



Protocol for Monitoring Environmental Contaminants in Bald Eagles (Version 2.0)

Great Lakes Inventory and Monitoring Network

Natural Resource Report NPS/GLKN/NRR—2019/1983



ON THE COVER

Nine-week-old bald eagle nestlings on a large nest in a cottonwood tree on the Mississippi River.
Photograph © Jim Campbell-Spickler, Eco-Ascension Research and Consulting.

Protocol for Monitoring Environmental Contaminants in Bald Eagles (Version 2.0)

Great Lakes Inventory and Monitoring Network

Natural Resource Report NPS/GLKN/NRR—2019/1983

Bill Route¹, Rebecca Key¹, William Bowerman², Karin Kozie³, and David VanderMeulen¹

¹ National Park Service
Great Lakes Inventory and Monitoring Network
2800 Lake Shore Dr. East
Ashland, Wisconsin 54806

² University of Maryland
Department of Environmental Science and Technology
1426 Animal Science Building
College Park, Maryland 20742

³ Northwoods Wildlife Consulting
29580 County Highway C
Washburn, Wisconsin 54891

August 2019

U.S. Department of the Interior
National Park Service
Natural Resource Stewardship and Science
Fort Collins, Colorado

The National Park Service, Natural Resource Stewardship and Science office in Fort Collins, Colorado, publishes a range of reports that address natural resource topics. These reports are of interest and applicability to a broad audience in the National Park Service and others in natural resource management, including scientists, conservation and environmental constituencies, and the public.

The Natural Resource Report Series is used to disseminate comprehensive information and analysis about natural resources and related topics concerning lands managed by the National Park Service. The series supports the advancement of science, informed decision-making, and the achievement of the National Park Service mission. The series also provides a forum for presenting more lengthy results that may not be accepted by publications with page limitations.

All manuscripts in the series receive the appropriate level of peer review to ensure that the information is scientifically credible, technically accurate, appropriately written for the intended audience, and designed and published in a professional manner.

This report received formal peer review by subject-matter experts who were not directly involved in the collection, analysis, or reporting of the data, and whose background and expertise put them on par technically and scientifically with the authors of the information.

Views, statements, findings, conclusions, recommendations, and data in this report do not necessarily reflect views and policies of the National Park Service, U.S. Department of the Interior. Mention of trade names or commercial products does not constitute endorsement or recommendation for use by the U.S. Government.

This report is available in digital format from the [Great Lakes Inventory and Monitoring Network website](#) and the [Natural Resource Publications Management website](#). If you have difficulty accessing information in this publication, particularly if using assistive technology, please email irma@nps.gov.

Please cite this publication as:

Route, B., R. Key, W. Bowerman, K. Kozie, and D. VanderMeulen. 2019. Protocol for monitoring environmental contaminants in bald eagles (version 2.0): Great Lakes Inventory and Monitoring Network. Natural Resource Report NPS/GLKN/NRR—2019/1983. National Park Service, Fort Collins, Colorado.

Contents

	Page
Figures.....	v
Tables.....	vii
Revision History Log.....	ix
Executive Summary	xi
Acknowledgments.....	xiii
1.0 Background and Objectives	1
1.1 Introduction	1
1.2 Goals and Objectives	3
1.3 Rationale for Monitoring Persistent, Bioaccumulative Toxicants.....	4
1.4 Toxic Compounds of Concern.....	7
1.5 Use of Biosentinel Species for Monitoring	9
1.6 Monitoring Questions.....	10
2.0 Monitoring Strategy	13
2.1 Regional Approach.....	13
2.2 Sampling Locations	13
2.3 Occupancy and Productivity Surveys.....	14
2.4 Sample Size	14
2.5 Sampling Frequency.....	16
2.6 Detection Limits, Transformations, and Summary Statistics	17
2.7 Data Analysis and Presentation	17
2.8 Advanced Data Analysis and Use of Ancillary Data	22
2.9 Interpretations and Recommendations to Managers	23
3.0 Overview of Sampling Methods	27
3.1 Field Season Preparations.....	27
3.2 Field Measurements and Sampling	27
3.3 Sample Preparation and Laboratory Analysis	28

Contents (continued)

	Page
4.0 Data Management	31
4.1 Overview of Database	31
4.2 Quality Assurance and Quality Control	31
4.3 Data Entry, Verification, and Certification	32
4.4 Data Sensitivity	33
4.5 Data Distribution	33
4.6 Metadata Procedures	34
4.7 Data Archival.....	34
4.8 Reports and Publications	34
5.0 Personnel Requirements and Training	37
5.1 Roles and Responsibilities.....	37
Project Manager.....	37
Field Personnel	38
Data Management Specialist	39
5.2 Crew Qualifications.....	39
5.3 Training Procedures.....	39
6.0 Operational Requirements.....	41
6.1 Annual Work Load and Schedule.....	41
6.2 Facility and Major Equipment Needs.....	44
6.3 Annual Costs	44
Literature Cited	47
Appendix A. List of Standard Operating Procedures	55
Appendix B. Summary Information on Selected Contaminants.....	57

Figures

	Page
Figure 1. Location of six study areas in Minnesota and Wisconsin where bald eagle nestlings were sampled for environmental contaminants, 2006–2015.	3
Figure 2. A simplified illustration of how anthropogenic contaminants enter the aquatic ecosystem.	5
Figure 3. Areas of Concern where toxic chemicals have been shown to cause reproductive problems in fish and wildlife.	7
Figure 4. Example of bar chart showing geometric mean levels (+/- 95% CI) of p,p' DDE in plasma of bald eagle nestlings in four study areas in the Great Lakes Network in 2006–2008, compared with published p,p' DDE thresholds.....	18
Figure 5. Example of fixed effects model results from Dykstra et al. (2019) showing the trend in concentrations of mercury in feather samples from bald eagle nestlings at Apostle Islands National Lakeshore (APIS) and the Lake Superior South Shore (LSSS) over time.	19
Figure 6. <i>Left panel:</i> Geometric mean concentrations of ΣPFCs in bald eagle nestlings, 2006–2011. <i>Right Panel:</i> Maximum concentrations of lead (Pb) in whole blood.	20

Tables

	Page
Table 1. List of acronyms used in this protocol.	1
Table 2. Water bodies in three parks of the Great Lakes Inventory and Monitoring Network with persistent, bioaccumulative toxicants that are either state-listed 303(d) impaired water bodies, listed as a Level I Substance in Appendix I of the Great Lakes Binational Toxics Strategy, or designated by state or federal regulators to be a new and emerging contaminant of concern.....	9
Table 3. Number of bald eagle nests that were successful and contained at least one nestling suitable for sampling contaminants in and directly adjacent to parks of the Great Lakes Network.	15
Table 4. Years in which bald eagle nestlings were sampled for environmental contaminants at four study areas in three parks from 2006 through 2015 and proposed sampling period in following years.....	16
Table 5. Threshold values for contaminants that may affect bald eagle populations.....	20
Table 6. Summary of quality assurance and quality control procedures for the management of monitoring data at the Great Lakes Inventory and Monitoring Network.....	31
Table 7. Chronology of bald eagle nesting biology in relation to flight and field sampling to monitor environmental contaminants in nestlings.	41
Table 8. Estimated mean hatching dates of bald eagle nestlings at the four primary national park study areas, based on the age of nestlings sampled each year.	42
Table 9. Projected timing of events, lead role, and effort required for conducting bald eagle monitoring of environmental contaminants at three parks in the Great Lakes Network.....	43
Table 10. Estimated budget, in 2015 dollars (the last year of sampling), for one year of fieldwork, sample analysis, data management, and reporting.	45

Revision History Log

The following table lists all edits and amendments to this document since the original publication date. Information entered in the log must be complete and concise. Users of this protocol and attached standard operating procedures should promptly notify the project leader and/or the data manager about recommended and required changes. The project leader must review and incorporate all changes, complete the revision history log, and change the date and version number on the title page and in the header of the document file. For complete instructions, please refer to SOP #11, Revising the Protocol.

Original publication name, version and date: Protocol for Monitoring Environmental Contaminants in Bald Eagles. Version 1.0. March, 2006.

Revision History Log*

Date and Number of Previous Version	Date and Number of Revision	Author(s) of Revision (with title and affiliation)	Location in Document and Description of Revision
March 2006, V 1.0	April 2008, V 1.1	Bill Route, lead investigator, NPS	Removed herring gull as a sentinel species and removed reference to six parks that are not being monitored under this protocol. Unable to obtain and cover costs of gull sampling and analysis. Other six parks are being monitored under a separate protocol using fish and dragonflies as sentinel species (Wiener et al. 2008).
April 2008, V 1.1	Dec. 2008, V 1.2; later published as NRR 2009/092 in 2009.	Bill Route, lead investigator, NPS	Addressed reviewer comments: minor edits throughout; removed power analysis from section 2.5 and replaced with illustrations of analysis and presentation using preliminary results of pilot work in 2006, 2007, and 2008. Power analysis was considered pointless by reviewers because contaminants data are highly variable. Data are more useful when analyzed and presented as bar charts with thresholds; trends lines and differences between areas with measures of variability; and assessments of spatial patterns with maps.
Jan. 2009, V 1.2	August 2019 V 2.0; published as NRR 2019/1983 in 2019.	B. Route and D. VanderMeulen	Updated literature; changed rotation to 3 years in a row every 10 years. Addressed reviewer comments

* Add rows as needed for each change or set of changes tied to an updated version number.

Executive Summary

This protocol details the objectives, study design, and reporting requirements for monitoring levels of targeted environmental contaminants in bald eagle (*Haliaeetus leucocephalus*) nestlings at three national park units in the western Great Lakes region of the United States. Detailed methods are in standard operating procedures. The protocol involves taking blood and feather samples from bald eagle nestlings to monitor trends in contaminants that are persistent, bioaccumulative, and toxic (PBT) to fish, wildlife, and humans. The monitoring will take place at Apostle Islands National Lakeshore (APIS), Mississippi National River and Recreation Area (MISS), and the St. Croix National Scenic Riverway (SACN). A companion protocol involves sampling dragonfly larvae and fish for mercury (Hg) at these and six other national parks in the region. Both programs are part of a nation-wide effort by the U.S. National Park Service (NPS) to provide park managers with scientifically sound data to manage natural resources under their care. The monitoring is funded and conducted by the NPS Great Lakes Inventory and Monitoring Network.

Human-made contaminants that are released into water, air, or on land often end up in aquatic systems through aerial deposition, runoff, and ground water. Bald eagles are high on the aquatic food web and are known to accumulate high levels of PBT contaminants. Hence, they sample the aquatic environment and act as integrators of human-made contaminants.

This protocol was implemented annually from 2006 through 2011 and repeated in 2014 and 2015 to provide a baseline of data on targeted contaminants. Ideally, the protocol will be repeated three years in succession with no more than ten years between sampling. Sampling will depend on funding levels and contributions from partners. The protocol targets six contaminants that have caused impairment to water bodies within the three parks, or which have been identified by regulatory agencies as contaminants of emerging concern (CEC). The six contaminants are:

- Lead
- Mercury (total)
- DDT (including DDE and DDD)
- PCBs (total and 75 congeners)
- PBDEs (total and 9 congeners)
- PFCs (total and 16 telomers)

In addition to these targeted contaminants, we measured concentrations of additional pesticides and CECs under special funding from the U.S. Environmental Protection Agency's (EPA) Great Lakes Restoration Initiative in 2010, 2011, 2014, and 2015. The sampling design can be easily expanded to include these and other contaminants as needed. Specifically, the monitoring program is designed to answer the following questions:

1. What is the direction and magnitude of change in concentrations of targeted contaminants in blood plasma and/or feathers from nestling bald eagles within the three parks?
2. What is the incidence of developmental deformities observed in live and dead nestlings of bald eagles within the three parks?
3. What is the direction and magnitude of change in the number of young produced per occupied bald eagle nest within the three parks?

Briefly, bald eagle nests, including new nests and those from previous years, are located by pilot/observer teams from either a fixed-wing aircraft or a helicopter in the spring of each year. In May or June, field teams visit all occupied nests that can be safely climbed. Sampling is timed to target nestlings when they are five to nine weeks old. Each occupied nest will contain one to three, very rarely four, nestlings. We take up to 11 mL of blood and three to four breast feathers from at least one, and often all, nestlings in each nest. Samples from one nestling are randomly selected and sent to an analytical laboratory. Samples from the remaining siblings are archived. All eaglets are measured, weighed, checked for general health, banded, and placed back in the nest. All handling and sampling data are recorded on standardized forms.

Blood samples are kept in a cooler on ice and then processed within eight hours. Processing involves centrifuging the whole blood and pipetting the resulting plasma into separate vials. The lab provides prewashed, chemically inert, and individually wrapped sample vials. The plasma is frozen within ten hours and kept frozen until delivery to the laboratory. Feather samples are kept dry in individually marked envelopes. Following the field season both plasma and feather samples are delivered to the designated lab where they are analyzed for concentrations of target contaminants.

Resulting data are quality checked, entered into the database, and stored on the GLKN server, with a backup copy of database kept off-site at Apostle Islands National Lakeshore.

Field efforts and preliminary results are summarized in administrative reports to federal and state agencies and as natural resource briefs to park managers. Two technical reports summarize initial findings (Route and Key 2009, Route et al. 2011) and journal articles have been published on the spatial and temporal patterns and trends in the legacy contaminants DDE, PCBs, and mercury (Dykstra et al. 2010, Dykstra et al. 2019), PBDEs (Route et al. 2014a), PFCs (Route et al. 2014b), and lead (Bruggeman et al. 2018).

Acknowledgments

This protocol was developed under the guidelines of the National Park Service, Vital Signs monitoring program (now Inventory and Monitoring Division, or IMD), and we thank past and current IMD leadership for their tireless efforts to make this program possible. The decision to monitor environmental contaminants was the result of numerous discussions and meetings with employees from the nine parks in the Great Lakes Network, especially the Technical Committee and Board of Directors, network staff, and numerous agency scientists. We thank all of them for their many hours in meetings, reviews, and informal discussions. Finally, we acknowledge C. DeSorbo, N. Duncan, R. Haro, A. Holdhusen, A. Jarding, M. Meyer, S. Miller, G. Rowell, M. Sieder, S. Strom, and J. Weiner for comments and suggestions that greatly improved the clarity and rigor of this protocol, and T. Gostonski for final edits and layout of the document.

1.0 Background and Objectives

1.1 Introduction

The National Park Service (NPS) has instituted a program to inventory and monitor natural resources at approximately 270 NPS units (parks) across the nation (Fancy 2004). The program is being implemented through 32 “networks” of parks that share common management concerns and geography. The Great Lakes Inventory and Monitoring Network (GLKN) includes nine parks in four states: Apostle Islands National Lakeshore (APIS), Grand Portage National Monument (GRPO), Indiana Dunes National Park (INDU), Isle Royale National Park (ISRO), Mississippi National River and Recreation Area (MISS), Pictured Rocks National Lakeshore (PIRO), Sleeping Bear Dunes National Lakeshore (SLBE), St. Croix National Scenic Riverway (SACN), and Voyageurs National Park (VOYA). See Table 1 for a list of all acronyms used in this protocol.

The purpose of the overall inventory and monitoring program is to identify and monitor indicators of park ecosystem health. These indicators, referred to as “Vital Signs”, are a select group of attributes that are particularly useful for understanding and managing NPS areas. The Great Lakes Network has developed a guiding document that details the goals, ecological context, and an implementation schedule for the Vital Signs selected by the nine parks (Route and Elias 2007). One of the Vital Signs identified by GLKN and its constituent parks is Trophic Bioaccumulation of Contaminants. Under this protocol, we will monitor environmental contaminants that are persistent, bioaccumulative, and toxic (PBT). This protocol, the associated standard operating procedures (SOPs; Appendix A), and quality assurance plan (Route and VanderMeulen 2018) provide the basis and methods for monitoring PBT contaminants in bald eagle (*Haliaeetus leucocephalus*) nestlings at three parks in the Great Lakes Inventory and Monitoring Network.

Table 1. List of acronyms used in this protocol.

Acronym	Definition
2,3,7,8-TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
APIS	Apostle Islands National Lakeshore
BEST	Biomonitoring of Environmental Status and Trends
CSDGM	Content Standard for Digital Geospatial Metadata
CU-DEQ	Clemson University and Michigan Department of Environmental Quality
DDE	Dichlorodiphenyldichloroethylene
DDT	Dichlorodiphenyltrichloroethane
DEQ	Department of Environmental Quality
DNR	Department of Natural Resources
ECCC	Environment and Climate Change Canada
EPA-STORET	Environmental Protection Agency Storage and Retrieval System
FGDC	Federal Geographic Data Committee
GLBTS	Great Lakes Binational Toxics Strategy

Table 1 (continued). List of acronyms used in this protocol.

