To: Neil Kmiecik, Biological Services Director

From: Kory Groetsch, Environmental Biologist

Re: Contaminant Levels in Juvenile Lake Sturgeon from Lake Superior.

Date: November 27, 2001

This memo is to provide a record of contaminant levels in juvenile lake sturgeon captured in Lake Superior during 1998. First, attached is an edited copy of the poster presentation entitled "Contaminants in Juvenile Lake Superior Lake Sturgeon Muscle" which I gave at the 2000 national meeting of the Society of Environmental Toxicology and Chemistry (SETAC) in Nashville, Tennessee (Attachment 1).

Also attached is the laboratory report from the Lake Superior Research Institute which contains results of mercury testing (see Table 15 in Attachment 2). Finally, results of testing lake sturgeon samples for chlorinated organics by EnChem, Inc. are provided (Attachment 3).

cc. John Colemann Environmental Section Leader
Bill Mattes, Great Lakes Section Leader

ATTACHMENT 1
National SETAC 2000 Poster entitled
Contaminants in Juvenile Lake Superior Lake Sturgeon Muscle

PREFACE

This research was presented at the 2000 National meeting of the Society of Toxicology and Chemistry in Nashville, Tennessee. The title of the poster was *Contaminants in Juvenile Lake Superior Lake Sturgeon Muscle* authored by Groetsch, K.J.*;Mattes, W.P. *;Quinlan**,** H.R.¥ from *Great Lakes Indian Fish and Wildlife Commission, Odanah, WI and ¥U.S. Fish and Wildlife Service, Ashland, WI.

ABSTRACT

Lake sturgeon (*Acipenser fulvescens)* are important to the culture of the Lake Superior Chippewa (i.e., Anishinaabe). Contaminant data on Great Lakes lake sturgeon including Lake Superior are almost non-existent, which makes it difficult to evaluate their contaminant load relative to environmental or human health guidelines. The objective of this study was to analyze the muscle tissue of eleven juvenile Lake Superior lake sturgeon for bioaccumulative chemical contaminants. The eleven juvenile sturgeon ranged in age from 3 to 9 years old. Skin-off muscle samples were individually homogenized and tested for mercury (0.04-0.11 ppm). The individual homogenates were composited into four samples based on the age of sturgeon. Concentrations of benzene hexachloride (2-6 ppb), hexachlorobenzene (1-2 ppb), dieldrin (10-25 ppb), total chlordane (3-22 ppb), total DDT (3-16 ppb), total PCBs (20-50 ppb), and toxaphene (50-250 ppb) were detected in the muscle tissue samples. Contaminant concentrations increased with age and length.

INTRODUCTION

Lake sturgeon (*Acipenser fulvescens)* are long lived (>50 years), native to Lake Superior, and consume mussels, snails, crustaceans, insect larvae, and small fish. Lake sturgeon are also an historically important fish to the Lake Superior Chippewa (i.e., Anishinaabe) and are still harvested today on a limited basis for cultural and consumption purposes.

Sturgeon populations suffered significant declines through the 1800s and have since remained at low abundances. Recently, tribal, state and federal agencies have been studying and attempting to restore historic populations.

Questions exist regarding the role chemical contaminants may have in the slow recovery of these Great Lakes sturgeon populations as well as human health risks related to consumption. Currently, limited chemical contaminant data exists for lake sturgeon, which impedes our ability to begin evaluating these ecological and human health questions.

The objective of this study was to determine the concentrations of total mercury, polychlorinated biphenyls, and a suite of chlorinated pesticides in the muscle tissue of 11 juvenile lake sturgeon.

METHODS

During a 1999 Lake Superior lake sturgeon population assessment, 11 juvenile sturgeon that died were used for contaminant testing. Age of sturgeon was interpreted from fin rays and the sex was determined by examination of the gonads by the U.S. Fish and Wildlife Service in Ashland, WI. A skin-off muscle tissue sample was homogenized from each fish. Equal weights of tissue from each ground sample were combined to form three similar aged composites and one individual sample (Table 1). Samples from individual sturgeon were analyzed for total mercury by the Lake Superior Research Institute, UW-Superior, Superior, WI by cold vapor analysis with a detection limit of 30 µg/kg. Chlorinated organic analyses by EN CHEM, Inc., Madison, WI were conducted on composite samples using GC-ECD (Table 2):

Soxhlet Extraction: *Test Methods for Evaluating Solid Waste* 3rd ed. SW846 Method 3540C.

Lipid Determination: *Standard Methods for Examination of Water and Wastewater*. 18th ed. Method 5520

Gel Permeation Chromatography: *Test Methods for Evaluating Solid Waste* 3rd ed. SW846 Method 3640A. (separate lipids from pesticides)

Silica Gel Cleanup: *Silica Gel Cleanup*, EPA SW_846 Method 3630C. (separate PCBs and pesticides)

GC-ECD Analysis: Test Methods for Evaluating Solid Waste, 3rd Ed. SW846 method 8000B &8081A

RESULTS

1. Of the 11 sturgeon, 5 were males, 3 were females, and the sex of 3 could not be determined. Ages ranged from 3 to 9 years (Table 1).

2. Fifteen of 37 investigated analytes were detected in the Lake Superior lake sturgeon muscle tissue samples (Table 2). Mercury, PCBs and toxaphene had the highest concentrations (Figures 1, 2, 5)

3. With the exception of dieldrin, concentrations in the smaller and younger fish were below the limit of quantification but above the limit of detection. In addition, two mercury analyses were below the limit of detection.

4. Significant positive correlations were found between the chemical concentrations in the muscle tissue and the age (3 to 9 years) and length (55 to 100 cm) of the fish (Note: For composites, the average age and average length were used in the regressions. Also, similar chemicals were combined as shown in Table 2) (Figures 1 - 5).

