

2019 Project Abstract

For the Period Ending June 30, 2022

PROJECT TITLE: Mercury and PFAS risk to Minnesota raptors

PROJECT MANAGER: Matthew Etterson

AFFILIATION: Hawk Ridge Bird Observatory, Duluth, MN

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FUNDING SOURCE: Environment and Natural Resources Trust Fund

LEGAL CITATION: M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 03c

APPROPRIATION AMOUNT: \$250,000

AMOUNT SPENT: \$211,703

AMOUNT REMAINING: \$38,297

Sound bite of Project Outcomes and Results

These results are a first look at polyfluoralkyl substances (PFAS) in Minnesota's Birds of Prey. Among vertebrates, birds appear to be most vulnerable to PFAS effects, which can result in reproductive failure. Birds of Prey are vulnerable to PFAS because of their position atop both aquatic and terrestrial food webs.

Overall Project Outcome and Results

Polyfluoralkyl substances (PFAS) are a class of chemicals used in industrial processes and fire suppression. Mercury (Hg) enters the environment from point-source releases due to industrial processes and through combustion of coal for power generation. Both Hg and PFAS are present locally at highly contaminated sites and ubiquitously due to atmospheric deposition. Due to their predatory nature, Birds of Prey are at unique and elevated risk of exposure to both PFAS and Hg which concentrate in animal tissues; with each link in their food chain, predators consume and concentrate these toxicants contained in their prey. We collected blood and feather samples from 355 birds of prey at two Minnesota locations, Hawk Ridge in Duluth, and The Raptor Center in St. Paul. We analyzed blood plasma for up to 40 PFAS chemicals and feathers for total mercury concentration. Our objectives were to (1) collect baseline data on exposure of MN raptors to Hg and PFAS and (2) to test specific hypotheses about patterns of exposure in relation to ecological variables such as diet, age, sex, and species identity. As expected, perfluorooctane sulfonate (PFOS) was the PFAS of highest concentration across all species and in each individual species, typically at concentrations ten or more times that of other PFAS. Bald Eagle (*Haliaeetus leucocephalus*) had the highest exposure, probably reflecting the greater representation of aquatic prey (fish) in its diet. However, Cooper's Hawks (*Accipiter cooperii*) had comparable exposures and they consume entirely terrestrial prey. Among ecological variables, species identity offered the greatest explanatory power, followed by a measure of species' tolerance of human activity, which may serve as a proxy for likelihood of exposure. These results will prove invaluable for understanding and managing both human and ecological exposures to PFAS and Hg in Minnesota.

Project Results Use and Dissemination

Dr. Etterson has presented results at internal meetings of the US Environmental Protection Agency's PFAS Working Group at the Great Lakes Toxicology and Ecology Division, Duluth, MN. Final PFAS results were received from the contract laboratory on 29 June 2022, just prior to the close of the project period and we expect dissemination activity to increase considerably over the next year. Dr. Ponder will present some results from this work at the upcoming International Ornithological Congress in Durban, South Africa, August 2022. We expect at least two manuscripts will be submitted to peer-reviewed journals for publication in the coming year.



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

Today's Date: 02 August 2022

Final Report

Project Completion Date: 30 June 2022

PROJECT TITLE: Mercury and PFAS risk to Minnesota raptors

Project Manager: Matthew Etterson

Organization: Hawk Ridge Bird Observatory

College/Department/Division:

Mailing Address: 6770 Haugen Lane

City/State/Zip Code: Duluth, MN 55803

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Email Address: metterso@d.umn.edu

Web Address: <https://www.hawkridge.org/>

Location: NE MN, Statewide

Total Project Budget: \$250,000.00

Amount Spent: \$211,703.29

Balance: \$38,296.71

Legal Citation: M.L. 2019, First Special Session, Chp. 4, Art. 2, Sec. 2, Subd. 03c

Appropriation Language: \$250,000 the first year is from the trust fund to the commissioner of natural resources for an agreement with the Hawk Ridge Bird Observatory to quantify the exposure and health risk of two environmental neurotoxins to Minnesota raptors.



Environment and Natural Resources Trust Fund (ENRTF)

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I. PROJECT STATEMENT:

We will quantify exposure to two environmental contaminants for 11 species of Minnesota raptors. Mercury and polyfluoralkyl substances (PFAS) threaten the health of raptor species in Minnesota and worldwide.

- Both PFAS and Hg are **neurotoxins** and are known to cause **reproductive failure** in birds.
- In Minnesota, PFAS has received considerable recent attention due to water contamination near the Twin Cities, raising **human health concerns**.
- Raptors are at **elevated risk** due to their position atop the food chain

With the knowledge gained through this work, we will be able to compare exposure in Minnesota raptors to tissue concentrations known to cause adverse effects in other bird species.

PFAS is one of a class of Persistent Bioaccumulative Toxins (PBTs) used in industrial processes and fire suppression. It is globally distributed through atmospheric transport. Mercury enters the environment from point-source releases due to industrial processes and through combustion of coal for power generation. Both Hg and PFAS are present locally at highly contaminated sites and ubiquitously due to atmospheric deposition. Raptors, due to their predatory nature, are at unique and elevated risk of exposure to PBTs, which, by definition, concentrate in animal tissues; with each link in their food chain, predators consume and concentrate the toxicants contained in their prey.

