DEPARTMENT OF NATURAL RESOURCES

WATERFOWL EXPOSURE TO PER- AND POLYFLUOROALKYL SUBSTANCES (PFAS) IN MINNESOTA, USA.

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SUMMARY OF FINDINGS

Prompted by increasing concerns of chemical contaminants in fish and game species commonly consumed by humans, the Minnesota Department of Natural Resources assessed if waterfowl are being exposed to for per- and polyfluoroalkyl substances (PFAS). Sera and tissue from 14 wild Canada geese (*Branta canadensis*) and 14 wild mallards (*Anas platyrhynchos*) were collected from two sites in Minnesota USA during summers 2022 and 2023; one site with decades of documented ground and surface water PFAS contamination and a control site with no known PFAS contamination. We found evidence of PFAS exposure in all birds sampled; however, hatch-year mallards raised at the known contaminated site had 360-7,000 times higher levels of one particular PFAS compound, perfluorooctane sulfonate (PFOS), compared to mallards at the control site. While the results suggest that not all waterfowl across Minnesota are exposed equally to PFAS, the elevated levels found in waterfowl near sites of known PFAS contamination may require additional attention. Further study into potential adverse effects of PFAS exposure to waterfowl survival and reproduction, and human health risks of game consumption is warranted.

INTRODUCTION

Chemical contaminants in the environment can affect the health of aquatic and terrestrial species and act as a route of exposure for people consuming fish and game species. Monitoring for chemicals in wild game species at known or suspected contaminated sites can provide valuable information on the potential for human exposure from consumption of local wildlife. Per- and polyfluoroalkyl substances (PFAS) are a large group of human-created chemicals used to repel water and grease, reduce friction and fire risk, and act as an insulator. They are of concern because they can easily move through soils, contaminate drinking water, do not break down in the environment, and many PFAS bioaccumulate in humans, fish, and wildlife (Death et al. 2021; ATSDR 2024). PFAS exposure is associated with adverse health outcomes in livestock, wildlife, and humans, including harmful effects on reproduction and development, liver, kidney, endocrine and immune systems, and behavior (Weiss et al. 2009; Tartu et al. 2014; Stahl et al. 2011; Dykstra et al. 2021).

While fish consumption advisories have been issued by states, tribes, and territories, mainly driven by levels of one specific PFAS, perfluorooctane sulfonate (PFOS) (GLCFCA 2019; MPCA 2021), understanding PFAS in game species (i.e., those targeted by humans for consumption) is only recently becoming a focus of research and monitoring efforts in the United States. A 2021 review of PFAS in terrestrial food chains found proximity to point sources of contamination is likely the main factor in elevated PFAS levels in game species (Death et al. 2021). Lab and field research has been on-going in non-game avian species (i.e., eagles, swallows, coastal and offshore birds), but these species are typically not consumed by people and sampling methods have varied between nestling plasma levels, egg collection, and analyzing various tissues including muscle and liver (Kannan et al. 2005; Custer et al. 2014; Wu et al. 2020; Dykstra et al. 2021). These avian studies have certainly documented PFAS exposure, but the effects are still not well understood. Tree swallows (*Tachycineta bicolor*) exposed to PFAS showed a negative correlation between PFOS burdens and hatching success (Custer et al. 2014), but field studies on tree swallows at a highly polluted site showed no demonstratable effects of high PFAS exposure on reproduction or most physiological responses (Custer et al. 2019).

OBJECTIVES

To understand if game species are exposed to PFAS in Minnesota USA (thereby presenting a potential exposure route to humans), researchers from three Minnesota state regulatory agencies developed a pilot study to determine whether the risk is present. Tissue and sera samples from two waterfowl species, Canada geese (*Branta canadensis*, CAGO) and mallards (*Anas platyrhynchos*, MALL), were collected at two sites, Lake Elmo Park Reserve (LEPR) and Thief Lake Wildlife Management Area (TLWMA), to determine if waterfowl, which spend a significant amount of time at the water's surface and consume multiple aquatic species, have detectable levels of PFAS in their tissue or organs. The first site, LEPR is of particular concern due to the high level of PFAS contamination that occurred over decades due to improperly handled manufacturing waste (MPCA 2021; Li and Gibson 2022; MDH 2024). The second site, TLWMA, acted as a control as no known PFAS contamination had been documented by Minnesota Pollution Control Agency in the area (Figure 1).