Table 1. Age (years) and length (cm) data for individual and composite juvenile Lake Superior sturgeon samples.

Composite Number	Sex	Age (years)	Mean Age per Composite	Total Length (cm)	Average Length per Composite
1	---	3	3.5	49	56
	F	3		53	
	M	4		59	
	---	4		63	
2	M	5	5	52	64
	F	5		64	
	M	5		75	
3	M	6	6.3	67	74
	---	7		76	
	F	6		80	
4	M	9	9	99	99

Table 2. Chemicals detected (●) in lake sturgeon muscle samples.

Chemical	Detected	Chemical	Detected	Chemical	Detected
Total mercury	●	4,4'-DDT		Methoxychlor	
Aroclor 1016		4,4'-DDE[c]	●	Hexachlorobenzene	●
Aroclor 1221		4,4'-DDD		Pentachloroanisole	
Aroclor 1232		2,4'-DDT		α-benzene hexachloride[f]	●
Aroclor 1242		2,4'-DDE		β-benzene hexachloride	
Aroclor 1248		2,4'-DDD[c,d]	●	δ-benzene hexachloride	
Aroclor 1254[a,b]	●	Cis-Chlordane[e]	●	γ-benzene hexachloride[f]	●
Aroclor 1260[a]	●	Trans-Chlordane[e]	●	Toxaphene	●
Aldrin		Cis-nonachlor[e]	●	Endosulfan	
Dieldrin	●	Trans-nonachlor[e]	●	Endosulfan sulfate	
Endrin		Oxychlordane[e]	●	Heptachlor epoxide[g]	
Endrin Ketone		Mirex		Heptachlor	

[a]: Summed and reported as Total PCBs
[b]: Aroclor 1254 was only detected in the oldest sturgeon sample.
[c]: Summed and reported as Total DDT + metabolites
[d]: 2,4'-DDD only detected in the oldest sturgeon sample
[e]:Summed and reported as Total Chlordane
[f]:Summed and reported as Total BHC
[g]:Possibly detected, peak interference

DISCUSSION

This study provided Lake Superior specific contaminant data on sturgeon muscle that may be beneficial in evaluating potential impediments to the rehabilitation of sturgeon populations and potential human health risks due to contaminants. The types and concentrations of chemicals detected were similar to those reported for similar aged Lake Superior whitefish and herring (Groetsch *et al.* 1999, Brooke *et al.* 1999). All of these contaminants biomagnify through the food chain. Lake Superior sturgeon, whitefish and herring feed at a similar trophic level which may explain the similar contaminant concentrations found in their muscle tissues.

Contaminant concentrations increased in a linear manner with age and length (Figures 1 - 5). If the apparent linear increase in contaminant concentrations continued for 50 years, the adult sturgeon concentrations of mercury and PCB's would reach 700 ppb and 600 ppb, respectively. These concentrations would exceed state and federal fish consumption advisory guidelines. The potential impact on sturgeon growth, reproduction, and survival is unknown and requires further study. It must be emphasized that this type of extrapolation beyond the age range of this data is highly speculative. Contaminant testing of adult sturgeon muscle is necessary to determine the contaminant levels and if predicted levels based on juvenile sturgeon are reliable.

Literature Cited:

Groetsch, K.J., L.T Brooke, and W.P Mattes. 1999. Comparing PCB, HCB, Lindane, and Mercury in Commercially Processed Filets from Lake Superior Lake Trout, Whitefish, and Herring to U.S. FDA Guidelines. Poster presented at National Society of Environmental Toxicology and Chemistry, Philadelphia, PA.

Brooke, L.T., K.J. Groetsch, and W.P. Mattes. 1999. Superior and Comparison of concentrations between Species, Capture Location, and Age at Capture with FDA Guidelines. Poster presented at National Society of Environmental Toxicology and Chemistry, Philadelphia, PA.