Preliminary studies of Hg in raptors at Hawk Ridge Bird observatory (HRBO; T. Keyel. 2016, MS Thesis, Univ. MN Duluth) showed increased exposure to Hg among Minnesota raptors known to eat birds. With this research we will expand our Hg studies to other species and perform similar research on PFAS. Our species list will consist of:

- | | |
|---|---|
| • Bald Eagle (<i>Haliaeetus leucocephalus</i>) | • Great Horned Owl (<i>Bubo virginianus</i>) |
| • Sharp-shinned Hawk (<i>Accipiter striatus</i>) | • Northern Saw-whet Owl (<i>Aegolius acadicus</i>) |
| • Cooper's Hawk (<i>Accipiter cooperii</i>) | • Merlin (<i>Falco columbarius</i>) |
| • Northern Goshawk (<i>Accipiter gentilis</i>) | • Peregrine Falcon (<i>Falco peregrinus</i>) |
| • Northern Harrier (<i>Circus hudsonicus</i>) | |
| • Red-tailed Hawk (<i>Buteo jamaicensis</i>) | |
| • Rough-legged Hawk (<i>Buteo lagopus</i>) | |

The work described in our proposal will help answer the following questions:

1. Are Minnesota's raptors exposed to PFAS and Hg?
2. How does exposure vary among species?
3. Do patterns of exposure differ among Minnesota's resident raptors and those that migrate through the state from elsewhere?
4. Are feathers (which are easier to collect) a reliable measure of blood concentrations of Hg and PFAS in raptors?

The effect of exposure to multiple environmental contaminants is an important and difficult topic in environmental toxicology. Individuals receiving safe exposures (i.e., below levels that cause adverse effects) to specific chemicals may nevertheless experience cumulative exposure to multiple chemicals that, in total, cause adverse effects. For example, previous work at The Raptor Center, has shown that almost all (90%) treated Bald Eagles have elevated lead, and our previous work at Hawk Ridge shows widespread exposure to mercury. The work we propose here will elucidate patterns of simultaneous exposure to both PFAS and Hg and give a more holistic picture of the risks faced by Minnesota's raptors exposed to multiple bioaccumulative contaminants.



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II. OVERALL PROJECT STATUS UPDATES:

AMENDMENT REQUEST 6 November 2019. We are requesting funds be shifted from the Personnel category to the Professional/Technical/Service Contracts category in order to better reflect what work will be completed in-house versus what work will be conducted by the Raptor Center. Specifically, we would like to move the Analyst Research Assistantships for graduate students (\$63,000) to a technical service contract to the University of Minnesota to fund graduate student work on the project. Also, we would like to move two items, the Raptor Center technician (\$5,000), and the Raptor Center supplies (\$1,000) to a technical service contract to the Raptor Center to support their work on collecting samples. This will reduce the Personnel budget to \$11,128 for Hawk Ridge Banding assistants. It will also reduce the equipment budget to \$5,093 (from \$6,093).

We are also requesting to modify language pertaining to sample sizes. In the proposal we state that we will obtain 20 samples of each species. Our efforts this Fall suggest that we will likely exceed 20 samples for some species and fall short for other species (e.g., the falcons are proving difficult to capture). While we have 2 more years to fill out these sample sizes we feel it would be prudent to indicate this potential asymmetry in sample size at this point. We also believe it would be wise to include an eighth species, Northern Harrier, which we can use to supplement samples when we don't achieve the full quota of 20 samples/species/year.

We are also requesting to combine our remaining equipment tools and supplies items into one budget line per LCCMR staff suggestion.

Amendment Approved by LCCMR **11/6/2019**

First Update January 15, 2020

Autumn 2019 was a successful first sampling season. In early September Raptor Center staff trained Hawk Ridge personnel in blood collection techniques. Collection continued at Hawk Ridge until the end of November. In total, for the 8 species currently listed on the grant, 262 blood samples were collected, 149 at Hawk Ridge and 20 at The Raptor Center. Additional samples were collected on 3 species, which we propose adding to the workplan with this amendment request. Sampling will continue year-round at The Raptor Center. At Hawk Ridge sampling will resume in March, with the onset of Spring migration. In December, an initial batch of 10 samples was sent to Axys/SGS for preliminary analysis and calibration. Results are expected back in early 2020. Pending successful analysis, 2019 samples will be sent in large quantities for analysis in February 2020. Paired feather samples were collected from all raptors sampled for blood and will be sent to Biodiversity Research Institute in Spring 2020 for analysis.

Spending has been quite modest to date, consisting only of supplies for tissue collection. We anticipate a sharp increase in spending as more and more samples are sent for analysis.

Amendment Request 15 January 2020. We are requesting inclusion of 3 additional species to our research. At Hawk Ridge we have captured more Northern Goshawks than expected (44), representing a valuable dataset on an important predator. Similarly, Great Horned Owl and Bald Eagle samples collected at The Raptor Center in Fall 2019 represent an important auxiliary dataset to our original set of 8 species. We also wish to add Spring migration season to our sampling frame to increase our representation of species, such as Merlin and Cooper's Hawk, which are trapped with greater frequency in Spring than Fall. To improve our sampling of American Kestrels we propose to sample Minnesota breeding Kestrels at sites around the Sax-Zim bog area, St. Louis County, MN in Summer 2020 and 2021.



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With this amendment request we are also requesting to reprogram \$6,000.00 from our Technical Service Contract with AxysSGS to our Personnel category to support hiring a raptor bander. The reason for this request is the recent (September 2019) increase in the U.S. Department of Labor minimum salary threshold from \$455/week to \$684/week. This will increase the expense of hiring a bander for the anticipated two seasons by a total of \$6,000. Pending approval, we will hire a banders for the Summer 2020 Kestrel work and for the Fall 2020 migration season. This reprogramming will reduce by about 15, the total number of samples we will be able to analyze.

Amendment Approved by LCCMR **2/8/2020**

Second Update June 15, 2020

The covid-19 pandemic prevented us from having a Spring or Summer field season. However, we are putting plans in place to conduct an Autumn season with appropriate social distancing measures in place to protect field personnel. We are hopeful that we can complete the project on the current schedule and are not requesting any amendment to our timeline at this point. We will review this decision after the Autumn 2020 field season.