Acronym	Definition
GLKN	Great Lakes Inventory and Monitoring Network
GRPO	Grand Portage National Monument
IADN	Integrated Atmospheric Deposition Network
IJC	International Joint Commission
IMS	Internet Mapping System
INDU	Indiana Dunes National Park
ISRO	Isle Royale National Park
MISS	Mississippi National River and Recreation Area
NBII	National Biological Information Infrastructure
NELAP	National Environmental Laboratory Accreditation Program
NPS	National Park Service
NPSTORET	National Park Service Storage and Retrieval System
PAH	Polycyclic aromatic hydrocarbon
PBDE	Polybrominated diphenyl ether
PBT	Persistent, bioaccumulative toxicant
PCB	Polychlorinated biphenyl
PCDD	Polychlorinated dibenzo-p-dioxin
PCDF	Polychlorinated dibenzofuran
PFC	Perfluorinated compounds
PFOS	Perfluorooctane sulfonate
PIRO	Pictured Rocks National Lakeshore
QA/QC	Quality assurance/quality control
RPD	Relative percent difference
SACN	St. Croix National Scenic Riverway
SLBE	Sleeping Bear Dunes National Lakeshore
SOLEC	The State of the Great Lakes Ecosystem Conference
SOP	Standard operating procedure
USEPA	U. S. Environmental Protection Agency
USFWS	U. S. Fish and Wildlife Service
USGS	U. S. Geological Survey
VOYA	Voyageurs National Park
WRD	Water Resources Division
WDNR	Wisconsin Department of Natural Resources
WSLH	Wisconsin State Lab of Hygiene

1.2 Goals and Objectives

The goal of this monitoring protocol is to collect data that will provide managers at APIS, MISS, and SACN with information on the temporal trends, spatial patterns, and, where feasible, the ecological effects of targeted, anthropogenic PBT contaminants in aquatic ecosystems as indicated by their bioaccumulation in bald eagle nestlings. The monitoring will include any new and emerging chemicals identified by regulatory agencies as being of concern for ecosystem and human health.

From 2006 through 2015 this protocol, under versions 1.0 and 1.1 (Route et al. 2009), was implemented at the three parks and at two adjacent study areas that were requested and paid for by collaborators. The adjacent study areas were Lake Superior South Shore (LSSS) and Pools 3 & 4 of the Mississippi River (Figure 1).

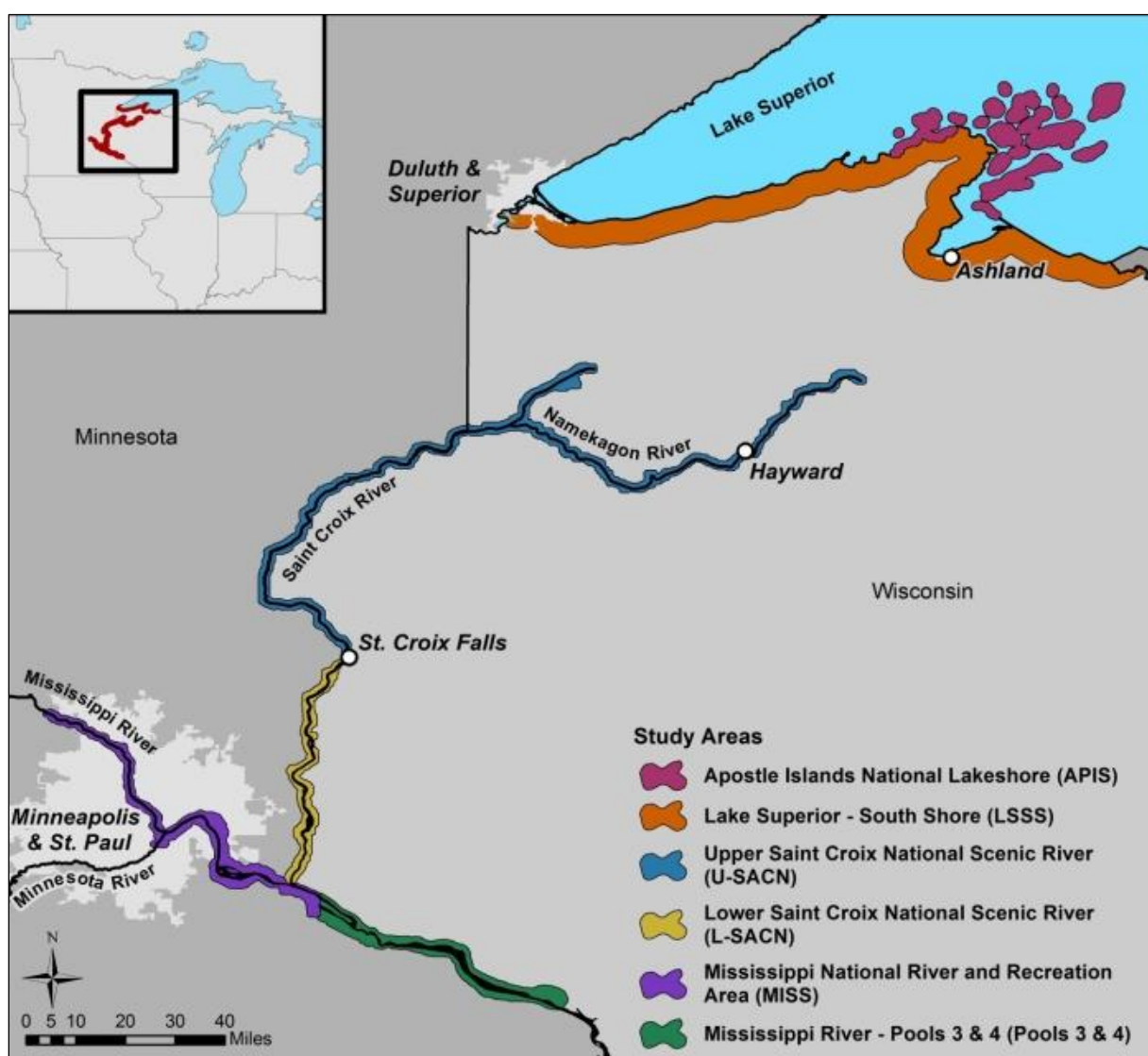


Figure 1. Location of six study areas in Minnesota and Wisconsin where bald eagle nestlings were sampled for environmental contaminants, 2006–2015. The LSSS and Pools 3 & 4 study areas are outside of NPS boundaries and were sampled and paid for at the request of partner agencies.

Contaminants accumulate in aquatic systems through wet and dry deposition from the air and through down-gradient transport in runoff, stream flow, and ground water. This monitoring protocol will focus on aquatic systems to increase the efficiency of sampling and the greater ability to observe actual effects in wildlife. We will focus on PBT contaminants that have been identified by state and/or federal regulatory agencies as contaminants that have caused impairment of one or more water bodies within a park. States are required to document causes of impairment to each water body by placing them on the 303(d) list, which is monitored by the U.S. Environmental Protection Agency (USEPA) under stipulations of the Clean Water Act.

In addition to these listed contaminants, many new and emerging chemicals are being released into the environment each year. The network will consult with the USEPA, the International Joint Commission (IJC), the state Departments of Natural Resources and/or Departments of Environmental Quality, the U.S. Geological Survey (USGS) Biomonitoring of Environmental Status and Trends (BEST) Program, the USGS Toxic Substances Hydrology Program, and natural resource staff from network parks to determine which new contaminants should be considered for monitoring under this protocol.

Specifically, the objectives of this protocol are threefold:

1. To monitor spatial patterns and temporal trends in exposure of bald eagles to targeted PBT contaminants in three parks of the Great Lakes Network;
2. Where feasible, to measure spatial patterns and temporal trends in reproductive outcomes and incidence of developmental deformities of bald eagles at the three parks, and to assess the relation of these variables to contaminant exposure;
3. To archive tissue (blood, egg, and feathers) from bald eagles at the three parks and periodically investigate exposure to new and emerging contaminants.

1.3 Rationale for Monitoring Persistent, Bioaccumulative Toxicants

The nine GLKN parks are within one of the areas in the U.S. where environmental contaminants have been shown to adversely affect wildlife reproduction and to cause developmental deformities (IJC 1989, Gilbertson et al. 1998). Many of these chemicals are known or hypothesized to cause similar reproductive and developmental problems in humans (Gilbertson and Carpenter 2004, Hites et al. 2004, Foran et al. 2005). The chemicals of greatest risk to wildlife and humans are those that are persistent, that bioaccumulate, and that are toxic. PBT contaminants originate from both point and non-point sources, and are transported into the NPS units by atmospheric, biotic, and water-borne routes (Figure 2).

A fraction of the PBT contaminants found in park units may be from past direct inputs or emissions in or near park boundaries. However, long-range atmospheric transport accounts for a significant fraction of many pollutants (e.g., Engstrom and Swain 1997).

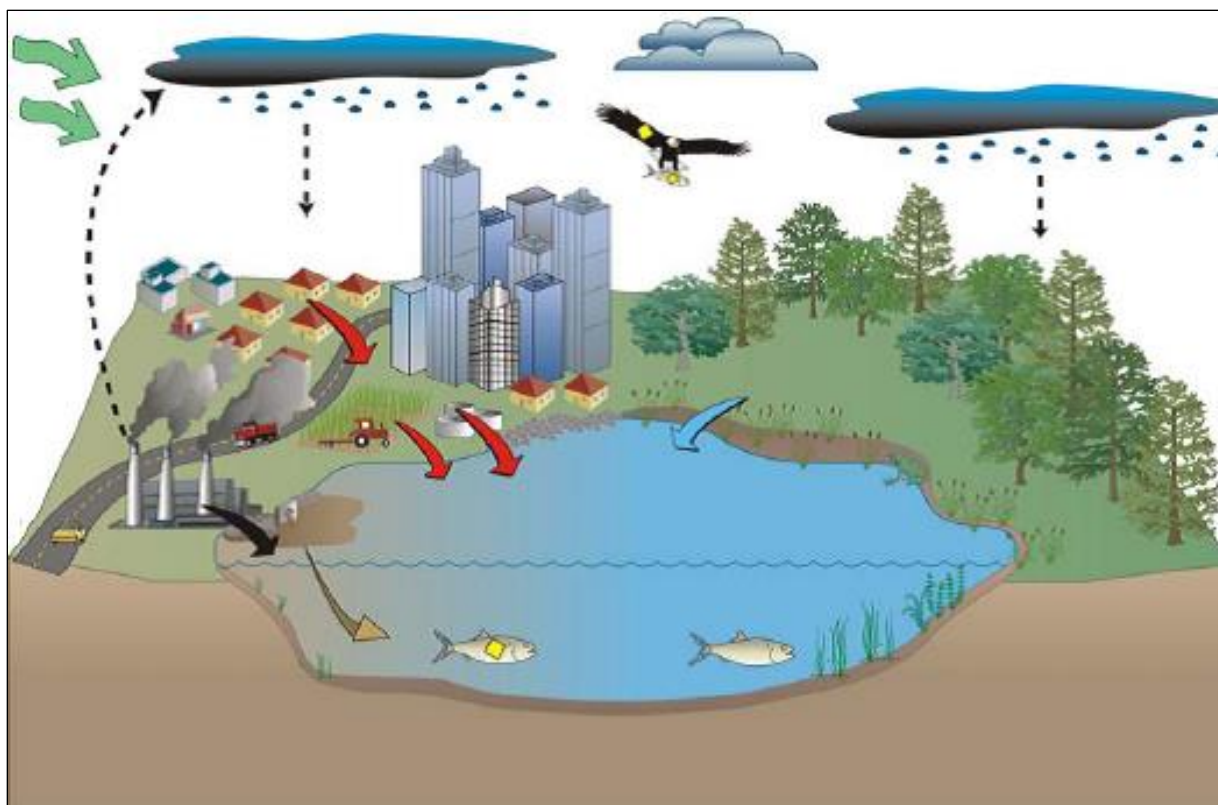


Figure 2. A simplified illustration of how anthropogenic contaminants enter the aquatic ecosystem. Prevailing winds (*green arrows*) drive polluted air masses over the landscape and precipitation deposits toxics (*black dashed arrows*) onto land and water. Rainwater runoff across urban, industrial, and agricultural lands (*red arrows*), combines with point-source pollution (*black arrow*) and sedimentation (*brown arrow*) to alter water quality. Some contaminants will bioaccumulate in higher trophic species (*yellow diamond* in fish and bald eagle). Effects of pollutants can be buffered by terrestrial and aquatic vegetation (*blue arrow*), or in the case of mercury, can be magnified by dry deposition off vegetative surfaces and subsequent production of toxic methylmercury in wetlands.

Reproductive failure and the resulting population decline of many species of birds began in 1947, with the introduction of the organochlorine pesticide DDT (dichlorodiphenyl-trichloroethane) in North America. DDE (dichlorodiphenyldichloroethylene), a metabolite of DDT, caused eggshell thinning and resulted in eggs breaking when adults attempted to incubate them. With the ban on DDT in 1972, many bird populations began to recover (Newton 1979, Grier 1982). Through at least the early 1980s bald eagle productivity was inversely correlated with DDE and PCB concentrations in eggs (Wiemeyer et al. 1984) and in blood plasma of nestlings (Bowerman et al. 2003). These chemicals may no longer limit bald eagle populations in the Lake Superior region (Dykstra et al. 2005); yet, recovery through the 1980s and 1990s was not uniform throughout the Great Lakes region. Bald eagles nesting along the shoreline of the Great Lakes and in Voyageurs National Park (VOYA) have lower reproductive success than inland populations in Minnesota, Wisconsin, and Michigan (Kozie and Anderson 1991, Grim and Kallemeyn 1995, Dykstra et al. 1998, Bowerman et al. 2003). The reasons for this difference in reproductive success are not fully understood, but, in the Great Lakes, may be attributed to prey abundance (Dykstra et al. 2010).

Many chemicals manufactured and released by humans have been studied and implicated in environmental pollution. Mercury has been used and released into the environment for centuries (Wiener et al. 2003), and its organic form, methylmercury, is persistent, biomagnifies in aquatic food webs, and is highly toxic (Evers 2005, Clarkson and Magos 2006, DeSorbo and Evers 2006, Mergler et al. 2007, Scheuhammer et al. 2007). Growing awareness of the hazards of mercury has led to reductions in emissions in the Great Lakes basin, yet it remains the most pervasive environmental contaminant in many aquatic ecosystems. Several states in the Great Lakes region, including those where GLKN parks are located, have issued statewide advisories for fish consumption in all lakes and rivers (Illinois, Indiana, Ohio, and Wisconsin) or for all lakes (Michigan, Minnesota) because of mercury contamination (USEPA 2005).

This protocol will serve APIS, MISS, and SACN—where there is now a solid baseline of data—and can be used to monitor contaminants in bald eagles at VOYA, ISRO, PIRO, and SLBE. A companion protocol will use dragonfly larvae and fish as the sentinel species for mercury in all nine park units (VanderMeulen et al. 2018).

Of the three focal parks for this protocol, APIS is the only one within the Great Lakes watershed. Annex 2 of the U.S./Canada Great Lakes Water Quality Agreement, administered by the IJC, defined 41 Areas of Concern where changes in the chemical, physical, or biological integrity of the system were sufficient to impair beneficial uses. Among the impairments listed were: (1) bird or animal deformities or reproductive problems, and (2) fish tumors or other deformities. In the 1970s when the Agreement was formulated, such impairments occurred at several locations across the Great Lakes (Figure 3; Aulerich et al. 1971, Gilbertson et al. 1976, Black 1983). Abnormalities in wildlife alerted biologists, human health professionals, and politicians to the potential effects on human health from chemicals such as DDT, PCBs, and dioxin. For mercury, public awareness and regulatory actions were prompted largely by human poisonings (including many fatalities) from consumption of mercury-contaminated fish, particularly in Minamata, Japan (Tsubaki and Irukyama 1977). The continued monitoring of PBT contaminants is important for scientific investigations and remedial actions.

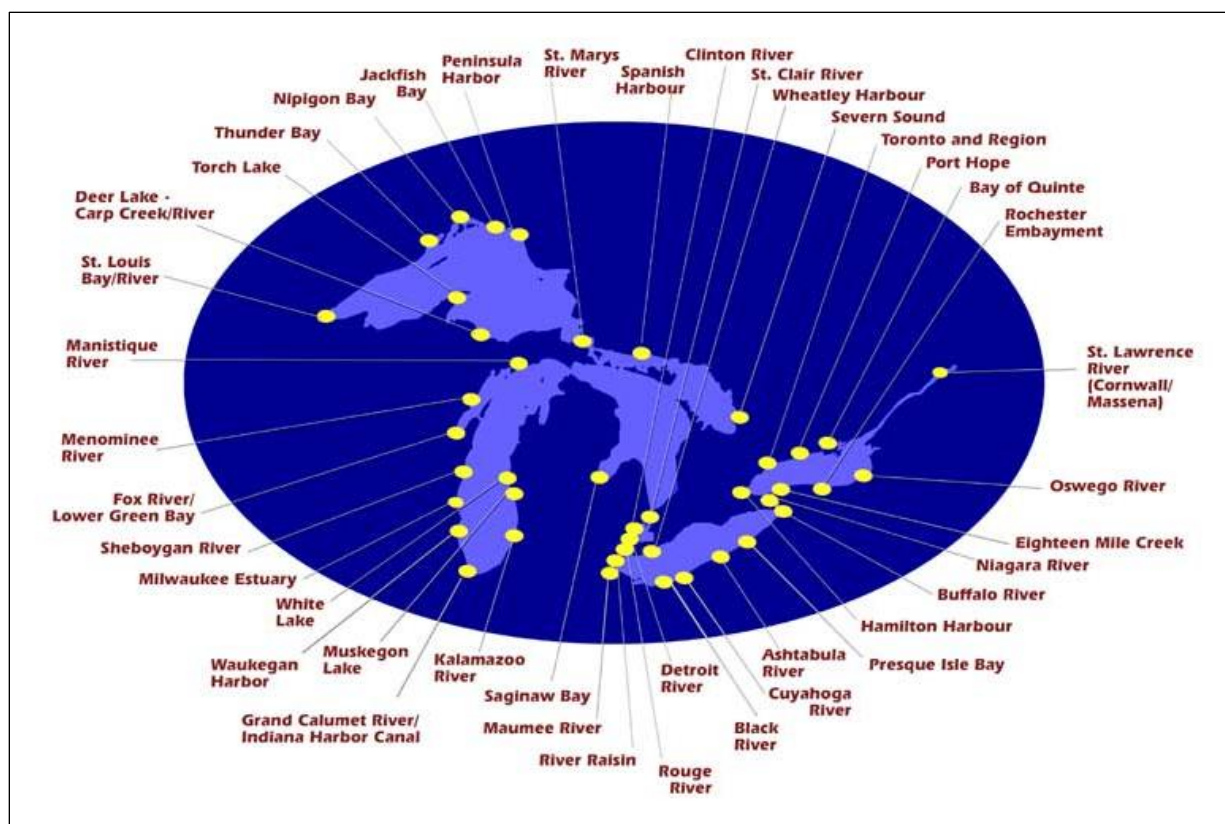


Figure 3. Areas of Concern where toxic chemicals have been shown to cause reproductive problems in fish and wildlife.