PFAS exposure may have potential lethal and sublethal effects on waterfowl, but those measurements or findings were beyond the scope of this study.

METHODS

Sample Collection

Unfledged, young-of-the-year birds, commonly called hatch-year birds, were targeted for collections as they would act as a proxy for local PFAS contamination given they cannot yet fly to other environments and be exposed to PFAS elsewhere. In 2022 and 2023, between June and August adult and hatch-year CAGO and hatch-year MALL were collected at LEPR; hatch-year MALL were collected August 2023 at TLWMA. During this pilot study, serum was collected (2023 only) to determine if antemortem sampling in waterfowl (i.e., blood draws) could replace lethal capture and sampling methods in the future. Waterfowl were live caught (CAGO = daytime, land-based corral traps; MALL = on-the-lake night lighting), with sex and age-class determined by size and cloacal examination (CAGO) or plumage (MALL). Whole blood was obtained from live birds through jugular or brachial blood draws and placed in 5ml polypropylene cryogenic vials with no preservative or anticoagulation agent; blood was spun for 15 min at 3,500 RPM. Serum was transferred to 2ml Thermo Scientific™ Nalgene™ cryogenic tubes and frozen upright at -20C within 6 hours of collection. Waterfowl were dispatched via cervical dislocation (Fair et al. 2023) and whole body weight recorded; staff plucked feathers from the breast area and collected breast tissue with skin attached (placed in labeled 4oz Whirl-pak®, weighed) and whole livers (placed in labeled 4oz Whirl-pak®, weighed). Tissue samples were kept cool and subsequently frozen at -20C within 6 hours of collection.

Laboratory Procedures

Tissue (20-50g provided to lab for homogenization) and sera (1ml provided to lab) were tested using isotope dilution via LC-MS/MS (modified Environmental Protection Agency PFAS method 1633). Samples were analyzed by two companies and combined reported on 54 unique PFAS analytes: SGS AXYS (British Columbia, Canada) in 2022 with 40 analytes and Eurofins

Environment Testing (California, USA) in 2023 with 46 analytes. Contaminant concentrations are presented in wet weight (ww).

RESULTS

Multiple PFAS analytes were found in every CAGO and MALL sampled, across all tissue types, and at both locations; PFAS results at LEPR were notably higher than TLWMA samples. Select PFAS analytes (PFOS, PFOA, PFDA, and PFHxS) displayed in Table 1 with median reported, ranges for remaining PFAS analytes available in Table 2, with CAS No, Acronyms, and compound names found in Table 3.

Across all species, PFAS were found at highest detection frequencies and concentrations in serum (26/46 analytes detected), followed by liver (22/54 analytes detected), and muscle tissues (19/54 analytes detected; Table 1). The level of PFOS found in tissue from hatch-year MALL at LEPR were 360-7,000 times higher than maximum concentrations found in hatch-year MALL at TLWMA (Table 1). At LEPR where both species were sampled, PFAS concentrations in CAGO were notably lower than in MALL; there was a significant difference in the average PFOS concentrations in the liver for CAGO (n=14, $\bar{x}$=140.3 ng/g) compared to MALL (n=9, $\bar{x}$=1775.6 ng/g; P<0.001). While sample sizes are small across species, study sites, age, and sex categories, some comparisons for PFOS in liver were made when possible. When comparing male (n=5) vs female (n=4) hatch-year MALL at LEPR, there is not a significant difference in average PFOS concentration for the liver (male $\bar{x}$=1676 ng/g, female $\bar{x}$=1900 ng/g). When comparing adult (n=7) vs hatch-year (n=7) CAGO at LEPR (both sexes included), there is not a significant difference in average PFOS concentration for the liver (adult $\bar{x}$=140.31 ng/g, hatch-year $\bar{x}$=140.23 ng/g). When comparing male (n=8) vs female (n=6) CAGO at LEPR (both age classes included), there is not a significant difference in average PFOS concentration for the liver (male $\bar{x}$ = 113.7 ng/g, female $\bar{x}$ =149.0 ng/g).