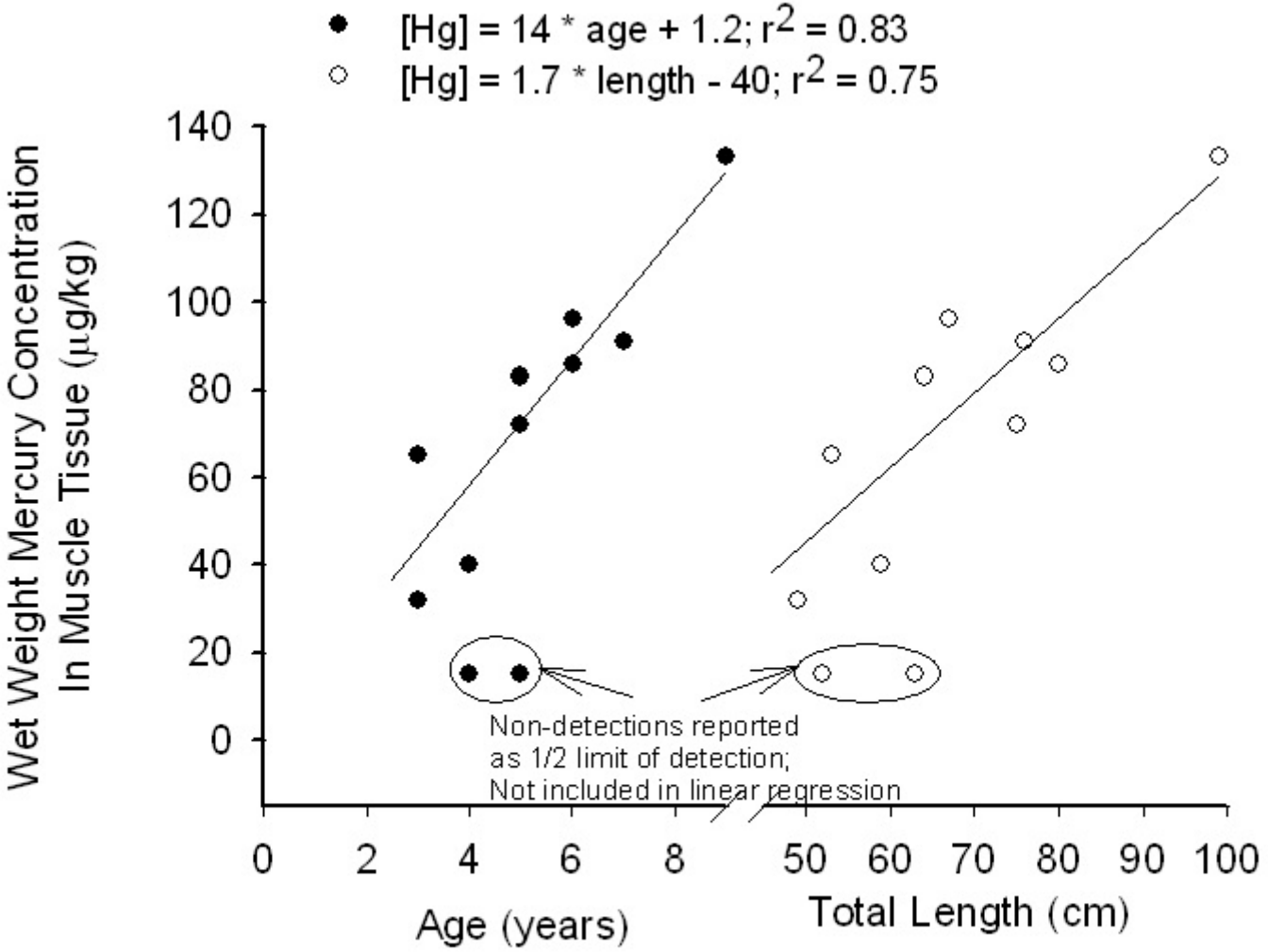

Figure 1. Linear regression of mercury concentrations in lake sturgeon muscle versus age (years) and length (cm).

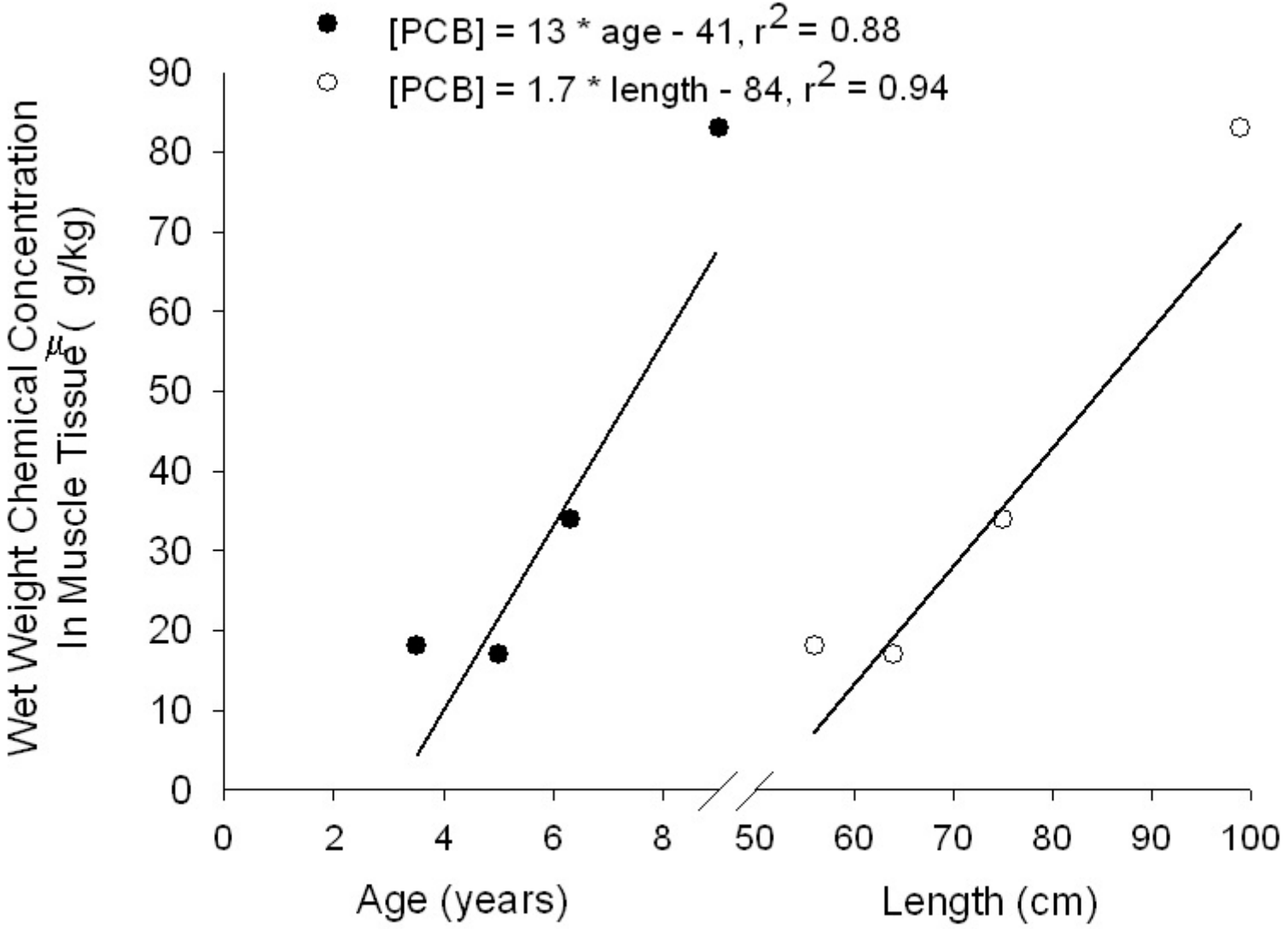

Figure 2. Linear regression of lake sturgeon muscle polychlorinated biphenyls (PCB) concentrations versus average age and length of the sturgeon samples.