With IJC leadership, the USEPA and Environment and Climate Change Canada (ECCC) developed the Great Lakes Binational Toxics Strategy (GLBTS; www.binational.net) to reduce PBT contaminants in the Great Lakes ecosystem. Efforts toward meeting the goals of this strategy are taken by federal (both U.S. and Canada), state, provincial, and tribal agencies, and non-governmental organizations, who voluntarily adopt indicators identified and developed through a series of meetings and reports involving over 300 scientists from around the world. The results of these monitoring efforts are presented every other year at The State of the Great Lakes Ecosystem Conference (SOLEC) and are presented broadly to stakeholders in a State of the Lakes Report online at www.epa.gov/glnpo/solec/.

1.4 Toxic Compounds of Concern

There are hundreds of anthropogenic compounds in water bodies of the three parks being monitored under this protocol. We have narrowed our scope to specific contaminants based on the following criteria:

1. Persistence, bioaccumulation, and toxicity to fish, wildlife, and humans.
2. Listing in section 303(d) of the federal Water Quality Act (including the cause of a fish consumption advisory).

3. Listing as a Level I Substance in Appendix I of the Great Lakes Binational Toxics Strategy.
4. Identification by state and federal authorities as a new and emerging chemical of concern.

All three parks have at least one water body listed as impaired under the state's 303(d) designation because of a bioaccumulative contaminant and/or listed as a Level I Substance in the Great Lakes Binational Toxics Strategy (Table 2).

We will periodically re-examine contaminants for inclusion in the monitoring protocol; however, annual monitoring of all chemicals in Table 2 would be cost prohibitive. Initially we will focus on the following six PBT contaminants:

- Lead
- PCBs (total and 75 congeners)
- Mercury (total)
- PBDEs (total and 9 congeners)
- DDT (including DDE & DDD)
- PFCs (total and 16 telomers)

These six chemical compounds, further described in Appendix B, account for most of the 303(d) and GLBTS listings or have been identified as new and emerging compounds of concern (Table 2). They are also some of the most toxic and pervasive compounds in the upper Midwest. Some of the remaining compounds, such as aldrin/dieldrin and chlordane, have either not been detected or have been found in low concentrations in Lake Michigan and Lake Superior (Roe et al. 2004).

Additionally, legacy compounds such as DDT and PCBs have been found to be near or below the estimated threshold levels for impairment (Dykstra et al. 2005, Dykstra et al. 2010, Dykstra et al. 2019) and may be monitored infrequently in future years.

Table 2. Water bodies in three parks of the Great Lakes Inventory and Monitoring Network with persistent, bioaccumulative toxicants that are either state-listed 303(d) impaired water bodies, listed as a Level I Substance in Appendix I of the Great Lakes Binational Toxics Strategy (GLBTS), or designated by state or federal regulators to be a new and emerging contaminant of concern. State 303(d) listings can be found at <http://oaspub.epa.gov/>.

Park	Water Body	Contaminants and Reasons for Monitoring
APIS	Lake Superior	Level I Substances from GLBTS: Aldrin/dieldrin Alkyl-lead¹ Benzo(a)pyrene {B(a)P} Chlordane Dichlorodiphenyltrichloroethane (DDT¹) & metabolites DDE¹ and DDD¹ Dioxins Hexachlorobenzene Mercury¹ Mirex Octachlororstyrene Polychlorinated biphenyl (PCB^{1,2}) PBDE^{1,3} and PFCs^{1,4} (New and emerging)
MISS	Mississippi River	PCBs¹ (State-listed 303(d)) PBDE¹ and PFCs¹ (New and emerging)
SACN	St. Croix Flowage Minong Flowage Yellow Lake Mud Hen Lake St. Croix River	Mercury¹ (State-listed 303(d)) Mercury¹ (State-listed 303(d)) Mercury¹ (State-listed 303(d)) Mercury¹ (State-listed 303(d)) Bioaccumulative toxics¹ (State-listed 303(d)) PBDE¹ and PFCs¹ (New and emerging)

¹ Toxicants chosen for initial monitoring (also in bold).

² PCB = polychlorinated biphenyl

³ PBDE = polybrominated diphenyl ethers

⁴ PFCs = perfluorinated compounds

1.5 Use of Biosentinel Species for Monitoring

For over four decades, fish and wildlife that bioaccumulate toxic compounds have been used for monitoring ecosystem quality in the Great Lakes (e.g., State of the Lakes 2005) and other regions of the world (Olsson et al. 2000, Whitehead et al. 2003, Hollamby et al. 2004). Many of the persistent contaminants in these ecosystems occur at biologically relevant concentrations, but some cannot be effectively monitored through sampling the water directly. The concentrations of contaminants in water are often measured in parts per trillion or even quadrillion, which are costly to quantify—if they can be detected at all. However, certain species of fish and wildlife atop aquatic food webs biomagnify these compounds to concentrations that can be readily measured. These sentinel species can be studied to detect changes in environmental quality and the potential for adverse effects for both wildlife and humans.

Species vary in their sensitivity to the same compound, and therefore the effects observed in one species may not be manifested in others, even when exposed to the same concentration. Yet, fish-eating wildlife share with humans a common aquatic food web and similar complex physiologies; both can suffer adverse effects controlled through a similar mechanism. Hence, we can make qualitative statements of risk to humans by monitoring contaminants in fish-eating birds such as the bald eagle.

We selected the bald eagle as a sentinel species for the following reasons:

1. Documented linkages between the reproductive health of bald eagles and PCBs, DDE, lead, and mercury allow us to relate concentrations of these target PBT contaminants in tissue samples to developmental deformities and/or impaired reproduction.
2. We have useful historical data on bald eagle population status and contaminant exposure from more than 30 years of monitoring in the Great Lakes and surrounding ecosystems (including past work at GLKN parks).
3. NPS is compelled to use the bald eagle as a sentinel species in monitoring programs when possible because this species was selected for monitoring under the joint U.S. Canadian Great Lakes Water Quality Agreement.
4. State, provincial, and federal partners are willing to share the costs of monitoring this species.
5. Bald eagles are readily sampled with proven, published methods.
6. The bald eagle is an iconic species that can arouse public interest and help motivate park managers and elected officials to address problems elucidated by this monitoring effort.

1.6 Monitoring Questions

The following questions are derived from our monitoring objectives, from the list of PBT contaminants, and from questions posed by park staff and other scientists (Route 2004). We have attempted to identify explicitly the contaminants, species, and tissues that will be monitored. We have excluded questions that will not be addressed through this monitoring program either due to prohibitive cost or because they will be addressed in other protocols.

This protocol is designed to answer the following questions for the parks being monitored:

1. What is the direction and magnitude of change in concentrations of target contaminants in nestling bald eagles?
2. What are the spatial patterns of target contaminants and are such patterns linked to ecosystem components or human development?
3. Is the direction and magnitude of change in bald eagle productivity correlated with levels of any target contaminant?

4. What is the incidence of addled eggs, developmental deformities, and mortality in bald eagle nestlings, and can these factors be linked to the target contaminants?

2.0 Monitoring Strategy

2.1 Regional Approach

Our sampling design builds upon other monitoring programs in the Great Lakes region. The bald eagle has been used to monitor levels of persistent chemicals in the aquatic environment of the upper Midwest for over 30 years (Kozie 1986, Best et al. 1990, Shapiro-Hurley 1990, Kozie and Anderson 1991, Ensor Giovengo 1997, Bowerman et al. 2003, Roe et al. 2004, Dykstra et al. 2005, Dykstra et al. 2010, Dykstra et al. 2019, Route et al. 2014a, Route et al. 2014b). The bald eagle was selected as the primary avian sentinel species by the Michigan Biosentinel Program, which has monitored the concentrations of organochlorine pesticides and PCBs in nestling eagle blood since 1986. Our protocol adopts the methods of the Michigan Biosentinel Program (Roe et al. 2004) and those used by the Wisconsin Department of Natural Resources (Meyer et al. 1993) for collecting and analyzing blood and feather samples from bald eagle nestlings.

2.2 Sampling Locations

Monitoring will occur at APIS, MISS, and SACN. Each park is a study area except that the St. Croix National Scenic Riverway (SACN) is divided into lower (L-SACN) and upper (U-SACN) study areas (see Figure 1). The SACN is a 250-mile-long river system, and the physical properties of the river and surrounding geology and human use make the two study area designations ecologically different. Additional study areas may occasionally be sampled to put these park areas in context.

Sampling will occur at the occupied nests of eagles that rely on the park's aquatic resources. Most sampling will occur within park boundaries, but we extend sampling to nests that are within 0.5 km of each park boundary. Eagles normally occupy one nest per year, but have alternate nests within a territory (See SOP #4, Occupancy and Productivity Surveys). (Note: an occupied "nest" and an occupied "territory" are considered the same.) Individual nesting territories can be identified by delineating logical breaks between nests or groups of nests and examining occupancy over years. A territory has only one occupied nest, though alternate, un-occupied nests can be in the territory.

Concentrations of organic compounds in blood and feathers of bald eagle nestlings are a direct reflection of contamination in aquatic prey captured by adults within a foraging area around their nest (Bowerman et al. 2002). Bowerman et al. (2002) calculated that growth from egg to five weeks, the minimum age for sampling, diluted any residual contaminants found in the eggs. Even a concentration of 100 parts per million (ppm) (wet weight) in an egg would be undetected in nestling blood plasma because of dilution (change in volume) through normal growth (W. W. Bowerman, Clemson University, Clemson, SC, unpublished data). Hence, contaminants in nestling blood plasma and feathers are derived from their diet obtained from the local environment.

The occupied nest may be within a park one year and outside the boundaries the next. This is especially true in long, narrow parks such as SACN and MISS with complex political boundaries and where alternate nests can be found on ridge tops or bluffs away from the river. Yet it is important to collect samples from the same territories each sample year to reduce bias. Nesting pairs mate for life and will use similar habitats and aquatic prey within their territory such that, regardless of nest location, samples from the same territory are not independent. To reduce sample bias, we will

attempt to sample the same territories each year and use analytical techniques that account for the lack of independence (see sub-section 2.6, Detection Limits, Transformations, and Summary Statistics). To ensure adequate sample size for APIS, we will include Madeline Island, which is not park-owned.

2.3 Occupancy and Productivity Surveys

The number of territories occupied by bald eagles (occupancy survey) is determined annually by analyzing data collected through aerial surveys by park partners using methods similar to Postupalsky (1983). Occupancy and productivity surveys (see below) can be attempted from the ground, but such surveys often take longer and the data are suspect due to the difficulty in seeing and counting nestlings in a nest 60 to 80 feet up in a tree (Fuller et al. 1995).

The Wisconsin Department of Natural Resources (WDNR) has flown occupancy surveys for both APIS and SACN using a relatively slow, maneuverable fixed-wing aircraft such as a Super Cub or Cessna 185. The WDNR has conducted these surveys since 1972 and we have reasonable expectation that they will continue in the future. At MISS, we will either conduct the occupancy surveys ourselves using fixed-wing aircraft certified by the U.S. Department of Interior Office of Aviation Services (e.g., VOYA pilot in 2014 and 2015), or we will accept data provided gratis by local partners (e.g., Mississippi Park Connection in 2016 and 2017). The Mississippi Park Connection works with the senior biologist at Three Rivers Regional Park in Minneapolis, who conducted these surveys by helicopter from 2006 through 2009 and again in 2014 and 2015. Use of a helicopter is preferable for nests near the Minneapolis/St. Paul International Airport.

These occupancy surveys will result in GPS locations of all occupied bald eagle nests within and directly adjacent (within 0.5 km) to the parks. A nest will be considered occupied if there is an adult incubating eggs, two adults at a nest, one or two adults near a repaired nest (e.g., egg cup, fresh lining material, green twigs, sticks with fresh breaks on top), or if eggs or young are observed (WDNR 2016).

Our partners also conduct separate aerial surveys to determine the number of young produced in each occupied territory (productivity survey). However, aerial productivity surveys are not always done annually; the State of Wisconsin cut funding for productivity surveys in many years, and Minnesota ended them in the early 2000s. Both occupancy and productivity are determined by on-the-ground field crews in years when blood and feather samples are collected from bald eagle nestlings (see sub-section 2.5, Sampling Frequency) .

2.4 Sample Size

The number of occupied bald eagle nests each year will vary among parks and between years, depending on habitat availability and health of the eagle population. The bald eagle population has grown in each study area since we began our contaminants monitoring program in 2006 (Table 3), but past surveys suggest that as few as three and as many as 30 nests in each park will have at least one nestling for sampling. However, based on bald eagle population growth we expect that sample size will exceed the maximums in Table 3 in future years.

Table 3. Number of bald eagle nests that were successful and contained at least one nestling suitable for sampling contaminants in and directly adjacent to parks of the Great Lakes Network. Data from 2006–2015 sampling under earlier versions of this protocol.

Park	Minimum and maximum # of successful nests in and within 0.5 km of the park	
	Min (year)	Max (year)
APIS	5 (2008)	13 (2015)
MISS	7 (2006)	30 (2014)
L-SACN	3 (2009)	13 (2012)
U-SACN	13 (2010)	18 (2015)
Total	28	74

Ground crews will use GPS coordinates from the occupancy survey to locate and sample occupied nests. We will attempt to get a complete census of all occupied nests; however, some nest trees will be unsafe to climb (e.g., dead tree or unstable nest structure), and some landowners will deny access. For these nests, ground crews typically can still make an on-site verification of nest occupancy and a minimum estimate of productivity.

We will collect blood and feather samples from at least one nestling at each occupied nest that is safe to climb. When there is more than one young in a nest we will randomly select one as the “primary sample” and attempt to collect samples from all siblings to save as archives. The number of young produced per active nest will vary from one to three and rarely four.

Normally, samples from only the one primary, randomly selected nestling will be analyzed for contaminants each monitoring year. The random selection will be by flip of a coin or random number at the nest site. Samples from siblings will be archived (serum in freezers at -20°C and feathers dried in envelopes) for future assessments of new and emerging contaminants, with frozen samples likely viable for 15 or more years (e.g., Custer et al. 2009).

Only one nestling will be sampled from a multiple-brood nest because of impending darkness, an approaching storm, or other circumstances that warrant haste. In these cases, we will sample the nestling nearest the climber as he/she enters the nest.

Our sampling effort is enhanced by the fact that each nestling integrates contaminants from a large number of prey. The adult eagles are effectively “sampling” the environment for us. For example, the average numbers of food items brought to a nest over two years on Outer Island (APIS) were 3.4 and 2.6 per day with two and one nestling, respectively, and the bulk of these prey items were fish (97% of 218 food items observed; Kozie 1986). Considering that we sample nestlings when they are five to nine weeks old (35 to 63 days), a single nestling will integrate contaminants from 90 to 160 prey items.

2.5 Sampling Frequency

Bald eagle nestlings were sampled in each of the core park study areas a minimum of six years and up to eight years between 2006 through 2015 (Table 4). This sampling effort provided a good baseline to characterize the mean concentrations and spatial patterns of the target contaminants at each of the study areas (Dykstra et al. 2010, Dykstra et al. 2019, Route et al. 2014a, Route et al. 2014b, Bruggeman et al. 2018). The recommended schedule is two years of sampling followed by two years off, then two on, two off, and so on through time. This sampling schedule would provide statistically robust data for long-term trends. However, if funds are not available, we recommend sampling as frequently as possible with a minimum of three years in a row followed by a seven year gap (three in every ten years). Hence, we recommend that the four core study areas be sampled again, minimally in 2023, 2024, and 2025. It will be important to sample at least three years in a row to provide a mean for the period and to minimize the potential for hitting an unusual year (e.g., poor reproduction, high flooding, etc.). This minimum revisit design will allow decadal comparisons to the initial baseline and should provide sufficient data for longer-term trends (Roe 2004, Roe et al. 2004, Dykstra et al. 2005) while economizing sampling costs and effort.

Table 4. Years in which bald eagle nestlings were sampled (X) for environmental contaminants at four study areas in three parks from 2006 through 2015 and proposed sampling period (S) in following years.

Year	APIS	MISS	L-SACN	U-SACN
2006	X	X	X	X
2007	X	X	X	X
2008	X	X	X	–
2009	–	X	X	–
2010	X	X	X	X
2011	X	X	X	X
2012	–	–	–	–
2013	–	–	–	–
2014	X	X	X	X
2015	X	X	X	X
2016	–	–	–	–
2017	–	–	–	–
2018	–	–	–	–
2019	–	–	–	–
2020	–	–	–	–
2021	–	–	–	–
2022	–	–	–	–
2023	S	S	S	S
2024	S	S	S	S
2025	S	S	S	S

2.6 Detection Limits, Transformations, and Summary Statistics

Samples below the laboratory's analytical limits of detection (LOD) will normally be assigned a value using Kaplan-Meier (Helsel 2012) or similar methods for replacing non-detections (see also Route et al. [2014b] for use of a Bayesian statistical framework). These methods are often used for survival analyses of right-censored data and contaminants data are left-censored. Programs in R (R Core Team 2015) such as package NADA (Helsel 2012, Lee 2015) flips the censored data, calculates means, medians, and standard deviations, then transforms estimates back to the appropriate scale. Values will then be checked for normality and log-transformed if necessary to correct for skewed distributions. If transformations will not adequately normalize distributions, non-parametric statistics will be used.

