Addendum to the GLIFWC Chippewa Ceded Territory Traditional Food Contaminant and Safety Report: A Scientific Evaluation of Possible Health Risks to Great Lakes Ojbwe Tribal Members from *manoomin* (wild rice), *zhiiwaagamizigan* (maple syrup) and *mizise* (wild turkey)

ANA SEDS Contract #90NA8320

By:

L.T. Brooke

Research Biologist

Lake Superior Research Institute (Retired)

Jim Thannum

Director of Planning and Development

Great Lakes Indian Fish & Wildlife Commission

Candace E. Kraft

ANA Environmental Biologist

Great Lakes Indian Fish & Wildlife Commission

March 31, 2020

Great Lakes Indian Fish & Wildlife Commission
Planning and Development Division
P.O. Box 9, 72682 Maple Street, Odanah, WI 54861
715-682-6619
www.glifwc.org

Table of Contents

List of Tables

List of Figures

List of Acronyms

AAS: Atomic Absorption Spectrometry

ANAB: ANSI-ASQ National Accreditation Board

ANSI: American National Standards Institute

ANA SEDS: Administration of Native Americans Social & Economic Development Strategies

BAL: Brooks Applied Labs

d.w.: Dry Weight

E.U.: European Union

GLIFWC: Great Lakes Indian Fish & Wildlife Commission

HACCP: Hazard Analysis and Critical Control Point

ICP-MS: Inductively Coupled Plasma-Mass Spectrometry

LOD: Limit of Detection

LOQ: Limit of Quantitation

LSRI: Lake Superior Research Institute

NRC-C: National Research Council-Canada

NIST: National Institute of Standards & Technology

MDL: Method Detection Limit

MRL_1: Method Reporting Limit

MRL_2: Maximum Residue Limit

Pace: Pace Analytical Services, LLC

ppm: parts per million = mg/L = mg/kg

QA/QC: Quality Assurance/Quality Control

RfD: Reference Dose

RPD: Relative Percent Difference

RSD: Relative Standard Deviation

SRM: Standard Reference Material

TEK: Traditional Ecological Knowledge

U.S. EPA: United States Environmental Protection Agency

U.S. FDA: United States Food & Drug Administration

w.w.: Wet Weight

INTRODUCTION

Ojibwe Bands entered into treaties with the United States government in 1836, 1837, 1842 and 1854. Within these treaties, the Bands ceded (sold) lands, mineral rights and timber to the United States but retained the right to harvest natural resources to continue traditional Ojibwe lifeways. These traditional Ojibwe lifeways incorporated seasonal harvests of fish, game, wild rice and other plant resources for spiritual, cultural, subsistence, and commercial purposes.

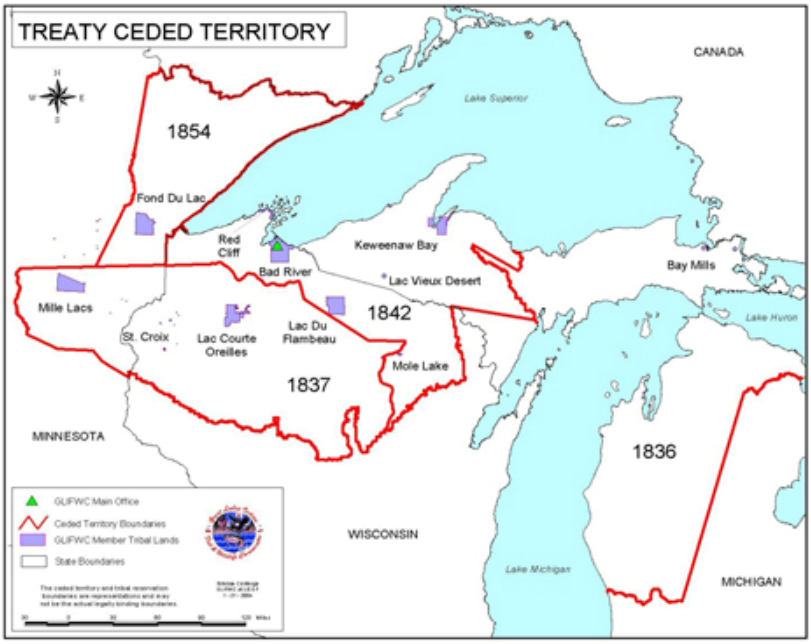

Figure 1: Boundaries of the territories ceded by the Ojibwe and year.

A series of federal and state court decisions re-affirmed off-reservation harvest rights of Ojibwe Bands including the 1981 *United States v. Michigan*, 1983 *Lac Courte Oreilles v. Wisconsin* and the 1999 U.S. Supreme Court Decision *Minnesota v. Mille Lacs Band*.

Eleven Ojibwe Bands in Minnesota, Wisconsin and Michigan established the Great Lakes Indian Fish and Wildlife Commission (GLIFWC) to implement court orders associated with the federal court decisions through inter-tribal protocols, delegated authorities and the development of tribal self-regulatory systems. The eleven Ojibwe Bands comprising the Great Lakes Indian Fish and Wildlife Commission include: *Nagaajiwanaag* (place were the water stops) commonly known as Fond du Lac, *Misi-zaagan'iganiing* (place of the big spread out lake) commonly known as Mille Lacs, *Wezaawaagami-ziibiing* (yellow river) commonly known as St. Croix, *Odaawaa-zaaga'iganing* (Ottawa Lake) commonly known as Lac Courte Oreilles, *Miskwaabikong* (place of steep rock of red material) commonly known as Red Cliff, *Mashkii-ziibing* (place of swampy river) commonly known as Bad River, *Waaswaaganing* (place of torch light) commonly known as Lac du Flambeau, *Zaka'aaganing* (place of torch-stick lake) commonly known as Sokaogon (Mole Lake), *Gete-gitgaaning* (old garden lake) commonly known as Lac Vieux Desert, *Gakiiwe'onaning* (or *Wiikwedong*) (place at the bay) commonly known as Keweenaw Bay, and *Ginoozhekaaning* (place of the pike) commonly known as Bay Mills. (GLIFWC 2018)

Traditional foods harvested by the Ojibwe have been documented to provide important sources of nutrition and have the potential to improve reservation health conditions. *"It is evident [from the above] that there is a relationship between the use of traditional Ojibwe food and the health and well-being of Band members. Band members recognize that traditional food is important for health, they would like to use more of it, and they wish this cultural knowledge to be taught to their children. Replacing some of the less-nutritious market food with traditional food will improve diet and nutrient*

intake, thus helping to prevent chronic disease; harvesting traditional food gives opportunity for activities in physical fitness and outdoor recreation; harvest and use of traditional food provides opportunity to experience, learn, and promote cultural activities·, it gives opportunity to develop personal qualities desired in Ojibwe culture such as sharing, self-respect, pride, self-confidence, patience, humility and spiritualty." (Kuhnlein 1995)

Federal courts have ruled State governments can only pre-empt tribal regulations governing the exercise of off-reservation treaty activities related to the harvest and sale of natural resources for conservation or safety purposes.

The *Voigt* and *Mille Lacs* cases both deferred a decision on the extent to which the States of Wisconsin and Minnesota, respectively, could regulate the off-reservation rights, including the sale and service of harvested food. (*Mille Lacs*, 124 F.3d 909). The issue of permissible state regulation was set for trial in subsequent phases and the parties later made stipulations to narrow the issues to be tried. The Deer Trial Stipulation provided that, as of 1989, the Tribes which were parties to *Voigt* did not have food regulations similar to enumerated provisions in Wisconsin law applicable to the processing of deer for human consumption, including the regulation of food processing and retail food establishments.(*Lac Courte Oreilles Band of Lake Superior Chippewa v. Wisconsin*, No. 74-C-313). The parties agreed, however, that Wisconsin law applied only until such time as a Tribe adopted "corollary regulations" and "employ[ed] trained and qualified personnel to enforce such regulations." (Pavel 2018)

Tribal leadership, and their communities, expressed desire to increase the availability of traditionally harvested foods within their communities and to integrate these foods into tribal community food systems. Tribes are particularly interested in increasing opportunities to sell traditionally harvested wild foods to the federal food programs operating within their reservation communities.

In response to community interest and tribal leadership directives, GLIFWC obtained a federal Administration of Native Americans Social & Economic Development Strategies (ANA SEDS) grant to develop a model traditional food code. The purpose of the tribal food code is to provide a regulatory structure governing the processing, distribution, labeling and sale of treaty harvested resources in a manner that effectively protects the health and safety of community members. Federal courts have ruled that Tribal off-reservation conservation codes must be based upon sound science to adequately protect natural resources. Similarly, tribal food codes must also be based upon sound science to adequately protect human health. In particular, food safety regulations need to identify and account for biological[1], chemical[2] and physical[3] food safety risks that are "reasonably likely" to occur.

GLIFWC utilized a systematic approach in identifying traditional Ojibwe foods of interest and potential food safety risks. GLIFWC first utilized a survey of tribal members and consultation with tribal elders and tribal leaders to identify and rank the 16 Ojibwe foods tribal members would most like

[1] Biological risks in food commonly consist of bacteria, virus, parasites and diseases such as Chronic Wasting Disease (CWD).
[2] Chemical risks in food commonly consist of lead, mercury and other heavy metals in addition to organochlorine chemicals such as PCBs and DDT.
[3] Physical risks in food commonly consist of metal fragments and glass.

to increase access to within their communities. Tribal members identified *manoomin* (wild rice), *zhiiwaagamizigan* (maple syrup) and *mizise* (wild turkey) through this process. (Kraft & Maroney 2018)

GLIFWC then conducted an extensive, peer review of scientific journals to ascertain possible food safety hazards associated with those Ojibwe foods identified as holding the greatest interest by tribal members in the survey. The scientific literature was compiled, analyzed and summarized in a report to identify potential biological, chemical and physical hazards to human health (Kraft 2018). Additional literature searches and analysis were then undertaken to verify which Ojibwe foods had limited or no published scientific research regarding chemical contamination. These foods were identified for further study.

Biology of Food Items for this Study and Identification of Potential Food Safety Concerns

manoomin (wild rice*, Zizania palustris*)

Manoomin is an annual grass that grows naturally in marshes, as well as on the fringes of lakes, streams, and rivers. These plants typically grow in calm, clear waters with soft, organic rich sediments at a relatively sensitive range of depths from 0.5 to 3 feet (Minnesota Department of Natural Resources 2018).

Manoomin has been a staple in the diet of native people in the upper Great Lakes region for over 1000 years (Johnson 1970). It has been an important component of the diet and the culture of the Ojibwe people since their immigration from the eastern seaboard into the heart of wild rice range at the west end of Lake Superior (Vennum 1988). With the arrival of Europeans, wild rice also became an important economic commodity, providing critical nutrition to the fur-trappers and traders moving into the area. Today, *manoomin* retains extraordinary significance to the Ojibwe, and is considered sacred food. The September moon is still referred to as Manoominike Giizis (the Rice Making Moon), and the harvest season is still celebrated with traditional pow-wows. (David 2013)

In addition to its value to Native Americans, wild rice provides a valuable food source for wildlife, and its presence increases the biological diversity of wetlands. *Manoomin* can also improve water quality by tying up nutrients and by decreasing the wind action across lakes that can suspend sediment particles and lead to water clarity and quality problems. (David 2013)

Unfortunately, wild rice is much less abundant than it was historically. The Great Lakes Indian Fish and Wildlife Commission (GLIFWC) conducts a *manoomin* (Zizania palustris) enhancement and research program in the territories ceded in the Treaties of 1836, 1837, and 1842 and works to restore the resource through cooperative projects with other natural resource agencies. (David 2013)

In 2014, tribal members were estimated to have harvested 18,605 pounds of *manoomin* in 520 trips. The total off-reservation harvest per active tribal license averaged 115 pounds. (David 2020)

Wild rice is commonly consumed by adults and children at tribal meals in cooked form as a side dish, or as an ingredient in casseroles or other main dishes. It is also sometimes ground into flour

or meal (GLIFWC 2014). It's general nutritional value surpasses cereal grains, such as wheat, oats, barley, and rye.

As an aquatic grain, or a cereal, wild rice contains more than 12 percent protein, is gluten free, and low in fat. It is also a good source of minerals, such as iron, potassium and phosphorus, as well as vitamins like thiamine, riboflavin and niacin. *Manoomin* has more overall nutrition than any other food in the Ojibwe diet. (*Manoomin* – Wild Rice the Good Berry).

Tribal members have experienced fish consumption advisories in ceded territory waters as these waters became polluted by mercury and organochlorine chemicals (i.e. PCBs, DDT, etc.) which bio-accumulated in fish tissue. (http://www.glifwc.org/Mercury/index.html)

As awareness of fish consumption advisories increased, tribal members became concerned pollutants could also enter the aquatic habitats growing *manoomin* (wild rice). These fears were augmented through scientific research, *"North American wild rice (Zizania aquatica L. and Z. palustris L.) grows in moderately soft and acidic freshwater wetlands. With the increasing pollution of such a habitat, there is some concern that the trace metal contents of the rice crop have become unduly elevated. This study finds moderately elevated levels of lead (0.5–11.5 µg/100 g dry wt.), cadmium (1.0–10.2 µg/100 g dry wt.) and arsenic (0.6–14.2 µg/100 g dry wt.) in 26 brands of wild rice sold in the United States".* (Nriagu 1995).

Tribal members have also historically utilized galvanized wash tubs to scorch wild rice as they process wild rice. Unfortunately, some of the galvanized wash tubs contain lead solder which necessitates testing to ensure food safety.

*zhiiwaagamizigan (*maple syrup*, boiled Acer spp. sap)*

Maple syrup is a traditional, Ojibwe sweetener made by evaporating sap from tapped *Acer spp.* trees, usually from sugar maples (*Acer saccharum*) or possibly red maple (*Acer rubrum*), due to the high sucrose concentration of sap from these particular species (Corbiere 2011). Sap (*ziinzibaadwadwaaboo*) is harvested by drilling through the bark and cambium into the sap wood of the tree and inserting a tube (spike) through which the sap drains into a pail. The sap is collected and dehydrated in an open pan with heat beneath the pan. The dehydration continues until the desired sugar concentration is accomplished. It was noticed during sample collection this particular traditional food is regularly bartered or gifted.

Lead contamination of maple syrup occurs predominantly from the use of lead-bearing processing equipment, and usually does not come from the sap itself (Ontario Ministry of Agriculture, Food, and Rural Affairs 2019). Under suitable conditions of acidity and temperature, lead mobilizes into sap when in contact with lead bearing materials (Willits and Tressler 1937). Therefore, concern existed that maple syrup may be contaminated with lead from old, inherited metal equipment used in processing and/or from lead-bearing mechanisms present in non-food grade equipment used by tribal members (Stilwell and Musante 1996; Willits and Tressler 1937; Ontario Ministry of Agriculture, Food, and Rural Affairs 2019).

Wisconsin regulatory rules expressly state that neither lead nor lead alloyed solder can be used in the assembly or repair of surfaces associated with food-contact for food sold in the private sector (Wisconsin Department of Agriculture, Trade and Consumer Protection 2019). This regulation could

potentially restrict the future sale of tribal maple syrup in retail establishments if lead-bearing equipment or repair material was inadvertently used by tribal harvesters.

*mizise (*wild turkey, *Meleagris gallopavo)*

Wild turkeys are large birds in the taxonomic order Galliformes that are four feet tall and weigh 18–24 pounds as adults. They are the largest wild bird in North America. Their young forage for food on the ground as soon as they are hatched. They feed on hard seeds and nuts as well as insects. They have a large and well developed gizzard (grinding organ at the entrance to the stomach) that grinds food prior to digestion (Wisconsin Department of Natural Resources 2015). Hunting of *miziseg* (turkeys) in the Wisconsin and Minnesota ceded territories has been a small but regular activity since the reaffirmation of off-reservation harvesting under the Voigt 1983 and Mille Lacs 1994 decisions. Once extirpated from the region, turkeys have again become established throughout much of the Wisconsin and Minnesota ceded territories and are now included in turkey management zones (David 2019).

Wild turkeys are customarily harvested with shotguns, presenting two, potential food hazards: physical and chemical. The possible physical hazard includes the presence of shot pellets or rifle bullet fragments embedded in turkey meat possibly causing damage to teeth or internal organs of the consumer. Most people attempt to remove visible pellets found in wild game when preparing meat for meal consumption (Food Standards Agency in Scotland 2012). However, placement of the pellets within meat may not always be visible during processing or before cooking.