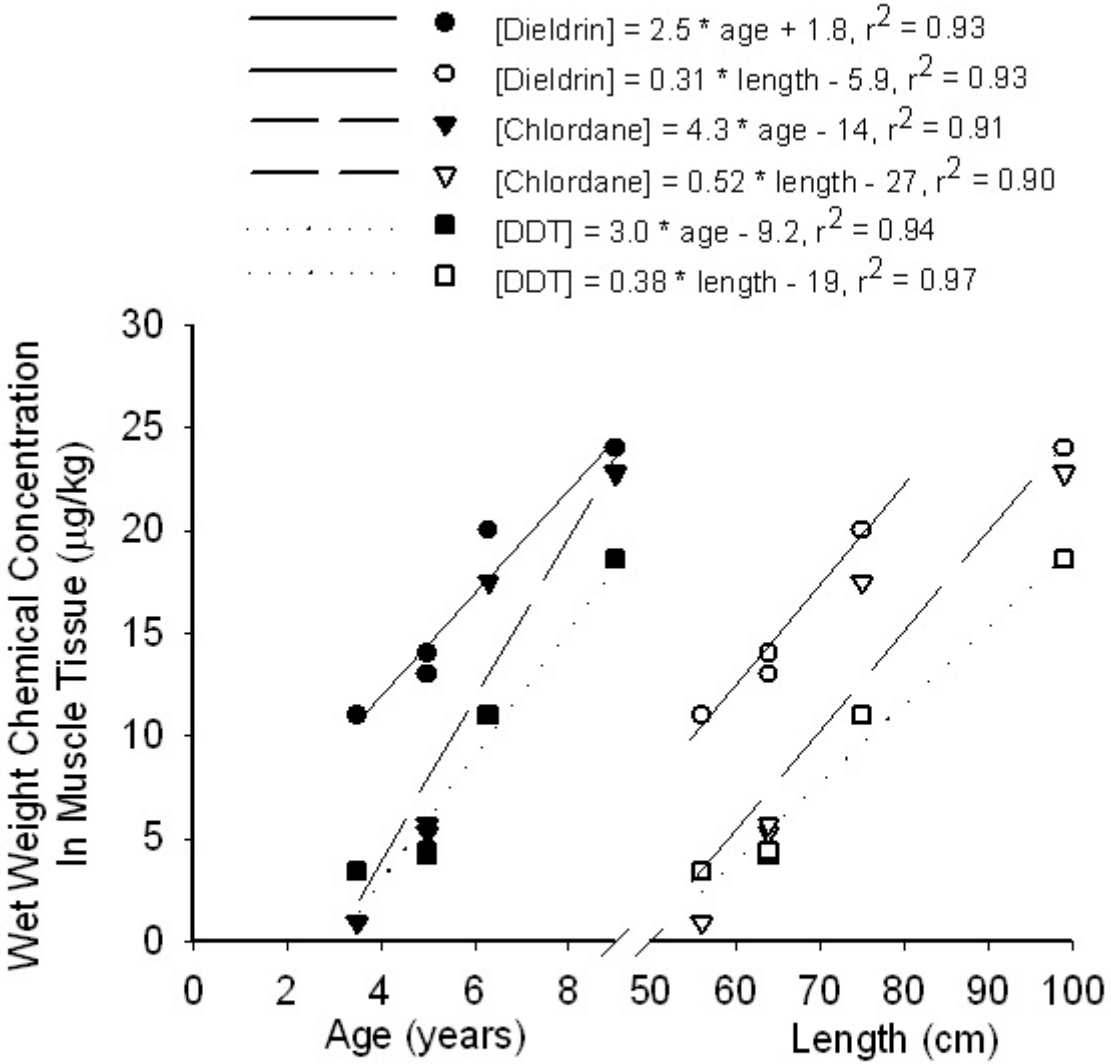

Figure 3. Linear regression of lake sturgeon muscle dieldrin,total DDT plus metabolites, and total chlordane concentrations versus average age and length of the sturgeon samples.