Occasionally we may elect to use simpler substitution methods such as half the LOD. This may be desirable when comparing current contaminant levels against historical results where the method should be consistent. Leith et al. (2010) provides a good example of where half the LOD performed well against Kaplan-Meier replacement methods and can be a valid approach for assessing contaminants data.

Raw or log-transformed results will provide arithmetic or geometric mean contaminant concentrations and measures of variability (95% confidence intervals, standard deviations, or standard errors). Geometric means are usually a better measure of central tendency for contaminants in biota, which tend to be log-normally distributed (Ginevan and Splitstone 2003).

2.7 Data Analysis and Presentation

Bar or line charts, scatter plots, and maps showing patterns of distribution across study areas will be used to compare levels of contaminants against known or hypothesized thresholds (Schmitt et al. 2008; Figures 4, 5, and 6). Thresholds have been established in the literature for the legacy contaminants (DDE and PCBs) (Table 5). However, very few studies have documented the lethal and sub-lethal effects of many other contaminants on bald eagles. This is due to the bald eagle being federally endangered or threatened since the 1970s, which has limited controlled studies of lethality. Information is particularly sparse for new and emerging contaminants. Thus, for some contaminants we will simply report the concentrations and the magnitude and direction of change. Although other protections such as the Bald and Golden Eagle Protection Act are still in place, removal of the bald eagle from the Federal Endangered Species List in 2007 has increased laboratory-based research and lead to the development of new thresholds. For example, Scheuhammer et al. (2008) found a significant relationship between mercury levels in the liver and certain neurochemicals in the brain of bald eagles. A more recent study by Rutkiewicz et al. (2011) assessed mercury in tissues (liver, brain, feathers) of adult bald eagles collected during this study and from investigators in other Midwestern states. Results suggest bald eagles in the Great Lakes region are exposed to mercury at levels capable of causing subclinical neurologic damage.

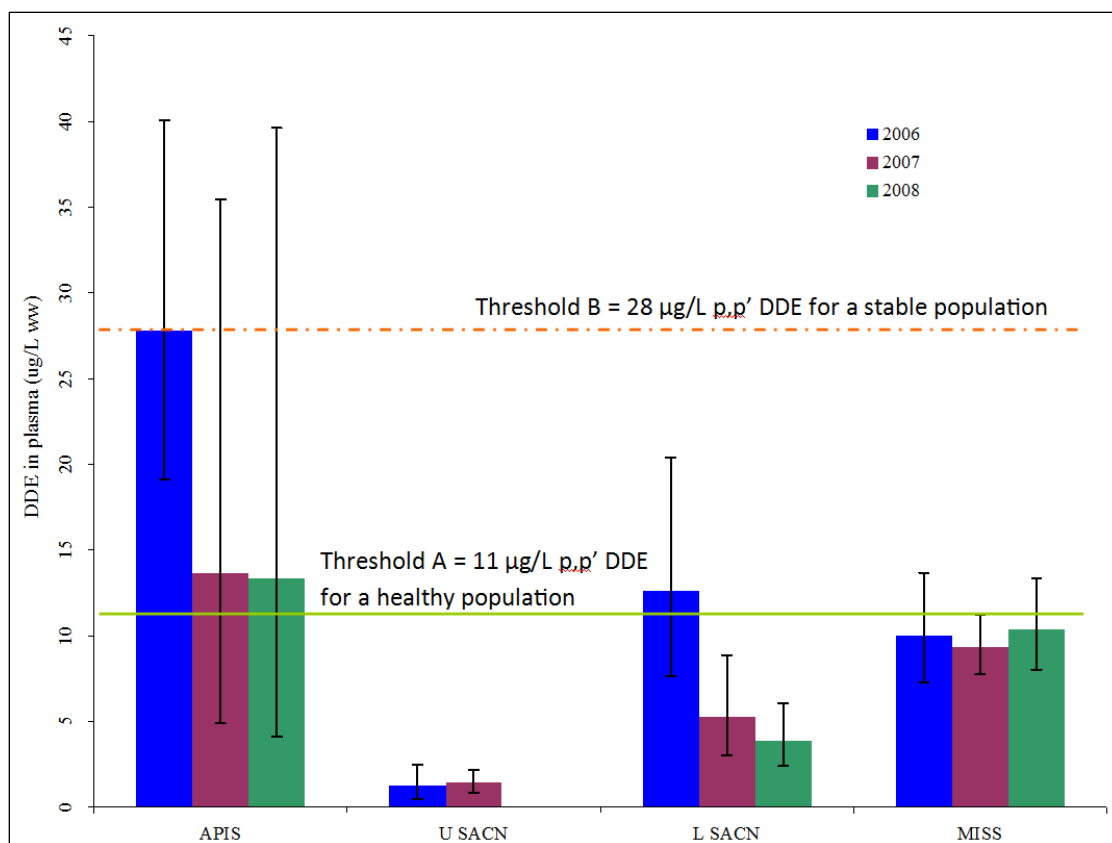


Figure 4. Example of bar chart showing geometric mean levels ($\pm$ 95% CI) of p,p' DDE in plasma of bald eagle nestlings in four study areas in the Great Lakes Network in 2006–2008, compared with published p,p' DDE thresholds. Threshold A is based on a healthy bald eagle population producing 1.0 young per occupied nest; B is based on a stable population of 0.7 young per occupied nest. Results from data collected during pilot work on this protocol. See Table 5 for references on thresholds.

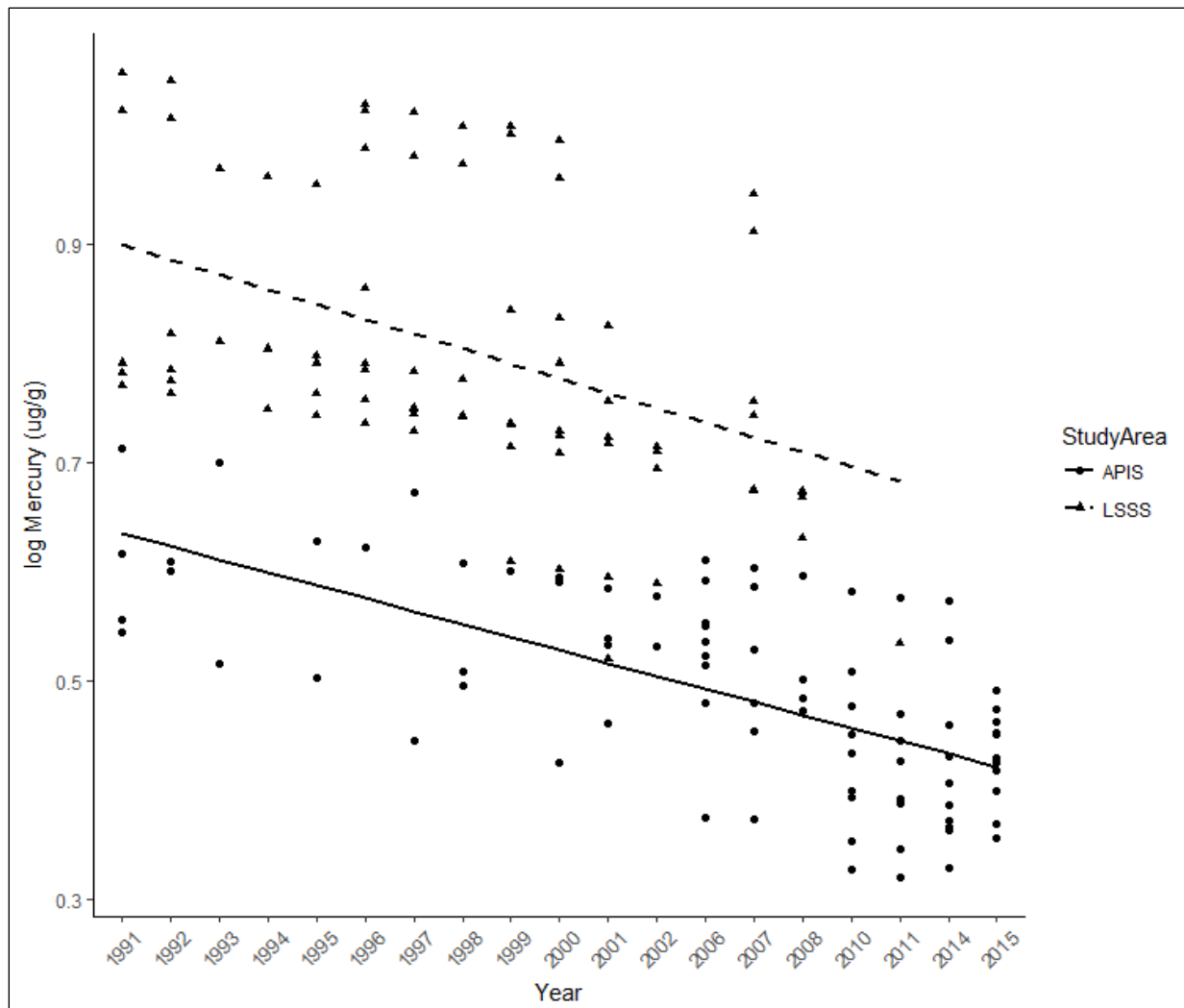


Figure 5. Example of fixed effects model results from Dykstra et al. (2019) showing the trend in concentrations of mercury in feather samples from bald eagle nestlings at Apostle Islands National Lakeshore (APIS) and the Lake Superior South Shore (LSSS) over time. Data are from the Wisconsin Department of Natural Resources (1991–2002) and this project (2006–2015); the regression line shows a significant downward trend over 24 years for LSSS and a non-significant decline for APIS. Note gap of three years between 2002 and 2006.

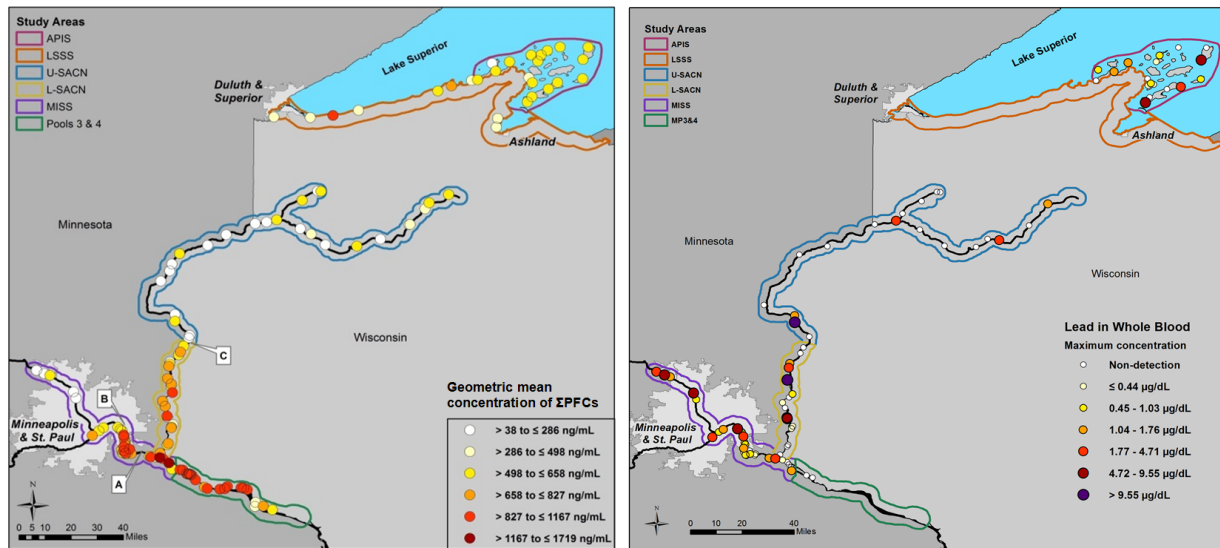


Figure 6. *Left panel:* Geometric mean concentrations of Σ PFCs in bald eagle nestlings, 2006–2011 (Route et al. 2014b). Values are modeled from measurements by three independent laboratories. **A** = location of 3M's PFC production facility; **B** = Minneapolis/St Paul WWTP and St. Paul Downtown Airport; **C** = the communities of St Croix Falls, WI, and Taylors Falls, MN. *Right Panel:* Maximum concentrations of lead (Pb) in whole blood. Values are the highest measured concentration in each territory from 2006 through 2015 (Bruggeman et al. 2018).

Table 5. Threshold values for contaminants that may affect bald eagle populations.

Variable / Contaminant	Threshold value ¹	Reference
Population productivity	1.0 young per occupied nest; >50% of occupied nests successful for a healthy population.	Wiemeyer et al. 1984
DDT	>0.0 µg/L in blood as an alert to parks for potential local hotspots.	This pesticide was banned in North America in 1972; any detections should be noted
DDE	11 ppb ww p,p'-DDE in plasma; NOAEC ² for healthy reproduction. This was updated to 11.4 ng/g in plasma as NOEL ³ for productivity.	Bowerman et al. 2003 for NOAEC; with update in Pittman et al. 2015 for NOEL.
	28 µg/kg DDE in nestling plasma, corresponding to 6 mg/kg DDE in added eggs and based on 0.7 young per active nest for a stable eagle population.	Elliot and Harris 2001/2002
	3.5 ppm ww p,p'-DDE in eggs; NOAEC ² for eggshell thinning effects; 15-16 mg/kg in added egg for near total reproductive failure.	Wiemeyer et al. 1984

¹ Values are as reported in the literature. Conversions: µg/kg = µg/L = ppb = .001 ppm

² NOAEC = No Observable Adverse Effect Concentration

³ NOEL = No Observable Effect Level

⁴ TRV = Toxicity Reference Value

Table 5 (continued). Threshold values for contaminants that may affect bald eagle populations.

Variable / Contaminant	Threshold value ¹	Reference
PCBs	190 µg/kg total PCBs in nestling plasma, corresponding to 20 mg/kg PCBs in addled eggs and based on 0.7 young per active nest for a stable eagle population.	Elliot and Harris 2001/2002
	4.0 ppm ww total PCBs in eggs; NOAEC ² for egg lethality	Wiemeyer et al. 1984
	33 ppb ww total PCBs in plasma; NOAEC ² based on 1.0 young per active nest for a healthy population. This was updated to 36.4 µg/kg in plasma as NOEL ³ for productivity.	Bowerman et al. 2003 for NOAEC and Pittman et al. 2015 for updated NOEL
PBDEs	Thresholds in plasma are currently unknown.	–
PFCs	Thresholds in plasma are currently unknown.	–
PFOS	1,700 ng/mL in blood plasma as a TRV ⁴ for a level IV fish-eating bird such as the bald eagle.	Newsted et al. 2005
Mercury	7.5 µg/g dw in nestling breast feathers	Jagoe et al. 2002
	20.0 µg/g in adult raptor primary feathers	Proposed by Scheuhammer and Norris 1996
Lead	Thresholds in feathers are currently unknown.	–
	20–60 µg/dL in whole blood considered subclinical; >100 µg/dL considered acute exposure for adult eagles. Bruggeman et al. (2018) suggest 5 µg/dL, which is the threshold for human children, be considered a preliminary threshold for nestlings.	Kramer and Redig 1997; Bruggeman et al. 2019

¹ Values are as reported in the literature. Conversions: µg/kg = µg/L = ppb = .001 ppm

² NOAEC = No Observable Adverse Effect Concentration

³ NOEL = No Observable Effect Level

⁴ TRV = Toxicity Reference Value

Regression analysis will be used to estimate the magnitude of change (i.e., slope of the regression line) in mean annual concentrations of each contaminant in each park over time (Figure 5).

Depending on variability within a geographic area, Roe et al. (2004) found that sampling as few as two nestlings per year from different locations can suffice to determine differences among areas, and they detected a 10% difference in concentrations of PCBs or DDE over time periods, with an alpha of 0.05 and power of 0.8. Our 2006–2015 sampling routine, with as few as three and as many as 30 samples per park per year, provided adequate statistical power to determine trends in all targeted contaminants for all study areas, including many individual congeners (Dykstra et al. 2010, Route et al. 2014a, Route et al. 2014b, Bruggeman et al. 2018).

Statistical power is enhanced in this study by sampling the same bald eagle territories, resulting in a repeated measures design. However, it cannot be assumed that these territories are independent, and data analysis must take this into account. The adults are often the same individuals who use the same area to acquire prey so that contaminants that accumulate in prey are likely correlated with feeding

territory. An important assumption in linear regression and analysis of variance (AOV) procedures is that observations are independent. We have employed mixed effects models to analyze samples grouped within territories (e.g., Dykstra et al. 2010). For mixed effects models, territory is identified as a random effect to account for correlations among samples within territory. To compare differences among study areas we used study area and year as fixed effects to account for annual variation in sample size and regional effects such as weather. We have also employed Bayesian modeling (Route et al. 2014b) where again we accounted for correlations between eagle territories (specifically, the correlated auto-aggressive model, *car.normal*, in WinBUGS).