The second possible hazard is lead contamination introduced by lead shot and is a possible chemical hazard from consumption of wild turkey meat. There is a large body of literature that exists to support this idea (Arnemo et al. 2016; Johansen et al. 2004; Pain et al. 2010; Tsuji et al. 1999). Lead is a well-studied element due to its known acute and chronic (lifetime) toxicity to the neurological system, particularly in children, pregnant women, and women of childbearing age (Agency for Toxic Substances and Disease Registry 2007). It is not known to what extent wild turkey meat retains this metal following harvest with lead ammunition, and after typical field dressing and butchering processes.

Research Objectives

Goals related to the chemical and/or physical contaminant inquiries regarding target Ojibwe foods:

- *Objectives regarding the inquiry into elemental contamination of wild rice*

1. Analyze total concentrations of nine elements in uncooked wild rice seeds gathered and processed within ceded territories by tribal harvesters (i.e., Pb, Zn, Cd, total Hg, Mg, total Cr, Cu, Se and As), and extrapolate these concentration results using moisture content information for cooked wild rice.
2. Analyze the proportion of wild rice's inorganic arsenic content in relation to total arsenic levels.
3. Compare elemental results to other published studies.
4. Compare elemental concentrations of Great Lakes Ojibwe harvested wild rice with regulatory action levels established to protect human health from excessive metal exposure.

 Objectives regarding the maple syrup lead inquiry

1. Analyze lead concentrations in maple syrup collected and processed within the ceded territories by tribal harvesters.
2. Compare lead concentrations in maple syrup with Canada's MDLs for lead in maple syrup and the State of California standard of 0.011 mg/kg.

 Objectives regarding wild turkey lead content

1. Evaluate how many pellets and/or fragments reside in wild turkey breast tissue
2. Analyze lead concentrations in individualized wild turkey breast tissue collected within the ceded territories by tribal harvesters.
3. Assess mean lead concentrations in wild turkey breasts per bird.
4. Compare lead concentrations in wild turkey breasts with regulatory action levels established to protect the human food supply and other wild game.

METHODS AND MATERIALS

Sample Collection

Several methods were used to disseminate information to GLIFWC-affiliated tribal harvesters regarding traditional foods to be collected for this study across the ceded territories. A call for samples was placed in the autumn 2018 edition of the *Mazina'igan*, a widely-distributed GLIFWC newspaper. Hard-copy, sample request posters were mailed to all member tribe offices, along with poster placement on GLIFWC's main webpage and social media accounts such as Facebook.

Known tribal harvesters and influential community members were subsequently contacted to request samples. Sample collection efforts were also announced at major GLIFWC meetings with tribal leadership for information dispersal. The various communication methods employed were used to reach the broadest Ojibwe audience, as well as diversify sample sources and methods used to harvest and/ or process samples within each of the 11 GLIFWC member tribes.

Most of the wild, traditional food samples came from within ceded territories (Fig. 1), with sample custody documented that included sample collection sites, how samples were harvested, and when collected by the tribal harvester. However, wild rice and maple syrup samples from institutions affiliated with tribal communities were also deemed acceptable. A select amount of comparable grocery store samples where tribal member would shop were also purchased for comparative analyses.

Wild rice sample collection

Forty wild rice samples were obtained by Ojibwe tribal harvesters within ceded territories; three commercial wild rice samples were purchased as comparative samples (Table 1). Package labels on cultivated (paddy) wild rice obtained from grocery stores indicated they were of Minnesota origin. Unfortunately, California grown paddy wild rice could not be found in the local grocery stores searched. Some of the wild rice from samples was selected at random and cooked to determine the moisture content of cooked wild rice.

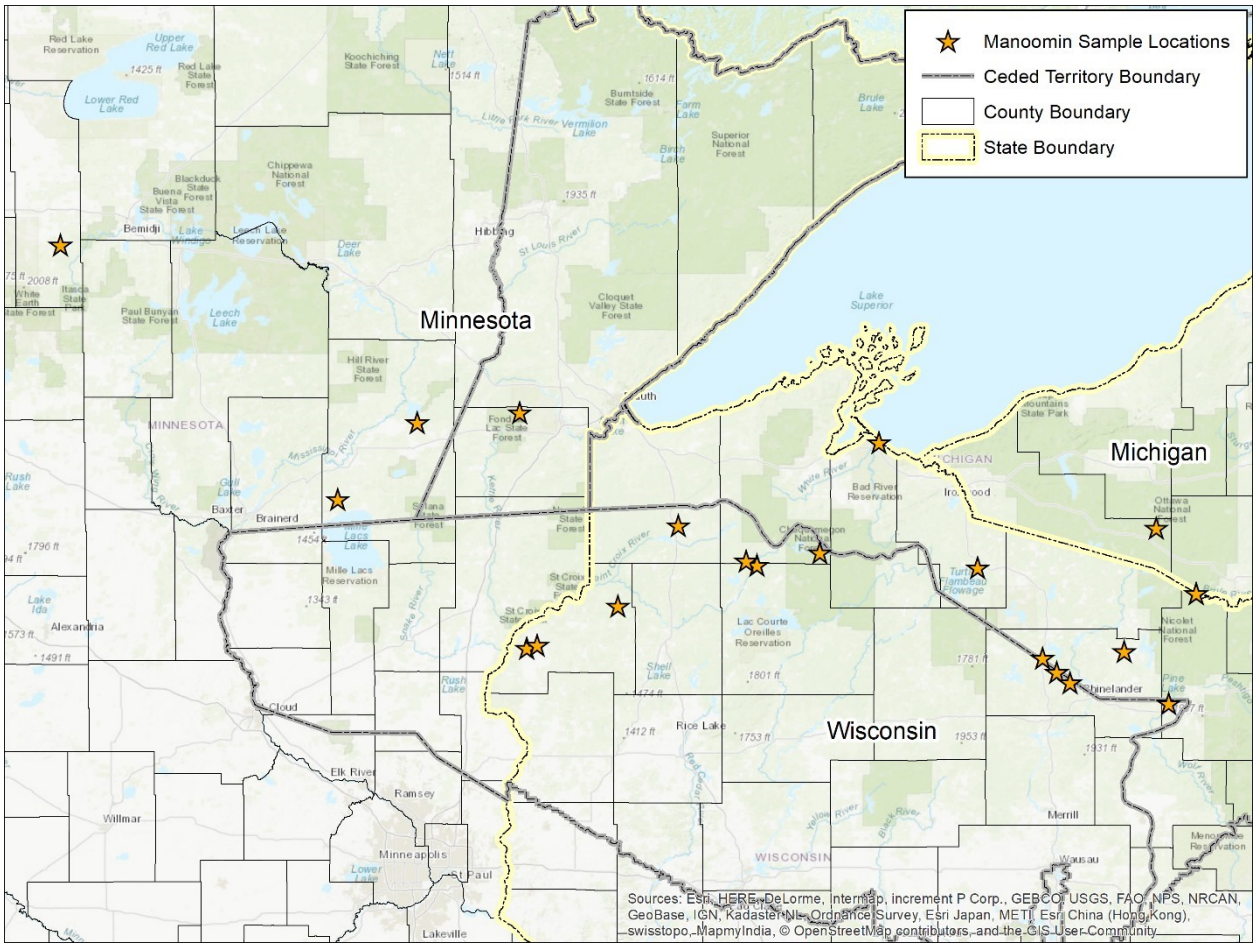

Figure 2. Wild rice samples for heavy metals testing were collected from natural beds located in the 1842, and 1854 treaty territories. However, three sample were collected outside ceded territory boundaries.

An additional wild rice sample was contributed by a Fond du Lac tribal member that was collected from Clearwater County Minnesota. Out of cultural respect and as a GLIFWC tribal member, that sample was also included for analyses, although technically outside the bounds of ceded territories. Some reservation localities experience a low abundance of wild rice resulting in samples obtained from both on-reservation and off-reservation harvest areas. A map of the general ceded territory boundaries (Fig. 2), with stars indicates wild rice source collection points identified by tribal harvesters. All samples were collected in northern regions of Minnesota, Wisconsin, and Michigan. Despite best staff efforts, it remained difficult to locate more samples from water bodies throughout the ceded territories. To increase sample size, wild rice purchased in previous years by GLIFWC was also submitted for analyses. "Finished" or "processed" wild rice throughout this document is defined as wild rice that has been dried, parched, threshed, and winnowed. "Unprocessed" or "green" rice refers to rice that has been collected but has not undergone any processing steps, therefore, containing more moisture.

Table 1. Wild rice sample locations identifying the water body, county, state of harvest and the number of samples from each site.

Lake / Water Body of Harvest	County of Harvest	Number of Samples
Wisconsin		
Bad River Sloughs	Ashland	2
Chippewa Lake	Bayfield	4
Cut-A-Way Dam	Douglas	1
Lee Lake	Rusk	1
Little Rice Lake	Forest	1
Little Turtle Flowage	Iron	1
Long Lake	Burnett	1
North Fork Flowage	Burnett	1
Pacwawong Lake	Sawyer	1
Phantom Lake Flowage	Burnett	1
Totagitic Lake	Bayfield	3
Rice Lake	Forest	5
Mixed Sources *		2
Michigan (Upper Peninsula)		
Brule River	Iron	1
Unknown Location**	Iron	1
Ontonagon River	Gogebic	1
Minnesota		
Deadfish Lake	St. Louis	1
Mallard Lake	Aitkin	1
Lake Minnewawa	Aitkin	1
Upper Rice Lake	Clearwater	1
Unknown Site & State		
		6
Cultivated Wild Rice from Unknown Harvest Locations		
MN paddies		3
Total		40

* Wild rice seeds from more than one source were combined by the harvester.
** Harvester wished to keep his source waterbody private to prevent others from harvesting there.

Maple syrup sample collection

Twenty-nine maple syrup samples were obtained by Ojibwe tribal harvesters within ceded territories and three commercial maple syrup samples were purchased as comparative samples.

The Ojibwe culture, as with many other indigenous tribes, customarily use items passed down to them from elders, e.g., harvesting or processing equipment. When possible, sample providers were asked whether they used any "old and/or inherited equipment from a previous generation," engendering a "yes" or "no" response. If the answer was "yes," more information regarding specific equipment was collected from the harvester. For purposes of this report, "old" is defined as having an age of more than 15 years old, i.e., one generation, and "inherited" means that it was passed down to the current harvester from a previous generation. This type of "old" and "inherited" equipment is more likely to have contained lead soldered connections or other non-food grade materials which come in contact with sap (International Maple Syrup Institute, 2015).

However, "old" and "inherited" are not necessarily interchangeable terms. An "old" piece of equipment may not necessarily be "inherited," but an "inherited" item is by default "old." If a processor did not classify their equipment as either "old and/or inherited," an assumption was made that the processor used all modernized equipment, even if not explicitly stated by them during sample collection.

In addition, finished wild rice and bottled maple syrup purchased from GLIFWC affiliated tribal harvesters in previous years were used to expand sample size and site selection. Scant or missing harvest and processing information remained a drawback to using samples from prior years. However, analyses of these samples provided valuable information of possible heavy metals exposure from consumption of those food items.

Wild turkey sample collection

Wild turkey samples were specifically collected in the fall 2018 and spring 2019 during tribal harvest seasons. Whole wild turkeys were collected from tribal hunters that contacted staff immediately upon harvest. The chain-of-custody record submitted with each bird contained information including the name of the harvester, shot size, type of shot, and gauge of shotgun used. Tribal harvesters submitting samples were required to freeze or refrigerate the birds no more than one hour after harvest. Whole birds were frozen by the harvester if turkey samples were held longer than 72 hours after harvest to maintain sample integrity. Whole wild turkeys were assigned a sample number and stored in an electronically-monitored freezer at ≤ -10°C until they underwent the breast removal process. Before breast removal, turkeys were thawed in a refrigerator at ~ 4°C for 48 to 72 hours.

Laboratories used for Processing and Analyzing Samples

Three laboratories were used for analysis of the metals of interest in this study for the assessment of chemical concentrations in the target Ojibwe foods. The laboratory at the University of Wisconsin-Superior, Superior, WI is a research laboratory of the Lake Superior Research Institute (LSRI) and was the primary laboratory for receiving and preparing samples for shipping to the other laboratories. LSRI ground the wild rice seeds and turkey breasts and analyzed a percentage of the wild

rice, turkey and maple syrup samples for moisture, and analyzed for the metals copper (Cu), magnesium (Mg), total mercury (Hg), and zinc (Zn) in wild rice. Pace Analytical (Pace) has its headquarters in Minneapolis, MN with many satellite laboratories of which one is located in Green Bay, WI and was utilized for this study. Pace analyzed samples for cadmium (Cd), chromium (Cr), lead (Pb), and selenium (Se). Brooks Analytical Labs (BAL) is located in Bothell, WA and specializes in trace metals analysis. BAL analyzed samples for arsenic (As); total and inorganic as well as the organic dimethylarsinic acid, and monomethylarsonic acid. BAL is an ANAB accredited analytical laboratory along with current certifications for environmental testing in eight states nationwide (BAL 2019b).[4] Specifically, BAL meets ANAB testing criteria for total and inorganic arsenic in biological tissues under the international standard ISO/IEC 17025:2005 using proprietary methods, processes, techniques, and standard operating procedures (BAL 2019b; ANSI 2019).

Sample processing and storage

Wild rice sample processing and storage

Upon arrival at GLIFWC's facility at Odanah, WI, plastic bags of wild rice seeds were labeled, a chain-of custody record was begun, and the sample placed in dry storage at room temperature (~18 to 21°C). Storing whole, finished wild rice seeds at an ambient temperature resembles typical finished wild rice storage by tribal consumers. Select aliquots of wild rice were cooked for later moisture analysis. These nonrandom cooked samples were chosen for moisture analysis based upon geographical location of harvest to represent the entire area from which samples were collected.

The cooked wild rice samples were prepared according to a standardized protocol developed at GLIFWC in a manner typical to that of tribal consumers. The resulting cooked wild rice was then stored in clean glass bottles in a temperature monitored freezer at ≤ -10°C. One moisture content sample included a can of grocery store purchased cooked paddy wild rice, drained and subsequently frozen. No other canned, commercial brands could be found in a variety of grocery stores explored. Both finished (uncooked) and cooked wild rice samples were then delivered to LSRI.

Maple syrup sample processing and storage

Labeled, sealed maple syrup bottles and the single maple sugar sample were placed in locked, dry storage at room temperature (~18 to 21°C), a typical storage procedure of these food items by tribal consumers. Glass maple syrup sample bottles were packed securely in plastic containers and delivered to LSRI. LSRI examined moisture content in all maple syrup samples and transferred an aliquot of each sample to critically clean bottles prior to delivering them to Pace in coolers on ice for lead analysis.

Wild turkey sample processing and storage

Before pectoral muscle removal from the birds, total length was measured from the end of the beak to the end of the tail. Right and left breasts of wild turkeys were extracted using a standardized protocol. "Right" and "left" breast orientation refers to the extractor's left and right sides with the

[4] ANSI-ASQ National Accreditation Board (ANAB) is an independent accreditation body that oversees the quality of laboratory test results for specific matrices, methods, and analytes, among other tasks (ANSI 2019).

bird's head facing away from the extractor. The breasts were separated at the sternum. Any shot pellets observed on or in breast tissue was manually removed using forceps, and accompanied the sample in a separate plastic bag for later analysis. After breast removal, samples were labeled with the sample number and an alphabetic letter (R for right or L for left) for each individual breast. Each breast was examined by x-ray to locate any hard objects obstructed from view by tissue and the objects removed for analysis. The breast samples were then returned to frozen storage and delivered to LSRI for tissue grinding and homogenizing. LSRI examined moisture content in 16 turkey breasts and transferred an aliquot of each sample to critically clean bottles prior to delivering them to Pace in coolers on ice for lead analysis.

All grinding equipment used in the preparation and analyses of samples were critically-cleaned with 0.1 M hydrochloric acid. All glassware and plastic labware that were not used in the homogenization process were critically-cleaned using a 10% solution of nitric acid. Due to a property of high solubility, maple syrup and maple sugar were not homogenized. Equipment and labware cleaned in any acidic solutions were subsequently rinsed with copious amounts deionized water. Decontamination procedures at Pace included similar critical-cleaning practices for reusable labware. BAL utilized proprietary critical-cleaning practices that third-party accreditation bodies have determined as acceptable.

