoid treated seeds  
Photo from Scientificbeekeeping.com