DISCUSSION

It was not surprising that the waterfowl from LEPR had higher PFAS values than TLWMA waterfowl, given LEPR has had ongoing PFAS contamination in both surface and groundwater for several decades (MPCA 2021; MDH 2024). However, we expected adult females to have less PFAS in general when compared to adult males, given maternal offloading of PFAS through placenta, milk, and eggs has been documented in birds, reptiles, and mammals (Kato et al. 2015; Pizzurro et al. 2019; Wilson et al. 2020; Beale et al. 2022,). Similarly, mother-to-offspring transfer of PFAS could also explain why the hatch-year waterfowl at TLWMA had detectable levels of PFAS, given there is no known contamination in the area. A study in Common Guillemot (*Uria aalge*) from the Baltic Sea showed the median concentration of PFOS was highest in eggs (325 ng/g wet weight (w wt)) followed by chick liver (309 ng/g w wt), and adult liver (121 ng/g w wt) (Holström and Berger 2008).

The levels of PFOS found in MALL and CAGO at LEPR were higher than PFOS found in the same species in a neighboring state: in 2013, Wisconsin documented at the Sheboygan River Area of Concern much lower PFOS levels in muscle of CAGO ($\bar{x}$=2.8 ± 3.4 ng/g) and MALL ($\bar{x}$=22.8 ± 16.6 ng/g; Strom 2013) when compared to our LEPR findings for CAGO ($\bar{x}$=38 ± 22 ng/g) and MALL ($\bar{x}$=959 ± 298 ng/g). Sharp et al. (2021) reported PFOS concentrations in ducks from Australian sites potentially impacted by with mean concentrations in muscle ranging from 15.2 - 28.0 ng/g. However, our PFOS levels were significantly lower than those found at the Holloman Airforce Base in New Mexico, USA. At the base, Witt et al. (2024) reported PFOS concentrations in various birds, including waterfowl, where PFOS concentrations in livers ranged from 14-38,000ng/g ($\bar{x}$=9,154 ng/g) while PFOS concentrations in the muscle ranged from 17-8,800 ng/g ($\bar{x}$=1,903 ng/g).

CONCLUSIONS

While documenting PFAS exposure in wildlife on a large scale is important, there is also a pressing need to understand how PFAS may be impacting wildlife, both individual fitness and long-term population impacts (i.e., effects on reproductive success). PFAS testing is complex, expensive, and labs able to conduct this research are limited. At the time of this study, each sample cost $470USD for analysis alone, notwithstanding the time, funding, and effort required for collecting samples from live birds. Consideration should be given to create a standardized sampling protocol for birds and mammals, and whether antemortem sampling can play a role in future contaminant surveillance. Though PFAS levels, particularly PFOS has been shown to be higher in the liver when compared to skeletal muscles (Holström and Berger 2008; Chen et al. 2018; Müller et al. 2011), wildlife researchers should agree on consistent tissues and collection methods to allow for the comparison of results to understand differences in exposure and potential lethal and sublethal effects on individuals and populations.

Given this study was a pilot to ascertain if waterfowl in Minnesota are exposed to PFAS, the sample size is small and the data should be interpreted with caution. Waterfowl in Minnesota are exposed to PFAS, however, information on sources of waterfowl exposure and duration of exposure is not available at this time. Given the migratory nature of waterfowl, broods reared in Minnesota may travel long distances down the Mississippi Flyway, thus delineating regions that may have higher consumption risks is challenging. Researchers only collected samples from two locations and the results suggest not all waterfowl across Minnesota are equally contaminated with elevated levels of PFAS, but waterfowl near sites of concern for PFAS may require additional attention. Waterfowl livers appear to have higher levels of PFAS than muscle tissue, but these levels may vary based on the amount of PFAS exposure each bird had and how each bird processes PFAS.

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Table 1. Tissue and sera results from waterfowl for four select per- and polyfluoroalkyl substances (PFAS), including PFOS, PFOA, PFDA, and PFHxS[1]. Samples were collected from Canada geese (*Branta canadensis,* n=14) and mallards (*Anas platyrhynchos*, n=14) at two study sites in Minnesota, USA during summers 2022 and 2023. Tissue results (liver and muscle) are presented in ng/g with serum results presented in ng/mL.