Third Update January 15, 2021

The time since our last update has been one of intensive sampling effort at both Hawk Ridge and at The Raptor Center. To date we have collected over 1,200 raptor serum samples and over 1,500 feather samples. Our project will now begin to focus more on chemical and statistical analysis than on sample collection, though collection will continue at both sites to bolster the numbers of less frequently sampled species.

Samples collected to date (14 January 2021) at Hawk Ridge and at The Raptor Center

Species	Serum Samples for PFAS			Feather Samples for Hg		
	Hawk Ridge	Raptor Center	Total	Hawk Ridge	Raptor Center	Total
Bald Eagle	0	152	152	0	107	107
Sharp-shinned Hawk	316	11	327	592	10	602
Cooper's Hawk	27	13	40	31	9	40
Northern Goshawk	119	0	119	151	0	151
Northern Harrier	26	1	27	31	1	32
Northern Saw-whet Owl	146	24	170	161	30	191
Great Horned Owl	2	83	85	5	59	64
Rough-legged Hawk	37	0	37	46	0	46
Red-tailed Hawk	97	71	168	112	54	166
Merlin	64	7	71	106	7	113
Peregrine Falcon	9	8	17	9	7	16
Total	843	370	1213	1244	284	1528

Preliminary findings from samples sent to SGS/Axys in March 2020 showed that samples with volumes less than 1 mL of blood serum often had levels of PFAS below the analytical reporting limits. Several of the raptor species on our list (Sharp-shinned Hawk, Northern Saw-whet Owl, Merlin, and American Kestrel) are physically too small to safely collect enough blood for a 1 mL serum sample. Thus, we decided to try combining serum for multiple individuals of the same species, age group, and sex, to make composite serum samples for PFAS analysis. In September 2020, we sent a preliminary set of 10 composite samples for analysis under SGS/Axys new analytical method, which includes more PFAS analytes (32 vs. 13) and has lower reporting limits. All the composite samples had levels of PFAS above the detection limit. Based on this finding, we have decided to composite serum samples for three of our smaller species (Sharp-shinned Hawk, Merlin, and Northern Saw-whet Owls) to obtain 1 mL samples for analysis. Further, based on this finding we propose to drop American Kestrel from the study (see amendment below).



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A further set of 61 composited samples was sent to SGS/Axys in December 2020 for analysis under the new analytical method and results are pending. Throughout the coming Winter and Spring Hawk Ridge Bird Observatory and The Raptor Center will work together to prioritize and analyze the existing inventory of serum samples. Feather samples for Hg analysis will be similarly processed.

A graduate student at the University of Minnesota Duluth was supported on a 50% Graduate Research Assistantship during the Autumn 2020 semester under the technical services contract with the University of Minnesota Duluth. The student helped to organize sample collection, manage personnel, enter data into the master database, and write this report. The expense associated with that expenditure is not reflected in the budget report as we have yet to receive an invoice from the University for that work. Thus the expense will appear in a future status update.

Amendment Request 15 January 2021:

Given the difficulty in obtaining sufficient volume of serum and the relative infrequency with which we capture American Kestrels, we propose to drop the species from the study and replace it with Rough-legged Hawk (*Buteo lagopus*). Early snowfall and cold temperature in October 2020 lead to a record autumn migration of Rough-legged Hawks through Duluth and we were able to collect 36 blood samples for PFAS analysis and 46 feather samples for Hg analysis. Rough-legged Hawk is a particularly interesting species of raptor to include in this study because it breeds in the arctic. By including Rough-legged Hawks we will be able to compare environmental contaminant exposure in arctic-breeding birds to those in our boreal/temperate biomes.

Amendment Approved by LCCMR 1/19/2021

Fourth Update June 15, 2021

During this project period no further sampling has been conducted at Hawk Ridge Bird Observatory. Project associated work has been focused on identification of final samples for PFAS and Mercury analysis this coming autumn and identifying gaps in our inventory that should be filled with limited Autumn sampling. This has involved coordination between Hawk Ridge Bird Observatory and The Raptor Center to plan for chemical analysis.

In working with MN DNR for our annual audit and in preparing this report we discovered that our amended budget from our 15 January 2021 status update was prepared incorrectly. The error was that the existing budget column (column C in the spreadsheet) was filled with the remaining budget, not the original budget, and the next column (D for expenses) was filled only with expenses incurred during that reporting period. The budget associated with this update has been filled correctly and a corrected version of our 19 January budget is also attached.

Fifth Update January 15, 2022

During this project period limited sampling occurred at both Hawk Ridge and The Raptor Center to fill gaps in samples collected. Two Graduate Student Analysts were hired to process samples and send for analysis at SGS/Axys. Two batches of samples totaling 88 samples were sent to SGS/Axys for PFAS analysis and results have been received. To date, results have been received for 236 individual birds of 12 species (see table below):



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Table. Cumulative PFAS samples analyzed up to 11 January 2022.

Species	Male	Female	Juvenile	adult	Total
Bald Eagle	21	17	10	28	38
Sharp-shinned Hawk	11	11	11	11	22
Cooper's Hawk	8	14	13	9	22
Nothorn Goshawk	18	22	22	18	40
Northern Harrier	3	8	8	3	11
Red-tailed Hawk	3	9	28	20	48
Rough-legged Hawk	5	5	10	0	10
Great Horned Owl	6	8	6	8	14
Northern Saw-whet Owl	4	9	9	4	13
Merlin	3	5	3	5	9
Peregrine Falcon	4	5	8	1	9

Sixth Update May 3, 2022

During this project period we have been processing the existing sample inventory. Since our last update an additional 152 samples have been sent to SGS/Axys for analysis for PFAS. Of those 152 samples, we have received results on 40 samples and paid the corresponding invoices (reflected in the budget for the current project period). The remaining 112 samples are currently undergoing analysis, with results expected in the coming days. Having paid for the 40 samples, our remaining PFAS budget with this update is \$56,099.00. However, that does not account for the 112 outstanding samples currently being analyzed at SGS/Axys. Once those invoices are paid, our SGS/Axys budget remaining will be \$7,939.00, which will pay for an additional 18 samples. These 18 samples will be sent to SGS/Axys in the second week of May, 2022. Significant progress was also made on Hg analysis during this project period, with 291 samples analyzed.