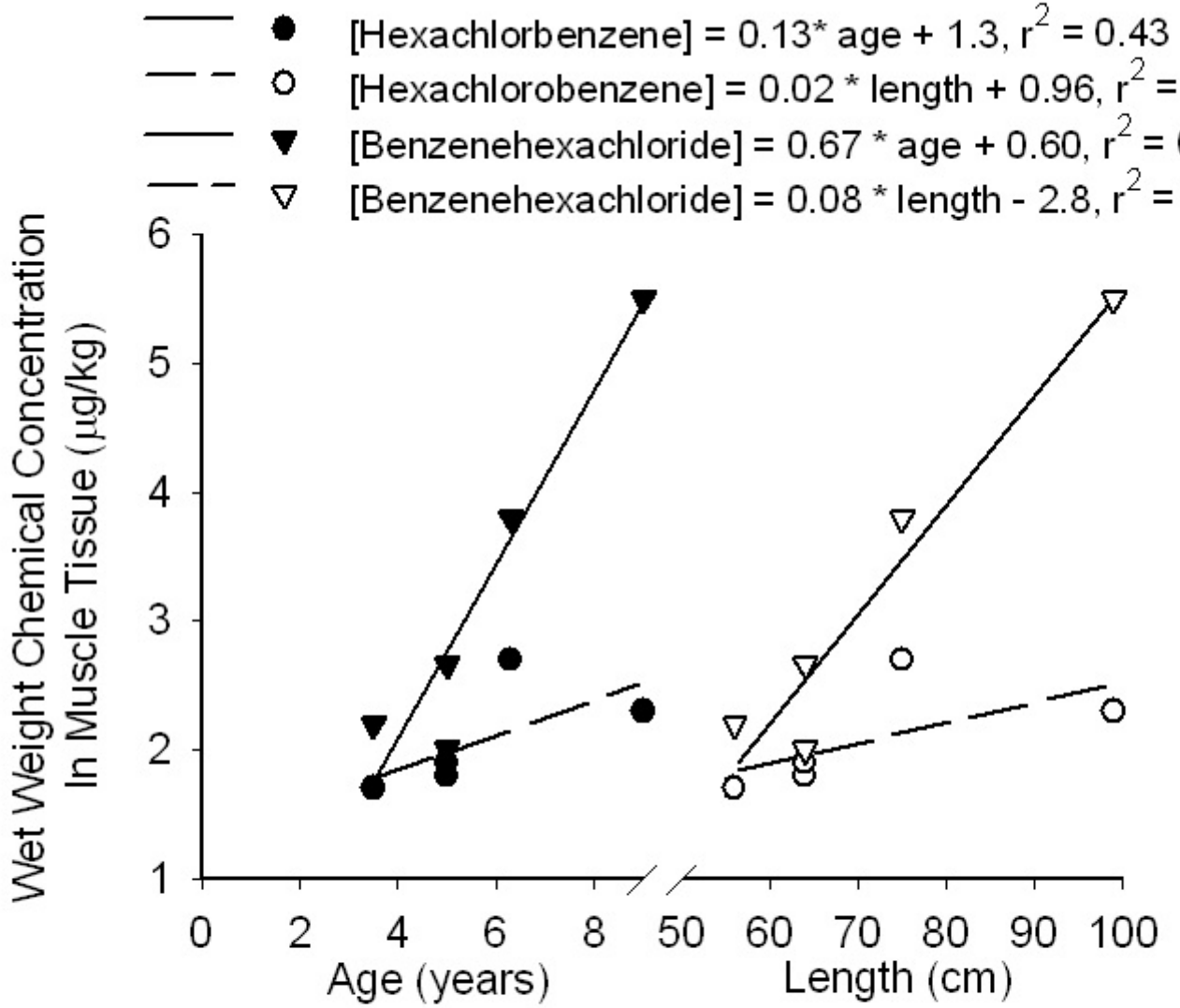

Figure 4. Linear regression of lake sturgeon muscle hexachlorobenzene and benzene hexachloride concentrations versus average age and length of the sturgeon samples.