Sample homogenization and storage

Wild rice sample homogenization and storage

Uncooked wild rice samples were ground into a fine powder at LSRI using a stainless-steel, commercial grinding blender. Homogenized aliquots were then placed in certified-clean, high-density polyethylene containers and secured in a locked cabinet at room temperature (~18-21 ºC). At the start of digestion at LSRI, samples were taken out of storage, the sample aliquot weighed with an analytical balance which had been verified daily with Class 1 weights.

Homogenized wild rice samples to be analyzed by Pace or BAL were stored in a locked cabinet at room temperature at LSRI until the time of transfer. Samples were kept in this manner to simulate as much as possible the typical storage method of wild rice flour by tribal households. Wild rice flour was transferred to Pace on ice and BAL with silica gel packs in coolers to preserve sample integrity in transit.

Maple syrup sample homogenization and storage

Labeled, sealed maple syrup glass bottles and the single maple sugar sample were placed in locked, dry storage at room temperature (~18 to 21°C), a typical storage procedure of these food items by tribal consumers. LSRI examined moisture content, and then shipped sample aliquots in coolers on ice to Pace for lead analysis.

Wild turkey sample homogenization and storage

Frozen wild turkey breast samples in individual plastic bags were transported to LSRI where the samples were homogenized. The night before the samples were to be processed, the breast samples

were removed from the freezer and were allowed to thaw at room temperature until the next morning. The sample was passed through the tissue grinder three times. A small amount of the initial tissue that passed through the grinder was discarded. The grinder attachment was disassembled after each breast sample was ground and the unit was washed according to the labware cleaning procedure. A subsample of each ground wild turkey breast was placed into a labeled, certified clean, high density polyethylene (HDPE) bottle and stored in a refrigerator until all samples were processed (three days). A sub-sample from each HDPE bottle was then placed into a certified clean four-ounce glass bottle received from Pace and shipped on ice to Pace with sample custody documentation.

Upon receipt at Pace, homogenized aliquots of wild rice flour, maple syrup, and wild turkey were placed in frozen storage until digestion commenced. A 1 g portion of sample was digested in nitric acid with subsequent additions of hydrochloric acid and hydrogen peroxide, diluted to a 50 mL volume with reagent water, and heated in a HotBlock®.

Tissue Moisture analysis

Wild rice tissue moisture analysis

Moisture content of samples was measured at LSRI. Moisture in both uncooked (finished) and cooked wild rice seeds were gravimetrically determined for 25% and 100% of samples, respectively. Since only a portion of the finished wild rice samples were analyzed for moisture, the samples were chosen randomly. Cooked wild rice samples were stored in a temperature monitored freezer until moisture analysis while the uncooked (finished) rice samples were stored at room temperature in a locked cabinet. The samples were placed into individual dried and weighed aluminum weighing pans. The pans were placed into an oven (60 ± 10 ºC) for ≥ 24 hours. Dried samples were cooled, and then weighed to three significant digits using an analytical balance. Randomized samples were placed back in the oven for a second period of equal time to confirm complete water loss. During moisture analysis of wild rice, one of the wild rice seed samples was analyzed in duplicate while two of the cooked seed samples were analyzed in duplicate.

Maple syrup moisture analysis

Moisture content in maple syrup was gravimetrically determined for all samples. Each sample was placed into individual, dried and weighed aluminum weighing pans. The pans were placed into an oven (60 ± 10 ºC) for 98 hours. Dried samples were cooled, and then weighed to three significant digits using an analytical balance. Randomized samples were returned to the oven for an additional 90 hours of drying before being weighed a second time. Then, samples were placed into the oven a third and final time, and allowed to dry for an additional 144 hours before being weighed. Maple sugar moisture analysis was similarly done.

Wild turkey tissue moisture analysis

Moisture content in wild turkey breast tissue was also gravimetrically determined by LSRI on ~26% of randomly chosen samples. Homogenized samples were individually placed into dried and weighed aluminum weighing pans. The pans were placed into an oven (60 ± 10 ºC) for 24 to 43 hours. Dried samples were cooled, and then weighed to three significant digits using an analytical balance.

Approximately 19% of randomized samples were placed back in the oven for another 24 hours to confirm complete water loss.

Radiographic Analysis of Wild Turkey Breasts

Ashland Area Veterinary Clinic (AAVC) is a small- and large-animal care clinic located in Ashland, WI. The clinic possesses and maintains a Summit Nova 360 x-ray unit, and provides digital radiographical services to its clients, as one component of animal care services. The clinic examined each wild turkey breast sample via x-ray (Fig. 3) using appropriate settings, and visually scanning subsequent images for dense matter.

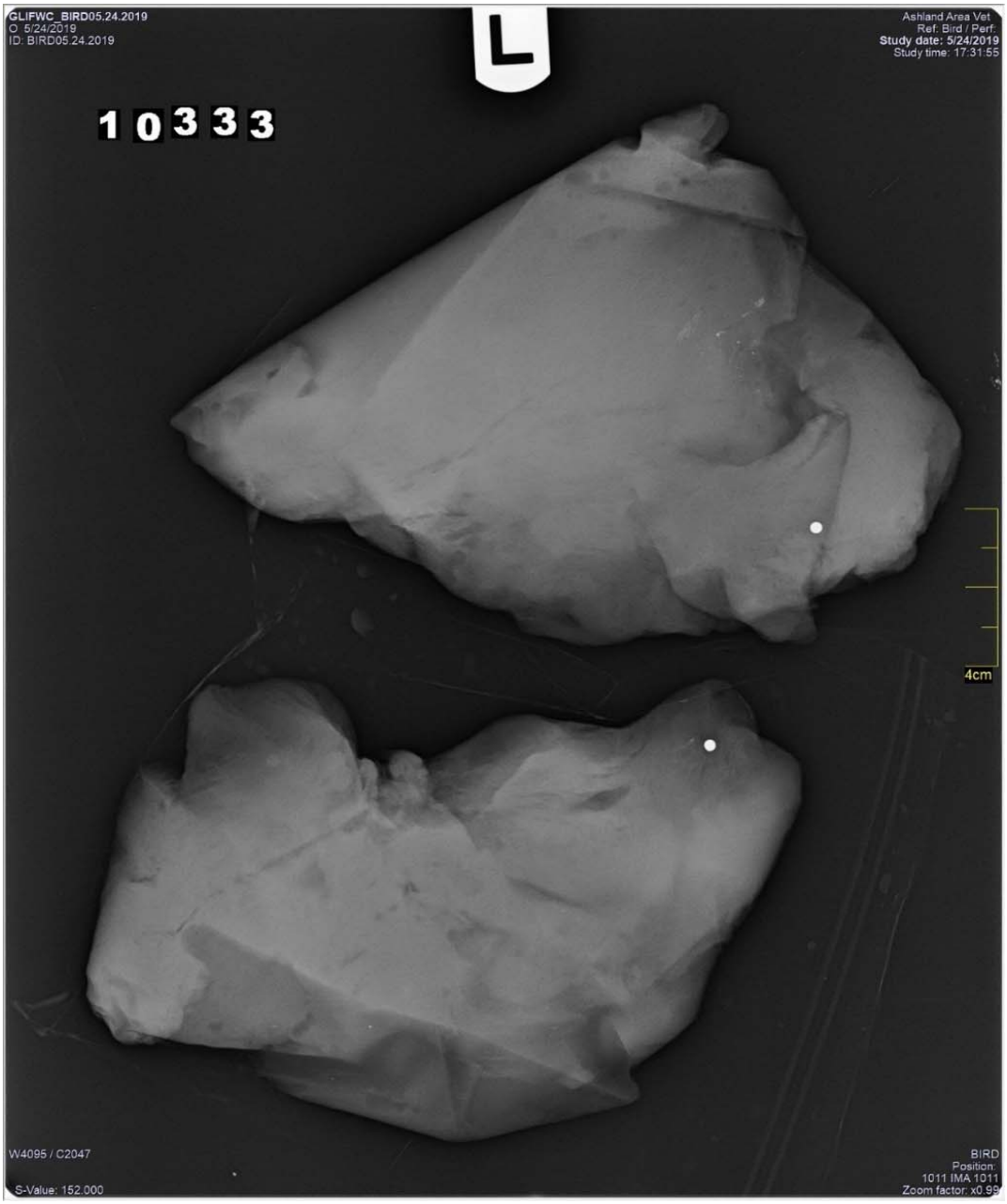

Figure 3. X-Ray radiograph image of a pair of wild turkey breast (pectoral muscle) samples showing hard metal objects embedded (white dots).

Shot pellets or larger fragments not initially observed at breast dissection were later removed utilizing radiographic images provided by AAVC. Once all conspicuous pellets and/or fragments were removed from the birds, the metallic pieces were tested for magnetism. Lead shot, bismuth-alloy, and some tungsten-matrix shot are nonmagnetic; steel and tungsten-polymer with iron in the shot possess magnetic qualities (Mann et al. 1994). Bird remains were always properly disposed of in a culturally-sensitive manner in accordance with Ojibwe customs, with certain salvageable parts saved for ceremonial purposes for tribal members upon request.

Shot Pellet/fragment Lead analysis

Shot and/or large fragments recovered from several turkey breast samples were analyzed to determine if the pellet was primarily lead or made of other metals. These metallic pieces were weighed, placed into a digestion vessel, with an addition of 3 mL of nitric acid, and then heated in a HotBlock® at 95 ± 5°C for 15 minutes. Samples were cooled and diluted to 50 mL of deionized water. Since not all of the samples were digested, remaining solid material was recovered, dried, and weighed. LSRI used the flame option on their Atomic Absorption Spectrometry (AAS) instrument for lead analysis. Lead percentages in the portions of the metal dissolved during digestions of pellets were calculated.

Quality Assurance and Quality Control (QA/QC)

Specific level of detection[5] (LOD) and level of quantitation[6] (LOQ) values were determined for each metal analyzed at LSRI, Pace, and BAL laboratories (Table 2).

Detection limits for Hg, Zn, Cu, and Mg are established annually at LSRI, with samples of each metal in particular matrices analyzed on a quarterly basis for calculation and/or adjustment. Detection limits for Pb, Cd, total Cr, and Se were determined by Pace, and utilized criteria they deemed as acceptable. BAL specializes in arsenic speciation analysis and has calculated their own detection limits for total and inorganic arsenic using proprietary methods. All analytes were measured as total of each metal except arsenic where inorganic was separated from total arsenic.

[5] Limit of detection (LOD) is the lowest level or amount of an analyte (analyte is the target chemical or element being measure i.e. mercury) which can reliably and feasibly be detected by the instrument used for measuring.
[6] Level of quantitation is a calculated value which factors in known and predefined bias or imprecision within the instrument used for measuring. LOQ represents the lowest reliable measurable concentration with known imprecision factored in.

Table 2. LOD and LOQ values for the ten analytes of interest and the analytical instrument used for analysis. LSRI used two Atomic Absorption Spectrometry (AAS) options: cold-vapor for Hg and flame for the other metals analyzed. Pace utilized Inductively Coupled Plasma-Mass Spectrometry (ICP-MS) for analyses. BAL used inductively coupled plasma collision reaction cell mass spectrometry (ICP-CRC-MS).

Analyte	LOD values* (mg/kg or ppm)	LOQ values** (mg/kg or ppm)	Analytical Instrument	Analytical laboratory
Zn	0.600	2.000	Flame AAS	LSRI; UW-Superior, WI
Mg	0.467	1.567		
Cu	0.433	1.443		
Hg	0.015	0.050	Cold-Vapor AAS	
Cd	0.014	0.047	ICP-MS	Pace Analytical Services; Green Bay, WI
Se	0.049	0.163		
Cr	0.088	0.293		
Pb	0.026	0.087		
Total As	0.003	0.011	ICP-CRC-MS	Brooks Analytical Labs, Bothell, WA
Inorganic As	0.004	0.012		

* As, Cd, Cr, Pb, and Se biota detection limits are based on 1 g tissue. Cu, Mg, and Zn biota detection limits are based on 3 g tissue. Hg biota detection limit is based on 0.232 g tissue.
** LOQ values are calculated as 10/3 of LOD.

During analyses at each laboratory, blanks, spikes, duplicates, as well as standard reference materials (SRM) from the National Institute of Standards & Technology (NIST) and/or the National Research Council of Canada (NRC-C), were utilized to ensure unbiased and accurate measurements. Where possible, an SRM was chosen that was of similar tissue make up to the samples being analyzed (i.e. rice flour and apple leaves are plant tissue and were analyzed concurrently with the wild rice). The NIST SRM, 1568b (rice flour) possessed certified concentrations for Cd, Zn, Mg, Cu, and Se, and was used at all three labs in conjunction with analysis of wild rice.

BAL analyzed blank spikes, matrix spikes, and duplicate spikes for each sample series. They also used As (III) and As (V) in separate matrix spike runs. Rice flour (NIST 1568b TM/SP) was also analyzed by BAL as a standard reference material. BAL only analyzed wild and commercial rice for total As, inorganic arsenic, monomethylarsonic acid (MMAs), and dimethylarsinic acid (DMAs).

Additionally, NIST SRM 1515 (apple leaves) and TORT-3 (lobster hepatopancreas) from NRC-C were used at Pace. TORT-3, a SRM routinely used at Pace, possessed certified concentrations for

Pb, Cd, Cr, and Se. The SRM used at LSRI for total Hg testing included DORM-4 (dogfish shark tissue) from NRC-C, as it had an appropriate certified concentration for that particular element.

Spikes, duplicates, and SRMs were evaluated during each analytical run for each metal analyzed at Pace and LSRI. Ten percent of the samples were analyzed in duplicate and ten percent of the samples were spiked with known concentrations. One reagent blank was run per digestion set for testing of the metals (other than mercury) analyzed by LSRI and Pace.

Analysis of Cu, Mg, Total Hg, and Zn

At LSRI a 3 g portion of ground sample was digested in nitric acid and 30% hydrogen peroxide, diluted to a 100 mL volume, and heated in a HotBlock®. The digested sample was filtered prior to analysis. Individually, Cu, Mg, and Zn analyses were performed using the flame option on a Perkin Elmer PinAAcle 900T AAS .

Mercury was analyzed for total Hg using 0.2 – 0.3 g of homogenized tissue samples digested with concentrated sulfuric and nitric acids in a HotBlock®. Potassium permanganate and potassium persulfate were used to convert organic mercury to inorganic mercury and stannous chloride converted inorganic mercury to elemental mercury which is analyzed by cold vapor AAS (Lobring and Potter 1991).

Analysis of Pb, Cd, Cr and Se

A slightly different digestion procedure was used at Pace than that used by LSRI. A 1 g portion of ground sample was digested in nitric acid with subsequent additions of hydrochloric acid and hydrogen peroxide, diluted to a 50 mL volume with reagent water, and heated in a HotBlock®. The digested sample was filtered prior to analysis. Pb, Cd, and Cr were analyzed as a multi-elemental suite utilizing ICP-MS.

Analyses of Total Arsenic and Arsenic Species

BAL digested the wild rice and commercial rice samples in a HotBlock® using known mass of tissue placed into a microwave digestion vessel and then aliquots of concentrated hydrogen peroxide and nitric acid were added to the samples. All samples at BAL were digested in sealed vessels at elevated temperature and pressure. BAL analyzed only wild and commercial rice samples for total arsenic, inorganic arsenic, dimethylarsinic acid (DMAs), and monomethylarsenic acid (MMAs).

The digestion method involves converting the As(V) to As(III) in the samples themselves, as well as the matrix spike samples. Inorganic arsenic was reported as the sum of As(III) and As(V). After digestion, the arsenic speciation analysis was performed using an Ion Chromatography (IC) ICP-MS. The ICP-MS used in that analysis was also equipped with an interference removal technology, collision reaction cell, to ensure data accuracy by reduction of polyatomic interferences and high sensitivity of results (BAL 2019a).

Arsenic is an element prone to polyatomic interferences (BAL 2019a). Polyatomic interferences are triggered by polyatomic ions formed from precursors with numerous sources, e.g., the sample

matrix, preparation reagents, and plasma gases (May & Wiedmeyer 1998). Thus, advanced interference removal technology was employed during total As analysis. The samples were analyzed on a triple quadrupole-equipped ICP-MS (BAL 2019a). Each sample for arsenic speciation and quantitation was extracted on a HotBlock® with an aliquot of trifluoroacetic acid (TFA) solution. Arsenic species were chromatographically separated on an ion exchange column and then quantified using inductively coupled plasma collision reaction cell mass spectrometry (ICP-CRCMS). Two sets of laboratory control samples and matrix spikes were prepared during arsenic speciation extraction to monitor for any potential interference.