LCCMR comments on previous updates have rightly noted that our project updates tend to be strongly focused on process and data collection activities, which is again true with this update. However, we are generating and analyzing results for review by our team and using these results to guide our choices for sample selection and analysis. Those reports are quite long, and we have been hesitant to insert them into this document. Therefore, we are taking the liberty of attaching our most recent internal report (PFAS_Report.html) to this document for review and to give a good sense for the results obtained so far.

Amendment Request May 3, 2022

As described immediately above, we have committed the majority of the original \$158,779 allocated for PFAS analysis under our Technical Services Contracts budget category and have just \$7,939 remaining. During this project period we also received our final invoice from the University of Minnesota Duluth for our graduate analyst positions hired in Fall 2021, which were billed at a much lower rate than expected because all students were on advanced status. Thus, we project a surplus of \$29,895.60 in the UM Technical Services Contract which we now request be transferred within the Technical Services Contract category to the SGS/Axys Technical Service Contract. This would allow us to analyze up to 69 additional samples.

Amendment Approved by LCCMR 5/25/2022

Final Report between project end (June 30) and August 15, 2022

Overall Project Outcomes and Results



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Polyfluoralkyl substances (PFAS) are a class of chemicals used in industrial processes and fire suppression. Mercury (Hg) enters the environment from point-source releases due to industrial processes and through combustion of coal for power generation. Both Hg and PFAS are present locally at highly contaminated sites and ubiquitously due to atmospheric deposition. Due to their predatory nature, Birds of Prey are at unique and elevated risk of exposure to both PFAS and Hg which concentrate in animal tissues; with each link in their food chain, predators consume and concentrate these toxicants contained in their prey. We collected blood and feather samples from 355 birds of prey at two Minnesota locations, Hawk Ridge in Duluth, and The Raptor Center in St. Paul. We analyzed blood plasma for up to 40 PFAS chemicals and feathers for total mercury concentration. Our objectives were to (1) collect baseline data on exposure of MN raptors to Hg and PFAS and (2) to test specific hypotheses about patterns of exposure in relation to ecological variables such as diet, age, sex, and species identity. As expected, perfluorooctane sulfonate (PFOS) was the PFAS of highest concentration across all species and in each individual species, typically at concentrations ten or more times that of other PFAS. Bald Eagle (*Haliaeetus leucocephalus*) had the highest exposure, probably reflecting the greater representation of aquatic prey (fish) in its diet. However, Cooper's Hawks (*Accipiter cooperii*) had comparable exposures and they consume entirely terrestrial prey. Among ecological variables, species identity offered the greatest explanatory power, followed by a measure of species' tolerance of human activity, which may serve as a proxy for likelihood of exposure. These results will prove invaluable for understanding and managing both human and ecological exposures to PFAS and Hg in Minnesota.

III. PROJECT ACTIVITIES AND OUTCOMES:

ACTIVITY 1 Title:

Activity 1: Quantify exposure and health risk to raptors at Hawk Ridge Bird Observatory

We will trap raptors at HRBO in Duluth MN in autumn 2019 and 2020. Our goal will be to trap and collect blood and feathers for an average of 20 individuals each of the eleven species listed above. PFAS samples will be analyzed at SGS Axys (www.axysanalytical.com/) and Hg samples will be analyzed by Biodiversity Research Institute (www.briloon.org).

For adult birds, blood samples will be taken for PFAS and Hg analysis and feathers will be sampled for Hg. Duplicate feather samples will be used to evaluate consistency among feathers in Hg residues. Blood samples will be used to evaluate the relationship between blood Hg and feather Hg. For PFAS analysis, only blood samples will be taken from adults.

For 1st year birds, both blood and feather samples will be taken to evaluate the relationship between blood PFAS and feather PFAS. Similar procedures will be followed for Hg. Paired Hg and PFAS samples will allow us to evaluate the relationship between feather residues and blood concentration for juvenile birds (for whom we are certain that all feathers were grown during the previous breeding season).

When body size does not permit sufficient amount of blood to be taken from a single individual (a possible constraint for American Kestrel and Saw-whet Owl), samples will be pooled across individuals for composite analysis. This might also require us to sample different individual birds for Hg versus PFAS for these two smaller species, again to avoid taking too much blood from a single individual.

ENRTF BUDGET: \$161,546.50

Outcome	Completion Date
1. Trap and sample an average of 20 individuals each of 8 raptor species.	1 December 2019



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2. Analyze 2019 samples for PFAS and Hg	1 February 2020
3. Repeat outcomes 1 & 2 in autumn/winter 2020/21 to augment samples from 2019/20	1 January 2021
4. Prepare publication for submission to peer-reviewed scientific journal	1 June 2022

First Update January 15, 2020

At Hawk Ridge, sampling far exceeded our expectations for some species (65 Sharp-shinned Hawks) and fell short for other species (1 American Kestrel). For the latter species we propose, with this update and amendment, to augment our database by collecting samples from an ongoing study of breeding Kestrels in and around Duluth, Minnesota. In total, 149 samples were collected from the original 8 species and an additional 44 samples were collected from the three additional species here proposed for inclusion, giving a total of 193 samples collected at Hawk Ridge.