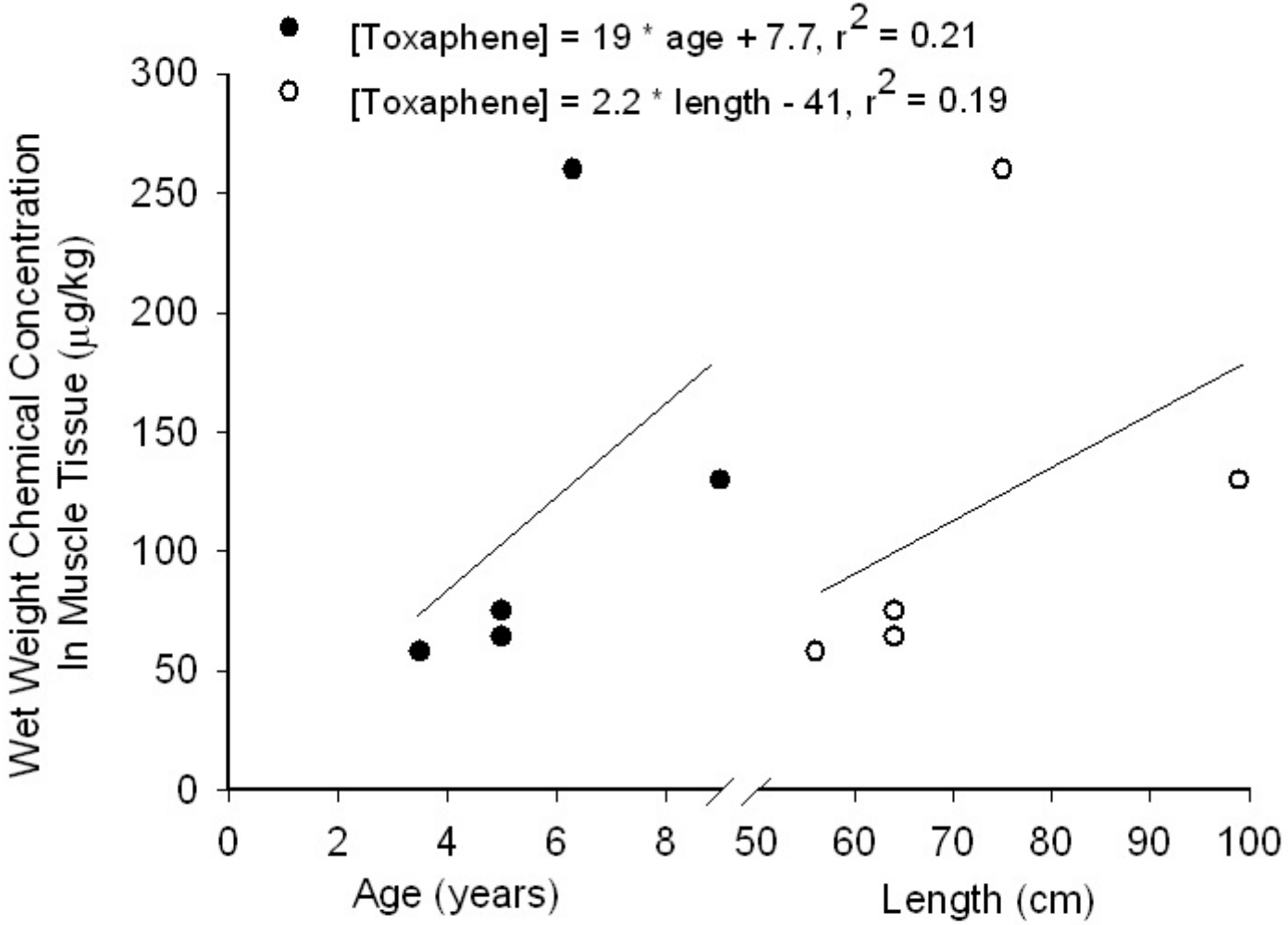

Figure 5. Linear regression of lake sturgeon muscle toxaphene concentrations versus the average age and length of the sturgeon samples.

ATTACHMENT 2

Lake Superior Research Institute report entitled

Analysis of 1998 Captured Walleye and Lake Sturgeon
from Ceded Territories for Total Mercury and Selenium

Analysis of 1998 Captured Walleye and Lake Sturgeon from Ceded Territories for Total Mercury and Selenium

for

Great Lakes Indian Fish and Wildlife Commission
P.O. Box 9
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by

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May 1999

Introduction

Fillets from walleye (*Stizostedion vitreum*) captured during the spring of 1998 from seventeen Wisconsin inland lakes within the ceded territories were analyzed for total mercury content. Samples of eggs and sperm (milt) were collected from walleye captured from Kentuck Lake in Wisconsin and analyzed for total mercury content. Walleye fillets from Lake Superior captured during the fall were analyzed for mercury and selenium content, and lake sturgeon (*Acipenser fulvescens*) incidentally captured in June 1998 from the Bad River by biologists of the U.S. Fish and Wildlife Service were analyzed for total mercury in the muscle tissues. The samples were delivered to the Lake Superior Research Institute (LSRI) of the University of Wisconsin-Superior for analysis. The analyses were conducted during June through February 1999.

Methods

At the time fish were captured, a tribal wardens or biologist was present to determine the sex and measure the total length of each fish. A tag with a unique number (i.e. fish identification number (FIT)) was attached through the mouth of each fish captured. The walleye were immediately placed on ice and frozen within 36 hr of capture. Tagged walleye from a single lake were placed into large plastic bags labeled with the lake nameLake sturgeon were eviscerated at the time of capture, sexed, measured for total length, placed individually into plastic bags, labeled, and frozen. Before delivery to the LSRI laboratory, the heads of the lake sturgeon were removed in order to collect otoliths to age the fish. At the LSRI laboratories, the walleye fillets and lake sturgeon were received with a list of the samples (chain of custody documentation) and examined for accuracy by GLIFWC and LSRI staff. The samples were stored in freezers at approximately -18 °C (-23 to -15) temperatures until removed and thawed for processing and analysis.

Prior to analysis of the fish tissue, all glassware, utensils, and grinders were cleaned according to the appropriate methods (Appendices B and C). Each day, the fish that would be processed were removed from the freezer and allowed to warm to a flexible, but stiff, consistency. Each fish had one fillet removed that was ground in a grinder three times with a small amount of the initial tissue which passed through the grinder collected and discarded (Appendix D). Skin was also removed from the fillet and discarded before grinding. A sub-sample of the ground tissue was placed into a glass vial and frozen until the mercury analysis was conducted. The grinder was disassembled after each fillet was ground and the unit was washed according to the grinder cleaning SOP (Appendix C).

Lake sturgeon were processed by cutting the section of the body trunk between the pectoral and pelvic girdles (transverse cut from the dorsal to ventral sides of the fish). The notochord, internal organs, and skin were removed from the steak before grinding the flesh in the same manner that the walleye were processed (Appendix D). Fat was not trimmed from the muscle and was included in the analysis. The length of the body section used for analysis ranged from 146 to 259 mm with the exception of fish identified as 06 which had most of its body missing and only 60 mm behind the head was used for analysis.

Samples of the fish tissues were weighed according to SOP SA/11 (Appendix E) for fish sample analysis in preparation for analysis. Solutions of mercury for making spikes of tissue and preparing the standards for analysis were prepared by the procedures in Appendix F Analysis was performed with an Instrumentation Laboratory Atomic Absorption Spectrophotometer Model Video 12 for the walleye from inland lakes and a Varian SpectraAA 200 Atomic Absorption Spectrophotometer for walleye from Lake Superior and the lake sturgeon according to Appendix G for cold vapor mercury determination.

Selenium was analyzed by Inductively Coupled Plasma (ICP) methods at the laboratories for EnChem, Inc., 525 Science Drive, Madison, WI. The method of analysis followed the U.S. EPA Method 6010B (Test Methods for Evaluating Solid Waste: Physical/Chemical Methods: Integrated Manual, U.S. Environmental Protection Agency, Office of Solid Waste and Emergency Response, June 1997, SW-846 final update III, PB97-156111). Prior to analysis, the samples were prepared through digestion at the LSRI laboratories with the methods described in Appendix H. Digested samples were shipped on ice to Enchem, Inc. and analyzed for selenium content.

Quality Assurance

Quality of analysis was monitored by four methods: Analysis of similar fish tissues before and after the tissue preparation process; analysis of the marine dogfish from the Canadian government that has a known concentration of mercury; duplicate analysis of fish tissue from the same fillet; and analysis of tissue with known concentrations that have been spiked with mercury. In addition, solutions with known concentrations of mercury were analyzed along with each batch of tissue that was analyzed. These analytical standard solutions contained 0, 25, 50, 100, 200, and 300 ng of mercury. They were prepared from a mercuric chloride stock solution.

