ENVIRONMENTAL CONTAMINANTS IN FISH DIETS OF LAKE SUPERIOR CHIPPEWA

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ABSTRACT

Walleye, lake trout, siscowet trout, and whitefish were obtained in two collections from Apostle Islands National Lakeshore (AINL) as a representative sample of fish consumed by the Red Cliff Band of Lake Superior Chippewa people and subsequently fed to laboratory rats in a neurotoxicity bioassay. Carp were collected from Little Lake Butte des Mortes, Green Bay, Wisconsin, to be used as a PCB positive control; and Pacific Salmon were analyzed for future use a a negative control species. All fish were prepared as fillets, ground into composite samples by species, analyzed for PCBs, mercury (Hg), and several other organochlorines.

For the first collection, the composite sample of walleye was 0.57 ug/g Hg which is slightly higher than the action level of 0.5 ug/g recommended by the State of Wisconsin for general human consumption. PCB analyses by gas chromatography with mass selective detection (MSD) of the walleye, lake trout and whitefish collected for the initial subchronic rodent study averaged <0.30 ug/g total PCBs, where the carp contained 3.00 ug/g total PCBs, and specific congeners 2,4,4' at 0.21 ug/g, 2,2',4,4' at 0.15 ug/g and 2,2',5,5' at 0.4 ug/g.

The second fish collection Hg levels were: walleye 0.58 ug/g; whitefish 0.06 ug/g siscowet trout 0.45 ug/g and carp 0.09 ug/g. Collection Two total PCB values were determined by GC-electron capture detector (ECD) following gel permeation chromatography (GPC) cleanup and resulted in: walleye 0.193 ug/g; whitefish 0.154 ug/g; siscowet trout 0.370 ug/g and carp 1.404 ug/g. All four

composite samples had total PCB concentrations well below the federal and state advisory limits for humans; however, the PCBs in all four species were above the no observable effect level (NOEL) for piscivorous wildlife.

Running Title: Chippewa and Fish Contaminants.

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INTRODUCTION

Toxic contamination of the Great Lakes basin is not a new issue. Although efforts of the past twenty years have made considerable strides in reducing discharges of contaminants and restoring health and viability to the lakes, persistent toxic substances continue to exert far reaching, adverse impacts throughout the ecosystem (IJC 1990).

Historical discharges of PCBs, chlorinated dioxins, and mercury are responsible for much of the existing contamination of the Great Lakes (Foran 1992), and biomagnification of these chemicals has been observed in the Great Lakes food chain. For example, PCBs in the waters of the Great Lakes are present in the low pg/g (parts per trillion) range and levels determined as recently as 1988 in the eggs of eagles from around Lake Erie were greater than 25.0 ug/g (parts per million). This is a biomaginfication factor of 25 million. Biomagnification factors for mercury and polynuclear aromatic hydrocarbons (PAHs) is less substantial, usually 1000 (Environment Canada 1991).

Contaminants are magnified to significant proportions in fish through bioconcentration and through absorption as water passes over the gills. The health effects on wildlife from the polluted fish they ingest are well documented (Environment Canada 1991). Contaminants have caused acute mortality and egg shell thinning in predatory birds, and the offspring of contaminated animals are often deprived of reproductive, endocrine, immune, nervous and digestive systems that function properly, as well as teratogenic effects such as deformed beaks observed in Great Lakes cormorants. A condition called bird wasting syndrome, attributed at least in part to contaminant body burdens, has caused high mortality among chicks for a number of years in a population of Forster's terns in Lake Michigan and appears to have caused the death of Bald Eagle chicks on the Ohio shoreline of lake Erie in the summer of 1991 (Colborn

1992). The 1990 level of PCBs in unhatched bald eagle eggs from around Lake Erie averaged 25.0 ug/g (ppm), which is roughly five times the 5.0 ug/g (ppm) "no effect" level associated with bald eagle reproductive success (Busch 1992). In addition, a large colony of ring-billed gulls from the Maumee Bay of Lake Erie failed to hatch a single egg in 1991, even though the colony contained 2000 nests and historically produced 2500 chicks (Glenn 1992).