For each contaminant, we will examine spatial patterns by mapping concentrations found in individual nestlings across the study areas (Figure 6). Categories (Bins, in GIS parlance) should be selected using the natural breaks feature in ArcGIS to remove bias. When combined with land-use data, these maps can help document point and non-point sources of pollution. For example, our data show that PFCs in eagles at MISS and lower SACN are primarily from point sources (left panel of Figure 6). These PFCs are globally distributed in the environment, but have been manufactured by 3M Corporation in St. Paul, Minnesota, since the 1950s. The company has a chemical plant (shown as *A* in Figure 6) and several waste disposal sites up river between *A* and *B*. Moreover, PFOS, which is one of the most common PFC isomers, has been used locally in chrome plating, and the effluent from this industry is run through the Metropolitan waste treatment plant (shown as *B* in Figure 6). A study in 2007 by the Minnesota Department of Health and the Minnesota Pollution Control Agency found that about 95% of the PFOS found in influent, effluent, and sludge from a wastewater facility in Brainerd, Minnesota, (just upstream from MISS) was from a chrome plating facility (Kelly and Solem 2007). The State of Wisconsin is investigating a similar facility along the lower St. Croix River below Taylors Falls (downstream from *C* in Figure 6).

By contrast, lead (Pb) levels in whole blood of nestlings may be associated with more dispersed sources (right panel of Figure 6). These patterns reflect more dispersed, random, ingestion of Pb fragments in prey or inadvertent ingestion of lead sinkers attached to fish brought to the nest (Bruggeman et al. 2018).

2.8 Advanced Data Analysis and Use of Ancillary Data

In addition to area comparisons and regression analysis (including non-parametric equivalents), monitoring data may be evaluated through Monte Carlo simulations, Bayesian analyses (e.g. Route et al. 2014b), and comparisons of period means. For the latter-most approach, one is often interested in comparing values before and after an important event (e.g., change in management policy, known toxic release, or reduction in emissions) and considers years within each of the two periods as replicates. Trends in parameters that are analyzed with respect to biotic and abiotic covariates may be examined and cause-effect relationships may be investigated more thoroughly by partners and collaborators (e.g., USGS or university investigators).

The data can be used to compare concentrations with water quality data, such as dissolved organic carbon (DOC) and pH, which are collected under a separate network protocol (Elias et al. 2015, Magdalene et al. 2016). These parameters are highly correlated with mercury. We will also consider using stable isotopes, such as lead, nitrogen, and carbon as ancillary data. Hebert and Weseloh (2006)

showed that dietary differences caused temporal variation in the trophic position of herring gulls. They concluded that adjusting for these changes using data from stable isotopes can provide more accurate assessments of trends in environmental contaminants. (For a review of stable isotopes and ecotoxicology, see Jardine et al. [2006] and Fry [2008].)

Chemical concentrations and other measures such as deformities may be analyzed collectively through multivariate ordinations (e.g., non-metric multidimensional scaling; NMS or NMDS) of resource conditions through time, following West and Yorks (2002). This approach effectively integrates information across many indicators and can suggest whether nesting pairs are all changing in the same direction in multidimensional ordination space. Furthermore, joint plots can be overlaid on the ordination to explore which variables correlate most strongly to the direction of changes.

2.9 Interpretations and Recommendations to Managers

This monitoring program is designed to document concentrations and monitor trends in targeted PBT contaminants in tissues of bald eagles. It is not designed for making conclusions about whether a contaminant has caused a biologically significant effect on wildlife or humans. Nonetheless, estimated threshold values are available in the literature for assessing population status and for comparing with documented cause-and-effect relationships for some of our targeted contaminants (see Table 5).

We will compare our data with these thresholds (see sub-section 2.7, Data Analysis and Presentation), which can be presented to park managers with recommendations. When interpreting results and making recommendations, the following guidelines will apply:

1. A decline in a bald eagle population, even when negatively correlated with trends in one or more contaminants, is not reason in itself for concluding there is a cause and effect relationship. Rather, exposure to contaminants constitutes one of many variables to be considered when assessing the health of the population. We may find that eagle populations are in decline, but that concentrations of the targeted contaminants are below known threshold values. Wildlife populations undergo natural fluctuations and we will be vigilant about assessing all natural and anthropogenic stresses. Indeed, another contaminant could be acting on the population and we may need to screen for new and emerging contaminants.
2. We will compare the concentrations of targeted contaminants directly with studies where cause-effect relationships have been documented (e.g., Table 5). The surrounding states, USEPA, and ECCC continue to develop and refine meaningful thresholds for various contaminants and we will continually update our table of thresholds.
3. We will be concerned if we find reduced embryo viability (addled eggs), chick wasting, and/or developmental deformities clustered in space and time, whether in concert with our suite of contaminants or not. Such observations are suggestive of impairment and could indicate that significant concentrations of developmental toxins are present.

To the extent possible, we will compare our temporal and spatial patterns in bald eagle contaminant levels with levels in the atmosphere surrounding the parks. Atmospheric transport and deposition is

an important pathway for contaminants to enter park ecosystems. Sources for atmospheric data include:

- The Integrated Atmospheric Deposition Network (IADN), a long-term monitoring program that has measured atmospheric concentrations of PCBs, DDE, and several other contaminants in air over the Great Lakes since 1990 (Sun et al. 2007) and;
- The National Atmospheric Deposition Program (NADP), which has been in operation since 1977 and includes a Mercury Deposition Network (MDN) with stations across the Great Lakes region.

Bald eagles generally forage in areas surrounding the nest during the chick-rearing period. Hence, contaminants found in eagles, which result from biomagnification in the food web, can be directly related to the park or adjacent environments. Stalmaster (1987) summarized the literature by stating that normal bald eagle territory size is about 1–2 km². In Minnesota and Michigan, territory size ranged from 1.09 to 1.55 km² (Mattsson 1974, Mahaffy 1981). Yet, some territories are larger and foraging areas can be greater than “defended” territories. For example, the defended territory of a pair of radio-tagged bald eagles in Saskatchewan, Canada, was 4 km², whereas the foraging area was 7 km² (Gerrard et al. 1992). Thus, we suspect bald eagles in our study areas will integrate contaminants from an area ranging from approximately 1 to 7 km² around the nest.

Furthermore, results from this monitoring will reflect contamination of the park’s aquatic environment. Prey remains at 285 nests in northern Minnesota, Michigan, and Wisconsin indicated that fish (mainly suckers [*Catostomus* spp. and *Moxostoma* spp.], northern pike [*Esox lucius*], bullheads [*Ictalurus* spp.], common carp [*Cyprinus carpio*], and bowfin [*Amia calva*]) were the most common summer food items (77%), followed by birds (15%; mainly waterfowl and gulls), and mammals (5%) (Bowerman 1993). Dunstan and Harper (1975) found that fish comprised 90%, birds 8%, and mammals 1% of prey remains at nests on the Chippewa National Forest in Minnesota. In inland waters of Wisconsin, fish comprised 84%, birds 6%, and mammals 7% of the prey remains at nests (P. Keasling graduate student, University of Wisconsin-Madison, and C. Sindelar, private contractor, WDNR, Waukesha, WI, unpublished data).

In summary, results from this monitoring program can be used to recommend that park managers:

1. Apprise state and federal regulators when/if concentrations of target contaminants are increasing over a period of years and/or when they approach, exceed, or decline below thresholds;
2. Investigate potential “hot spots” where comparatively high levels of one or more contaminants are found;
3. Work with researchers and other science partners to assess causes of contamination and possible remedies;

4. Educate park visitors about the sources, pathways, and effects of contaminants in the environment, such as PCB and PBDE releases from burn-barrels and other poor waste disposal practices;
5. Educate park visitors about state and federal health advisories, such as fish and waterfowl consumption advisories based on mercury and PCB levels;
6. Work with researchers and other science partners to assess eagle population declines, high rates of egg or nestling mortality, disease, deformities, or other problems uncovered as a result of our examining bald eagle nestlings and monitoring their populations.

3.0 Overview of Sampling Methods

3.1 Field Season Preparations

Field season preparations are described in detail in SOP #2, Training, Certifications, and Permits, and SOP#3, Preseason Preparations. The project manager will obtain all necessary state and federal permits to handle and band bald eagles. Occupancy surveys will be scheduled and conducted in early spring each year (late May for southern nests and early June for more northerly nests). The resulting survey data will be obtained from the pilot/observer teams and then mapped to assist in locating each occupied nest from the ground. Once nest sites are determined, park and network personnel will visit and observe a number of occupied nest sites from the ground to determine the approximate age of nestlings in order to estimate sampling dates. Cooperators will then be contacted, notified of sampling dates, and transportation (boats, vehicles) arranged. Private landowners also will be contacted for permission to access their property.

At minimum, the handling crew will consist of a climber and handler. Climbers should be hired (or contracted) and should complete all safety training and certifications (SOP #10, Safety) well in advance of field operations. Primary handlers must be experienced and should secure all sampling materials and prepare all field equipment prior to the initiation of fieldwork.

3.2 Field Measurements and Sampling

Handling, measuring, and collecting blood, feather, and egg samples from nestling bald eagles is described in detail in SOP #1, Nestling Bald Eagle Handling, Measuring, and Sample Collection. After the age of the nestling(s) is determined to be within guidelines and the tree is judged to be safe, the climber will climb to the nest and lower the nestling(s) to the ground in specially designed bags. The handler will first weigh the nestling(s) before removing it from the bag(s) and will then record both total and tare weight. After removal, measurements of bill length and depth, and length of hallux claw will be taken using calipers, and the foot pad and eighth primary feather measured with a ruler (Bortolotti 1984a, 1984b, 1984c). Using a 10 cc plastic syringe, 10–12 cc of blood is drawn from the brachial vein. One cc (equivalent to 1 milliliter [1 mL]) of this whole blood is transferred to a labeled, 2 mL glass vacutainer with 7.5% solution of EDTA for analysis of select metals (primarily lead and mercury). The remaining blood is then transferred to a labeled glass 10 mL vacutainer and both are placed in a zip-lock bag and put on ice packs in a cooler. The last few drops of blood from the syringe are put on a 4" × 4" piece of gauze, placed in a small paper envelope, labeled, and put in the cooler. This small blood sample is dried and kept as a DNA sample to positively identify the nestling's sex. Finally, three to four breast feathers are collected from the nestling(s) by pulling them out at the base of the feather. The feathers are placed in a labeled envelope, which is sealed and placed in the cooler.

Before returning the nestling(s) to the nest, each bird is examined for deformities, wounds, or unusual markings, ear maggots, and fullness of crop. The eaglet is banded using #9 USGS rivet bands. Data sheets are checked to ensure all data was collected and that the data sheet is legible. While the nestling(s) are being returned to the nest, tree species, tree and nest condition, and GPS

coordinates of the tree are recorded, and the nest tree's height and diameter-at-breast-height are measured.

3.3 Sample Preparation and Laboratory Analysis

The following process may differ depending on targeted contaminants and the laboratory doing the final measurements of concentrations. Detailed information on sample processing, handling, and shipping can be found in SOP #7, Sample Handling. It is important to correspond with the laboratory to ensure the correct vials are being used. From 2006 through 2015, the laboratory selected the vials, acid-washed and/or sterilized them, and sent them to us for each season's fieldwork.

Each evening the 10 mL vacutainers are centrifuged for 8–12 minutes or until fully separated. One milliliter (1 mL) of serum is then drawn off using a glass pipette and placed in a vial provided by the lab for PFC analysis. The remaining serum is drawn off and placed in a separate vial provided by the lab for PCBs, PBDEs, and DDE, DDD, DDT analysis. Vials are labeled and frozen in an upright position. The remaining red blood cells in the original vacutainer, 2 mL purple-top vials to be analyzed for metals, and the DNA sample are also frozen. Envelopes containing DNA blood blots and feathers are labeled and stored in a dry, secure place.

All whole blood and plasma samples are kept frozen and hand delivered to the analytical lab in a portable freezer or shipped on dry ice in coolers with custody seals. If samples are shipped, they should be sent FedEx overnight and the lab contacted to ensure they are secured on the receiving end. [Note: In 2006, we shipped samples on dry ice and determined it was not worth the time, expense, and worry of shipping that quantity of expensive samples. Thereafter, we drove samples directly to the laboratory in Madison, Wisconsin.] The lab will retain the original chain of custody forms and send copies to GLKN. Original data sheets are archived at the Great Lakes Network Office.

All samples collected in the field are individually labeled with an assigned GLKN sample number and logged on field data sheets (SOP #7, Sample Handling). Original field data forms are retained in a binder at the GLKN office, a scanned version is archived in the bald eagle project folder, and data are entered into an MS Access database (for details see SOP #10, Data Management).

The diagnostics lab determines the concentrations of target contaminants using methods approved by GLKN. When possible we will use the same lab to ensure comparable data are collected through time. At minimum, contract labs will use methods consistent with or substantially better than those used by the Wisconsin State Laboratory of Hygiene (WSLH) from 2006–2015 as follows (detailed descriptions of methods are available upon request):

- PCBs and DDT — Gas chromatograph equipped with an electron-capture detector (ESS ORG Method 1810).
- PBDEs — Gas chromatograph-mass spectrometer operating in the negative ion mode (ESS ORG Method 1810).
- PFCs — Turbo ion spray, triple quadrupole mass spectrometer in the negative ionization mode (WSLH Method 1470).

- Mercury – Cold vapor atomic absorption (ESS INO Method 540.4).
- Lead — Inductively coupled plasma spectroscopy (ESS INO Method 620.2).

4.0 Data Management

4.1 Overview of Database

The GLKN Bald Eagle Bioaccumulatives Database is a Microsoft (MS) Access database with a front-end and back-end. The front-end is a user interface that provides access to queries, forms, reports, and visual basic code for underlying function of the database. The user interface consists of various forms to help the user enter and validate data, along with the ability to query the data for various quality assurance/quality control (QA/QC) checks and final reports. The back-end consists of numerous lookup and data storage tables, which are linked to the front-end.

Changes to the design or structure of the database can only be made by GLKN data management staff. Prior to any structural changes to the database, a copy with the most recent version number is stored in the “Archive” subdirectory. Each new version of the database is assigned a sequentially higher number. Version numbers allow the project managers and data management staff to track changes to the database over time. Changes to any record in the database are documented in the record itself and must include a description of pre- and post-edit changes, especially changes made to the data post-verification.

The data tables for this project consist of three general types: *standard tables* that describe the specifics (who, what, when) of the data collection, *project-specific tables*, and *lookup tables*. The standard tables are based on the Natural Resources Database Template standards, but have been added to or altered to contain fields or descriptions that meet project objectives.

4.2 Quality Assurance and Quality Control

Quality assurance and quality control (QA/QC) procedures are detailed in SOP #8 and in the Quality Assurance Plan (QAP; Route and VanderMeulen 2018). Steps are taken at all stages of the program—from protocol documentation through data collection, entry, and analysis—to ensure quality. These steps are highlighted throughout the protocol (e.g., section 4.3), in most of the protocol SOPs, and re-iterated and expanded upon in the QAP. The crucial procedures are summarized in Table 6. Additionally, each analytical laboratory is required under contract to have written QA/QC standards that meet high scientific standards, such as those promulgated by USEPA and the federal National Environmental Laboratory Accreditation Program (NELAP).

Table 6. Summary of quality assurance and quality control (QA/QC) procedures for the management of monitoring data at the Great Lakes Inventory and Monitoring Network.

Procedure	Description
Instrument calibration logs	Each instrument, such as GPS and weighing scales, are calibrated annually.
Field forms	Field forms are required by USEPA for all water quality related data. Copies will be placed in site binders and originals kept on file indefinitely.
Manual data entry	Field data will be manually entered into computer forms that look similar to field forms to reduce transcription error.
Data verification	All (100%) manual entries from hardcopy field forms will be checked. Errors will be corrected and documented.

Table 6 (continued). Summary of quality assurance and quality control (QA/QC) procedures for the management of monitoring data at the Great Lakes Inventory and Monitoring Network.

Procedure	Description
Data validation	Data validation is the checking of the data for missing values and against known ranges for outliers. This will be done during verification (above) and through queries within GLKN's master database.
Data qualification codes	Data will be coded to qualify its relative ability to represent or estimate the values being measured.
Laboratory QA/QC	QA/QC at the analytical lab includes matrix spikes and surrogate standards added to all samples to monitor analytical error. Method blanks are also analyzed after every 10 samples to check instrument calibration and contamination. Each individual sample is coded for exceedances based on these QA/QC procedures.
Data certification	Data sets will be individually certified before being used for reports and publications or released to others. Data certification differs between datasets as detailed in SOP #8 and the QAP.
Data archiving	Field data forms and associated records will be archived at the GLKN Ashland (WI) office in 3-ring binders numbered consecutively by year. Electronic data are validated, assessed for quality, certified, and maintained in a MS Access database. An authoritative copy of the database is available in the NPS IRMA Data Store, and a copy of the certified data is publicly available from IRMA. A working copy of the database is stored on the GLKN server, and a backup copy of the MS Access database is kept off-site at APIS.

4.3 Data Entry, Verification, and Certification

Detailed instructions for data entry procedures are given in SOP #9, Data Management. Two general classes of data will be collected. The first is field observations and measurements that are recorded on field forms by observers conducting occupancy surveys via helicopter or fixed-wing aircraft, and by field crews in years when eagle nestlings are sampled for contaminants. When eagle nestlings are sampled, certain elements of the field form will be pre-filled and printed from the database for nests visited in past years. This will help field teams get to the right tree (pre-filled GPS locations) and reduce multiple versions of nest names (especially alternates) that have plagued other bald eagle monitoring programs. The second class of data is the results of testing performed by contract analytical laboratories. Import routines have been created in MS Access and Excel to bring laboratory results, which are provided to GLKN in MS Excel, directly into GLKN's master database.

The database has drop-down menus and data range limits to reduce errors. After data are entered into the database, they are re-checked by one person reading 100% of the entries from the database while a second person checks against the original field forms. In addition, a suite of data checks and QA/QC queries are run to compare the entered data with expected formats, accepted data ranges, duplicate sample numbers, and missing values. Data are flagged for further review by an expert if they fall outside the expected range for a parameter based on previous records and scientific literature. These procedures protect the integrity of the data and allow the history of each data record to be traced. Data verification means that the data have been correctly entered for the period of

record. Verification does not indicate that the data are ready for distribution, only that the process of having the quality of the data assured is underway.