Data analysis

All metal concentrations from LSRI and Pace Lab were expressed on a wet-weight (w.w.) basis upon reporting to GLIFWC, and later converted to dry weight for comparison to BAL concentrations which were reported as dry weight results. The conversion formula used was $C_d = (C_w / P_s) \times 100$, where C_d represents dry weight concentration, C_w is the wet weight concentration, and P_s is percent solids. Percent solids, or P_s was calculated for each analyte analyzed by Pace and LSRI by subtracting the percent moisture from 100.

A simple substitution method was utilized for values that were below either the LOD or LOQ values when calculating summary statistics. Where values were below the LOD value for each particular metal analyzed, a value of zero was used in its place. When values were below the LOQ value for each particular metal analyzed, the substituted value used was that element's LOQ value divided by the square root of two. Division of the LOQ values by the square root of two (1.414) reduced the LOQ values by 29.3%.

Total and inorganic arsenic, as well as copper, were calculated using the simple substitution method. Only one sample analysis for each of these analytes resulted in a value less than the LOD. Pace originally analyzed the rice samples for total arsenic and measured values above the LOQ in four samples. As a result, the samples were sent to BAL for the more sensitive analytical measurements reported for total arsenic. BAL also analyzed for the analytes of organic arsenic, monomethylarsoinic acid and dimethylarsinic acid. The analysis of total arsenic by Pace was not used although in general agreement with the BAL results.

All values for magnesium and zinc were above LOQ values, so no substituted values were necessary to assess summary statistics for those elements. However, due to measured values of <LOD, descriptive statistics were unable to be generated for Pb, Cd, total Hg, total Cr, and Se for wild rice.

RESULTS

Moisture analysis

Wild rice moisture analysis results

Ten of the finished wild rice seed samples were analyzed for moisture content. Moisture analysis occurred immediately following rice homogenization at LSRI. Moisture values ranged from 6.1 to 11.7%, with a mean value of 7.8 ± 1.8 % moisture. The data obtained by drying and weighing

the samples twice indicates that drying for 24 hours remained sufficient in removing moisture from the samples. Eleven cooked wild rice samples were measured for moisture content. Mean and standard deviation of moisture content in cooked rice was 59.2 ± 7.3 %. The relative percent difference for the three samples measured in duplicate ranged from 0.2 to 0.7% (Polkinghorne et al. 2019a).

Maple syrup moisture analysis results

Maple syrup samples obtained from tribal harvesters had moisture values that ranged from 24.4 to 52.5% with a mean value of 32.5 ± 7.0 % moisture. Commercial obtained maple syrup samples had measured moisture of 27.3 ± 3.3 % moisture. Higher variability found in the tribal samples likely reflects the culinary preferences of the processors and/or intended recipients of the syrup (Table 5). The moisture value for the single maple sugar sample was 0.7%.

Wild turkey moisture analysis results

Percent moisture was measured in 16 wild turkey breast samples. Moisture values ranged from 69.0 to 73.4% with a mean value of 71.0 ± 1.1%. Moisture analysis took place immediately following homogenization of the turkey breasts. The data obtained by drying and weighing the samples twice indicates that drying for 24 hours is sufficient to remove the moisture from the samples. Two of the samples were analyzed in duplicate, yielding relative percent differences of 0.4 and 0.5%.

Metals Measured in Wild and Commercial Wild Rice

Copper, magnesium, and zinc are elements essential to plant growth and, consequently, were the most abundant metals quantified in processed wild rice seeds. Copper in finished wild rice ranged from 1.013 to 5.683 mg/kg d.w. with mean and standard deviation values of 2.299 ± 1.520 mg/kg d.w. in the 37 samples measured (Table 3). Magnesium in wild rice ranged from 712.6 to 1108.7 mg/kg d.w. with a mean of 923.5 ± 97.4 mg/kg d.w. Zinc values ranged from 18.98 to 70.72 mg/kg d.w. with a mean of 34.98 ± 3.30 mg/kg d.w. A Totagatic Lake (WI) sample possessed the highest zinc concentration.

Total mercury had two values greater than the LOQ of 0.050 mg/kg and they ranged from 0.061 to 0.064 mg/kg d.w., there were three samples with total mercury concentration greater than the LOD of 0.015 mg/kg but less than the LOQ. The remaining 31 samples had total mercury values less than the LOD.

Only three samples of commercial wild rice seeds were analyzed and they had higher concentrations of copper (mean of 4.013 ± 3.159 mg/kg d.w.), magnesium (1009.2 ± 14.57 mg/kg d.w.), and zinc (55.04 ± 2.53 mg/kg d.w.) than wild rice. All three samples of commercial wild rice had total mercury concentrations less than the LOQ of 0.050 mg/kg.

Pace analyzed the 40 samples of wild rice and commercial wild rice seeds for lead (Table 3), cadmium, chromium, and selenium (Table 4). Lead in wild rice seeds had two measured concentrations (0.063, 0.26) above the LOD of 0.026 mg/kg. Of the two samples, one sample was

less than the LOQ of 0.087 mg/kg and other sample had a lead concentration value of 0.26 mg/kg d.w. The remainder of the samples were less than the LOD. The lead sample with the highest lead concentration (Table 3) was collected from the Bad River Sloughs (sample 3A). A second sample was collected from Bad River Sloughs (sample 20A) and the lead concentration measured in that sample was less than the LOD. All commercial wild rice samples had lead concentrations less than the LOD.

Cadmium in wild rice seeds had three measured concentrations (0.021, 0.024, 0.079 mg/kg w.w.) greater than the LOD of 0.014 mg/kg and the remainder were less than the LOD (Table 4). Commercial wild rice seeds had cadmium concentrations measured above the LOD but less than the LOQ of 0.047 mg/kg in all three samples.

Chromium had two measured values for wild rice (0.094, 0.120 mg/kg w.w.) above the LOD of 0.088 mg/kg but less than the LOQ of 0.293 mg/kg (Table 4). Commercial wild rice had all chromium values below the LOD of 0.088 mg/kg. Of valence states of chromium compounds, hexavalent chromium, or Cr(VI), most negatively impacts human health (NIH 2018). The proportion of hexavalent chromium from total chromium in wild rice was not measured.

Selenium in wild rice seeds was not measured at concentrations above the LOD of 0.049 mg/kg in any of the 41 samples of wild and commercial rice (Table 4).

Pace also measured total arsenic in the wild and commercial rice samples (Table 4) and in general had good agreement with the BAL results (Table 3) for total arsenic concentrations. The wild and commercial rice samples prepared at LSRI were shipped to BAL for speciation of arsenic and the resulting concentrations of each arsenic analyte.

BAL measured arsenic as total, inorganic, dimethylarsenic acid (DMAs) and monomethylarsonic acid (MMAs) in the wild and commercial rice samples. DMAs had five measured values above the Method Detection Limit (MDL_1) of 0.005 mg/kg and the remainder were below the Method Reporting Limit (MRL_1) of 0.011 mg/kg. All measured values for MMAs were below the MDL of 0.005 mg/kg d.w. Inorganic arsenic was the major analyte in samples with measured concentrations above the MRL_1 of 0.012 mg/kg and represented 66.3 % of the arsenic measure in wild rice (Table 3). Inorganic arsenic represented all (101.7 %) the arsenic in commercial rice samples. Total arsenic in the 17 wild rice samples with measured concentrations above the LOQ ranged from 0.023 to 0.108 mg/kg d.w. with a mean of 0.047 ± 0.026 mg/kg d.w. Commercial rice samples had higher total arsenic concentrations than wild rice. The mean concentration in commercial rice was 0.114 ± 0.057 mg/kg d.w.

Table 3. Measured, wild rice elemental concentrations (dry weight) with water body source of sample.

Sample Number	Source Water Body Location	LSRI				Pace	BAL		
		Cu (mg/kg)	Mg (mg/kg)	Zn (mg/kg)	Total Hg (mg/kg)	Pb (mg/kg)	InOrg As (mg/kg)	Total As (mg/kg)	% of InOrg As of Total As
1A	Unknown	1.013*	992.4	32.65	<0.015**	<0.026**	0.038	0.064	59.8
2A	Mixed Source (WI)	1.013	1039.1	43.53	<0.015	<0.026	0.008*	0.008*	
3A	Bad River Sloughs (WI)	1.013	1031.5	28.09	<0.015	0.260	0.021	0.030	69.5
4A	Mixed Source (WI)	2.397	995.7	32.65	<0.015	<0.026	0.024	0.037	64.5
5A	Long Lake (WI)	1.608	974.4	33.65	<0.015	<0.026	0.008	0.008	
6A	Chippewa Lake (WI)	1.013	904.6	36.66	<0.015	<0.026	0.008	0.008	
7A	North Fork Flowage (WI)	2.028	968.5	40.13	<0.015	<0.026	0.038	0.055	68.8
8A	Phantom Lake Flowage (WI)	1.013	930.8	46.75	<0.015	<0.026	0.075	0.105	71.4
9A	Pacwawong Lake (WI)	2.798	984.8	31.89	<0.015	<0.026	0.008	0.008	
10A	Upper Rice Lake (MN)	2.061	1052.1	53.04	<0.015	<0.026	0.023	0.046	50.4
11A	Lake Minnewawa (MN)	1.013	938.2	27.66	<0.015	<0.026	0.016	0.027	59.5
12A	Mallard Lake (MN)	1.013	892.6	35.90	<0.015	<0.026	0.019	0.035	54.4
13A	Rice Lake (WI)	4.698	827.7	31.61	<0.015	<0.026	0.008	0.008	
14A	Totagitic Lake (WI)	1.822	836.2	70.72	<0.015	<0.026	0.008	0.008	
15A	Chippewa Lake (WI)	1.013	798.3	36.88	<0.015	<0.026	0.008	0.008	
16A	Totagitic Lake (WI)	3.415	869.6	40.30	<0.015	0.062	0.016	0.008	
17A	Ontonagon River (MI)	3.872	901.3	38.40	<0.015	<0.026	0.058	0.066	88
18A	Lee Lake (WI)	1.013	954.4	43.82	<0.015	<0.026	0.008	0.023	51.5
19A	Chippewa Lake (WI)	1.013	770.1	39.15	<0.015	<0.026	0.008	0.008	
20A	Bad River Sloughs (WI)	1.013	879.6	23.43	<0.015	<0.026	0.030	0.038	79.8

21A	Unknown	1.013	937.1	42.73	<0.015	<0.026	<0.004**	0.008	
22A	Unknown	1.013	953.4	18.98	<0.015	<0.026	0.021	0.025	85
23A	Unknown	1.013	772.2	43.71	<0.015	<0.026	0.008	0.008	
24A	Rice Lake (WI)	3.279	770.2	26.14	<0.015	<0.026	0.008	0.008	
25A	Rice Lake (WI)	2.657	805.9	32.10	<0.015	<0.026	0.017	0.028	59.9
26A	Totagitic Lake (WI)	5.456	874.2	52.93	<0.015	<0.026	0.008	0.008	
27A	Unknown	1.013	811.3	42.95	<0.015	<0.026	0.017	0.027	62.7
28A	CutAway Dam (WI)	3.330	972.3	33.52	<0.015	<0.026	0.017	0.008	
29A	Rice Lake (WI)	1.714	972.9	26.57	<0.015	<0.026	0.008	0.008	
30A	Brule River (MI)	3.364	1108.7	36.47	<0.015	<0.026	0.008	0.008	
31A	Rice Lake (WI)	5.683	712.6	33.62	0.035*	<0.026	0.008	0.008	
32A	unknown (MI)	4.913	826.5	43.38	0.061	<0.026	0.040	0.050	79.8
33A	Chippewa Lake (WI)	1.013	894.2	36.64	0.064	<0.026	0.008	0.008	
34A	Little Rice Lake (WI)	1.013	764.3	36.60	<0.015	<0.026	<0.004	0.008	
35A	Little Turtle Flowage (WI)	5.152	921.9	34.71	0.035	<0.026	0.021	0.028	75.3
40A	Deadfish Lake (MN)	3.796	1084.6	39.05	0.035	<0.026	0.050	0.108	46.3
41A	Unknown	3.796	854.7	37.31	0.035	<0.026	0.008	0.008	
	Mean	2.299	923.5	34.98	0.007***	0.009***	0.018	0.026	66.3
	Std. Dev.	1.520	97.4	3.30	0.017	0.045	0.016	0.026	12.4
Commercial Grown Wild Rice									
36A	Unknown (MN)	6.247	1019.5	53.25	0.035	<0.026	0.164	0.177	92.7
37A	Unknown (MN)	5.434	1022.8	59.54	0.035	<0.026	0.093	0.099	93.8
38A	Unknown (MN)	1.779	998.9	56.83	0.035	<0.026	0.144	0.066	*118.5***
	Mean	4.013	1009.2	55.04	0.035	<0.026	0.134	0.114	101.7
	Std. Dev.	3.159	14.6	2.53	0	0	0.037	0.057	14.6

* Value used when LOQ was reported (LOQ / square root of 2).
** Value used when LOD was reported (0 was used when calculating statistics).
*** Values were less than LOD values due to the use of 0 in place of the LOD.
**** Value is acceptable when the relative percent difference (RPD) is <20%.

Table 4. Concentrations (mg/kg) of four metals (wet weight) in wild and commercial rice seeds measured by Pace.

Wild Rice				
Sample ID	Cd	Cr	Se	As (Total)
1A	<0.014	<0.087	<0.050	0.071
2A	<0.013	<0.081	<0.046	<0.028
3A	<0.014	<0.087	<0.050	<0.030
4A	0.024	<0.088	<0.100	0.080
5A	<0.013	<0.080	<0.046	<0.027
6A	<0.014	<0.086	<0.049	<0.029
7A	<0.014	<0.087	<0.050	<0.056
8A	<0.013	<0.080	<0.046	0.093
9A	<0.014	<0.088	<0.051	<0.030
10A	<0.013	<0.079	<0.045	0.041
11A	<0.014	<0.088	<0.051	0.034
12A	<0.014	<0.086	<0.049	0.048
13A	<0.013	<0.082	<0.047	<0.028
14A	<0.014	<0.086	<0.049	<0.029
15A	<0.014	<0.088	<0.050	<0.030
16A	0.021	<0.079	<0.045	0.042
17A	<0.010	0.120	<0.047	0.078
18A	<0.011	<0.085	<0.049	<0.029
19A	<0.010	<0.081	<0.047	<0.028
20A	<0.010	<0.079	<0.045	0.033
21A	<0.010	<0.084	<0.048	<0.029
22A	<0.011	<0.088	<0.050	0.034
23A	<0.011	<0.086	<0.049	<0.029
24A	<0.011	<0.086	<0.049	<0.029
25A	<0.010	<0.080	<0.046	0.036
26A	0.079	<0.088	<0.051	0.130
27A	<0.010	0.094	<0.048	0.039
28A	<0.010	<0.083	<0.047	<0.028
29A	<0.010	<0.080	<0.046	<0.027
30A	<0.010	<0.082	<0.047	<0.028

31A	<0.014	<0.088	<0.051	<0.030
32A	<0.013	<0.084	<0.048	0.047
33A	<0.014	<0.085	<0.049	<0.029
34A	<0.014	<0.087	<0.050	<0.030
35A	<0.014	<0.088	<0.050	0.071
40A	<0.014	<0.086	<0.049	0.056
41A	<0.013	<0.082	<0.047	<0.028
Commercial Rice				
36A	0.040	<0.084	<0.048	0.180
37A	0.031	<0.083	<0.048	0.097
38A	0.033	<0.083	<0.046	0.120

Lead measured in maple syrup and maple sugar

Lead concentrations were measured in 29 samples of maple syrup harvested by tribal members in the ceded territories (Table 5). Only one sample had a lead concentration measured above the LOQ of 0.087 mg/kg. The lead concentration in the sample was 0.27 mg/kg and the harvester identified the equipment used as "old" or "inherited" metal equipment. The single maple sugar sample had a lead concentration less than the LOD of 0.026 mg/kg. Commercial maple syrup samples were analyzed for lead content but none had lead concentrations measured above the LOD (Polkinghorne et al. 2019b).

Table 5. Lead concentrations and moisture content in maple syrup and maple sugar. Sample number, harvest year, state and county of harvest, and the equipment used by harvester or processor. LOD and LOQ values for lead were 0.026 mg/kg and 0.087 mg/kg, respectively.