Second Update June 15, 2020

No spring or summer sampling has been possible due to the covid-19 epidemic. A total of 89 samples collected during Autumn 2019 were sent to Axys/SGS for PFAS analysis. Similarly, 83 feathers samples were sent to BRI for Hg analysis. Results of both PFAS and Hg analyses have been returned and are pending statistical analysis. A further set of approximately 70 samples will be sent to Axys/SGS for PFAS analysis following completion of the reimbursement process through MN DNR for the first set (total cost of analyses ≈ \$34,000.00). A contract has been drawn up with the University of Minnesota Duluth (pending) to hire the graduate student analyst beginning Fall semester 2020.

Third Update January 15, 2021

Autumn 2020 was a successful second sampling season at Hawk Ridge. Following a strict Covid-19 protocol, we were able to collect samples from the end of August through early December 2020. During the fall field season, we collected 676 serum samples from 11 species of raptors and 1,007 feather samples for Hg analysis. Additional samples were collected on Rough-legged Hawk, which we propose adding to the workplan with this amendment request.

Fourth Update June 15, 2021

During this project period no further sampling has been conducted at Hawk Ridge Bird Observatory. Project associated work has been focused on identification of final samples for PFAS and Mercury analysis this coming autumn and identifying gaps in our inventory that should be filled with limited Autumn sampling.

Fifth Update January 15, 2022

During this project period limited sampling was conducted at Hawk Ridge to increase the sample size available for species with limited representation, primarily Cooper's Hawks, Merlins, and Peregrine Falcons. Ten additional Cooper's Hawks were captured, of which five provided sufficient serum for analysis without compositing. Seven additional Peregrine Falcons were captured, of which five provided sufficient serum for analysis without compositing. Seven additional Merlin samples were collected, of which five provided sufficient serum for analysis. These samples will be analyzed during Spring 2022.

Sixth Update May 3, 2022

During this project period samples from underrepresented species were chosen from the Hawk Ridge inventory and sent for analysis at SGS/Axys. These included 10 Cooper's Hawks, 8 Merlin, and 5 Peregrine Falcons, for a total of 23 samples. These results, when received, will greatly improve our resolution on exposure estimates for these three species. Pending approval of our amendment request, additional samples from Hawk Ridge will be composite samples for our smaller species, Northern Saw Whet Owl, Sharp-shinned Hawk, and Merlin.

Final Report Summary



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A total of 204 samples from 9 species from Hawk Ridge were processed for PFAS analysis (Table F1). Sample size for individual species at Hawk Ridge ranged from a minimum of 10 (Rough-legged Hawk) to a maximum of 43 (Northern Goshawk) (Table F1). Across all species at Hawk Ridge, perfluorooctane sulfonate (PFOS) was the PFAS of greatest concentration, followed by perfluorononanoic acid (PFNA) and perfluoroundecanoic acid PFUnA (Table F2). Within species PFOS was also the PFAS of greatest concentration, though the next most abundant chemical in serum varied among species. Note that age and sex categories may not sum to “Total” value because some individuals could not be identified to age and/or sex.

Table F1. Final sample size for all PFAS samples from Hawk Ridge

Code	Species	Total	Male	Female	Juvenile	adult
SSHA	Sharp-shinned Hawk	26	13	13	15	11
COHA	Cooper's Hawk	32	13	19	21	11
NOGO	Northern Goshawk	43	20	23	25	18
NOHA	Northern Harrier	10	3	7	7	3
RTHA	Red-tailed Hawk	37	1	0	21	16
RLHA	Rough-legged Hawk	10	5	5	10	0
NSWO	Nothern Saw-whet Owl	15	4	10	10	5
MERL	Merlin	17	4	13	7	10
PEFA	Peregrine Falcon	14	6	8	12	2

Table F2. Concentrations of 13 most common PFAS in avian serum collected at Hawk Ridge and The Raptor Center

	Mean	sd	cv
PFOS	22.03	59.27	2.69
PFNA	3.08	3.83	1.24
PFUnA	2.21	2.08	0.94
PFHxS	1.79	5.36	2.99
PFBA	1.74	3.63	2.09
PFDA	1.67	1.92	1.15
PFDoA	0.94	0.89	0.95
PFOA	0.77	1.39	1.80
PFBS	0.60	0.88	1.48
PFHpA	0.53	0.76	1.44
PFPeA	0.52	0.36	0.69
PFHxA	0.38	0.41	1.09
PFOSA	0.37	0.41	1.10

Sample analysis for this project was greatly impacted by the 2021 Highly Pathogenic Avian Influenza (HPAI) outbreak in Minnesota in Spring 2021, which resulted the quarantine of approximately 90 samples by the Canadian government. By the time clearance was received for sample shipping, there was insufficient time remaining to guarantee analysis would be complete by the 30 June 2022 project deadline. This resulted in approximately \$38,000 of the project budget being unspent. It also resulted in a significantly reduced sample size for several species, including Sharp-shinned Hawk, Northern Saw-whet Owl, and Merlin.



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In addition to the PFAS analysis, 232 individual raptors were sampled for Hg (Figure F1). In contrast to PFOS, falcons had the highest Hg concentrations in feathers among birds sampled at Hawk Ridge. Hg processing is ongoing as part of an ongoing Hg study at Hawk Ridge and this dataset is expected to grow.