After data verification, a number of quality control and quality assurance steps are taken for certification. All data are provisional until they are certified. Data certification must be completed before data are shared with outside entities. The process of certification can differ depending on the data set of interest. In all cases, a Data Certification Request Form is initiated and completed by the data specialist and project manager respectively. In brief, the data certification process is as follows:

1. After data verification (see SOP #9, Data Management), program staff begins the certification process by starting a “Project Data Certification Form.” The staff fills in the relevant information on the validation process and quality control procedures conducted manually and within the database. The staff person then signs, either by printing and manually signing or by signing digitally, the draft form stating that they started to procedure and are submitting it to the principal investigator.
2. The principal investigator (project lead) determines whether a quality assessment (QA) needs to be completed. If so, the analyst performs the assessment and completes a QA report. If no assessment needs to be completed, an explanation should be included in the certification report.
3. If through the assessment staff finds data that need to be qualified, censored, or corrected, the analyst works with the data specialist or project staff to correct hardcopy forms and/or the database as necessary.
4. After all corrections are made to the field forms and/or the database, the data manager or project staff may need to submit a revision of the Project Data Certification Form with descriptions of changes. Once completed, the project lead approves and dates the Project Data Certification Form.

4.4 Data Sensitivity

The justification for this declaration of sensitivity is that the data deals with “rare, threatened, endangered, or commercially valuable species” (National Parks Omnibus Management Act of 1998). Bald eagles are protected under the American Bald and Golden Eagle Protection Act (U.S.C. 668-668c) of 1940. Individual nest locations are provided to state and federal managers, but only the territory centroid is used in generally available reports and data sets. Protected data are released in limited cases only with park superintendent approval. Personal information (e.g., contact information for landowners) is redacted from all forms and databases.

4.5 Data Distribution

GLKN has a number of different avenues for data distribution depending on the audience served and the degree of analysis and customization needed by the end-user. See Hart and Gafvert (2006) for more details. Individual data requests can be accommodated by developing queries to produce reports of the desired information. Depending on the dataset, the data may be accompanied by the

quality assurance report. The project manager, in consultation with the data manager, will determine when it is appropriate to attach quality assurance reports to the dataset.

4.6 Metadata Procedures

Metadata allows potential data users to evaluate the quality and usefulness of the data based on the process under which it was collected and maintained. In this respect, all of the protocol documentation, including this narrative and SOPs, is part of a dataset's metadata. A reference to the appropriate version of these documents is part of the metadata. Although perhaps obvious, all data must have an associated value for the date and time they were collected.

The Geospatial Data Act of 2018 mandates federal agencies use metadata “*consistent with international standards and protocols*” using guidance of the Federal Geographic Data Committee. Bald eagle contaminants monitoring meets the definition of geospatial data and, thus, GLKN is responsible for documenting all public datasets using metadata that meets that standard. Consult the GLKN Data Management Plan (Hart and Gafvert 2006) for additional details about metadata procedures and requirements.

Although it is not required, we will make every effort to complete Biological Data Profiles (www.fgdc.gov/standards/status/sub5_2.html) for appropriate data sets and add associated metadata to the National Biological Information Infrastructure (NBII; www.nbii.gov/datainfo/metadata) Clearinghouse.

See Chapter 8, Data Documentation, of GLKN's Data Management Plan (Hart and Gafvert 2006) for more details on the network's overall strategy for metadata generation, management, and distribution.

4.7 Data Archival

Data archival serves two primary functions: it provides a source from which to retrieve a copy of any dataset when the primary dataset is lost or destroyed and it provides a data record that is an essential part of the QA/QC process. Original hard-copy data are archived at the GLKN office. Electronic data files are maintained in a logical folder structure on the GLKN file server. All data related to this protocol including that sent by analytical laboratories should be stored on the GLKN central server as soon as possible.

Data are validated, assessed for quality, certified, and maintained in GLKN's database. An authoritative copy of the database is available in the NPS IRMA Data Store, and a copy of the certified data is publicly available from IRMA. A working copy of the database is stored on the GLKN server, and a backup copy of the MS Access database is kept off-site at Apostle Islands National Lakeshore.

4.8 Reports and Publications

Field data summaries are provided to the U.S. Fish & Wildlife Service (USFWS) and state agencies each year as stipulated by agency permits. These summary reports are also provided to each park. Highlights of annual results, such as nest productivity and points of interest, will be provided through the network's biannual newsletter, *The Current*. Natural Resource Briefs will be developed when

significant new data or findings of interest are available that require the attention of park managers, other scientists, or the public. The primary audience for these briefs is park resource managers, superintendents, and interpreters, but they find their way to the public as well. Briefs are widely read, and park staff find them to be an effective way of communicating our results.

More comprehensive reports and scientific journal articles will occur as sufficient data and full analyses are available to present patterns and trends in targeted contaminants and the overall eagle population. An initial data summary report (Route and Key 2009) and a technical report (Route et al. 2011) helped document methods and report on initial baseline results. These reports were followed by a series of publications to present significant patterns and trends on each of the targeted contaminants: Dykstra et al. 2010 and Dykstra et al. 2019 (mercury, PCBs, and DDE), Route et al. 2014a (PBDEs), Route et al. 2014b (PFCs), and Bruggeman et al. 2018 (lead).

Going forward, the network will focus on a technical report for the NPS series that will present a comprehensive summary of all data collected through 2015. This should be followed by updated findings in scientific journals where sufficient new data or novel analyses are available. The target audience of technical reports and publications will be park management and the broader scientific community.

5.0 Personnel Requirements and Training

5.1 Roles and Responsibilities

This monitoring program is funded primarily by GLKN, who also takes primary responsibility for program administration, data collection, management, analysis, and reporting. The three parks where the monitoring takes place assist with logistics, fieldwork, and interpretation of results. GLKN works closely with the Wisconsin Department of Natural Resources (WDNR) and the Minnesota Department of Natural Resources. WDNR conducts annual nest occupancy surveys for eagles nesting on the Wisconsin portions of the parks (all of APIS and over 75% of SACN). The WDNR has over 30 years of experience monitoring contaminants in bald eagles and their review of data quality, as well as consultation on its interpretation has been important in the startup of this program. The Mississippi Park Connection, a not-for-profit organization based in Minneapolis, conducts helicopter occupancy surveys along the Mississippi River (100% of MISS) and provides those data to GLKN in-kind. Climbing of nest trees has been done under contract and was through Eco-Ascension Research and Consulting during the initial years from 2006 through 2015. Specific responsibilities for the principals are as follows:

Project Manager

The project manager is an NPS biologist who has overall responsibility for program administration, data collection, and reporting. The project manager coordinates activities with other cooperators and park staff. Specifically, the project manager:

- Ensures all permits are acquired prior to field work;
- Documents changes to this protocol and standard operating procedures;
- Coordinates field schedules and associated activities with cooperators, contractors, and park staff;
- Ensures field personnel and contractors have sufficient support and training;
- Ensures QA/QC measures are followed and performs periodic review of quality standards for field, laboratory, and office work;
- Contracts for climbing services and for analytical laboratories;
- Ensures laboratory results meet specified minimum detection limits, reproducibility of replicate samples, and other performance indicators;
- Supervises or performs data entry and ensures that data are verified, validated, and certified;
- Ensures data are analyzed fully using appropriate statistical procedures;
- Reports results to parks, partners, and other scientists in a timely manner.

The project manager also works with the GLKN data manager and data specialist to:

- Create, and periodically update, metadata on the master MS Access database;
- Develop data verification and validation measures for quality assurance;
- Provide training in the use of database software and quality assurance procedures;
- Coordinate changes to field forms and user interface for the project database;
- Identify sensitive information requiring special consideration prior to distribution;
- Ensure regular archiving of metadata, original field data, databases, reports, summaries, and other products;
- Provide automated data queries and output from the master Access database that can be used in statistical and graphical software and written reports.

Field Personnel

At a minimum, the field crew will include an eagle handler and a tree climber. These two can efficiently capture, handle, and take samples from eaglets at two to four nests per day. The handler is normally an NPS employee and is responsible for coordinating field activities. The responsibilities of this two-person field crew include:

- Completing all required training, certifications, and permit applications;
- Assembling field equipment and supplies and ensuring they are up-to-date and in working order;
- Collecting data and samples according to the most recent protocol, SOPs, and QA/QC procedures;
- Climbing nest trees and acquiring samples in a manner that is safe to the climber, ground crew, and eaglets;
- Ensuring samples are processed and frozen within 24 hours of collection;
- Packing and shipping samples to analytical laboratory within maximum holding times;
- Maintaining accurate field data records and sample handling notes including explanations of all deviations from standard procedures;
- Maintaining and calibrating equipment according to SOPs and manufacturers' directions;
- Communicating with the project manager and USFWS when there are significant deviations from sampling protocols or injury to humans or birds;
- Entering and quality checking field data into databases.

Data Management Specialist

Data management is the shared responsibility of the field crew, project manager, and the GLKN data manager. Typically, field personnel are responsible for data collection. The data management specialist is responsible for database design, data entry, QA/QC, data security, and data archiving. The data management specialist, in collaboration with the project manager, also develops data entry forms, data upload and download tools, and other database features (as part of quality assurance) and automates routine data summaries through queries and report generation.

5.2 Crew Qualifications

The eagle handler must have a bachelor's or advanced degree in biology, chemistry, or a related physical or biological science. Field experience and training with handling and taking blood samples from bald eagle nestlings is mandatory (see sub-section 5.3 below for training procedures). The handler must be permitted (directly or under sub-permit) on a valid scientific collecting permit from the USFWS. Additionally, at least one crew member, normally the handler, must have a valid banding permit from the USGS or be listed as a sub-permittee under a Master Bird Bander. Prior leadership experience and good decision-making skills are highly desirable, as is experience with boats, motors, and canoes.

Those authorized to be the lead climber to retrieve nestlings from nests must pass one of the following criteria:

1. Have prior experience safely climbing and retrieving eaglets from 100 or more separate nest trees under permit from a state or federal agency; OR
2. Have obtained two days of instruction from a professional climber including the use of ropes, knots, safety equipment, and climbing technique, AND must have mentored with an experienced climber while climbing to at least 10 nests with nestlings and safely transporting them to the ground.

5.3 Training Procedures

All crew members who are authorized to handle bald eagles must be under valid state and federal permits, including both a USFWS Bald Eagle Scientific Collecting Permit and a USGS Banding Permit. Additionally, those individuals authorized to collect blood must either mentor with a trained, experienced professional for the first 10 nestlings or undergo training from one of the following: the Raptor Rehabilitation and Research Program at the University of Minnesota; the Raptor Education Group, Inc. in Antigo, Wisconsin; or the Small Animal Clinical Sciences Facility, College of Veterinary Medicine at Michigan State University.

As stated in section 5.2 (Crew Qualifications) those authorized to climb nest trees must either have extensive previous experience or get training in combination with mentoring. Training will be provided by individuals who possess an extensive record of safe climbing. The U.S. Forest Service provides a tree climbing certification course.

Before data collection, field personnel must become familiar with the SOPs and the equipment to be used in the field. Specific training procedures are covered in SOP #2, Training, Certifications, and Permits, and will include the following:

- Review of this protocol and all SOPs with special attention to human safety, emergency contacts, and safe handling of nestlings;
- Calibration, operation, and maintenance of equipment;
- Current status of avian influenza virus (H5N1) and how to identify sick birds and handle birds and other potentially bio-hazardous material safely;
- Methods for measurements and sample collection;
- Methods for handling and preserving samples;
- Completion of field data forms, sample labels, chain-of-custody forms, and analytical lab request forms;
- Completion of field and calibration logbooks;
- NPS-specific training as applicable (e.g., boat operation, computer use, radios, charge card, travel).

6.0 Operational Requirements

6.1 Annual Work Load and Schedule

Bald eagle nestlings must be sampled when they are between five and nine weeks old. At five weeks they can thermoregulate in a reasonable range of weather conditions without continuous attention from an adult. At ages older than nine weeks there is increasing concern that the nestling will fledge prematurely (i.e., jump from the nest). This five to nine week sampling window drives all fieldwork. Bald eagle nesting chronology varies by latitude and year (weather) (Table 7).

Table 7. Chronology of bald eagle nesting biology in relation to flight and field sampling to monitor environmental contaminants in nestlings. For each activity, 1 (dark gray) indicates peak activity, 2 (both light and dark gray) indicates the full chronological range for each activity.

Activity	January		February		March		April		May		June		July	
Adults on territory	2	–	–	–	–	–	–	–	–	–	–	–	–	–
Mating and eggs layed	–	2	1, 2	1, 2	1, 2	2	–	–	–	–	–	–	–	–
Incubation period	–	–	2	1, 2	1, 2	1, 2	2	–	–	–	–	–	–	–
Hatching	–	–	–	–	–	2	1, 2	1, 2	2	–	–	–	–	–
Occupancy flight	–	–	–	–	–	1, 2	1, 2	–	–	–	–	–	–	–
Productivity flight	–	–	–	–	–	–	–	–	–	1, 2	–	–	–	–
Field sampling	–	–	–	–	–	–	–	–	–	1, 2	1, 2	1, 2	–	–
Fledging	–	–	–	–	–	–	–	–	–	2	1, 2	1, 2	1, 2	1, 2

Normally the field sampling begins in mid-May for southerly areas (MISS and lower SACN) and will continue through late June in the more northerly areas (upper SACN and APIS). The WDNR and Mississippi Park Connection flights to determine nest occupancy and nestling age will assist with determining field season dates. However, dates will vary depending on late-winter and spring weather patterns. From our 2006–2014 data, and back-calculating from the estimated age of nestlings when handled, we estimate that 6–8 April is the average hatching date for a normal year at MISS (Table 8). That would mean, on average, nestlings would be six-weeks-old around 18–20 May at MISS and ready for sampling. However, hatching date has varied widely. In 2014, for example, nestlings in all study areas hatched up to 15 days late compared to normal years due primarily, we believe, to a late winter storm that set eagle nesting back. APIS is particularly difficult to predict due to the severe weather and ice conditions that can affect early nesting behavior and success on Lake Superior.

Table 8. Estimated mean hatching dates of bald eagle nestlings at the four primary national park study areas, based on the age of nestlings sampled each year. M = MISS (also in green), L = L-SACN (also in pink), U = U-SACN (also in orange), and A = APIS (also in blue).

Year	April																														May																			
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20
2014														L							M					U									A															
2011								M							U															A																				
2010				M						L					U												A																							
2009								M			L																																							
2008									M	L																									A															
2007							M				L			U																																				
2006				L	M									U																					A															

The estimated workload and schedule shown in Table 9 are based on three years of pilot work at APIS, MISS, and SACN.

Table 9. Projected timing of events, lead role, and effort required for conducting bald eagle monitoring of environmental contaminants at three parks in the Great Lakes Network.

Tasks	Dates	Lead ²	Person-hrs
Apply for permits (6 mo in advance)	November of prior year	PM	8
Secure contracts and agreements	December of prior year	PM	20
Order field supplies	March	PM	4
Planning logistics	February through mid-May	PM	30
Occupancy flight for MISS	¹ Early to mid-March	MISS	8
Occupancy flight for SACN	¹ Early April	WDNR	16
Occupancy flight for APIS	¹ Late April or early May	WDNR	6
Prepare field maps and forms	Following occupancy surveys	DMS	24
Field sampling at MISS	¹ Mid-May	PM/C	60
Field sampling at SACN	¹ Early to mid-June	PM/C	90
Field sampling at APIS	¹ Mid- to late June	PM/C	40
Prepare, deliver/ship samples to lab	Late June to early July	DMS/PM	24
Clean and put away field equipment	July	PM	8
Data entry and verification	July	DMS	40
Analytical lab work on samples	August through November	Lab	
Data analysis, tables, and graphs	October through December	PM	80
Prepare USFWS collecting report	January of year following	DMS/PM	2
Prepare USFWS banding reports (2)	January of year following	DMS/PM	4
Prepare Annual Investigator Reports (3)	March of year following	PM	4
Total	–	–	506

¹ Dates marked depend on weather, which dictates nesting chronology.

² APIS = Apostle Islands National Lakeshore; C = Climber; DMS = Data Management Specialist; MISS = Mississippi National River and Recreation Area; PM = Project Manager; SACN = St. Croix National Scenic Riverway; USFWS=U.S. Fish and Wildlife Service; WDNR = Wisconsin Department of Natural Resources.

Bald eagle sampling will be conducted by one or two crews. Depending on the experience of individual crew members, a crew will consist of two to four individuals (see sub-section 5.1, Roles and Responsibilities). Close coordination between flight staff after occupancy and productivity surveys and the project manager will be essential to ensure efficiency (reduce potential for hiking to nest trees that are not occupied) and safety of the birds (decrease chances of nestlings jumping from the nest). The field season ends in late June after samples are collected at APIS. All of the accumulated samples are then inventoried, logged in the database, and packaged for submission to

the analytical laboratory. Normally we will transport the samples by vehicle in a portable freezer directly to the lab. This is the best way to ensure they arrive in good condition. Occasionally we may need to ship samples on dry ice. Analytical work by the lab can take between three and twelve months depending on their workload.

Analysis and reporting on results is the most time consuming part of the program. State and federal agencies, as well as park staff and partners, require the information in a variety of reports and formats. In addition to routine reporting formal and informal presentations, briefing statements, and other personal communication to convey the information will occur as well.