Sample #	Year of Harvest	State	County	Pb Levels (mg/kg d.w.)	Moisture Content (%)	Usage of Old and/or Inherited Metal Equipment
5A	2018	WI	Bayfield	<0.026*	29.8	No
7A	2019	WI	Bayfield	<0.026	31.9	No
8A	2019	WI	Bayfield	<0.026	29	No
13A	2019	WI	Bayfield	<0.026	37.2	No
24A	2019	WI	Bayfield	<0.062**	26.4	Yes
6A	2019	WI	Sawyer	<0.026	28.4	No
12A	2019	WI	Bayfield	<0.026	45.65	No
14A	2019	WI	Sawyer	<0.026	27.1	Yes
15A	2019	WI	Sawyer	<0.026	32.9	No
33A	2019	WI	—	<0.026	24.6	—
9A	2019	WI	Burnett	0.27	28.9	Yes

10A	2019	WI	Ashland	0.062**	52.5	Yes
11A	2019	WI	Ashland	<0.026	31.9	Yes
16A	2019	WI	Ashland	<0.026	27.2	Yes
20A	—		—	<0.026	26.9	—
34A	2019	WI	Ashland	<0.026	40.3	No
17A	2019	MN	Carlton	<0.026	37.3	No
18A	2019	MN	Carlton	<0.026	30.9	No
19A	2019	Iron	WI	<0.026	31.7	No
27A	2018	Vilas	WI	<0.026	42.2	No
28A	2019	Vilas	WI	<0.026	24.4	Yes
21A	2019	WI	WI	<0.026	27.8	No
22A	2019	WI	WI	<0.026	34	No
32A	2019	WI	WI	<0.026	28.2	Yes
25A	2019	MI	Baraga	<0.026	29.1	No
25C***	2019	MI	Baraga	<0.026	0.7	No
26A	2019	MI	Baraga	<0.026	27.1	No
29A	2019	MN	Mille Lacs	<0.026	36.3	Yes
30A	2019	MN	Mille Lacs	<0.026	46	Yes
31A	2019	MI	Chippewa	0.062**	27.4	No
Commercial Maple Syrup						
1A	—	—	WI	<0.026	22.5	—
2A	—	—	MI	<0.026	28.5	—
4A	—	—	WI	<0.026	28.6	—
23A	2019	—	WI	<0.026	29.7	—

— indicates missing and/or unknown information.
* LOD value.
** LOQ value.
*** Sample 25C is a corresponding sugar sample with 25A.

Lead concentrations measured in wild turkey breasts

Sixty wild turkey breast samples from 30 birds were analyzed for lead concentrations. Only twelve of the breasts had lead concentrations measured above the LOD of 0.026 mg/kg. Ten of the breasts had concentrations between the LOD and LOQ of 0.087 mg/kg. Two breasts had high lead concentrations of 15.9 and 16.2 mg/kg d.w. Wild turkey sample number 10394 had been shot with a high power rifle using a single bullet and did not have a lead concentration above the LOD. Ten breast samples had pellets or metal fragments found in the tissue. Five of the breasts containing metal pellets

or fragments did not have lead concentrations measured above the LOD indicating that all pellets were removed or did not contain lead. Six breast samples had measured lead concentrations without detected pellets or fragments.

A small percentage (8.3 %) of total breast samples that did not have pellets/fragments detected had measured lead values above the LOD value of 0.026 mg/kg d.w. It is possible that x-ray analysis did not identify microscopic fragmentation too small for observation. Wild turkeys are omnivorous, but primarily eat vegetation and are, therefore, unlikely to ingest lead in their diets.

Table 6. Lead concentrations in wild turkey breast samples grouped by size of shot used for harvest with sample identification number, county and state of harvest, total length of bird, and shotgun used with shot size of ammunition.

Sample Number w/turkey breast side (left/right)	County of Harvest	State of Harvest	Bird* Length (in.)	Gun	Shot Size	Shot Type	# of shot fragments noted & shot/bullet fragmentation notes	Lead Concentrations (mg/kg d.w.)
Samples harvested with copper plated lead shot								
10334L	Burnett	WI	32	20 gauge	5 shot	copper plated lead	0	<0.026
10334R	Burnett	WI	32	20 gauge	5 shot	copper plated lead	0	<0.026
10398L	Burnett	WI	30	20 gauge	5 shot	copper plated lead	0	<0.026
10398R	Burnett	WI	30	20 gauge	5 shot	copper plated lead	0	<0.026
10399L	Ashland	WI	26	20 gauge	5 shot	copper plated lead	0	<0.026
10399R	Ashland	WI	26	20 gauge	5 shot	copper plated lead	0	<0.026
10400L	Ashland	WI	31	Unknown	5 shot	copper plated lead	0	<0.026

10400R	Ashland	WI	31	Unknown	5 shot	copper plated lead	0	<0.026
Lead Level by reported shot size								
223 Caliber								
10394L**	Ashland	WI	---	223 rifle	Unknown	Unknown	1 lg. gray fragment and small fragments	<0.026
10394R**	Ashland	WI	---	223 rifle	Unknown	Unknown	small microscopic fragments	<0.026
00 Buck								
10336L	Ontonagon	MI	33	12 gauge	00 Buck	Unknown	0	<0.026
10336R	Ontonagon	MI	33	12 gauge	00 Buck	Unknown	0	<0.026
10337L	Ontonagon	MI	32	12 gauge	00 Buck	Unknown	1 large pellet found (nonmagnetic & gray)	<0.026
10337R	Ontonagon	MI	32	12 gauge	00 Buck	Unknown	0	0.100
#4 shot								
10388L	Iron	MI	34	12 gauge	4 shot	Unknown	0	<0.026
10388R	Iron	MI	34	12 gauge	4 shot	Unknown	0	<0.026
10389L	Iron	MI	32	12 gauge	4 shot	Unknown	0	<0.026
10389R	Iron	MI	32	12 gauge	4 shot	Unknown	0	<0.026
#5 shot								
10338L	Gogebic	MI	34	12 gauge	5 shot	Unknown	0	<0.026
10338R	Gogebic	MI	34	12 gauge	5 shot	Unknown	0	<0.026
10339L	Gogebic	MI	29	12 gauge	5 shot	Unknown	0	<0.026
10339R	Gogebic	MI	29	12 gauge	5 shot	Unknown	0	<0.026
10340L	Gogebic	MI	33	12 gauge	5 shot	Unknown	0	<0.026
10340R	Gogebic	MI	33	12 gauge	5 shot	Unknown	0	<0.026
10341L	Gogebic	MI	35	12 gauge	5 shot	Unknown	1 small fragment found	<0.026
10341R	Gogebic	MI	35	12 gauge	5 shot	Unknown	0	<0.026

10342L	Gogebic	MI	31	12 gauge	5 shot	Unknown	0	<0.026
10342R	Gogebic	MI	31	12 gauge	5 shot	Unknown	0	<0.026
10343L	Gogebic	MI	32	12 gauge	5 shot	Unknown	0	<0.026
10343R	Gogebic	MI	32	12 gauge	5 shot	Unknown	0	<0.026
10387L	Bayfield	WI	36	Unknown	5 shot	Unknown	2 large pellets found (nonmagnetic)	0.110[J]
10387R	Bayfield	WI	36	Unknown	5 shot	Unknown	0	<0.026
10390L	Gogebic	MI	36	Unknown	5 shot	Unknown	microscopic fragmentation found	1.41
10390R	Gogebic	MI	36	Unknown	5 shot	Unknown	0	<0.026
10393L	Gogebic	MI	36	Unknown	5 shot	Unknown	0	<0.026
10393R	Gogebic	MI	36	Unknown	5 shot	Unknown	0	<0.026
10395L	Bayfield	WI	40	Unknown	5 shot	Unknown	0	<0.026
10395R	Bayfield	Wi	40	Unknown	5 shot	Unknown	0	<0.026
#6 shot								
10333L	Ashland	WI	34	20 gauge	6 shot	Unknown	3 large pellets found (all nonmagnetic & gray) and small fragments found	2.83
10333R	Ashland	WI	34	20 gauge	6 shot	Unknown	1 large pellet (nonmagnetic & gray)	0.260[J]
#8 shot								
10344L	Ashland	WI	32	Unknown	8 shot	Unknown	0	0.45
10344R	Ashland	WI	32	Unknown	8 shot	Unknown	0	<0.026
10391L	Ashland	Wi	33	Unknown	8 shot	Unknown	0	<0.026
10391R	Ashland	WI	33	Unknown	8 shot	Unknown	0	<0.026
10392L	Ashland	WI	34	Unknown	8 shot	Unknown	0	1.00
10392R	Ashland	WI	34	Unknown	8 shot	Unknown	0	<0.026
Unknown Shot Size								
10331L	Gogebic	MI	33	Unknown	Unknown	Unknown	microscopic fragments found on x-ray	16.2
10331R	Gogebic	MI	33	Unknown	Unknown	Unknown	0	<0.026

10332L	Gogebic	MI	22	Unknown	Unknown	Unknown	0	0.380
10332R	Gogebic	MI	22	Unknown	Unknown	Unknown	0	0.240[J]
10335L	Vilas	WI	36	Unknown	Unknown	Unknown	0	<0.026
10335R	Vilas	WI	36	Unknown	Unknown	Unknown	0	<0.026
10345L	Ashland	WI	37	12 gauge	Unknown	Unknown	0	<0.026
10345R	Ashland	WI	37	12 gauge	Unknown	Unknown	0	<0.026
10346L	Ashland	WI	37	12 gauge	Unknown	Unknown	0	<0.026
10346R	Ashland	WI	37	12 gauge	Unknown	Unknown	0	<0.026
10396L	Ashland	WI	38	Unknown	Unknown	Unknown	0	<0.026
10396R	Ashland	WI	38	Unknown	Unknown	Unknown	0	<0.026
10397L	Ashland	WI	37	Unknown	Unknown	Unknown	0	15.9
10397R	Ashland	WI	37	Unknown	Unknown	Unknown	2 large fragments	2.38

* Bird length measured from tip of beak to end of tail.
** Killed with a centerfire 223 rifle
[J] Value between the LOD and LOQ

The current study examined birds that were harvested as meat intended for human consumption. This investigation also operated under the assumption that most birds were harvested using lead-containing ammunition, or some combination of metals including lead, due to the lower cost and high commercial availability of lead-based ammunition. According to Winchester Ammunition (2017), the best shot size to use for wild turkey hunting is either No. 4, No. 5, or No. 6 (Fig. 4). All wild turkeys acquired for this study were harvested with a firearm. Unfortunately, archery (bow or crossbow) harvested samples were unable to be obtained from tribal harvesters as a control group. Although turkey hunters typically aim for the bird's head, other factors, such as gun gauge, shot size, metallic composition of ammunition, distance, and shooting skill, all ultimately influence shot placement. Gathering birds from different hunters helped reflect this variability. However, turkey hunting remains either a niche or opportunistic harvest for tribal hunters, so the overall number of hunters obtaining this particular avian species was expected to be relatively low.

From the limited firearms data gathered from Ojibwe harvesters, shot size 5 appeared to be the most common shot size employed, although shot sizes 4, 6, and 8, as well as double-0 buckshot were also reportedly used. Not all firearms information was able to be collected from the hunter. In addition, not all tribal harvesters were able to provide reliable data regarding firearm caliber and/or shot size used. All pellets/fragments were tested for magnetism and all were nonmagnetic, indicating that the shot was composed primarily of either lead, iron, tungsten, or bismuth alloy. Color of pellet/fragments was documented for further inquiry (Mann et al. 1994). An observation was made of the data ranked by shot size (Table 6) that the smaller the shot size there was a tendency for more samples to contain a measurable lead concentration. The speculation is that the x-ray analysis did not identify the smaller shot and it remained in the tissue during lead analysis.

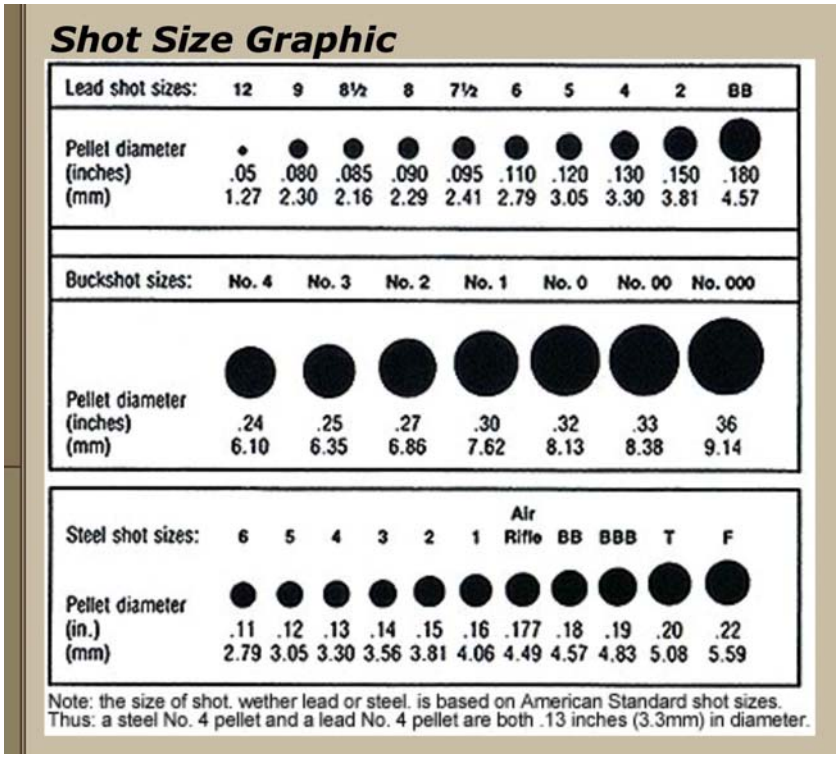

Figure 4. Description of shot size used in shotgun ammunition.

Lead percentages in the shot pellets or fragments that dissolved during digestion ranged from less than 1% to 93%. The greater the lead percentages from the dissolved pellets/fragments after digestion indicate that those samples were predominately composed of lead, not another metal. Furthermore, the possibility remains that pellets collected from some wild turkey breasts came from more than one firearm.

Ten wild turkeys had metal embedded in one or both of their pectoral muscles. Metal from five of the breast samples were analyzed for lead. Two breast samples had two shot pellets analyzed and one had three pellets analyzed for lead concentration. Three other breast samples had single pellets/fragments analyzed. Not all metal in pellets or fragments dissolved during digestion. Remaining metallic material was weighed, and resulting solutions were analyzed by flame AAS for lead content. Lead concentrations in solutions varied widely from <1 to 93 percent of weight (Table 7). Pellet color was also noted for comparison with lead concentrations. Color did not correlate well with lead concentrations. Gray color is the color of lead and the highest lead concentrations were in gray colored pellets/fragments. Copper color was the color of the lowest lead concentration in pellets/fragments. However, four of the gray colored pellets/fragments had intermediate concentrations of lead that matched two of the copper colored pellets/fragments. Turkey number 10394L was harvested with a rifle and the gray colored fragments were likely the lead portion of the projectile. The likelihood is that much of the shot used to kill wild turkeys was not pure lead rather a mixture of metals such as copper, bismuth, tungsten, or iron.

Table 7. Lead analysis of shot pellets found in wild turkey breasts.

Sample ID	Initial Sample Weight (mg)	Final Sample Weight (mg)	Weight of Portion Dissolved (mg)	Pb Conc. in Solution* (mg/L)	Lead Dissolved (mg)	% Lead in Portion of Shot That Dissolved	Qualitative Pellet / Fragment Color
10333L-1	124	45	79	1450	72.5	92	Gray
10333L-2	133	61	72	590	29.5	41	Gray
10333L-3	128	50	78	720	36	46	Gray
10333R	124	54	70	460	23	33	Gray
10337L	227	122	105	720	36	34	Gray
10387L-1	150	70	80	580	29	36	Copper
10387L-2	158	91	67	430	21.5	32	Copper
10394L	67	14	53	990	49.5	93	Gray
10397R-1	21	0	21	0.2	0.01	<1	Copper
10397R-2	11	0	11	7.7	0.39	4	Copper

* Solution was 20 mL in volume; therefore the concentration was divided by 20 to achieve the dissolved lead concentration.