				PFOS		PFOA		PFDA		PFHxS	
Site	Species	Tissue	N	Range	Median	Range	Median	Range	Median	Range	Median
Lake Elmo Park Reserve	Canada Goose	Liver	14	78 – 200	150	0 – 1	0.5	0 – 3	1.3	0 - 0.6	0.3
		Muscle	14	13 – 72	31	0 - 0.8	0.1	0 - 0.5	0.3	0 - 0.9	0.2
		Serum	11	92 – 1900	180	0.6 – 51	2	0.94 – 28	1.4	0 – 35	0.5
	Mallard	Liver	9	760 – 2300	1800	3 – 39	19	8 – 50	34	1 – 22	14
		Muscle	9	360 – 1400	948	2 – 22	12	4 – 22	14	1 – 15	13
		Serum	7	1500 – 5000	2900	7.3 – 110	57	16 – 94	51	1 – 72	15
Thief Lake Wildlife Management Area	Mallard	Liver	5	0 – 13	4.1	0	-	0.2 – 1	0.3	0 - 0.2	0
		Muscle	5	0.2 – 1	0.4	0	-	0 - 0.3	0	0	-
		Serum		Not analyzed.							

[1]PFOS = Perfluorooctanesulfonic acid, PFOA = Perfluorooctanoic acid, PFDA = Perfluorodecanoic acid, PFHXS = Perfluorohexanesulfonic acid.

Table 2. Tissue and sera results (range, min-max) for 54 per- and polyfluoroalkyl substances (PFAS) collected from Canada geese (*Branta canadensis,* n=14) and mallards (*Anas platyrhynchos*, n=14) at two study sites in Minnesota, USA during summers 2022 and 2023. Tissue results (liver and muscle) are presented in ng/g with serum results presented in ng/mL.

	Lake Elmo Park Reserve						Thief Lake Wildlife Management Area	
	Canada Goose			Mallard			Mallard	
PFAS Compound[1]	Liver (n=14)	Muscle (n=14)	Serum (n=11)	Liver (n=9)	Muscle (n=9)	Serum (n=7)	Liver (n=5)	Muscle (n=5)
10:2 FTS	0 - 0.11	0[2]	0	0 - 0.1	0 - 0.1	0	0 - 0.1	0
11Cl-PF3OUdS	0	0	0	0	0	0	0	0
3:3 FTCA	0	0	na[3]	0	0	na	na	na
4:2 FTS	0	0	0	0	0	0	0	0
5:3 FTCA	0	0	0	0	0	0	0	0
6:2 FTCA	0	0	0	0	0	0	0	0
6:2 FTS	0	0	na	0	0	na	na	na
6:2 FTUCA	0	0	0	0	0	0	0	0
7:3 FTCA	0	0	0	0	0	0	0	0
8:2 FTCA	0	0	0	0	0	0	0	0
8:2 FTS	0	0	0 - 0.1	0 - 0.1	0	0	0	0
9Cl-PF3ONS	0	0	0	0	0	0	0	0
ADONA	0	0	0	0	0	0	0	0
EtFOSA	0	0	na	0	0	na	na	na
EtFOSAA	0	0	0 - 1.2	0.5 - 3.9	0.3 - 2.1	0.6 - 3	0	0
EtFOSE	0 - 9.9	0	na	0 - 4.5	0	na	na	na
FOUCA	0	0	0	0	0	0	0	0
HFPO-DA (GenX)	0	0	0	0	0	0	0	0
Hydro-PS Acid	0	0	0	0	0	0	0	0
MeFOSA	0	0	na	0	0	na	na	na
MeFOSAA	0	0	0	0 - 0.2	0	0 - 0.1	0	0
MeFOSE	0	0	na	0	0	na	na	na
NFDHA	0	0	0	0	0	0	0	0
PFBA	0 - 3.4	0	0 - 2	0.7 - 3.9	0 - 0.6	0.7 - 2.5	0 - 0.8	0
PFBS	0	0	0 - 0.1	0	0	0 - 0.1	0	0
PFDA	0 - 2.7	0 - 0.5	0.9 - 28	8 - 50	3.6 - 22	16 - 94	0.2 - 1	0 - 0.3
PFDoA	0	0	0 - 0.2	0.2 - 0.5	0 - 0.3	0.2 - 0.6	0	0
PFDoS	0	0	0	0	0	0	0	0
PFDS	0 - 0.3	0	0 - 0.8	0.3 - 1.2	0 - 0.4	0 - 0.7	0	0
PFECA G	0	0	0	0	0	0	0	0
PFECHS	0.2 - 0.9	0 - 0.3	0.8 - 10	2 - 12	1.2 - 4.4	6.5 - 23	0	0
PFEESA	0	0	0	0	0	0	0	0
PFHpA	0	0 - 0.7	0 - 0.1	0	0	0 - 0.2	0	0
PFHpS	0 - 1.2	0 - 0.3	0.6 - 17	2.2 - 24	1.2 - 9	11 - 35	0	0