The Great Lakes basin acts as an environmental sink collecting toxic contaminants from adjacent and remote regions of the country and concentrating them, either in sediments or indirectly within the ecosystem (Glenn 1992). Polychlorinated biphenyls and mercury are two of eleven contaminants identified by the International Joint Commission as critical toxic pollutants in the Great Lakes (IJC undated). The persistence and long retention time of these chemicals in the lakes, combined with biomagnification, poses a serious threat to wildlife and humans.

For example, PCBs are persistent highly lipophilic environmental contaminants previously used in industry, that cause a variety of adverse physiological and reproductive effects in wildlife and humans (Jacobson *et al.*, 1984). PCBs were banned in the 1970's, however they are continuing to bioaccumulate in the aquatic food chain and cause serious contamination in lake sediments, benthos, fish, and consumers of the contaminated fish. Neurotoxic effects have been observed across several species of animals including humans, and the human nervous system appears to be extremely sensitive to this compound (Glenn 1992). A 1990 mink feeding study using food (fish) from Saginaw Bay of Lake Huron resulted in reduced survival of mink fetuses and death in adult mink. There is also evidence from trapping records that mink are rare in areas where habitat is suitable (such as northern Lake Michigan and portions of Lake Huron) except that contamination levels are elevated (Blankenship 1992). The State of New York has established a no observable effect level for PCBs in the

diet of piscivorous wildlife of 1.5____??? (EPA, 1992).

The advisory limits for human consumption are considerably higher (2.0 ppm for total PCBs and 0.5 ppm for mercury) and are based in part upon the assumption that humans consume much smaller amounts of contaminated fish than piscivorous wildlife. The Red Cliff Band of Lake Superior Chippewa like many other Ojibway people rely heavily upon the Great Lakes fisheries for commercial and subsistence livelihoods. Therefore, compared to the general population, the EPA along with many other agencies assumes that the aboriginal populations consume more fish and are receiving more persistent environmental contaminants in their diets.

This study was undertaken to determine what levels of contaminants were in the diets of the Red Cliff tribe. To meet this objective, sufficient quantities of each species were collected, analyzed, and subsequently fed to laboratory rats in neurotoxicity bioassays. The rodent studies and human epidemiology data are being reported elsewhere. The predominant species consumed were walley, lake trout, and whitefish (Figure 1). Studies by Daly (1992) have shown Pacific salmon to be an effective negative control when used in rodent feeding studies, therefore we purchased and analyzed salmon for the subsequent studies. Little Lake Butte des Mortes (LLBM) carp were reportedly high in total PCB concentrations and the State of Wisconsin Department of Natural Resources (WDNR) personnel provided carp for the present studies. The carp were used as a positive control species in the subsequent rodent bioassays.

In this report, we list the types and levels of contaminants in the various species. We also compare the contaminants in the composite fish samples to individual fish data provided by the WDNR.

METHODS

Fish Collection and Preparation

Walleye (*Stizostedion vitreum*), whitefish (*Coregonus clupeaformis*), lake trout (*Salvelinus namaycush namaycush*), and siscowet trout (*Salvelinus namaycush siscowet*) were collected during routine monitoring of the tribal commercial fishery and assessment of gillnets during the 1991 and 1992 seasons. Two fish collections were received by the Lake Superior Research Institute (LSRI) from the Red Cliff Fisheries Department. The first collection occurred in June and July of 1991, and contained frozen walleye, lake trout and whitefish fillets. The second collection occurred in October, November and December of 1991 and contained walleye, lake trout, whitefish and siscowet trout fillets. All fish were collected from management unit WI-2 (Figure 2) of Lake Superior and assigned an individual sample number.

The following data were recorded for each fish: length (measured to the nearest tenth of an inch), weight (measured to the nearest ounce), scale samples, fin clips, and the presence of lamprey wounds. In addition, otoliths were obtained from the lake trout over 23.0 inches long and aged using the crack and burn method (Casselman 1983).