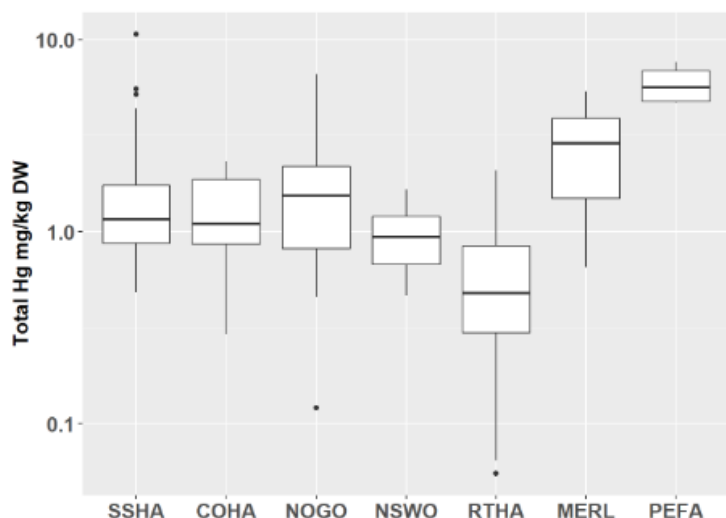


Figure F1. Hg concentrations in feathers of raptors sampled at Hawk Ridge (N=232)

An obvious question (outside the stated objectives of this proposal) is whether these exposures represent a health risk to these birds. Based on reported effect concentrations by Ackerman et al. (2016), and taking into account the differences between feather and blood Hg, these Hg exposures are below any known effect concentrations for birds. For PFAS, conclusions about health risk are premature. Very few PFAS have been tested for avian toxicity in controlled laboratory settings. Field-based effects estimates suggest the potential for adverse effects on reproduction, but the exposure concentrations at which effects are reported are inconsistent among studies (Custer 2021). Concentrations found here for Bald Eagle are lower than serum concentrations reported by Route et al. (2014) for breeding birds in MN and WI. As our knowledge of PFAS and their ecological effects accumulates, these data will prove invaluable.

Ackerman, J.T., C.A. Eagles-Smith, M.P. Herzog, C.A. Hartman, S.H. Peterson, D.C. Evers, A.K. Jackson, J.E. Elliott, S.S. Vander Pol, and C.E. Bryan (2016). Avian mercury exposure and toxicological risk across western North America: A synthesis. *Science of the Total Environment* 568: 749-769.

Custer, C. 2021. Linking field and laboratory studies: Reproductive effects of perfluorinated substances on avian populations. *Integrated Environmental Assessment and Management* 17:690–696.

Route, W.T., R.E. Russell, A.B. Lindstrom, M.J. Strynar, and R.L. Key. 2014. Spatial and Temporal Patterns in Concentrations of Perfluorinated Compounds in Bald Eagle Nestlings in the Upper Midwestern United States. *Environmental Science and Technology* 48:6653–6660.

Activity 2: Quantify exposure and health risk to raptors treated at The Raptor Center

With our Raptor Center partners, we will sample blood and feathers from injured raptors treated at the Raptor Center. Blood and feather tissue samples for an average of 20 individuals of each of the 11 raptor species listed above will be collected and analyzed as described under Activity 1.

For adult birds, blood samples will be taken for PFAS and Hg analysis and feathers will be sampled for Hg. Duplicate feather samples will be used to evaluate consistency among feathers in Hg residues. Blood samples will be used to evaluate the relationship between blood Hg and feather Hg. For PFAS analysis, only blood samples will be taken from adults.



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For 1st year birds, both blood and feather samples will be taken to evaluate the relationship between blood PFAS and feather PFAS. Similar procedures will be followed for Hg. Paired Hg and PFAS samples will allow us to evaluate the relationship between feather residues and blood concentration for juvenile birds (for whom we are certain that all feathers were grown during the previous breeding season).

ENRTF BUDGET: \$88,453.50

Outcome	Completion Date
1. Obtain an average of 20/species of raptors treated at the Raptor Center, St. Paul, MN	31 December 2019
2. Submit 2019 samples for PFAS and Hg analysis	31 January 2020
3. Repeat outcomes 1 & 2 in autumn/winter 2020/21	31 January 2021
4. Prepare publication for submission to peer-reviewed scientific journal	1 June 2022

First Update January 15, 2020

A total of 20 samples from the 8 target species were collected in Fall 2019 at The Raptor Center. This is well below our target of 20 samples/species. However, two species not present in our original 8, gave us the opportunity collect substantial opportunistic data (37 birds), which we are proposing to include in our study with this amendment and update. With patients continuously admitted to The Raptor Center (unlike the seasonal sampling at Hawk Ridge), we expect to be able to make up for the autumn shortfall. We also have access to an archive of plasma samples at The Raptor Center which may be used to augment samples for species for which sample size is limited.

Cumulative list of all samples collected up to 15 January 2020

	Hawk Ridge	Raptor Center	Total
Sharp-shinned Hawk	65	0	65
Cooper's Hawk	9	1	10
Northern Goshawk	44	0	44
Bald Eagle	0	35	35
Northern Harrier	7	0	7
Red-tailed Hawk	27	10	37
Northern Saw-whet Owl	25	4	29
Great Horned Owl	0	12	12
American Kestrel	1	0	1
Merlin	11	3	14
Peregrine Falcon	4	2	6
Total expanded list	193	67	260

Second Update June 15, 2020

Selection of samples from The Raptor Center for chemical analysis is pending statistical analysis of residues in the recently received results for birds sampled at Hawk Ridge Bird Observatory. Once statistical analysis is complete, samples from The Raptor Center will be selected and sent for immediate analysis by Axys/SGS and BRI. A contract for the collection of samples at The Raptor Center is in draft form and will be submitted to the University for approval.

Third Update January 15, 2021

Due to COVID-19 restrictions, The Raptor Center was unable to collect samples in the spring and summer of 2020. Because these seasons see the highest numbers of Cooper's Hawks and Peregrine Falcons admitted to



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The Raptor Center, we plan to make up for the shortfall in 2021. Sampling at The Raptor Center resumed in September 2020.