Discussion

GLIFWC has undertaken this project to comply with the FDA food safety requirements that incorporate Hazard Analysis and Critical Control Point (HACCP) to determine if certain tribal wild harvested foods are safe for child nutrition under the National School Lunch Program (NSLP) and sale of these foods to the public. HACCP guidance is concerned with points during food acquisition and processing that identify potential biological, chemical and physical risks that may contribute to unsafe food.

National and International Health Standards

The United States Food & Drug Administration (U.S. FDA), and other health organizations worldwide, have established Maximum Residue Limit (MRL_2) to halt the flow of excessively contaminated foods from entering the human food chain. According to Australia's Agriculture Victoria (2019), a MRL_2 is "the maximum concentration of a chemical residue that is legally permitted to be present in food…" Any concentration above a MRL_2 is deemed unacceptable by the responsible regulatory agency. The use of a Canadian MRL_2 are utilized in this document, where a U.S. MRL_2 is not available, as they are considered comparable to U.S. standards. Also, commerce occurs regularly between these countries.

The U.S. Environmental Protection Agency (U.S. EPA) established health standards in foods, and other modes of ingestion exposure, as oral Reference Doses (RfD) to protect human health. A

RfD, according to the U.S. EPA (2018), is "an estimate … of a daily oral exposure to the human population (including sensitive subgroups) … likely to be without an appreciable risk of deleterious effects during a lifetime." The RfD is useful in gauging potential toxicity of foods (among other routes of intake) from chemicals, heavy metals, and other elements, as in the case of cooked wild rice (U.S. EPA 2005; 1991).

Table 8: MRL_2 values from various health organizations for rice, rice products and maple syrup.

Arsenic (Inorganic)*	
United States	
0.1 ppm	Infant rice cereal (draft guidance)
European Union	
0.1 ppm	Rice destined for the production of food for infants & young children
0.25 ppm	Parboiled & husked rice
0.3 ppm	Rice waffles, rice wafers, rice crackers, & rice cakes
World Health Organization	
0.35 ppm	Hulled rice
Lead	
.011 ppm	Maple syrup State of California labeling standard
.500 ppm	Maple syrup Canadian standard
≥.500 ppm	Maple Syrup is considered adulterated 3715.59 of the Revised Ohio Food Code
European Union	
0.25 ppm	Legumes & pulses (non-rice food staples)
Cadmium	
World Health Organization	
0.4 ppm	Polished rice
Copper	
United States	
10 ppm	Rice (African rice, Nerica hybrids, & Indian/wild rice)
*Total As, Zn, Mg, Cr, and Se do not have comparable MRL_2 values for rice, and are therefore not included in this table. No total Hg MRL_2 exists, but the MRL_2 for methylmercury in rice (*Oryza spp.*) and wild rice is 0.01 ppm. However, no published information exists regarding the proportion of methylmercury from total Hg in wild rice.	

Wild Rice

Arsenic concentration in naturally occurring wild rice is one of the primary concerns of this investigation. Arsenic is an element that occurs in the environment from both natural and man-made sources. Arsenic is not an essential element for plant or human growth. Inorganic arsenic (As III) is considered more toxic to humans than organic arsenic (As V) according to U.S. FDA (2016a). Rice (*Oryza sativa*) and rice-based products tend to have higher levels of inorganic arsenic than do other cereal crops such as wheat and barley. Though wild rice (*Zizania palustris*) is not a true rice but an

aquatic grass, it is commonly served as a rice and little information about the concentration of inorganic arsenic wild rice is available. The U.S. FDA has set action level of 0.1 mg/kg in rice.

Table 9. Comparison of metal concentrations (mg/kg d.w.) in wild rice and processed wild rice from four investigations.

	Cu Mean ± Std. Dev. (n=)	Mg Mean ± Std. Dev. (n=)	Zn Mean ± Std. Dev. (n=)	T. Hg Mean ± Std. Dev. (n=)	Pb Mean ± Std. Dev. (n=)	T. As Mean ± Std. Dev. (n=)	Cd Mean ± Std. Dev. (n=)	Cr Mean ± Std. Dev. (n=)	Se Mean ± Std. Dev. (n=)
Nriagu and Lin 1995 (Processed) seed)	8.7 ±0.32 (26)	--	23.2 ±0.78 (26)	--	0.042 ±2.58 (26)	0.066 ±3.89 (26)	0.053 ±1.48 (26)	–	--
Bennett et al. 2000 (unprocessed seed w/hulls)	5.27 (29)	1175 (44)	43.9 (44)	0.035 (7)	0.96 (32)	0.11 (4)	0.021 (24)	0.49 (3)	0.21 (3)
Brooke et al. 2004* (unprocessed seed)	1.65 ±0.61 (36)	879.4 ±107.7 (36)	22.4 ±5.91 (36)	0.002** ±0.002 (32)	0.063 ±0.033 (36)	<0.054** ±0.004 (36)	<0.163** ±<0.002 (36)	0.403 ±0.124 (36)	<0.058** ±0 (36)
This Study (processed seed-Tribal)	2.30 ±1.52 (37)	923.5 ±97.4 (37)	34.98 ±3.30 (37)	0.007** ±0.017 (37)	0.009** ±0.045 (37)	0.026 ±0.026 (37)	0.003** ±0.014 (37)	0.006** ±0.025 (37)	<0.049** ±0 (37)
This Study (Commercial grown & processed seed)*	4.013 ±3.159 (3)	1009.2 ±14.6 (3)	55.04 ±2.53 (3)	0.035 ±0.017 (3)	<0.026 ±0 (3)	0.114 ±0.057 (3)	0.035 ±0.005 (3)	<0.084** ±0 (3)	<0.048** ±0 (3)

* Value used when LOQ was reported = (LOQ / square root of 2).
** When an LOD was reported, 0 was used when calculating statistics.

In 1997 and 1998, GLIFWC, in cooperation with the University of Wisconsin–Madison, collected whole wild rice plants from northern Wisconsin to measure several heavy metal elements in roots, stems, leaves, and seeds. This study identified the location of these metals within the plants (Bennett et al. 2000). Wild rice roots contained the highest concentrations of arsenic, cadmium, chromium, lead and selenium while rice seeds (included hulls) contained the highest concentrations of copper and zinc. Stems and leaves had concentrations intermediate to the roots and seeds of these elements. Mercury and magnesium were evenly distributed in the plants.

In 2004, GLIFWC collected wild rice plants from eight water bodies near potential metal mining sites. The roots and seeds (included hulls) were removed and analyzed for metals. Samples were collected and processed according to a U.S. EPA-approved quality assurance project plan. The results of that study were reported to the U.S. EPA Region V Environmental Justice Department (Brooke et al., 2004). Both of these GLIFWC affiliated research studies examined unprocessed wild rice seeds, with the encasing hull. They agreed in rank order of concentrations of the nine measured metals; however, the 1998 study reported higher concentrations of each metal. In slight contrast, this current study evaluated elemental concentrations in processed (without hulls and beards) wild rice seeds.

Another study (Nriagu and Lin 1995) of wild rice measured metal concentrations in finished (processed) wild rice and long-grain commercial brown or white rice. The rice samples (n=26) were purchased from retail stores with labeling naming sources as from Minnesota, California, and Saskatchewan, Canada. Some of the samples were pure wild rice and some were mixtures of wild rice and commercial brown and white rice. Concentration of six metals (cadmium, lead, total arsenic copper zinc and iron) were measured.

Inorganic arsenic was an average of 66.7% of the total arsenic in the three paddy wild rice seed samples tested in the U.S. FDA (2013) rice and rice product study. Arsenic has been found in white rice grown in the U.S. (Lamont 2003). In our study, where arsenic was detected in concentrations near or above the LOQ value, the average proportion of inorganic arsenic in total arsenic was calculated at 66.3% in tribal harvested rice and 101.7% in the three commercial wild rice samples (Table 3). This study agrees with the U.S. FDA study that indicates when arsenic is present in wild rice, inorganic arsenic is the most dominant species.

The current study of 37 measured samples of tribal harvested wild rice had a mean and standard deviation of inorganic arsenic of 0.018 ± 0.016 mg/kg d.w. Total arsenic had a mean and standard deviation of 0.026 ± 0.026 mg/kg d.w. in the same study. The earlier studies by Bennett et al. (2000), Brooke et al. (2004), and Nriagu and Lin (1995) found total arsenic seed concentrations of 0.11, 0.054 and 0.066 mg/kg d.w., respectively (Table 8). The U.S. FDA has set a MRL_2 for rice and rice products of 0.10 mg/kg. Australia has determined a limit of 1.0 mg/kg arsenic in cereals.

Table 10. Comparison of tribal and commercial harvested wild rice samples for total and inorganic arsenic content.

Inorganic Arsenic Concentrations (mg/kg d.w.)		
	Tribal Harvested Wild Rice (n=37)	*Commercial Wild Rice (n=3)*
Mean	0.018	0.134
Standard Deviation	0.016	0.037
Maximum	0.075	0.164
Minimum	0.000	0.093
Total Arsenic Concentrations (mg/kg d.w.)		

	Tribal Harvested Wild Rice (n=37)	*Commercial Wild Rice (n=3)*
Mean	0.026	0.144
Standard Deviation	0.026	0.057
Maximum	0.108	0.177
Minimum	0.000	0.066

Mean inorganic arsenic concentrations in tribal wild rice are considerably (87%) lower when compared to mean inorganic arsenic commercial wild rice concentrations (Table 9). The mean total arsenic concentration (0.026 mg/kg d.w.) in tribal wild rice is only 18% of the concentration in commercial rice of 0.144 mg/kg d.w. It must be cautioned that the data for commercial wild rice is derived from only three samples. Ingestion of cooked tribal wild rice or commercial wild rice is not likely to impact human health due to the volume of rice needed to be consumed to exceed safe limits (Table 10).

However, arsenic compounds present in the cooking water, and the acidity of the recipe used when cooking, among other unknown factors, would also likely impact the final arsenic content of cooked wild rice, and subsequent dietary arsenic intake. Additionally, while rinsing and cooking other rice varieties in excess water may potentially lower inorganic arsenic content in wild rice (Raab et al. 2009), this cooking method would likely not be necessary based upon inorganic arsenic results of tribal obtained wild rice.

Table 11. Consumption of wild and commercial harvested rice needed to exceed the adult oral RFD (U.S. EPA 1991; 2005).

Oral Reference Doses (RfD) for Cooked Wild Rice*			
Element	*RfD (mg/kg-day)*	*Consumption/day (lbs.)*	*Consumption/day (oz.)*
Inorganic Arsenic (As) Tribal Harvest	0.0003	4.12	65.8
Inorganic Arsenic (As) Commercial Harvest	0.0003	0.25	4.01
Cadmium (Cd)**	0.001	3.13	50.0
Zinc (Zn)	0.03	0.13	2.12
Magnesium (Mg)	11	1.84	29.41

* Maximum daily consumption was derived using a body weight of 70 kg (~154 lbs.) and a cooked moisture value of 60% for wild rice. Consumption/day = [(RfD mg/kg/day X Body weight kg) / (Mean element concentration mg/kg)] X 1.60 (percent moisture).

** Highest value measured above the LOD of 0.079 mg/kg was used to calculate consumption/day.

Wild rice has been documented to contain lead in unhulled seeds (Pip 1993; Bennett et al. 2000, Brooke et al. 2004) and processed wild rice (Nriagu and Lin 1995). The Bennett et al. (2000) study

found a mean lead concentration in unprocessed wild rice seeds with attached hulls at 0.94 mg/kg d.w. Brooke et. al. (2004) found the mean lead concentration in unhulled wild rice seeds of 0.063 mg/kg d.w., and Nriagu and Lin (1995) measured a mean lead concentration of 0.042 mg/kg d.w. The European Union (E.U.) has set a lead MRL_2 of 0.25 mg/kg for legumes, but does not have one established for rice. The U.S. FDA has not set a lead action limit for either rice or legumes. The U.S. EPA (1988) has not determined a RfD for lead or its inorganic compounds because as a known neurotoxin at extremely low blood levels, particularly in children, it was deemed inappropriate to establish one.

However, the highest lead value from this study is from the Bad River Slough of 0.26 mg/kg d.w. and is between the average lead concentrations in unprocessed wild rice seeds with attached hulls from the two previous northern Wisconsin studies (Bennet et al. 2000, Brooke et.al. 2004). A second sample from the Bad River Slough had no detectable lead concentration indicating possible processing contamination. The only detectable lead value found in this study remained comparable to the E.U.'s legume action limit of 0.25 ppm. However, legumes are not taxonomically related to rice, but provided the only comparable action level from either national or international health organizations. The U.S. EPA (1988) decided it was not appropriate to establish a RfD, so cooked wild rice information could not be calculated for this heavy metal.

Magnesium is an essential element for plant and animal growth (Cakmak 2013) and these results indicate that natural wild rice is also a rich source of dietary magnesium. Bennet et al. (2000) measured a mean concentration in unhulled wild rice seeds of 1152 mg/kg d.w. and Brooke et al. (2004) measured a mean concentration of 879.4 mg/kg d.w. In the current study, magnesium values ranged from 712.58 to 1,108.72 mg/kg d.w. with a mean of 923.5 mg/kg d.w. An adult would have to eat 1.84 lbs. of cooked wild rice per day in order to exceed the magnesium RfD (Table 10).

Copper is another essential element for plant and animal growth. Bennett et al. (2000) found an average copper concentration of 5.27 mg/kg d.w. in unfinished wild rice seeds, Brooke et al. (2004) measured a mean concentration of 1.65 mg/kg d.w. in unfinished wild rice. Copper concentrations in finished tribal wild rice seeds in this study ranged from 1.013 to 5.68 mg/kg d.w. with an average of 2.23 ± 1.52 mg/kg d.w. and Nriagu and Lin (1995) measured copper concentrations at a mean value of 8.7 mg/kg (Table 8). All were below the 10 mg/kg copper action level for rice established by the U.S. FDA. Commercial wild rice in the current study had a 42.7% higher mean copper concentration than tribal harvested wild rice with a mean concentration of 4.0 mg/kg d.w. (Table 3).

Six of the 40 analyzed wild rice samples had cadmium values that were greater than the LOD value of 0.014 mg/kg, but all of those concentrations were less than the LOQ value of 0.047 mg/kg. The cadmium values are comparable (Table 8) to average unhulled wild rice seed findings from northern Wisconsin of 0.021 mg/kg d.w. (Bennett et al. 2000) and <0.163 mg/kg d.w. (Brooke et al. 2004). Nriagu and Lin (1995) found a mean concentration of 0.053 mg/kg d.w. in 26 samples of wild rice and mixed wild rice with brown and white rice. A typical adult would have to ingest over 3.13 lbs. of cooked wild rice per day to exceed the cadmium RfD (Table 10).

Selenium is an essential element for plant and animal growth. Concentrations in all wild rice samples remained less than the LOD value of 0.049 mg/kg in this study. Bennett et al. (2000) found an

average concentration of 0.21 mg/kg d.w. in unfinished wild rice seeds, and Brooke et al. (2004) measured selenium in unhulled wild rice seeds of <0.058 mg/kg d.w. There is no action level or RfD for selenium.

Zinc is essential for plant and animal growth. This study shows that natural wild rice is a rich source of dietary zinc. Bennett et al. (2000) measured a mean value of 43.9 mg/kg d.w. for unhulled wild rice, Brooke et al. (2004) measured a mean value of 22.4 mg/kg d.w. for unhulled wild rice, and Nraigu and Lin (1995) measured a mean of 23.2 mg/kg d.w. in wild rice and mixed wild, brown, and white rice (Table 8). A mean concentration for zinc of 34.98 mg/kg d.w. for unfinished wild rice and 55.04 mg/kg d.w. for finished wild rice was measured in this study (Table 3). Some concern exists regarding elevated zinc concentrations in finished wild rice may be introduced during processing. The parching process did not influence zinc concentrations as the levels are fairly comparable between parched (processed) and unparched (unprocessed) samples demonstrated from this study and the earlier studies (Table 8). Zinc safe concentrations have not been determined by U.S. FDA, but a RfD has been established by U.S. EPA of 0.3 mg/kg/day. However, a typical adult would have to ingest 0.13 pounds of cooked wild rice per day to exceed the RfD for zinc (Table 10).

Chromium compounds exist in either of two valence states (III and VI). Chromium (III) is an essential element for plant and animal life. Hexavalent chromium, or Cr (VI), most negatively impacts human health (National Institute of Health 2018). Bennet et al. (2000) and Brooke et al. (2004) reported mean chromium concentrations of 0.49 and 0.40 mg/kg d.w., respectively. This study did not differentiate the valence state for chromium. Only two wild rice samples were above the LOD value of 0.088 mg/kg total chromium, yet both values were less than its LOQ value of 0.293 mg/kg. The proportion of hexavalent chromium to total chromium in wild rice is not presently known. An MRL_2 for chromium has not been established.