Fish were filleted according to traditional tribal method. Lake trout and whitefish were filleted with the skin attached, but the whitefish were scaled. Walleye samples were filleted and processed with the skin removed. One fillet from each fish was wrapped in aluminum foil (prerinsed with acetone and hexane) and later was analyzed for PCBs. The fillet was wrapped a second time in regular foil and frozen. The second fillet was placed into a Ziplock sealed plastic freezer bag and frozen for mercury analyses.

Pacific salmon (pink salmon, humpback salmon (*Oncorhynchus gorbuscha*),

was purchased through two local vendors, Lake Superior Fish Company and Captain AJ's, Superior, WI. The salmon were frozen, with heads and entrails removed, it was then filleted and skinned prior to being processed.

Carp (*Cyprinus carpio*) from LLBM near Green Bay, Wisconsin, was collected by WDNR scientists (Dr. Mike Meyer and Mr. Tim Doelger) by electro-fishing, wrapped whole in aluminum foil, frozen, and sent to LSRI to be used as a positive control in rodent feeding studies. The fish were weighed to the nearest ounce, measured, filleted (skin removed), and processed.

Prior to preparing the fish, a Waring commercial food grinder (model #FGC60) was dismantled and the auger components were treated with the following: Liquinox soap and water wash, deionized water rinse, 1% hydrochloric acid rinse, deionized water rinse, acetone, methylene chloride, hexane, and deionized water. The food grinder was cleaned with this method prior to each species or food group being processed. Mid-section dorsal to ventral 1.5 -2.0 inch wide portions of each individual fillet were archived in aluminum foil prerinsed with acetone and hexane. The remaining muscle tissue was pooled by species and collection number, and ground three (3) times to ensure homogeity. Sample analyses were completed on the composite sample of each species. The composite samples were then mixed with ground Certified Purina Laboratory Rat Chow at a rate of 30% fish/70% lab rat chow and used as food stock in the rodent neurotoxicity studies.

Chemical Analyses Total PCBs and Other Organochlorines

Extraction of Chlorinated Hydrocarbons from Fish Tissue. The tissue was rinsed with deionized water, patted dry, and ground to a fine consistency. A portion of the ground tissue was transferred to a tared glass jar and the weight recorded. Dry sodium sulfate was added at five times the tissue weight. This was then mixed to form a homogeneous mixture which was covered

and stored frozen overnight. More sodium sulfate was added the next day if the mixture appeared moist. The mixture was blended to a homogeneous powder.

The powder was transferred to a pre-extracted glass wool thimble and the weight of sample mixture was recorded. A glass wool pad was placed on top of the mixture in the thimble to prevent splashing. The sample was spiked with a known amount of three PCBs after placing in a soxhlet extractor. The sample was extracted with methylene chloride for 24 hours. The extract was concentrated by Rotovap and under nitrogen to about 1 mL and placed in a capped vial for GC-MSD analysis or gel permeation chromatography (GPC) fractionation.

Gas Chromatography-Mass Selective Detector (GC MSD). During Year 1 of the study, the composite fish samples were analyzed using a GC-MSD. Samples were stored frozen until analysis. Following florisil cleanup, total PCB analysis was performed with a Hewlett Packard 5971A Mass Selective Detector monitoring the M+ and M+2 ions of the isotope clusters characteristic of the homolog classes. The limit of detection for total PCB concentrations was 300 ng/g (parts per billion). The limit of detection for individual congeners was 3.0 ng/g (parts per billion).

Gel Permeation Chromatography (GPC cleanup). Toluene was added to the vials and the samples were chromatographed on a 27 cm x 25 mm glass column containing Bio-Rad S-X2 Biobeads using methylene chloride as the mobile phase. The high molecular weight fraction was wasted and the portion just prior to and after toluene elution, containing the PCBs, was collected. The collected portion was concentrated by Rotocap and under nitrogen for the next phase of clean-up.

Iso-octane was added to the vials containing the samples and they were chromatographed on a 25 cm x 9.4 mm Whatman Magnum 9 PAC (polar amino-cyano) 10 um column. Fractionation was obtained with a binary gradient of hexane and

methylene chloride with methanol column clean-up. The portion eluting after toluene was collected and concentrated by Rotovap and under nitrogen to a volume of about 0.5 mL. The sample was then ready for GC analysis.