Fourth Update June 15, 2021

During this reporting period, sampling resumed at The Raptor Center.

As noted above, Raptor Center personnel are coordinating with Hawk Ridge personnel to identify important samples for analysis with remaining analytical budget.

Fifth Update January 15, 2022

During this reporting period, sampling continued at The Raptor Center in an effort to catch up on sample numbers. Fifty raptors were sampled between June 1st and Dec 31st with largest sample sizes for bald eagles (22) and red-tailed hawks (10). Other species sampled during fall 2021 include great horned owls (5), Cooper's hawks (3), sharp-shinned hawks (3), American kestrels (3), merlins (2), and peregrine falcons (2).

Sixth Update May 3, 2022

During this sampling period, efforts focused on analysis of the inventory of samples available from The Raptor Center. Of the 152 samples sent for analysis during this project period, 129 were Raptor Center samples. While results are still mostly pending from SGS/Axys, we will have a robust dataset of 153 samples analyzed from the raptor center. Contingent upon approval of the amendment requested in this status update additional samples will be analyzed, including more Bald Eagle samples (the species with the highest exposure in our data set).

Cumulative Raptor Center samples sent for analysis

Species	Samples sent
Bald Eagle	73
Cooper's Hawk	5
Red-tailed Hawk	31
Great Horned Owl	41
Peregrine Falcon	3
Total	153

Final Report Summary

Final Report between project end (June 30) and August 15, 2022

A total of 151 samples from 5 species from The Raptor Center were processed for PFAS analysis (Table F3). Sample size for individual species at The Raptor ranged from a minimum of 3 (Peregrine Falcon) to a maximum of 72 (Bald Eagle) (Table F3). Across all species at The Raptor Center, perfluorooctane sulfonate (PFOS) was the PFAS of greatest concentration, followed by perfluorononanoic acid (PFNA) and perfluoroundecanoic acid PFUnA) (Table F2, above). Within species PFOS was also the PFAS of greatest concentration, though the next most abundant chemical in serum varied among species. Note that age and sex categories may not sum to "Total" value because some individuals could not be identified to age and/or sex.

Table F3. Final sample size for all PFAS samples from The Raptor Center

Code	Species	Total	Male	Female	Juvenile	adult
BAEA	Bald Eagle	72	35	37	22	50
COHA	Cooper's Hawk	5	2	2	1	4
RTHA	Red-tailed Hawk	30	9	14	10	18
GHOW	Great Horned Owl	41	10	25	8	32
PEFA	Peregrine Falcon	3	3	0	3	0



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This activity was greatly impacted by the covid-19 pandemic, which closed the Raptor Center facility during parts of 2019 and 2020, effectively preventing sampling from occurring. This explains why fewer samples were collected at The Raptor Center compared to Hawk Ridge where sampling occurs outside and was not as affected by the pandemic. Feather samples from The Raptor Center are still undergoing processing at our cooperating laboratory and Hg results are expected soon.

IV. DISSEMINATION:

Description:

Scientific publications: We expect that this project will produce at least 1 peer reviewed journal article focusing on avian tissue residues for PFAS and Hg.

Presentations: Results will be disseminated through local, regional, and national conferences.

Publicly available data will be hosted on the Hawk Ridge Bird Observatory website. Descriptions of main findings will be posted to the HRBO website and disseminated electronically through HRBO electronic newsletters to members.

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 January 15, 2020

No work on dissemination occurred during this reporting period. In the subsequent period we intend to publish short communications in the newsletters of our respective organizations (The Raptor Center and Hawk Ridge Bird Observatory) for the benefit of our membership. We do not anticipate scientific publications prior to the conclusion of sampling in 2020/2021.

Second Update June 15, 2020

No work on dissemination occurred during this reporting period.

Third Update January 15, 2021

No work on dissemination occurred during this reporting period.

Fourth Update June 15, 2021

No work on dissemination occurred during this reporting period.

Fifth Update January 15, 2022

M. Etterson presented the seminar "Raptors and Contaminants" at the University of Minnesota Duluth Biology Seminar Series as part of the Hawk Ridge Annual Hawk Weekend Festival. The seminar contained four slides showing preliminary data from this study. The seminar was presented 17 September 2021.

Sixth Update May 2, 2022

During this reporting period, two presentations on this work and preliminary results were given to the PFAS working group at the US Environmental Protection Agency, Great Lakes Toxicology and Ecology Division, Duluth MN. The presentation dates were 24 February 2022 and 31 March 2022. These presentations were not open to the public and were intended to share results and solicit advice from experts in the field.

Final Report between project end (June 30) and August 15, 2022



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To date, dissemination has focused largely on seeking peer review from colleagues with expertise in ecotoxicology to facilitate course-corrections and interpretation of data as it is generated. Dr. Etterson presented a seminar on “Raptors and Contaminants” to the Biology Department of the University of Minnesota Duluth (17 September 2021) that included some preliminary data from this study. Dr. Ponder will present a seminar “The human footprint: Environmental contaminants impacting raptors” to the International Ornithological Congress in Durban, South Africa (17 August 2022).

The final round of PFAS analytical samples was received on 29 June 2022. Thus, dissemination activities will continue beyond the closing date for the project. These will include further analysis of data and publication of results in peer-reviewed journals. The authors have taken note of acknowledgement requirements for LCCMR-funded projects and will ensure that all such articles are properly credited and shared with LCCMR staff.

V. ADDITIONAL BUDGET INFORMATION:

A. Personnel and Capital Expenditures

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

Explanation of Use of Classified Staff: N/A

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

Instructions - Section A.: delete the budget table. Update FTEs funded to reflect final accurate accounting.