6.2 Facility and Major Equipment Needs

The GLKN sampling crew will be primarily stationed in Ashland, but park staff and facilities will be used when necessary. Most travel will be accomplished with one GSA leased vehicle. Motor boats will be required to access nests along the Mississippi and lower St. Croix rivers as well as nests on the Apostle Islands. GLKN's 18-foot Lund works well for the MISS and lower portions of the SACN. SACN has boats that require less draft for accessing nests on sections between St. Croix Falls and the High Bridge. Canoes are readily available at GLKN and SACN for sampling the upper St. Croix and Namekagon rivers, though it is often more efficient to simply hike to each nest. GLKN has a 20-foot, twin-engine Hewescraft that can safely navigate Lake Superior to sample the Apostle Islands. APIS staff can also provide assistance when needed.

Blood samples from bald eagles must be frozen within 24 hours (usually within 10 hrs) of being collected. The primary method of freezing and maintaining frozen samples during fieldwork are portable freezers. These work on regular 120V systems when staying in hotels and can plug into 12V battery systems in vehicles when moving between areas. When necessary, adequate freezers are available at the following strategic locations:

- The St. Croix Watershed Research Station, Marine on the St. Croix, MN
- SACN Headquarters in St. Croix Falls, WI
- Crex Meadows State Wildlife Area, Grantsburg, WI
- GLKN Office, Ashland, WI

The WSLH in Madison will provide long-term archival of biological samples at -20°C in freezers that are protected with alarms and backup power. Red blood cells that are often discarded by many investigators will be kept frozen at the GLKN office. These samples may prove useful in the future with advances in analytical methods. Unused feather samples will be kept dried in envelopes at the GLKN office for archival. All archived samples will be cataloged and individually marked.

6.3 Annual Costs

Initial equipment has been purchased and is available for future sampling. A centrifuge, a chest freezer, two portable freezers, and two GPS devices were the largest investments in equipment. In addition, boats, motors, and canoes have been acquired by GLKN to support all programs.

Excluding wages for GLKN staff, about \$136,000 would be required for fieldwork and full analysis of 60 nestlings across the four study areas (Table 10). This assumes one nestling from each territory is measured for the targeted contaminants by the WSLH at the 2017 rate. The WSLH has analyzed past samples of bald eagle plasma and feathers, including early WDNR work (e.g., Dykstra et al. 2005) and those collected under this program from 2006 through 2015. Continuing with WSLH in the future greatly enhances the comparability with this past work. The WSLH is also accredited by NELAP. The laboratory analysis is the single most expensive cost. Investigators could alter sample size and/or drop expensive analytes such as PCBs that have been declining consistently and are well documented.

Table 10. Estimated budget, in 2015 dollars (the last year of sampling), for one year of fieldwork, sample analysis, data management, and reporting. Assumes a GS-13 project manager at 20% time and sampling of 120 nestlings, 60 of which are analyzed for targeted contaminants with the remaining samples archived.

Category	Item	Details	Estimated Cost
NPS wages	GLKN program manager	20% of GS-13/5	16,900.00
	GLKN boat operator	5 days of GS-9	960.00
	GLKN data management	20% of GS-9/5	9,800.00
	Sub-total	–	\$27,660.00
NPS travel	Vehicle lease & gas	2 weeks MISS to APIS	900.00
	Boat fuel	MISS, L-SACN, APIS	240.00
	Per diem, program manager	3 weeks, including weekends	1,400.00
	Per diem, boat operator	5 days/nts @ MSP rate	450.00
	Sub-total	–	\$2,990.00
Contractors	Audubon biologist, MISS only	Negotiated service contract	2,500.00
	Analytical laboratory	60 samples @ \$1,500 each	90,000.00
	Statistical consultation	Negotiated service contract	10,000.00
	Tree climbers	Negotiated service contract	27,000.00
	Genetic analysis	120 samples @ \$20 each	2,400.00
	Diagnostic and vet services	One case	150.00
	Occupancy surveys	In-kind	0.00
	Sub-total	–	\$132,050.00
Supplies and equipment	Equipment replacement	If needed	500.00
	Supplies	Misc. supplies	300.00
	Sub-total	–	\$ 800.00
Total	–	–	\$163,500.00
Total excluding NPS wages	–	–	\$135,840.00

The second largest expense is contractual tree climbers. Climbing nest trees and handling the nestlings in the nest is also the most dangerous part of the monitoring effort. It is critical to hire experienced, safe climbers to retrieve the young for sampling. The estimated cost for an experienced climber is based on the 2015 negotiated contract.

Literature Cited

- Auer, C. 2000. Phaseout of PFOS. Interagency memo. U.S. Environmental Protection Agency. 6 September 2000.
- Aulerich, R. J., R. K. Ringer, H. L. Seagran, and W. G. Youatt. 1971. Effects of feeding coho salmon and other Great Lakes fish on mink reproduction. *Canadian Journal of Zoology* 49:611–616.
- Best, D. A., M. Gilbertson, and H. Hudson. 1990. Proceedings of the expert consultation meeting on bald eagles. International Joint Commission, Windsor, Ontario, Canada.
- Black, J. J. 1983. Field and laboratory studies of environmental carcinogenesis in Niagara River fish. *Journal of Great Lakes Research* 9:326–334.
- Bortolotti, G. R. 1984a. Sexual size dimorphism and age-related size variation in bald eagles. *Journal of Wildlife Management* 48:72–81.
- Bortolotti, G. R. 1984b. Physical development of nestling bald eagles with emphasis on the timing of growth events. *The Wilson Bulletin* 96:524–542.
- Bortolotti, G. R. 1984c. Criteria for determining age and sex of nestling bald eagles. *Journal of Field Ornithology* 5:467–481.
- Bowerman, W. W. 1993. Regulation of bald eagle (*Haliaeetus leucocephalus*) productivity in the Great Lakes basin: An ecological and toxicological approach. Dissertation. Michigan State University, East Lansing, Michigan.
- Bowerman, W. W., A. S. Roe, M. Gilbertson, D. A. Best, J. G. Sikarskie, and C. L. Summer. 2002. Using bald eagles to indicate the health of the Great Lakes environment. *Lakes and Reservoirs: Research and Management* 7:183–187.
- Bowerman, W. W., D. A. Best, J. P. Giesy, M. C. Shieldcastle, M. W. Meyer, S. Postupalsky, and J. G. Sikarskie. 2003. Associations between regional differences in polychlorinated biphenyls and dichlorodiphenyldichloroethylene in blood of nestling bald eagles and reproductive productivity. *Environmental Toxicology and Chemistry* 22:371–376.
- Bruggeman J. E., W. T. Route, P. T. Redig, and R. L. Key. 2018. Patterns and trends in lead (Pb) concentrations in bald eagle (*Haliaeetus leucocephalus*) nestlings from the western Great Lakes region. *Ecotoxicology* 27:605–618.
- Christensen, V. G., S. P. Wente, M. B. Sandheinrich, and M. E. Brigham. 2006. Spatial variation in fish-tissue mercury concentrations in the St. Croix River basin, Minnesota and Wisconsin, 2004. Scientific Investigations Report 2006-5063. U.S. Geological Survey, Reston, Virginia.
- Clarkson, T. W., and L. Magos. 2006. The toxicology of mercury and its chemical compounds. *Critical Reviews in Toxicology* 36:609–662.

- Custer, T. W., K. Kannan, L. Tao, A. R. Saxena, and B. Route. 2009. Perfluorinated compounds and polybrominated diphenyl ethers in great blue heron eggs from Indiana Dunes National Lakeshore, Indiana. *Journal of Great Lakes Research* 35:401–405.
- DeSorbo, C. R., and D. C. Evers. 2006. Evaluating exposure of Maine's bald eagle population to mercury: Assessing impacts on productivity and spatial exposure patterns. Report BRI 2006-02. Biodiversity Research Institute, Gorham, Maine.
- Dunstan, T. C., and J. F. Harper. 1975. Food habits of bald eagles in north-central Minnesota. *Journal of Wildlife Management* 39:450–455.
- Dykstra, C. R., M. W. Meyer, D. K. Warnke, W. H. Karasov, D. E. Anderson, W. W. Bowerman, and J. P. Giesy. 1998. Low productivity rates of Lake Superior bald eagles: Low food delivery rates or environmental contaminants? *Journal of Great Lakes Research* 24:32–44.
- Dykstra, C. R., M. W. Meyer, P. W. Rasmussen, and D. K. Warnke. 2005. Contaminant concentrations and reproductive rate of Lake Superior bald eagles, 1989–2001. *Journal of Great Lakes Research* 31:227–235.
- Dykstra, C. R., W. T. Route, M. W. Meyer, and P. W. Rasmussen. 2010. Contaminant concentrations in bald eagles nesting on Lake Superior, the upper Mississippi River, and the St. Croix River. *Journal of Great Lakes Research* 36:561–569.
- Dykstra, C. R., W. T. Route, K. Williams, and R. L. Key. 2019. Trends and patterns of PCB, DDE, and mercury contamination in bald eagle nestlings in the upper Midwest. *Journal of Great Lakes Research*. Available at: <https://doi.org/10.1016/j.jglr.2019.01.010>.
- Elias, J., R. Axler, E. Ruzycki, and D. VanderMeulen. 2015. Water quality monitoring protocol for inland lakes: Great Lakes Inventory and Monitoring Network, Version 1.1. Natural Resource Report NPS/GLKN/NRR—2015/1027. National Park Service, Fort Collins, Colorado.
- Elliott, J. E., and M. L. Harris. 2001/2002. An ecotoxicological assessment of chlorinated hydrocarbon effects on bald eagle populations. *Reviews in Toxicology* 4:1–60.
- Engstrom, D. R., and E. B. Swain. 1997. Recent declines in atmospheric mercury deposition in the Upper Midwest. *Environmental Science and Technology* 31:960–967.
- Ensor Giovengo, K. 1997. Contaminant exposure of bald eagles via prey at Voyageurs National Park, Minnesota, 1993. Project completion report. U.S. Fish and Wildlife Service, Twin Cities Field Office, Bloomington, Minnesota.
- Evers, D. C. 2005. Mercury connections: The extent and effects of mercury pollution in northeastern North America. Biodiversity Research Institute, Gorham, Maine.
- Fancy, S. 2004. An overview of vital signs monitoring and its central role in natural resource stewardship and performance management. Unpublished National Park Service document.

Available at: <http://npshistory.com/publications/interdisciplinary/im/vital-signs-overview.pdf> (accessed 30 July 2019).

- Foran, J. A., D. O. Carpenter, M. C. Hamilton, B. A. Knuth, and S. J. Schwager. 2005. Risk-based consumption advice for farmed Atlantic and wild Pacific salmon contaminated with dioxins and dioxin-like compounds. *Environmental Health Perspectives* 113:552–556.
- Fry, B. 2008. *Stable Isotope Ecology*. Springer Science+Business Media, LLC, New York, New York.
- Fuller, M. R., J. S. Hatfield, and E. L. Lindquist. 1995. Assessing ground-based counts of nestling bald eagles in northeastern Minnesota. *Wildlife Society Bulletin* 23:169–174.
- Gerrard, J. M., A. R. Harmata, and P. N. Gerrard. 1992. Home range and activity of a pair of bald eagles breeding in northern Saskatchewan. *Journal of Raptor Research* 26:229–234.
- Gilbertson, M., and D. O. Carpenter. 2004. An ecosystem approach to the health effects of mercury in the Great Lakes basin ecosystem—Introduction. *Environmental Research* 95:240–246.
- Gilbertson, M., G. A. Fox, and W. W. Bowerman. 1998. Designing the environmental results workshop: Historical context, causality, and candidate species. *Environmental Monitoring and Assessment* 53:17–55.
- Gilbertson, M., R. D. Morris, and R. A. Hunter. 1976. Abnormal chicks and PCB residue levels in eggs of colonial birds on the lower Great Lakes (1971–73). *Auk* 93:434–442.
- Ginevan, M. E., and D. E. Splitstone. 2003. *Statistical Tools for Environmental Quality Measurement*. Chapman and Hall/ CRC Press, Boca Raton, Florida.
- Gorski, P. R., L. B. Cleckner, J. P. Hurley, M. E. Sierzen, and D. E. Armstrong. 2003. Factors affecting enhanced mercury bioaccumulation in inland lakes of Isle Royale National Park, USA. *Science of the Total Environment* 304:327–348.
- Grier, J. W. 1982. Ban of DDT and subsequent recovery of reproduction in bald eagles. *Science* 218:1232–1235.
- Grim, L. H., and L. W. Kallemeyn. 1995. Reproduction and distribution of bald eagles in Voyageurs National Park, Minnesota, 1973–1993. Biological Science Report 1. National Biological Survey, International Falls, Minnesota.
- Hart, M., and U. Gafvert (editors). 2006. Data management plan: Great Lakes Inventory and Monitoring Network. Technical Report GLKN/2006/20. National Park Service, Great Lakes Inventory and Monitoring Network, Ashland, Wisconsin.
- Hebert, C. E., and D. V. Weseloh. 2006. Adjusting for temporal changes in trophic position results in reduced rates of contaminant decline. *Environmental Science and Technology* 40:5624–5628.

- Helsel, D. R. 2012. Statistics for Censored Environmental Data Using Minitab® and R, 2nd edition, vol. 77. John Wiley & Sons, Hoboken, New Jersey.
- Hites, R. A., J. A. Foran, D. O. Carpenter, M. C. Hamilton, B. A. Knuth, and S. Schwager. 2004. Global assessment of organic contaminants in farmed salmon. *Science* 303:226–229.
- Hollamby, S., J. Afema-Azikuru, J. G. Sikarskie, J. B. Kaneene, W. W. Bowerman, S. D. Fitzgerald, K. Cameron, A. R. Gandolf, G. N. Hui, C. Dranzoa, and W. K. Rumbeiha. 2004. Mercury and persistent organic pollutant concentrations in African fish eagles, Marabou storks, and Nile tilapia in Uganda. *Journal of Wildlife Diseases* 40:501–514.
- Hylander, L. D., and M. Meili. 2005. The rise and fall of mercury: Converting a resource to refuse after 500 years of mining and pollution. *Critical Reviews in Environmental Science and Technology* 35:1–36.
- IJC (International Joint Commission). 1989. Fourth biennial report under the Great Lakes Water Quality Agreement of 1978 to the governments of the United States and Canada and the state and provincial governments of the Great Lakes Basin. Windsor, Ontario.
- Jagoe, C. H., A. L. Bryan, H. A. Brant, T. M. Murphy, and I. L. Brisbin. 2002. Mercury in bald eagle nestlings from South Carolina, USA. *Journal of Wildlife Diseases* 38:706–712.
- Jardine, T. D., K. A. Kidd, and A. T. Fisk. 2006. Applications, considerations, and sources of uncertainty when using stable isotope analysis in ecotoxicology. *Environmental Science and Technology* 40:7501–7511.
- Kelly, J., and L. Solem 2007. Identification of a major source of perfluorooctane sulfonate at a wastewater treatment plant in Brainerd, Minnesota. Available at: www.health.state.mn.us/divs/eh/hazardous/topics/pfcs/index/html.
- Kozie, K. D. 1986. Breeding and feeding ecology of bald eagles in Apostle Islands National Lakeshore. Thesis. University of Wisconsin, Stevens Point, Wisconsin.
- Kozie, K. D., and R. K. Anderson. 1991. Productivity, diet, and environmental contaminants in bald eagles nesting near the Wisconsin shoreline of Lake Superior. *Archives of Environmental Contaminants and Toxicology* 20:41–48.
- Kramer, J. L., and P. T. Redig. 1997. Sixteen years of lead poisoning in eagles, 1980–95: An epizootiologic view. *Journal of Raptor Research* 31:327–332.
- Lee, L. 2015. Package NADA version 1.5-6. Nondetects and data analysis for environmental data. Available at www.R-project.org/ (accessed 12 February 2015).
- Leith, F. L., W. W. Bowerman, M. R. Wierda, D. A. Best, T. G. Grubb, and J. G. Sikarske. 2010. A comparison of techniques for assessing central tendency in left-censored data using PCB and

- p,p'DDE contaminant concentrations from Michigan's Bald Eagle Biosentinel Program. *Chemosphere* 80:7–12.
- Magdalene, S., D. R. Engstrom, J. Elias, D. VanderMeulen, and R. Damstra. 2016. Large rivers water quality monitoring protocol: Great Lakes Inventory and Monitoring Network. Version 1.1. NPS/GLKN/NRR—2016/1262. National Park Service, Fort Collins, Colorado.
- Mahaffy, M. S. 1981. Territorial behavior of the bald eagle on the Chippewa National Forest. Thesis. University of Minnesota, St Paul, Minnesota.
- Mattsson, J. P. 1974. Interaction of a breeding pair of bald eagles with sub-adults at Sucker Lake, Michigan. Thesis. St. Cloud State University, St. Cloud, Minnesota.
- Mergler, D., H. A. Anderson, L. H. M. Chan, K. R. Mahffey, M. Murray, M. Sakamoto, and A. H. Stern. 2007. Methylmercury exposure and health effects in humans: A worldwide concern. *Ambio* 36:3–11.
- Meyer, M. W., D. E. Anderson, W. H. Karasov, R. Brander, and J. Van Stappen. 1993. Factors controlling Great Lakes bald eagle productivity: Annual progress report – 1992. Unpublished report to the Great Lakes Protection Fund, Chicago, Illinois.
- Newsted, J. L., P. D. Jones, K. Coady, and J. P. Giesy. 2005. Avian toxicity reference values for perfluorooctane sulfonate. *Environmental Science and Technology* 23:9357–9362.
- Newton, I. 1979. *Population Ecology of Raptors*. Buteo Books, Vermillion, South Dakota.
- Olsson, A., K. Ceder, A. Bergman, and B. Helander. 2000. Nestling blood of the white-tailed sea eagle (*Haliaeetus albicilla*) as an indicator of territorial exposure to organohalogen compounds—An evaluation. *Environmental Science and Technology* 34:2733–2740.
- Pittman, H. T., W. W. Bowerman, L. H. Grim, T. G. Grubb, W. C. Bridges, and M. R. Wierda. 2015. Using nestling plasma to assess long-term spatial and temporal concentrations of organochlorine compounds in bald eagles within Voyageurs National Park, Minnesota, USA. *Chemosphere* 123:79–86.
- Postupalsky, S. 1983. Techniques and terminology for surveys of nesting bald eagles. Appendix D in J. W. Grier, J. B. Elder, F. J. Gramlich, N. F. Green, J. V. Kussman, J. E. Mathisen, and J. P. Mattsson, editors. *Northern states bald eagle recovery plan*. U.S. Department of Interior, U.S. Fish and Wildlife Service, Twin Cities, Minnesota.
- R Core Team. 2015. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. Available at www.R-project.org/ (accessed 16 January 2015).
- Renner, R. 2000. PBDE Polybrominated diphenyl ether. What fate for brominated fire retardants? *Environmental Science and Technology* 34:222–236.