Gas Chromatography Electron Capture Detector (GC-ECD). In order to lower the limits of detection, during the second year of the study, all samples were analyzed using the GPC cleanup and GC-ECD detection. The cleaned extracts were analyzed for PCBs (either total or congener-specific) by gas-liquid chromatography, utilizing a Hewlett-Packard Model 5880A gas chromatograph equipped with an electron-capture detector and a 60 m DB-5 capillary column. Congener identification/quantitation was accomplished utilizing a Nelson analytical data system equipped with a Turbochrome II software package. In addition to the specific congeners of PCBs, numerous other organic contaminants were quantified including: chlordanes, dieldrin, heptachlorabenzene, dichloro_____(DDT and its degradation products). **[[list all]]**

Chemical Analysis of Total Mercury

Total mercury analyses were performed by cold vapor atomic absorption spectrometry on an Instrumentation Laboratory Video 12 aa/ae spectrophotometer with methods adopted from Lobring *et al.* (1991).

The samples were digested to convert all forms of mercury to inorganic mercury ions. The procedure involves the following steps. Approximately 0.2 - 0.3 g of fish tissue were weighed into acid washed BOD bottles for each sample. A series of mercury standards were prepared with each set of tissue samples. Reagent blanks and spikes were also prepared with each set of tissue samples. The blanks, standards, and spikes received the same treatment as tissue samples. To each bottle was added 1.0 mL of concentrated nitric acid and 4.0 mL of concentrated sulfuric acid. The samples were heated in a water bath at 80-90^{o}C for 60 minutes or until the tissue was dissolved. The samples

were cooled to room temperature and 15.0 mL of 5% potassium permanganate solution and 8.0 mL of 5% potassium persulfate solution was added to each sample and swirled. The samples were allowed to sit overnight, oxidizing the organic forms of mercury to inorganic mercury ions.

To each sample was added 10.0 mL of 10% hydroxylamine hydrochloride-10% sodium chloride solution and swirled until no purple coloration of potassium permanganate remained. Just prior to analysis, 5.0 mL of 10% stannous chloride solution was added to the sample and the flask was immediately attached to the aeration system. The absorbance of the sample was monitored for 60 seconds and the maximum absorbance of the sample recorded as the response. The aeration system was purged of mercury before the next sample was analyzed.

Quantifying Total Lipids

Total lipids in fish tissues were determined by extracting one to two grams of tissue once with 2:1 chloroform/methanol and repeated with an additional aliquot of 100% chloroform. The extract was filtered to remove particulates then evaporated to dryness and placed in an oven for one hour. Following the drying period, the residues were placed in a dessicator to cool and subsequently weighed on an analytical balance. Percent lipid was then calculated on a wet weight basis.

RESULTS

All fish were collected from the western end of Lake Superior, management unit WI-2 (Figure 2) by the Red Cliff Fisheries department. The first collection contained twenty-two walleye from grid area 1405 with a mean length of 53.3 cm (21 inches) and mean weight of 1.6 kg (3.5 pounds). Forty-two whitefish from grid areas 1209, 1309, and 1310 were collected with a mean length 46.5 cm (18.3 inches) and mean weight 0.9 kg (2.0 pounds). Finally, twenty-one lake trout from grid areas 1209, 1308 and 1309 were collected with

mean length of 55.1 cm (21.7 inches) and mean weight of 1.7 kg (3.7 pounds) ***(Table 1)***.

In the second collection, twelve walleye were received from grids 1306 and 1307 and had a mean length of 58.7 cm (23.1 inches) and a mean weight of 2.4 kg (5.2 pounds). Eight whitefish were collected from grid 1311 with a mean length of 47.0 cm (18.5 inches) and mean weight of 0.86 kg (1.9 pounds). Nine lake trout were received from grid 1307 with mean length of 71.6 cm (28.2 inches) and a mean weight of 3.0 kg (6.6 pounds). Finally, thirty-two siscowet trout were received from grid 1211 with mean length 52.3 cm (20.6 inches) and a mean weight of 1.3 kg (2.8 pounds) (Table 1).