Enter Total Estimated Personnel Hours for entire duration of project: 2,800	Divide total personnel hours by 2,080 hours in 1 yr = TOTAL FTE:1.35
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B. Other Funds

Update **Section B. Other Funds** to reflect final accurate accounting of other funds used and for what purpose. This will help us accurately account for leverage of ENRTF funding.

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: 0	Divide total contract hours by 2,080 hours in 1 yr = TOTAL FTE: 0
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VI. PROJECT PARTNERS:

A. Partners outside of project manager’s organization receiving ENRTF funding

Name	Title	Affiliation	Role
Dr. Julia Ponder	Executive Director	The Raptor Center, University of MN	Sampling injured raptors



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

Name	Title	Affiliation	Role
Dr. David Evers	Chief Scientist, Executive Director	Biodiversity Research Institute	Hg analysis

VII. LONG-TERM- IMPLEMENTATION AND FUNDING:

This proposal is a part of a larger effort to understand the influence of persistent bioaccumulative toxic chemicals on Minnesota's birds of prey. Results of this work will allow us to evaluate whether and which of Minnesota's raptor species are exposed to these dangerous contaminants. Exposure concentrations will be compared to concentrations known to cause effects in other birds to determine whether remediation is needed to protect our iconic birds of prey.

VIII. REPORTING REQUIREMENTS:

- The project duration is 3 years and will begin on July 1, 2019 and end on June 30, 2022.
- Periodic project status update reports will be submitted January 1 and June 1 of each year.
- A final report and associated products will be submitted between June 30 and August 15, 2022.

IX. SEE ADDITIONAL WORK PLAN COMPONENTS:

- A. Budget Spreadsheet
- B. Visual Component or Map
- C. Parcel List Spreadsheet
- D. Acquisition, Easements, and Restoration Requirements
- 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. 03c

Project Manager: Matthew Etterson

Project Title: Mercury and PFAS risk to Minnesota raptors

Organization: Hawk Ridge Bird Observatory

Project Budget: \$250,000.00

Project Length and Completion Date: 3 Years, 30 June 2022

Today's Date: 05/01/22

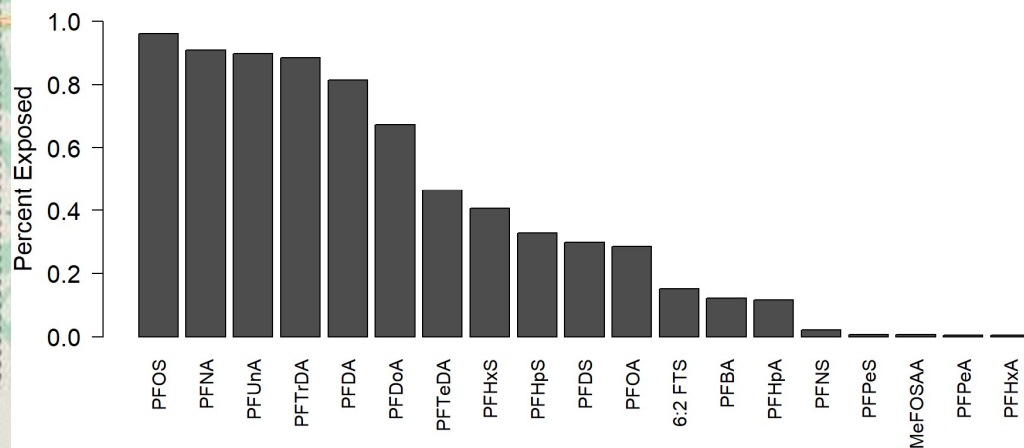


ENVIRONMENT AND NATURAL RESOURCES TRUST FUND BUDGET		Budget 5/1/22	Amount Spent	Balance
BUDGET ITEM				
Personnel (Wages and Benefits)				
Raptor Bander \$8,564 (88% Salary, 12% Benefits) 21% FTE (11/52 weeks) x 2 seasons		\$ 17,128	\$ 17,128	\$ -
Professional/Technical/Service Contracts				
Axys/SGS contract for PFAS analysis		\$ 188,675	\$ 150,410	\$ 38,265
Analysts/Research Assistantships - University of MN Duluth		\$ 33,104	\$ 33,104	\$ -
Raptor center technical contract for collection of blood & feathers for raptor center		\$ 6,000	\$ 6,000	\$ -
Equipment/Tools/Supplies				
Envelopes for feathers & supplies (vials & needles & syringes) for blood sampling for Hawk Ridge (~\$1k), 10 nylon 127mm mesh mist nets, 12m length @ \$109.25 ea (~\$1k), Tissue shipping expenses (incl. dry ice preservation during shipping) (~\$2k), Portable centrifuge for blood serum extraction (~1k) & laboratory supplies		\$ 5,093	\$ 5,061	\$ 32.11
Capital Expenditures Over \$5,000				\$ -
Fee Title Acquisition				\$ -
Easement Acquisition				\$ -
Professional Services for Acquisition				\$ -
Printing				\$ -
Travel expenses in Minnesota				\$ -
Other				\$ -
COLUMN TOTAL		\$ 250,000	\$ 211,703	\$ 38,296.71

OTHER FUNDS CONTRIBUTED TO THE PROJECT	Status (secured or pending)	Budget	Spent	Balance
Non-State:		0.00	0.00	0.00
State:		0.00	0.00	0.00
In kind: Hg analysis provide by Biodiversity Research Institute	pending	26400.00	0.00	26400.00

PAST AND CURRENT ENRTF APPROPRIATIONS	Amount legally obligated but not yet spent	Budget	Spent	Balance
Current appropriation:			0.00	0.00
Past appropriations:			0.00	0.00

Percent of birds of prey sampled at Hawk Ridge and The Raptor Center 2019-2021 exposed to 19 PFAS



Hawk Ridge

Minneapolis
The Raptor Center