A commercial canned tuna fish (*Thunnus* sp.) sample was used as check on the grinding process. One portion of each can was transferred directly to a sample bottle. The second portion was processed through the grinding process in the same manner as the walleye and lake sturgeon fillets. This check was made to ensure that no contamination or loss of mercury or selenium was occurring in the grinding process. Analysis of the canned tuna fish from two occasions coincident with the analysis of inland lake caught walleye gave 87.8 ±4.6% agreement (Table 1). Analyses that coincided with the analysis of Lake Superior caught walleye and Bad River lake sturgeon gave 92.1 ±8.6% agreement for mercury and 91.5 percent agreement for selenium (Table 2).

Table 1. Percent Agreement of Procedural Blank Samples [Commercially Purchased Tuna Fish(*Thunnus* sp.) Samples Before and After Grinding] for Mercury Analyses Conductedwith Inland Lake Caught Walleye.

Date of Analysis	Before Grinding (µg Hg/g tissue)	After Grinding (µg Hg/g tissue)	Percent Agreement
7/9/98	<0.034	0.051	n.a.
8/18/98	0.072	0.079	91.1
8/31/98	0.091	0.077	84.6

Table 2. Percent Agreement of Procedural Blank Samples [Commercially Purchased Tuna Fish (*Thunnus* sp.) Samples Before and After Grinding] for Mercury and Selenium Analyses Conducted with Lake Superior Caught Walleye and Lake Sturgeon.

Date of Analysis	Before Grinding (µg/g tissue)	After Grinding (µg/g tissue)	Percent Agreement
12/30/98	0.055	0.056	98.2[a]
2/12/99	0.062	0.072	86.1[a]
3/24/99	0.751	0.821	91.5[b]

[a] Results for mercury analysis.
[b] Results for selenium analysis.

Analysis of the dogfish (*DORM-1, Squalus acanthias*) tissue (certified reference material from National Research Council Canada, Ottawa, Ontario) of known concentration was conducted twenty-seven times during the analysis of the inland lake walleye fillets (Table 3) and two times during the analysis of the Lake Superior walleye and lake sturgeon analyses (Table 4). The expected mercury concentration value for the dogfish tissue was 0.798 ±0.074 µg/g. The grand mean and standard deviation for the analysis during the inland lake walleye study was 0.803 ±0.039 µg/g and 0.804 ±0.089 µg/g during the analysis of the Lake Superior walleye and Bad River lake sturgeon. The expected and measured mercury values varied by 0.623 percent. This result was considered adequate agreement with the expected value.

Table 3. Results of Mercury Analysis (µg/g) of Dogfish Shark Tissue Supplied by the NationalResearch Council Canada (DORM-1) that was Coincident with the Analysis of InlandLake Caught Walleye. The Tissue has a Known Concentration of Mercury of 0.798µg Hg/g ±0.074.

Date of Analysis	#1	#2	#3	Mean	Std.Dev.
6/12/98	0.768	0.737	0.794	0.766	0.029
7/9/98	0.828	0.773	0.826	0.809	0.031
7/14/98	0.747	0.687	0.928	0.787	0.125
7/21/98	0.795	0.814	0.855	0.821	0.031
7/29/98	0.732	0.906	0.897	0.845	0.098
8/11/98	0.655	0.750	0.944	0.783	0.147
8/14/98	0.861	0.784	0.911	0.852	0.064
8/18/98	0.727	0.736	0.736	0.733	0.005
8/31/98	0.681	0.884	0.915	0.827	0.127
			Grand Mean	0.803	0.039

Table 4. Results of Mercury Analysis (µg/g) of Dogfish Shark Tissue Supplied by the National Research Council Canada (DORM-1) that was coincident with the analysis of Lake Superior Caught Walleye and Bad River Caught Lake Sturgeon. The Tissue has a Known Concentration of Mercury of 0.798 µg Hg/g ±0.074.

Date of Analysis	#1	#2	#3	Mean	Std.Dev.
12/30/98	0.935	0.724	0.897	0.852	0.112
2/12/99	0.748	0.750	0.768	0.755	0.011
		Grand Mean		0.804	0.089

Walleye tissues from seventeen fish captured from inland lakes of the ceded territories were analyzed twice. They were processed as two separate samples of the same fish. Agreement between two mercury analyses of the same fish was 89.4 ±8.8 percent (Table 5). Agreement between two mercury analyses for Lake Superior captured walleye was measured in three fish and averaged 87.0 ±3.5 percent (Table 6). Agreement between two selenium analyses for Lake Superior captured walleye was 93.1 ±6.1 percent (Table 7). Duplicate analyses for mercury and selenium in lake sturgeon were not measured.

Table 5. Percent Agreement Between Duplicate Analysis for Mercury Content in Skinless Fillet Tissue of Walleye Captured from Ceded Territories Inland Waters during 1998.

Date of Analysis	Sample Capture Location and Identification	Percent Agreement
6/12/98	East Chippewa Flowage 680	97.8
6/12/98	Parent Lake 3293	98.2
7/9/98	Crab Lake 838	77.2
7/9/98	Gogebic Lake 624	77.1
7/14/98	Nelson Lake 632	80.4
7/14/98	Turtle-Flambeau Flowage 812	100.0
7/21/98	Bearskin Lake 1469	86.4
7/29/98	Namekagon 1389	99.7
7/29/98	Kentuck 2062 E	78.9
8/11/98	Kentuck 1185	92.8
8/11/98	Mille Lacs 861	88.1
8/14/98	Bass Lake 1014	95.0
8/14/98	Sherman Lake 848	76.1

8/18/98	Big Lake 383	84.5
8/18/98	Upper Eau Claire 660	96.8
8/31/98	Pelican Lake 2091	96.2
8/31/98	West Chippewa Flowage 688	94.1
	Mean	89.4 ±8.8

Table 6. Percent Agreement Between Duplicate Analysis for Mercury Content in Skinless Fillet Tissue of Walleye Captured from Lake Superior during the Fall of 1998.