The first shipment of LLBM carp contained 20 fish with a mean length of 55.9 cm (22.0 inches) and mean weight of 2.22 Kg (4.9 pounds). Shipment two of carp contained 15 fish with a mean length of 63.5 cm (25.0 inches) and mean weight of 3.08 Kg (6.8 pounds).

Heads and entrails were removed from the Pacific salmon prior to purchase, therefore, length and weight data are unavailable.

Total mercury and total PCB concentrations, in the fish composite samples are summarized in Table 1.

Polychlorinated Biphenyls

Total PCB concentrations in the WI-2 management area walleye, lake trout and whitefish and in the Pacific salmon from collection one were all below the detection limit of 0.30 ug/g GC MSD (Table 1). The LLBM carp was the only food stock from the first fish collection which contained concentrations greater than the detection limit, with total PCB concentration of 3.0 ug/g. ***Table 2*** lists the concentrations of the neurotoxic congeners of interest in the collection one food stocks.

Second collection total PCB concentrations (using GC ECD) were: walleye

0.193 ug/g; whitefish 0.154 ug/g; siscowet trout 0.370 ug/g; salmon 0.017 ug/g and carp 1.404 ug/g. Quantities of lake trout from Collection One were sufficient to complete the subsequent rodent feeding studies, therefore, additional lake trout were not collected and were not available for the GC-ECD analyses of PCBs and other organochlorines. However, the Collection One lake trout extract (without GPC cleanup) was passed through the GC ECD to produce a chromatogram. The lake trout chromatogram was used to qualitatively compare to the siscowet trout from Collection Two.

Total Mercury

Total mercury concentrations in the Collection One fish composites were: walleye 0.570 ug/g; lake trout 0.190 ug/g; whitefish 0.055 ug/g; Pacific salmon 0.040 ug/g; and LLBM carp 0.080 ug/g (Table 1). In the second fish collection composites, total mercury values were: walleye 0.580 ug/g; whitefish 0.060 ug/g; siscowet lake trout 0.450 ug/g; Pacific salmon 0.040 ug/g; and LLBM carp 0.090 ug/g (Table 1). As explained earlier, lake trout were not available from the Collection Two for mercury analyses.

Other Contaminants and Lipid Results

The second collection composite fish samples were run on GC-ECD allowing an organochlorine screen to be conducted on the following composites: walleye; salmon; siscowet trout (fat trout); carp; whitefish; and whitefish liver. ***Table 3*** lists some of the other contaminants that were quantified in the samples. The chromatograms contained not only PCB contamination, but also detectable levels were reported for chlordanes, hexachlorobenzene, dieldrin, p,p'-DDE, and p,p'-DDT.

Lipid determination was conducted on second collection samples and the average percent recoveries were: salmon 5.3%; walleye 3.4%; siscowet (fat trout) 17.5%; carp 8.4%; whitefish 6.8%; and whitefish liver 5.5% lipid.

Comparisons of Contaminant From Other Studies

Results of the analyses performed by the LSRI on composite samples of fillets from three species of fish (walleye, lake trout, and whitefish) collected from the management unit WI-2 in Lake Superior were compared with Wisconsin Departmentent of Natural Resources (WDNR) contaminant values for individual fillets of Lake Superior fish. LSRI values are consistent with WDNR determinations ***(Figures 4 through 6)***.

LSRI and the Great Lakes Indian Fish and Wildlife Commission (GLIFWC) routinely analyze fish fillets for total mercury and other persistant contaminants. Data from 1990 was reported by Gerstenberger *et al.* (1993), and those results indicated that the composite samples of Lake Superior fish (0.6 ppm walleye and 0.4 ppm siscowet) used in the present study are relatively low in mercury compared to many smaller inland northern Wisconsin lakes. Fish collected from inland Wisconsin lakes are routinely found to have from 1-2 ug/g mercury.

LSRI also analyzed nine addition siscowet trout for GLIFWC and our composite sample data for the contaminants in siscowet trout were confirmed ***(Table 4)***. The individual siscowet trout had similar levels of chlordanes, PCBs, and DDT(DDE) compared to the Red Cliff composite sample.