PFAS Compound[1]	Lake Elmo Park Reserve						Thief Lake Wildlife Management Area	
	Canada Goose			Mallard			Mallard	
	Liver (n=14)	Muscle (n=14)	Serum (n=11)	Liver (n=9)	Muscle (n=9)	Serum (n=7)	Liver (n=5)	Muscle (n=5)
PFHxA	0	0	0 - 0.1	0	0	0	0	0
PFHxDA	0	0	0 - 0.8	0	0	0 - 0.1	0	0
PFHxS	0 - 0.6	0 - 0.9	0.5 - 35	1.3 - 22	1.1 - 15	5.9 - 72	0 - 0.2	0
PFMBA	0	0	0	0	0	0	0	0
PFMPA	0	0	na	0	0	na	na	na
PFNA	0 - 0.7	0	0.3 - 9.7	2.3 - 15	0.9 - 5.2	5.1 - 26	0.6 - 2.6	0.2 - 0.7
PFNS	0	0	0 - 1.9	0.7 - 2.9	0 - 0.9	1.3 - 5.8	0	0
PFO5DoA	0	0	0	0	0	0	0	0
PFOA (br)[4]	0	0	0	0	0 - 0.59	0 - 0.18	0	0
PFOA (ln)[4]	0.2 - 1.2	0 - 0.8	0.6 - 51	2.8 - 39	1.5 - 22	7.1 - 110	0	0
PFOA (total)	0 - 1.2	0 - 0.8	0.6- 51	2.8 - 39	1.5 - 22	7.3 - 110	0	0
PFOS (br)	17 - 45	4.2 - 20	16 - 220	190 - 880	64 - 270	180 - 570	0 - 5.1	0 - 0.3
PFOS (ln)	67 - 160	12 - 53	76 - 1700	570 - 1500	290 - 1100	1300 - 4500	0 - 11	0.2 - 0.9
PFOS (total)	78 - 200	13 - 72	92 - 1900	760 - 2300	360 - 1400	1500 - 5000	0 - 13	0.2 - 1.1
PFOSA	0	0	0 - 4.8	0.3 - 3.2	0.1 - 0.7	0.1 - 0.2	0	0
PFPeA	0	0	na	0	0	na	na	na
PFPeS	0	0	0 - 1.6	0 - 1.1	0 - 0.6	0.2 - 4.1	0	0
PFTeDA	0	0	0 - 0.3	0	0	0	0	0
PFTrDA	0	0	0 - 0.1	0	0	0.1 - 0.12	0	0
PFUnA	0 - 0.3	0	0.1 - 1.7	0.8 - 3.5	0.4 - 1.8	1.2 - 5.4	0.3 - 1.0	0 - 0.4

[1] Compound names, CAS numbers, and associated acronyms for each compound are displayed in S Table
[2] A value of “0” indicates no detections were reported for that tissue and compound combination
[3] A value of “na” indicates that compound was not analyzed, depending on the year and lab/equipment used.
[4]Br=branched, Ln=linear

Table 3. Table with full PFAS list, including full chemical name, acronyms, and CAS numbers.