- Roe, A. S. 2004. Spatial and temporal analyses of environmental contaminants and trophic status of bald eagles in the Great Lakes region. Dissertation. Clemson University, Clemson, South Carolina.
- Roe, A., A. Birrenkott, W. Bowerman, D. Bush, and J. Sikarskie. 2004. Michigan wildlife contaminant trend monitoring: Year 2002 annual report – nestling bald eagles. Michigan Department of Environmental Quality Report MI/DEQ/WD-04/024. MDEQ, East Lansing, Michigan.
- Route, B. 2004. Process and results of selecting and prioritizing vital signs for the Great Lakes Network. Technical Report GLKN/2004/05. National Park Service, Great Lakes Inventory and Monitoring Network, Ashland, Wisconsin.
- Route, B., and J. Elias, editors. 2007. Long-term ecological monitoring plan: Great Lakes Inventory and Monitoring Network. Natural Resource Report NPS/GLKN/NRR—2007/001. National Park Service, Fort Collins, Colorado.
- Route, B., and R. Key. 2009. Contaminants in bald eagle nestlings at Apostle Islands National Lakeshore, Mississippi National River and Recreation Area, and the St. Croix National Scenic Riverway: Data summary, 2006–2008. Natural Resource Data Series NPS/GLKN/NRDS—2009/001. National Park Service, Fort Collins, Colorado.
- Route, B., and D. VanderMeulen. 2018. Quality assurance plan for monitoring environmental contaminants in bald eagle nestlings: Great Lakes Inventory and Monitoring Network. Natural Resource Report NPS/GLKN/NRR—2018/1848. National Park Service, Fort Collins, Colorado.
- Route, B., W. Bowerman, and K. Kozie. 2009. Protocol for monitoring environmental contaminants in bald eagles, version 1.2: Great Lakes Inventory and Monitoring Network, Natural Resource Report NPS/GLKN/NRR—2009/092. National Park Service, Fort Collins, Colorado.
- Route, B., P. Rasmussen, R. Key, M. Meyer, and M. Martell. 2011. Spatial patterns of persistent contaminants in bald eagle nestlings at three national parks in the upper Midwest: 2006–2009. Natural Resource Technical Report NPS/GLKN/NRTR—2011/431. National Park Service, Fort Collins, Colorado.
- Route, W. T., C. R. Dykstra, P. W. Rasmussen, R. L. Key, M. W. Meyer, and J. Mathew. 2014a. Patterns and trends in brominated flame retardants in bald eagle nestlings from the upper Midwestern United States. *Environmental Science & Technology* 48:12516–12524.
- Route, W. T., R. E. Russell, A. B. Lindstrom, M. H. Strynar, and R. L. Key. 2014b. Spatial and temporal patterns in concentrations of perfluorinated compounds in bald eagle nestlings in the upper Midwestern United States. *Environmental Science & Technology* 48:6653–6660.
- Rutkiewicz, J., D. Nam, T. Cooley, K. Neumann, I. Bueno Padilla, W. Route, S. Strom, and N. Basu. 2011. Mercury exposure and neurochemical impacts in bald eagles across several Great Lakes states. *Ecotoxicology* 20:1669–1676.

- Scheuhammer, A. M., and S. L. Norris. 1996. The ecotoxicology of lead shot and lead fishing weights. *Ecotoxicology* 5:279–295
- Scheuhammer, A. M., M. W. Meyer, M. B. Sandheinrich, and M. W. Murray. 2007. Effects of environmental methylmercury on the health of wild birds, mammals, and fish. *Ambio* 36:12–18.
- Scheuhammer, A. M., N. Basu, N. M. Burgess, J. E. Elliot, G. D. Campbell, M. Wayland, L. Champoux, and J. Rodrigue. 2008. Relationships among mercury, selenium, and neurochemical parameters in common loons (*Gavia immer*) and bald eagles (*Haliaeetus leucocephalus*). *Ecotoxicology* 17:93–101.
- Schmitt, C. J., W. G. Brumbaugh, J. M. Besser, J. E. Hinck, D. E. Bowles, L. W. Morrison, and M. H. Williams. 2008. Protocol for monitoring metals in Ozark National Scenic Riverways, Missouri. Version 1.0. U.S. Geological Survey Open-File Report 2008–1269.
- Shapiro-Hurley, S. 1990. Persistent toxic chemicals. In D. A. Best, M. Gilbertson, and H. Hudson, editors. Proceedings of the expert consultation meeting on bald eagles. International Joint Commission, Windsor, Ontario, Canada.
- Sorensen, J. A., L. W. Kallemeyn, and M. Sydor. 2005. Relationship between mercury accumulation in young-of-the-year yellow perch and water-level fluctuations. *Environmental Science and Technology* 39:9237–9243.
- Stalmaster, M. 1987. *The Bald Eagle*. Universe Books, New York, New York.
- State of the Lakes (SOLEC). 2005. State of the Great Lakes 2005. U.S. Environmental Protection Agency and Environment Canada. Available at: www.epa.gov/glnpo/solec/solec_2004/technical_final/index.html (accessed 30 March 2007).
- Sun, P., I. Basu, P. Blanchard, K. A. Brice, and R. A. Hites. 2007. Temporal and spatial trends of atmospheric polychlorinated biphenyl concentrations near the Great Lakes. *Environmental Science and Technology* 41:1131–1136.
- Tsubaki, T., and K. Irukyama (editors). 1977. *Minamata Disease*. Elsevier, Amsterdam, Netherlands.
- USEPA (U.S. Environmental Protection Agency). 2005. 2004 national listing of fish advisories. Fact Sheet EPA-823- F- 05-004. U.S. Environmental Protection Agency, Office of Water, Washington, D.C.
- VanderMeulen, D. D., B. Route, J. Wiener, R. Haro, K. Rolffhus, M. Sandheinrich, S. J. Nelson, A. Klemmer, C. Eagles-Smith, and J. Willacker. 2018. Protocol for monitoring mercury in dragonfly larvae and fish (version 1.0): Great Lakes Inventory and Monitoring Network. Natural Resource Report NPS/GLKN/NRR—2018/1726. National Park Service, Fort Collins, Colorado.
- WDNR (Wisconsin Department of Natural Resources). 2016. Bald eagle and osprey nest survey protocol – Version 1.1. Wisconsin Department of Natural Resources, Madison, Wisconsin.

- West, N. E., and T. P. Yorks. 2002. Vegetation responses following wildfire on grazed and ungrazed sagebrush semi-desert. *Journal of Range Management* 55:171–181.
- Whitehead, M. A., A. H. Birrenkott, K. A. N. Simpson, G. A. Torrao, K. J. Bader, V. B. Masterov, and W. W. Bowerman. 2003. Potential impacts of oil exploration on sea eagles inhabiting coastal regions of the Russian Far East. Pages 387–396 *in* B. Helander, M. Marquiss, and W. Bowerman, editors. *Sea eagle 2000*. Swedish Society for Nature Conservation, Stockholm, Sweden.
- Wiemeyer, S. N., T. J. Lamont, C. M. Bunck, C. R. Sindelar, F. J. Gramlich, J. D. Fraser, and M. A. Byrd. 1984. Organochlorine pesticide, polychlorobiphenyl, and mercury residues in bald eagle eggs—1969–1979—and their relationships to shell thinning and reproduction. *Archives of Environmental Contaminants and Toxicology* 13:529–549.
- Wiener, J. G., D. P. Krabbenhoft, G. H. Heinz, and A. M. Scheuhammer. 2003. Ecotoxicology of mercury. Pages 409–463 *in* D. J. Hoffman, B. A. Rattner, G. A. Burton, Jr., and J. Cairns, Jr., editors. *Handbook of Ecotoxicology*, 2nd edition. CRC Press, Boca Raton, Florida.
- Wiener, J. G., B. C. Knights, M. B. Sandheinrich, J. D. Jeremiason, M. E. Brigham, D. R. Engstrom, L. G. Woodruff, W. F. Cannon, and S. J. Balogh. 2006. Mercury in soils, lakes, and fish in Voyageurs National Park: Importance of atmospheric deposition and ecosystem factors. *Environmental Science and Technology* 40:6261–6268.
- Wiener, J., R. Haro, K. Rolfhus, M. Sandheinrich, and B. Route. 2008. Protocol for monitoring and assessing methylmercury and bioaccumulative organic contaminants in aquatic food webs. Technical Report Number GLKN/2008/ Version 1.0. National Park Service, Great Lakes Inventory and Monitoring Network, Ashland, Wisconsin.

Appendix A. List of Standard Operating Procedures

- SOP #1. Nestling Bald Eagle Handling, Measuring, and Sample Collection
- SOP #2. Training, Certifications, and Permits
- SOP #3. Preseason Preparations
- SOP #4. Occupancy and Productivity Surveys
- SOP #5. Egg Measurements
- SOP #6. Methods for Analyzing and Presenting Bald Eagle Productivity and Contaminants Data
- SOP #7. Sample Handling
- SOP #8. Quality Assurance and Certification of Bald Eagle and Contaminants Data
- SOP #9. Data Management
- SOP #10. Safety
- SOP #11. Revising the Protocol

Appendix B. Summary Information on Selected Contaminants.

Organochlorine Compounds

This group includes DDT and its metabolites (breakdown products such as DDE), several persistent pesticides such as chlordane, dieldrin/aldrin, and heptachlor, and PCBs including many congeners (congeners are chemically related compounds with slightly different formulations). PCBs and DDT are listed as contaminants causing impairment of water bodies in seven and six respectively of the nine parks in the Great Lakes Inventory and Monitoring Network. PCBs and DDT have been steadily declining in the Great Lakes and elsewhere in the region since they were banned in the 1970s. These contaminants are often referred to as “legacy” contaminants because production has been banned; yet stocks can still legally be used (at least for PCBs) and they continue to persist and re-enter the food chain when contaminated sediments are disturbed by dredging and extreme weather events. Bowerman et al. (2003) showed that bald eagle productivity and success rates were inversely correlated to PCB and DDE levels in blood of nestling bald eagles around the Great Lakes and in Voyageurs National Park. However, Dykstra et al. (2005) argue that, at least as of 2001, concentrations of these two contaminants in blood from bald eagles on the Wisconsin shoreline of Lake Superior (including samples from the Apostle Islands) are near or below threshold levels. Still, these and other organochlorine contaminants continue to be near or above the levels believed to be safe for wildlife and human consumption of fish (Dykstra et al. 2005). It is likely that synergistic effects, weather, prey availability, and other stressors confound any clear cause-effect relationship. Nonetheless, attendees to a binational workshop on chemical integrity of the Great Lakes contend that continued monitoring of these legacy chemicals is needed to assess the health of the Great Lakes ecosystem (State of the Lakes 2005).

DDT, DDE, and DDD

Dichlorodiphenyldichloroethylene (DDE) is a metabolite, or breakdown product, of a synthetic pesticide known as dichlorodiphenyltrichloroethane (DDT). DDT was first introduced for widespread use as an insecticide just after World War II. Most uses of DDT were banned in Canada in 1969 under the Pest Control Products Act. Three years later, they were banned in the United States. However, the use and the sale of existing stocks of DDT products were allowed until the end of 1990. Unfortunately, DDT is still used in many parts of the world (especially in developing countries) mainly for tsetse fly control and to reduce insect damage to crops. According to estimates from the World Health Organization, Mexico and Brazil each used almost 1,000 tons of DDT in 1992.

DDE, the most persistent DDT metabolite, is routinely detected or encountered instead of DDT. DDE is produced in most animals when the body attempts to metabolize or digest DDT. DDE is also highly fat soluble. For these reasons, top predators, such as bald eagles, are more likely exposed to DDE than DDT from the food they consume. Very little DDT has been found in Great Lakes bald eagle eggs, except during periods of high use of this pesticide in the early 1970s.

PCBs

Polychlorinated biphenyls (PCBs) have been in use since 1929. There are 209 possible types of PCBs, termed “congeners,” which differ slightly from each other in their chemical and physical properties. A small number of these congeners are highly toxic and probably account for most PCB-induced toxicity in animals. PCBs, like DDT and dieldrin, are organochlorine compounds which persist for a long time once released into the environment. However, unlike the pesticides DDT and dieldrin, PCBs were not deliberately released into the environment. PCBs are extremely stable molecules, a characteristic that make them desirable for industrial uses. Their low flammability made them useful as lubricants and as fire retardants in insulating and heat-exchanging fluids used in electrical transformers and capacitors. They have also been used as plasticizers, waterproofing agents, and in inking processes used to produce carbonless copy paper. Since 1977, regulations have been in place in Canada and the United States to ban the import and manufacture of PCBs, and tight restrictions are in place for the storage and destruction of all PCB-containing wastes.

Heavy Metals

Heavy metals of concern in the Great Lakes Network parks include mercury, alkyl-lead, zinc, and cadmium. Mercury is a particularly pervasive and highly toxic heavy metal found in waters across the Network and the globe (for excellent reviews, see Evers [2005] and Wiener et al. [2003]). Mercury is a naturally occurring element found in rock. Mercury was mined for manufacturing and production of consumer products (fungicides in seed grain, thermometers, lamps, dental fillings) and is also a by-product of coal-burning power generation and solid waste incineration. Growing awareness of its hazards led to discontinuation or reductions in most uses beginning in the 1960s; however, significant anthropogenic releases of mercury still occur (Evers 2005, Hylander and Meili 2005). State and federal regulatory agencies list water bodies as impaired from mercury in eight of nine parks in the Great Lakes Network; this has spurred research in Voyageurs (Ensor Giovengo 1997, Sorensen et al. 2005, Wiener et al. 2006), Isle Royale (Gorski et al. 2003), St. Croix (Christensen et al. 2006), and more recently Apostle Islands (J. G. Wiener, University of Wisconsin-La Crosse, unpublished data).

New and Emerging Contaminants

Perfluorinated compounds (PFCs), including perfluorooctane sulfonate (PFOS), polycyclic aromatic hydrocarbon (PAH), and polybrominated diphenyl ether (PBDE) are three large groups of chemicals that are emerging as contaminants of concern in the Great Lakes region and around the world (Renner 2000). The 3M Company, stationed in St. Paul, Minnesota, near the Mississippi National River and Recreation Area, phased out production of PFOS, the most prevalent form (telomer) of PFC, in 2000 after studies showed that it was highly toxic, persistent, and bioaccumulative in the environment (Auer 2000). PFOS was widely used in Scotchgard™ products that served as water, oil, and grease repellents for garments and other fabrics, as well as use in fire-fighting foams. In February 2006, an employee of the Minnesota Pollution Control Agency released information that fish in the Mississippi River, near the 3M plant, contained the highest levels of PFOS ever recorded (<http://news.minnesota.publicradio.org/projects/2005/02/toxitraces/>). PFOS has been found widely in humans and in wildlife, especially fish-eating birds (Auer 2000). PBDEs are widely used as flame retardants in plastic components of computers and televisions, circuit boards, vehicle upholstery, and

many textiles. There is increasing concern that these chemicals are ubiquitous in the environment (human breast milk to marine mammals) and that low-level exposure may be detrimental to human and animal health (Renner 2000).

Nine PBDE congeners and 16 PFC telomers being screened for in this protocol:

PBDE #28
PBDE #47
PBDE #66
PBDE #85
PBDE #99
PBDE #100
PBDE #138
PBDE #153
PBDE #154
PERFLOURO-N-TETRADECANOIC ACID
PERFLOURO-N-TRIDECANOIC ACID
PERFLUORO-1-BUTANESULFONATE
PERFLUORO-1-DECANESULFONATE
PERFLUORO-1-HEPTANESULFONATE
PERFLUORO-1-HEXANESULFONATE
PERFLUORO-1-OCTANESULFONATE
PERFLUORO-N-BUTANOIC ACID
PERFLUORO-N-DECANOIC ACID
PERFLUORO-N-DODECANOIC ACID
PERFLUORO-N-HEPTANOIC ACID
PERFLUORO-N-HEXANOIC ACID
PERFLUORO-N-NONANOIC ACID
PERFLUORO-N-OCTANOIC ACID
PERFLUORO-N-PENTANOIC ACID
PERFLUORO-N-UNDODECANOIC ACID

The Department of the Interior protects and manages the nation's natural resources and cultural heritage; provides scientific and other information about those resources; and honors its special responsibilities to American Indians, Alaska Natives, and affiliated Island Communities.

NPS 920/160133, August 2019

National Park Service
U.S. Department of the Interior



[Natural Resource Stewardship and Science](#)

1201 Oakridge Drive, Suite 150
Fort Collins, CO 80525