Discussion

The LS lake trout and whitefish apparently have relatively low contaminant levels well below FDA and Wisconsin advisory levels (Table 1) but above the NOEL for piscivorous wildlife (EPA, 1992). It should be noted that whitefish livers are considered a delicacy in the region, and due to the levels of contaminants identified in that tissue, it should be consumed with caution.

The lake trout had lower than expected levels of PCBs which may be explained in part that a preponderance of the lake trout are hatchery fish. As the hatchery-originated fish grow larger they will increase their contaminant burdens, and the subsequent health risk posed to consumers will increase.

The carp samples ranged from 1.404 to 3.0 ug/g of total PCBs with relatively large amounts of the alledged neurotoxic congeners. The differences in the total PCBs was likely due to the differences in filleting techniques (amount of fat removed), in the extraction method (GPC versus florisil) and GC analysis procedures (MSD versus ECD) and individual fish differences.

Mercury levels determined in LS walleye were slightly higher than advisory limits (0.6 ppm). It is likely that these fish were caught after migrating from the St. Louis river where walleye are known to have relatively high levels of mercury contamination (Schram *et al.*, 1992). Unfortunately, it is common to find walleye with 1-2 ppm mercury burdens in many small lakes of the upper Great Lakes.

Obviously, the bioaccumulation of persistent food chain contaminants and the resulting risks to human health cannot be completely discussed without considering the additive and/or synergistic effects of the various toxicants. The small amount of DDT detected in siscowet trout indicates that although this pesticide has been banned from production for nearly two decades, fresh sources are still impacting the Great Lakes.

It is important that the reader remember that similar contaminant patterns will be found in many other freshwater and oceanic fish. The lower Great Lakes typically are much higher in contaminant burdens than LS. However, contaminated fish is not unique to the Great Lakes. For example, mercury burdens in the midwest are not necessarily greater than elsewhere in the world. For example, a recent paper from Italy (Rezoni, 1992) reported that many saltwater species that are popular for human consumption from the Medi-

terranean region contained greater than 0.5 ug/g (ppm) mercury concentrations (*e.g.* lobster, shrimp, turbot, amberjack and swordfish).

It was beyond the financial resources of this project to attempt to measure the co-planar PCBs, dioxins, or other Great Lakes contaminants. However, the contaminants we measured, plus the ones we did not quantify, will continue to contribute to adverse health effects resulting from fish consumption. Reproductive problems have been noted for Apostle Island bald eagles which feed one step higher on the food chain than humans. They consume sea gulls which are reportedly even more contaminated than the LS fish described herein (Brander, pers. comm.). The composite samples for the LS and LLBM fish were above the NOEL established for piscivorous wildlife in the State of New York. Therefore, if substantial amounts of the diet consists of these fish, one could expect adverse health effects.

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[[*Cathy - Check references*]]

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15.0 APPENDICES

FISH CONTAMINANT DATA
TOTAL MERCURY AND TOTAL PCB

COMPOSITE SAMPLE DATA FISH COLLECTION - 1
(Samples run GC MSD)

	Sample Size N	Mean Length (cm)	Mean Weight (Kg)	Total Mercury (ug/g)	Total PCB (ug/g)
Lake trout	21	55.1	1.7	0.190	<0.300
Walleye	21	53.3	1.60	0.570	<0.300
Whitefish	42	46.5	0.90	0.055	<0.300
Carp	20	55.9	2.22	0.080	3.00
Salmon	NA	NA	NA	0.040	<0.300

COMPOSITE SAMPLE DATA FISH COLLECTION-2

(Samples run GC ECD; GPC Cleanup)

Lake trout	9	71.6	3.00	NA	NA
Walleye	12	58.7	2.40	0.580	0.193
Whitefish	8	47.0	0.86	0.060	0.154
WF liver	NA	NA	NA	0.130	0.244
Carp	15	63.5	3.08	0.090	1.404
Salmon	NA	NA	NA	0.040	0.017
Siscowet	32	52.3	1.27	0.450	0.370