Name	CAS #	Abbreviation
11-chloroeicosafluoro-3-oxaundecane-1-sulfonic acid	763051-92-9	11Cl-PF3OUdS
2H-Perfluoro-2-decenoic acid	70887-84-2	FOUCA
3:3 perfluorohexanoic acid	356-02-5	3:3 FTCA
4:2 fluorotelomersulfonic acid	757124-72-4	4:2 FTS
5:3 perfluorooctanoic acid	914637-49-3	5:3 FTCA
6:2 Fluorotelemer unsaturated acid	70887-88-6	6:2 FTUCA
6:2 Fluorotelomer carboxylic acid	53826-12-3	6:2 FTCA
6:2 fluorotelomersulfonic acid	27619-97-2	6:2 FTS
7:3 perfluorodecanoic acid	812-70-4	7:3 FTCA
8:2 Fluorotelomer carboxylic acid	27854-31-5	8:2 FTCA
8:2 fluorotelomersulfonic acid	39108-34-4	8:2 FTS
9-Chlorohexadecafluoro-3-oxanonane-1-sulfonate	756426-58-1	9Cl-PF3ONS
Br-perfluorooctanesulfonic acid	1763-23-1 (Br)	PFOS (br)[1]
Br-perfluorooctanoic acid	335-67-1 (Br)	PFOA (br)
Dodecafluoro-3H-4,8-dioxanonanoic acid	919005-14-4	ADONA
FTS 10:2 acid	120226-60-0	10:2 FTS
Hexafluoropropylene oxide dimer acid	13252-13-6	HFPO-DA (GenX)
Hydro-PS Acid	749836-20-2	Hydro-PS Acid
N-Ethylperfluorooctanesulfonamide	4151-50-2	EtFOSA
N-Ethylperfluorooctanesulfonamidoacetic acid	2991-50-6	EtFOSAA
N-Ethylperfluorooctanesulfonamidoethanol	1691-99-2	EtFOSE
N-Methylperfluorooctanesulfonamide	31506-32-8	MeFOSA
N-Methylperfluorooctanesulfonamidoacetic acid	2355-31-9	MeFOSAA
N-Methylperfluorooctanesulfonamidoethanol	24448-09-7	MeFOSE
Perfluoro(2-ethoxyethane)sulfonic acid	113507-82-7	PFEESA
Perfluoro-3,5,7,9,11-pentaoxadodecanoic acid	39492-91-6	PFO5DoA
Perfluoro-3,6-dioxaheptanoic acid	151772-58-6	NFDHA
Perfluoro-3-methoxypropanoic acid	377-73-1	PFMPA
Perfluoro-4-ethylcyclohexanesulfonic acid	133201-07-7	PFECHS
Perfluoro-4-isopropoxybutanoic acid	801212-59-9	PFECA G
Perfluoro-4-methoxybutanoic acid	863090-89-5	PFMBA
Perfluorobutanesulfonic acid	375-73-5	PFBS
Perfluorobutyric acid	375-22-4	PFBA
Perfluorodecanesulfonic acid	335-77-3	PFDS
Perfluorodecanoic acid	335-76-2	PFDA
Perfluorododecanesulfonic acid	79780-39-5	PFDoS
Perfluorododecanoic acid	307-55-1	PFDoA
Perfluoroheptanesulfonic acid	375-92-8	PFHpS
Perfluoroheptanoic acid	375-85-9	PFHpA

Name	CAS #	Abbreviation
Perfluorohexadecanoic acid	67905-19-5	PFHxDA
Perfluorohexanesulfonic acid	355-46-4	PFHxS
Perfluorohexanoic acid	307-24-4	PFHxA
Perfluorononanesulfonic acid	68259-12-1	PFNS
Perfluorononanoic acid	375-95-1	PFNA
Perfluorooctanesulfonamide	754-91-6	PFOSA
Perfluorooctanesulfonic acid (total)	1763-23-1	PFOS (total)
Perfluorooctanesulfonic acid (ln)	1763-23-1 (ln)	PFOS (ln)[1]
Perfluorooctanoic acid (total)	335-67-1	PFOA (total)
Perfluorooctanoic acid (ln)	335-67-1 (ln)	PFOA (ln)
Perfluoropentanesulfonic acid	2706-91-4	PFPeS
Perfluoropentanoic acid	2706-90-3	PFPeA
Perfluorotetradecanoic acid	376-06-7	PFTeDA
Perfluorotridecanoic acid	72629-94-8	PFTrDA
Perfluoroundecanoic acid	2058-94-8	PFUnA