Wild rice seed samples were analyzed for total mercury concentrations. Mercury is a neurotoxin especially when it is in the organic methylmercury form. Measurement of methylmercury is a difficult measurement and not usually done. In the current study, a majority of the total mercury values remained below the LOD value of 0.015 mg/kg. Four samples had mercury concentrations between the LOD the LOQ value of 0.050 mg/kg. Three samples had measured total Hg concentrations at or above the LOQ value. The highest total mercury concentration of 0.064 mg/kg d.w. came from Chippewa Lake in Wisconsin. The other mercury value above the LOQ value came from an unknown site in Michigan with a concentration of 0.061 mg/kg d.w. Deadfish Lake, Minnesota, had a concentration of 0.035 mg/kg d.w. which was at the LOQ value for mercury.

Mercury concentrations in the current study and in previous studies were similar. Bennett et al. (2000) measured a mean concentration of 0.035 mg/kg d.w. in eight water bodies which equalled the concentration of total mercury in the current study for commercial wild rice (Table 8). Brooke et al. (2004) measured a mean concentration of 0.002 mg/kg d.w. in wild rice seeds from eight different water bodies which is similar to the mean mercury value measured in this study of 0.007 mg/kg d.w. The samples for the previous studies were unfinished (not parched) and the current study wild rice seeds were finished (parched) showing that the parching process does not significantly reduce the total mercury content of the seeds. The previous studies examining dried

wild rice seeds, including hulls, from northern Wisconsin that possessed similar average total mercury concentrations to the Deadfish Lake sample at 0.035 mg/kg d.w. and lower concentrations than the other two samples from Chippewa Lake and Iron County, Michigan samples. The proportion of methylmercury in total Hg of wild rice is not presently known.

Maple Syrup

Maple syrup is a traditional, Ojibwe sweetener made by evaporating sap from tapped *maple* trees, usually from sugar maples (*Acer saccharum*), due to the high sucrose concentration in the sap (Corbiere 2011). Lead contamination of maple syrup is a concern and occurs predominantly from the use of lead-bearing processing equipment. Lead does not come from the sap itself (Ontario Ministry of Agriculture, Food, and Rural Affairs 2019). Under suitable conditions of acidity and temperature, lead mobilizes into sap when in contact with leadbearing materials (Willits and Tressler 1937). Hence, concern exists that maple syrup may be contaminated with lead from old, inherited metal equipment used in processing and/or from lead containing equipment present in nonfood grade equipment used by tribal members (Stilwell and Musante 1996; Willits and Tressler 1937; Ontario Ministry of Agriculture, Food, and Rural Affairs 2019).

Wisconsin rules expressly state that neither lead or lead-alloy solder can be used in the assembly or repair of surfaces associated with food sold in the private sector (Wisconsin Department of Agriculture, Trade and Consumer Protection 2019). Several other states have similar rules/guidance for commercial maple Syrup production. The State of California has set a maximum limit for lead in maple syrup of 0.011 mg/kg enforced by their Proposition 65 law (Table 11)[7]. This regulation could potentially hinder the future sale of tribal maple syrup in retail establishments if lead-bearing equipment or repair material was inadvertently used by tribal harvesters. It could require a lead warning label on maple products sold in California. Lead contamination can be eliminated from maple syrup using the guidance offered by the maple syrup organizations of Canada and the U.S. (International Maple Syrup Institute, 2015; Manufacturers of Maple Sugaring Equipment, 2002).

Past, peer-reviewed studies have only examined large-scale, commercial maple syrup enterprises. Lead concentrations in sap samples from plastic commercial tanks in Connecticut ranged from less than 0.0005 to 0.0054 mg/kg, with an average of 0.0011 mg/kg. Whereas, the lead content in all grove sap samples using both plastic and metal equipment ranged from <0.0005 to 0.019 mg/kg, averaging 0.0026 mg/kg. Higher values corresponded with samples procured from galvanized receptacles. The lead content of resultant maple syrup samples from State of Connecticut producers ranged from 0.038 to 0.948 mg/kg, averaging 0.291 mg/kg (Stilwell and Musante 1996).

Unintended sap contamination in the Stilwell and Musante (1996) study was caused by a bronze gear pump used in sap transfer steps. This contamination resulted in an average lead level of 0.019 mg/kg in sap from the terminal stainless-steel storage tanks (Stilwell and Musante 1996). The U.S. FDA does not have a specific regulatory guideline for lead in maple syrup, but Canada's MRL_2 is 0.5 mg/kg w.w. (Health Canada 2018).

[7] October 1, 2014 the Superior Court of the State of California, County of Humboldt stipulated a consent judgment in the case between Mateel Environmental Justice Foundation vs Anderson's Maple Syrup, Inc. et al. and set the lead limit for maple syrup at 11 ppb requiring that the lead content in syrup be measured before bottling to assure compliance.

This study included 29 maple syrup samples from tribal harvesters. Twenty-seven samples had lead concentrations measured as <LOD of 0.027 mg/kg. Commercial processed maple syrup (four samples) had the same result. One sample of maple syrup had a measured concentration of lead between the LOD and LOQ of 0.091 mg/kg. A single sample had a lead concentration measured above the LOQ at 0.27 mg/kg. The harvester/processer reported using old or inherited equipment. Lead was not detected in the single maple sugar sample. Moisture concentrations in the syrup samples varied and reflects the personal preference of the processor. It would be safe to conclude that maple syrup gathered and processed using plastic pails and new welded stainless steel evaporators would be safe for human consumption.

Wild Turkey

Concern for consuming wild turkey meat is due to the possibility of lead contamination from the methods commonly used to harvest wild turkeys. Published studies on metal contamination have not been found for wild turkeys although some x-ray and lead analyses data exist regarding other avian species. Three, peer-reviewed research studies were compared regarding lead contamination of other game bird tissues. A study on the effects of white-tailed deer (*Odocoileus virginianus*) also showed the effects of lead containing ammunition on the contamination of rifle harvested wild game (Hunt et al. 2009). They fed rifle-killed deer that had been commercially processed with the removal of tissues around the wound channel to swine and measured their blood lead concentrations. Swine fed meat from these deer had significantly elevated lead blood concentrations in two days after feeding began.

Scheuhammer et al. (1998) collected nearly 4000 game birds from every province in Canada harvested mostly by shotgun using lead shot. The samples included 44 avian species (mostly waterfowl) with the pectoral muscles analyzed for lead. Ten gram portions of each right pectoral muscle were pooled (827 pools of 1-12 birds per pool) by species and geographic location and analyzed the tissue for lead. Pectoral muscle tissue from both the right and left sides of some birds were examined, with visually detectable pellets or fragments removed before x-ray and lead analyses. Random, individual birds from various pools were also analyzed for lead content.

Lead concentrations averaged ~4.7 ± 43 mg/kg d.w. for all 827 sample pools. There were 735 sample pools with lead concentration averaging ≤2 mg/kg d.w. and 92 sample pools from the upper 10 percentile of lead concentrations that averaged 40 ± 125 mg/kg d.w.

In addition, two American woodcocks (*Scolopax minor)* sampled in the Scheuhammer et.al. (1998) study had measured lead concentrations of 14.2 and 844 mg/kg (d.w.) in the right breasts. In two spruce grouse *(Falcipennis canadensis) samples*, no lead was detected in one bird's left breast and its corresponding right breast possessed a lead level of 104 mg/kg (d.w.). The other spruce grouse had high lead concentrations in each of its breasts. X-rays were also taken of selected samples before lead analysis and, in some instances, indicated lead fragment and/or pellet presence. Scheuhammer et al. (1998) concluded "This form of dietary lead exposure in people is completely unnecessary, and can be avoided by the use of non-toxic shot for hunting."

Greenland seabirds were evaluated by x-ray and subsequently analyzed for lead content. Shot pellets were removed from tissue if visually observed or detected in x-ray. Using common eiders (*Somateria mollissima*) drowned in fishing nets as a control group and common eiders harvested by

shotgun as the experimental group, common eiders and thick-billed murres (*Uria lomvia)* were boiled according to a recipe common to the region. Whole, right pectoral breasts were removed and analyzed for lead content (Johansen et. al. 2004). Among eiders shot, one to 42 pellets were found radiographically in the whole bird and 0 to 3 pellets were found in breast tissue alone. Among eiders drowned, 0 to 3 pellets were found radiographically in whole birds, and 0 to 1 pellet was found in breast tissue alone. The average lead concentration in shotgun-harvested common eiders was 6.1 mg/kg w.w., and the mean lead concentration for drowned eiders were 0.14 mg/kg w.w. Murres harvested by shotgun had 0 to 12 pellets embedded within the whole bird, and 0 to 5 pellets in breast tissue alone. Murres harvested by shotgun had an average lead concentration in breast tissue of 0.73 mg/kg w.w. (Johansen et al. 2004).

Common eiders are overall larger than thick-billed murres, at about 20-28 inches and 18 inches, respectively (Cornell Laboratory of Ornithology 2017a; 2017b). In contrast, adult wild turkeys are 43 to 45 inches long (Cornell Laboratory of Ornithology 2017c). Therefore, due to their size, eiders would theoretically be more comparable in size to wild turkey than thick-billed murres, although the turkey is roughly twice the size as eider.

In another study, Pain et.al. (2010) examined a wide range of variables potentially impacting lead levels in European wild fowl meat, such as radiographic pellet presence and cooking methods. Through qualitative identification techniques regarding the composition of recovered pellets, 91% of birds were identified as having been shot with lead ammunition, rather than ammunition composed of non-lead materials. These qualitative methods included: notation of the pellet's color and malleability, determinations of magnetism and melting points, as well as chemical analysis for metal in shot. Nitric acid combined with potassium iodide were used to determine if the shot contained lead or bismuth alloys. Known pellet types were used as positive controls

In the study by Pain et.al. (2010), two groups of birds with approximately the same amount of pellets detected via x-ray were sampled from each of six game bird species examined. Afterwards, entire birds were cooked using either an acidic or nonacidic recipe which included the whole bird. After cooking, the edible muscle tissue was removed from the skeleton and analyzed for lead content. Grocery store chicken breasts were cooked using the acidic or nonacidic recipes, as control groups. Cooked game bird samples exceeding the E.U. lead action limit of 0.1 mg/kg w.w. for red grouse *(Lagopus lagopus scotica)*, ring-necked pheasant *(Phasianus colchicus*), and red-legged partridge (*Alectoris rufa*) by an estimated 6%, 8%, and 20%, respectively. Of these three species, ring-necked pheasants would be the most comparable in size to wild turkey (Ring-necked pheasant measure a length of nearly 20 to 28 inches), although wild turkey is roughly twice the size and four times heavier than pheasant (Cornell Laboratory of Ornithology 2017c). A significant positive correlation was found between the number of shot detected by x-ray and lead concentrations in total muscle tissue of cooked birds, even upon removal of shot pellets and/or fragments after cooking.

Pain et. al. (2010) did not find any statistically significant difference between lead concentrations in game meat cooked in acidic and non-acidic recipes. The researchers did not measure the pH of the cooking broth, but categorized acidic and non-acidic recipes based upon primary ingredients added. This finding contrasts with a previous study, in which lead levels increased when cooked as part of an acidic recipe (Mateo et al. 2007), and is possibly due to the pH levels of the

recipes used in the different studies. The current study did not examine cooked wild turkey breast tissue. However, wild turkey is typically prepared in a nonacidic manner by Ojibwe tribal harvesters.

The potential exists for physical harm to consumers from metal contamination in wild turkey from shotgun shot. A U.S. FDA (2005) investigation of hard and/or sharp foreign objects in foods prompted guidance to protect human health. "The investigation Board found that foreign objects that are less than 7 mm (0.276 in. which is about the diameter of no. 4 buckshot) maximum dimension, rarely cause trauma or serious injury except in special risk groups such as infants, surgery patients, and the elderly." Shot would not exceed the 7 mm size needing guidance for safe consumption of wild turkey.

The Canadian MRL_2 for lead in fish tissue is 0.5 mg/kg (Health Canada 2014), but the E.U.'s lead action limit for farmed poultry meat is 0.1 mg/kg. The U.S. FDA does not have an action limit for lead in poultry tissue. As in the case of maple syrup, Canada's MRL_2 for lead in fish tissue is used to evaluate lead concentrations in relation to possible impact on human health.

In our study, not all analyzed breast tissue containing pellets and/or fragments possessed lead concentrations above the LOD or LOQ values. Out of the 60 total breast samples from 30 different wild turkeys, four breast samples with pellets or fragmentation found in them possessed lead concentration values under the LOD value of 0.026 mg/kg. Two tissue samples containing pellets/fragments had lead levels under the LOQ value of 0.087 mg/kg (Table 6). Forty percent of total wild turkey breast samples where metallic material or fragments were found generated lead values over the LOQ value, with two of those values above the Canadian MRL_2 (0.5 mg/ kg) for lead in fish tissue.

Five (8.3%) of total breast samples where no pellets/fragments were discovered had detectable lead values above the LOQ value of 0.087 mg/kg, and ranged from 0.240 to 15.9 mg/kg d.w. It is possible that x-ray analysis did not capture microscopic shot fragmentation too small for observation. Wild turkeys are omnivorous, but primarily eat vegetation and are, therefore, unlikely to bio-accumulate significant concentrations of lead in their muscle tissues. Nonetheless, further investigation using wild turkey samples harvested with non-lead containing methods should be conducted to determine the sources of lead exposure in wild turkeys other than lead shot.

Ultimate lead concentrations in individual pectoral tissue samples were highly variable, ranging from 16.2 mg/kg d.w. to levels below the detection limit of 0.026 mg/kg. Notably, microscopic fragmentation was associated with the highest lead value from the 60 breast samples (10331-L). The mean lead concentration in tissue samples of wild turkey with concentrations above the LOD is 3.44 mg/kg d.w. This highest lead value in breast tissue is over thirty-two times the Canadian MRL_2 in fish muscle (0.5 mg/kg). This is a concerning finding, and warrants a change in ammunition used to harvest wild turkey. Smaller shot (size 8) may have resulted in higher lead concentrations due to greater difficulty finding the shot by sight or in x-ray examination. Due to the permanent impacts of lead exposure on children's cognitive and neurological development, harvest techniques need to be evaluated. In total, three individual samples contained lead concentrations greater than the Canadian MRL_2.

Conclusion

Wild rice (*manoomin*), maple syrup (*zhiiwaagamizigan*), and wild turkey (*mizise*) were all analyzed to better understand potential chemical and/or physical contamination associated with these traditional, Ojibwe foods.

Wild rice

Finished wild rice seeds harvested and processed by Ojibwe tribal members do not contain amounts of lead, zinc, cadmium, total mercury, copper, magnesium, total chromium, selenium, and total and inorganic arsenic concentrations that would be of negative impact to human health, in either cooked or dry form. Naturally-derived wild rice met current and/or proposed MRL_2 or a RfD identified by national health organizations.

While limited to three samples, testing results of cultivated (commercial) wild rice was found not to contain amounts of lead, zinc, cadmium, total mercury, copper, magnesium, total chromium, selenium, and total arsenic concentrations that would be of negative impact to human health. Two of the three cultivated (commercial) wild rice samples tested above the 0.1 ppm action level proposed by the U.S. FDA as being harmful to infants consuming cereals. An extensive review of arsenic in rice and rice products has been developed by the U.S. FDA (2016) in developing a risk assessment of consuming these products. Additional research is needed before any definitive conclusions can be made regarding health risks to infants in relation to inorganic arsenic levels in cultivated wild rice.

Consumption of wild rice, harvested and processed by tribal members, does not pose a chemical risk to tribal communities and should be made available to tribal members being served in federally-funded food programs to improve reservation health conditions.

Maple Syrup

Maple sap harvested and processed by Ojibwe tribal members into syrup does not contain lead concentrations that would be harmful to human health using the Canadian MRL_2 for lead in maple syrup. Only one out of 29 tribally-obtained maple syrup samples analyzed contained a detectable lead concentration, yet the sample did not exceed Canada's MRL_2 of 0.5 ppm for lead in maple syrup.

Approximately 1/3 of tribal harvesters and processors indicated they used "old and/or inherited" equipment in procuring or processing of maple sap into syrup. Despite the intrinsic sentimentality of inherited equipment, use of modernized equipment by tribal members will further ensure undetectable concentrations of lead in maple syrup. Therefore, maple syrup processed by tribal members does not pose a chemical risk, from lead, to tribal communities and should be made available to tribal members served in federally-funded food programs, as well as made available for retail sale within their tribal communities. Using the guidelines produced by maple syrup producing organizations will eliminate lead contamination (Health Canada 2018; International Maple Syrup Institute 2015, Manufacturers of Maple Sugaring Equipment 2002).

Wild Turkey

Wild turkey breast meat should be further studied, as results from this study indicate that ammunition used to harvest the birds was the main source of lead in the meat. The potential for lead contamination of wild turkey breasts due to lead ammunition usage represents a greater human health hazard than that of natural contamination. If wild turkey meat is eaten, a consideration of the irreversible neurological impacts lead exposure can have on sensitive populations, such as children, must be considered.

With regards to metallic contamination of wild turkey breast meat, harvesting of this avian species with smaller size No. 8 and No. 6 shot increased lead content found in the breast meat. Furthermore, turkey harvested with larger size No. 5 copper coated lead shot were found to test below laboratory detection limits. The risk of lead contamination from shot pellets can further be reduced by tribal hunters utilizing either crossbow, archery, or waterfowl shotgun ammunition which is lead free. It should also be noted that the risk of fragments in breast meat, cannot be completely eliminated because it cannot be ascertained that the bird had not been shot before and subsequently survived.

While bow or crossbow usage by tribal harvesters would assist in reducing physical risk, all submitted samples were harvested with a firearm. Crossbow or bow archery harvesting appears to be a harvest technique that not many tribal hunters use today. Future research involving lead contamination of wild turkeys from lead-bearing ammunition would require a control group of turkeys harvested by archery or shotgun with waterfowl ammunition.

For Ojibwe members wishing to harvest with firearms, using the recommend shot sizes for wild turkey hunting, either No. 4, No. 5, or No. 6, would assist in keeping meat beneath the U.S. FDA hard and/or sharp foreign object threshold of 7 mm. However, shot pellets or fragments may not always be visually detectable during normal processing or meal preparation. Therefore, care should be exercised to remove any metallic components before consumption.

Acknowledgements

The author would like to thank the tribal harvesters that submitted samples for analysis. Dr. Sara Moses from GLIFWC and Christine Polkinghorne from LSRI provided extensive technical assistance in preparing Quality Assurance Project Plans (QAPPs) and review and explanation of testing results. Ben Wozniak from BAL provided valuable technical guidance in the production of this document. Dr. Jonathan Gilbert and Miles Falck from GLIFWC proofread drafts and supplied constructive comments for structural improvement. Owen (Maroney) Schwartz from GLIFWC compiled MRL_2 for comparable foods from national and international health organizations and assisted in editing. Zoongee (Leith) Mayotte assisted in sample collection. LaTisha Coffin, ANA SEDS Coordinator, assisted in project coordination. Funding for this research was provided through ANA SEDS grant No. 90NA8320.

References

Agency for Toxic Substances and Disease Registry. 2007. "Toxic Substances Portal: Lead." *United States Centers for Disease Control & Prevention.* https://www.atsdr.cdc.gov/phs/phs.asp?id=92&tid=22.

Agriculture Victoria. 2019. "Maximum Residue Limits." *The State of Victoria.* http://agriculture.vic.gov.au/agriculture/farm-management/chemicals/chemical-residues

ANSI National Accreditation Board. 2019. "About ANAB. https://anab.ansi.org/

Arnemo, J. M., O. Andersen, S. Stokke, V. G. Thomas, O. Krone, D. J. Pain, and R. Mateo. 2016. "Health and Environmental Risks from Lead-Based Ammunition: Science Versus Socio-Politics." *EcoHealth* 13: 618622.

Bennett, J. P., E. Chiriboga, J. Coleman, and D. M. Waller. 2000. "Heavy Metals in Wild Rice from Northern Wisconsin." *The Science of the Total Environment* 246: 261-69.

Brooke, L.T., C.N. Polkinghorne, H.J. Saillard, and T.P. Markee. 2004. Metal Concentrations in Wild Rice Roots and Seeds, Mollusks, Crayfish, and Fish Collected from Various Wisconsin Water Bodies in the Autumn of 2003. Lake Superior Research Institute, university of Wisconsin-Superior, Superior, WI. Final Report to the Great Lakes Fish and Wildlife Commission, Odanah, WI. 35 pp.

Brooks Applied Labs. 2019a. Arsenic Speciation. https://brooksapplied.com/services/trace-elements-speciation/arsenic-speciation-as/

_____. 2019b. Certifications, Licenses, & Permits: Laboratory Accreditation Certificates. https://brooksapplied.com/resources/certificates-permits/

Cakmak, I. 2013. Magnesium in crop production, food quality and human health. *Plant Soil* 368, 1–4 https://doi.org/10.1007/s11104-013-1781-2

Corbiere, A. 2011. "Ninaatigwaaboo (Maple Tree Water): An Anishinaabe History of Maple Sugaring." *Carlton University*. https://carleton.ca/grasac/story/maple-exhibit/.

Cornell Lab of Ornithology. 2017a. "Common Eider." *Cornell University.* https://www.allaboutbirds.org/guide/Common_Eider/id.

_____. 2017b. "Thick-Billed Murre." *Cornell University.* www.allaboutbirds.org/guide/Thick-billed_Murre/id.

_____. 2017c. "Wild Turkey." *Cornell University.* https://www.allaboutbirds.org/guide/Wild_Turkey/lifehistory

David, P. 2013. Manoomin (wild rice) Enhancement and Research in the Ceded Territories in 2010. Great Lakes Indian Fish and Wildlife Commission. Administrative Report 13-1. 4 pp

David, P. 2020. Manoomin (Wild Rice) Abundance and Harvest in Northern Wisconsin in 2014. Great Lakes Indian Fish and Wildlife Commission. Administrative Report 20-01. 20 pp.

David, P. 2019. 2018 Mizise (Turkey) Harvest in the Wisconsin in the Wisconsin and Minnesota 1837 and 1842 Ceded Territories. GLIFWC Administrative Report. 3 pp.

Food Standards Agency in Scotland. 2012. "Habits and Behaviours of High-Level Consumers of Lead-Shot Wild Game Meat in Scotland." *Harris Interactive*.

Government of Canada. 2020. List of contaminants and other adulterating substances in food. https://www.canada.ca/en/health-canada/services/food-nutrition/food-safety/chemical-contaminants/ contaminants-adulterating-substances-foods.html

GLIFWC (Great Lakes Indian Fish and Wildlife Commission). 2014. "*Mino Wiisinidaa! (Let's Eat Good!): Traditional Foods for Healthy Living."* GLIFWC: Odanah, WI.

GLIFWC (Great Lakes Indian Fish and Wildlife Commission). 2018. *"Ojibwe Treaty Rights."* GLIFWC: Odanah, WI.

Health Canada. 2018. Preventative Controls for Maple Products-Lead Residues. *Canadian Food Inspection Agency*. http://www.inspection.gc.ca/food/general-food-requirements-and-guidance/preventive-controlsfood-businesses/maple-products/eng/1510678697138/1510678888805.

_____. 2014. Appendix 3: Canadian Guidelines for Chemical Contaminants and Toxins in Fish and Fish Products. *Fish Products Standards & Methods Manual*. Canadian Food Inspection Agency.

Hunt, W.G., R.T. Watson, J.L. Oaks, C.N. Parish, K.K. Burnham, R.L. Tucker, J.R. Belthoff, and G. Hart. 2009. Lead bullet fragments in venison from rifle-killed deer: Potential for human dietary exposure. PloS ONE 4(4): e5330. https://doi.org/10.1371/journal.pone.0005330.

International Maple Syrup Institute. 2015. North American Good Manufacturing Practices to Avoid Lead Contamination of Maple Syrup. 4 pp. https://agnr.osu.edu/sites/agnr/files/imce/pdfs/maplesyrup/GMPForLeadMarch2015.pdf

Johnson, E. 1970. Preliminary notes on the historic use of wild rice. The Minnesota Archaeologist, Vol XXX, Number 2

Johansen, P., G. Asmund, and F. Riget. 2004. "High Human Exposure to Lead through Consumption of Birds Hunted with Lead Shot." *Environmental Pollution* 127: 125-9.

Kraft, C. E. 2018. Unknown Biological, Chemical, & Physical Risks in Identified Traditional Foods: Companion Document to 2018 Traditional Food Contaminant and Food Safety Report. Submitted to ANA SEDS in Year 1 of project.

Kraft, C.E., and O.H. Maroney. 2018. Traditional Food Contaminant and Food Safety Report, Version 1.0. Submitted to ANA SEDS Year 1 of project.

Kuhnlein, H.V. 1995. Ojibwe Health and Traditional Food Use. Centre for Nutrition and Environment of Indigenous Peoples, McGill University, Montreal.

Lamont, W.H. 2003. Concentration of Inorganic Arsenic in Samples of White Rice from the United States. *Journal of Food Composition and Analysis* 16: 687-95.

Lac Courte Oreilles Band of Lake Superior Chippewa v. Wisconsin, No. 74-C-313 (W.D. Wis. 1989) (Deer Trial Stipulation).

Lobring, L.B., and B.B. Potter. 1991. Method 245.6, Revision 2.3: Determination of Mercury in Tissues by Cold Vapor Atomic Absorption Spectrometry. Method from U.S. EPA, Office of Research & Development, Environmental Monitoring Systems Laboratory.

Mann, M-J., E.O. Espinoza, R.M. Ralston, R.K. Stroud, M.D. Scanlan, and S.J. Strauss. 1994. Shot Pellets: An Overview. *AFTE Journal* 26 (3). 18 pp.

Mille Lacs, 124 F.3d 909; *Lac Courte Oreilles Band of Lake Superior Chippewa Indians v. Wisconsin*, 653 F. Supp. 1420, 1435 (W.D. Wis. 1987).

Manufacturers of Maple Sugaring Equipment (LMEA). 2002 Standards on Maple Equipment Intended for the Production of Maple Syrup. 19 pp and Appendices. http://www.internationalmaplesyrupinstitute.com/uploads/7/0/9/2/7092109/__lmea__standards_on_maple_equipment.pdf

Mateo, R., M. Rodriguez-de la Cruz, D. Vidal, M. Reglero, and P. Camarero. 2007. "Transfer of Lead from Shot Pellets to Game Meat During Cooking." *Science of the Total Environment* 372: 480-5.

Manoomin – Wild Rice the Good Berry, Great Lakes Indian Fish and Wildlife Commission, http://www.glifwc.org/publications/pdf/Goodberry_Brochure.pdf

May, T.W. and R.H. Weidmeyer. 1998. A Table of Polyatomic Interferences in ICP-MS. https://pubs.er.usgs.gov/publication/70020866.

Minnesota Department of Natural Resources. 2018. Wild Rice (*Zizania palustris*). www.dnr.state.mn.us/aquatic_plants/emergent_plants/wildrice.html.

National Institute of Health. 2018. Hexavalent Chromium. *National Toxicology Program. https://www.niehs.nih.gov/health/materials/hexavalent_chromium_508.pdf*

Nriagu, J.O., and T-S. Lin. 1995. Trace Metals in Wild Rice Sold in the United States. *The Science of the Total Environment* 172: 223-228.

Ontario Ministry of Agriculture, Food, and Rural Affairs. 2019. Best Production Practices for Producing Safe, Quality Maple Syrup.

Pavel, M.J. 2018, Food Code Guidance Report, Sonosky, Chambers, Sachse, Endreson & Perry, LLP, p. 5-6

Pain, D.J., R.L. Cromie, J. Newth, M.J. Brown, E. Crutcher, P. Hardman, L. Hurst, R. Mateo, A. A. Meharg, A.C. Moran, A. Raab, M.A. Taggart, and R.E. Green. 2010. Potential Hazard to Human Health from Exposure to Fragments of Lead Bullets and Shot in the Tissues of Game Animals. *PLoS ONE* 5 (4), e10315. 17 pp.

Pip, E. 1993. "Cadmium, Copper, and Lead in Wild Rice Seeds from Central Canada." *Archives of Environmental Contamination & Toxicology* 24: 179-81.

Polkinghorne, C.N., Beesley, K.M., and Markee, T.P. 2019a. Metals Concentrations in Finished Wild Rice Seeds
Collected from Various Water Bodies within the Ceded Territories. Lake Superior Research Institute, University of Wisconsin-Superior, Superior, WI. Final Report to the Great Lakes Fish and Wildlife Commission, Odanah, WI. 20 pp.

----------. 2019b. Lead Concentrations in Maple Syrup made from Sap Harvested within the Ceded Territories. Lake Superior Research Institute, University of Wisconsin-Superior, WI. Final Report to the Great Lakes Indian Fish and Wildlife Commission, Odanah, WI. 14 pp.

Raab, A., C. Baskaran, J. Feldmann, and A.A. Meharg. 2009. Cooking Rice in a High Water to Rice Ratio Reduces Inorganic Arsenic Content. *J. Environ. Monit.* 11: 41-44.

Scheuhammer, A.M., J.A. Perrault, E. Routhier, B.M. Braune, and G.D. Campbell. 1998. "Elevated Lead Concentrations in Edible Portions of Game Birds Harvested with Lead Shot." *Environmental Pollution* 102: 251-7.

Stilwell, D.E. and C.L. Musante. 1996. Lead in Maple Syrup Produced in Connecticut. *Journal of Agricultural and Food Chemistry* 44(10): 3153-3158.

Superior Court of the State of California, County of Humboldt. Mateel Environmental Justice Foundation vs Anderson's Maple Syrup, Inc., et al. October 1, 2014. Stipulated Consent Judgement. https://oag.ca.gov/system/files/prop65/judgments/2014-00281J2359.pdf

Tsuji, L.J.S., E. Nieboer, J.D. Karagatzides, R.M. Hanning, and B. Katapatuk. 1999. Lead Shot Contamination in Edible Portions of Game Birds and its Dietary Implications. *Ecosystem Health* 5 (3): 183-92.

U.S. Environmental Protection Agency. 2018. Basic Information about the Integrated Risk Information System..

_____. 2005. Zinc & Compounds; CASRN 7440-66-6. Integrated Risk Information System: Advanced Chemical Assessment Summary. .

_____. 1991. Arsenic, inorganic (CASRN 7440-38-2). Integrated Risk Information System: Advanced Chemical Assessment Summary.https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/ 0278_summary.pdf

_____. 1988. Lead & Compounds (Inorganic); CASRN 7439-92-1. Integrated Risk Information System Chemical Assessment Summary. https://cfpub.epa.gov/ncea/iris/iris_documents/documents/subst/0277_summary.pdf#nameddest=rfd

U.S. Food and Drug Administration. 2013. Analytical Results from Inorganic Arsenic in Rice & Rice Products Sampling.

_____. 2016a. Supporting Document for Draft Guidance for Industry on Inorganic Arsenic in Rice Cereals for Infants. 9 pp. https.www.fda.gov/media/97121/download.

_____. 2016b. Arsenic in rice and rice products: Risk assessment report. 269 p. www.fda.gov/media/96071/download.

_____. 2005. Compliance Policy Guidance Sec. 555.425 Foods, Adulteration Involving Hard or Sharp Foreign Objects. 3 pp. www.fda.gov/regulatory-information/search-fda-guidance-documents/cpg-sec-555.

Vennum, Jr., T. 1988. *Wild Rice and the Ojibway People.* 350 pp. Minnesota Historical Society Press. 345

Willits, C.O. and C.J. Tressler Jr. 1937. Sources of Lead in Maple Syrup and a Method for its Removal. *Journal of Food Science* 3(5): 449-452.

Winchester Ammunition. 2017. Is there a Best Shot Size for Turkey? https://winchester.com/Blog/2017/03/isthere-a-best-shot-size-for-turkey

Wisconsin Department of Agriculture, Trade and Consumer Protection. 2019. Rules for Maple Syrup Processing. *Division of Food & Recreational Safety*. https://datcp.wi.gov/Documents/RulesForMapleSyrupProcessors.pdf

Wisconsin Department of Natural Resources. 2015. Ecology of Wild Turkeys in Wisconsin: A Plan for their Management, 2015-2025. Bureau of Wildlife Management. https://dnr.wi.gov/files/PDF/pubs/WM/WMO585.pdf.