[1]Br=branched, Ln=linear

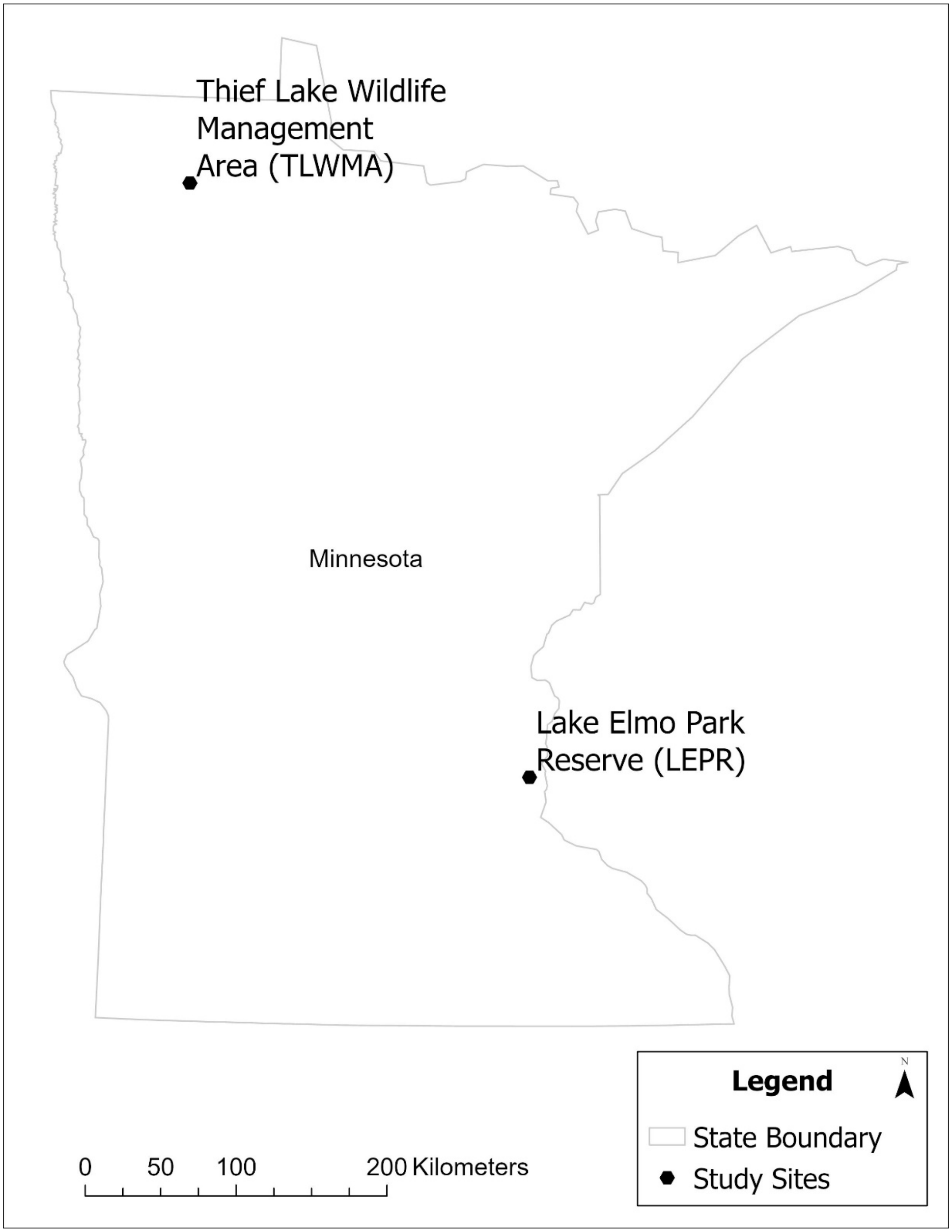

Figure 1. Location of two study sites in Minnesota, USA, Lake Elmo Park Reserve and Thief Lake Wildlife Management Area. Tissue and sera samples from two waterfowl species, Canada geese (*Branta canadensis,* n=14) and mallards (*Anas platyrhynchos*, n=14) were collected during summers 2022 and 2023 to assess per- and polyfluoroalkyl substances (PFAS) levels.