Date of Analysis	Sample Identification	Percent Agreement
12/30/98	Walleye 3002	83.1
12/30/98	Walleye 3014	87.8
2/12/99	Walleye 3032	90.0
	Mean	87.0 ±3.5

Table 7. Percent Agreement Between Duplicate Analysis for Selenium Content in Skinless Fillet Tissue of Walleye Captured from Lake Superior during the Fall of 1998.

Sample Identification	Percent Agreement
Walleye 3015	88.8
Walleye 3027	97.4
Mean	93.1 ±6.1

Digested tissues from seventeen skinless walleye fillets from inland waters of the ceded territories that had analyzed mercury values were spiked with a known quantity of mercury and analyzed for recovery of the spiked mercury (Table 8). Grand mean and standard deviation of the recovery was 99.1 ±11.7 percent. The recovery of mercury spiked into Lake Superior captured walleye was 77.1 ±6.7 percent (Table 9), and was 77.3 ±2.5 percent for Bad River captured lake sturgeon (Table 10). Selenium was spiked into walleye captured from Lake Superior and averaged 107.5 ±3.7 percent (Table 11).

The minimum detection limit for mercury was seven ng for the method used in this study (Appendix G). Analyses of sample sets were considered acceptable when the mean value obtained for the dogfish reference samples from a set fell within the expected (0.798 ±0.074 µg/g) limits for the reference sample.

Table 8. Percent of Mercury Recovered from Walleye Samples Spiked with a Known Quantity of Mercury Coincident with the Analysis of Inland Lake Caught Walleye.

Date of Analysis	Sample Location and Identification	Spike #1	Spike #2	Spike #3	Mean	Std. Dev.
6/12/98	East Chippewa Flowage 680	65.7	106.2	95.9	89.3	21.0
6/12/98	Parent Lake 3293	138.1	94.2	93.1	108.5	26.7
7/9/98	Crab Lake 838	106.6	93.9	91.4	97.3	8.2
7/9/98	Gogebic Lake 624	76.4	54.9	75.3	68.9	12.1
7/14/98	Nelson Lake 632	110.3	100.8	118.8	109.9	9.0
7/14/98	Turtle-Flambeau Flowage 812	103.8	84.4	81.2	89.8	12.2
7/21/98	Bearskin Lake 1469	126.5	110.5	34.5	90.5	49.5
7/21/98	Upper St. Croix 1485	117.9	99.5	92.6	103.3	13.1
7/29/98	Namekagon 1389	126.2	91.8	87.3	101.8	21.3
8/11/98	Kentuck 1185	95.4	130.5	103.2	109.7	18.4
8/11/98	Mille Lacs 861	114.7	106.2	119.6	113.5	6.8
8/14/98	Bass Lake 1014	81.7	111.2	121.1	104.7	20.5
8/14/98	Sherman Lake 848	114.9	103.6	0*	109.3	8.0
8/18/98	Big Lake 383	78.2	81.3	97.7	85.7	10.5
8/18/98	Upper Eau Claire 660	94.3	92.6	87.5	91.5	3.6
8/31/98	Pelican Lake 2091	85.9	107.7	116.4	103.3	15.7
8/31/98	West Chippewa Flowage 688	112.6	87.6	124.3	108.2	18.7
				Grand Mean	99.1	11.7

* Sample lost.

Table 9. Percent of Mercury Recovered from Skinless Walleye Fillet Samples Spiked with a Known Quantity of Mercury Coincident with the Analysis of the Lake Superior Walleye

Date of Analysis	Sample	Spike #1	Spike #2	Spike #3	Mean	Std.Dev.
12/30/98	W 3002	72.1	25.9	110.3	69.4	42.3
12/30/98	W 3014	99.9	61.4	80.7	80.7	19.3
2/12/99	W 3032	69.3	108.2	66.4	81.3	23.3
				Grand Mean	77.1	6.70

Table 10. Percent of Mercury Recovered from Skinless Bad River Lake Sturgeon Fillet Samples Spiked with a Known Quantity of Mercury.

Date of Analysis	Sample Identification	Spike #1	Spike #2	Spike #3	Mean	Std. Dev.
2/12/99	Sturg- 06	53.8	87.4	90.6	77.3	20.5

Table 11. Percent Recovery of Selenium from Skinless Fillet Walleye Samples Spiked with a Known Quantity of Selenium.

Sample Identification	Percent Recovery
Walleye 3015	110.1
Walleye 3027	104.9
Mean	107.5 ±3.7

Results

Skinless fillets of 159 walleye from seventeen lakes in Wisconsin, Minnesota, and Michigan were analyzed for total mercury content (Table 12). The fish were measured for total length in the laboratory before filleting and sexed during the filleting process. Total mercury concentrations ranged from 0.035 to 1.579 µg/g (parts per million) in muscle tissue from the samples.

Table 12. Concentrations (Parts per Million) of Mercury (Hg) from Skinless Walleye Fillets Captured from Inland Waters of Ceded Territories during the Spring of 1998.

Lake of Capture	Sample Number	Length (Inches)	Sex	µg Hg/g of tissue
Ballard L.	1	13.2	M	0.371
Ballard L.	2	14.3	M	0.458
Ballard L.	3	14.5	M	0.508
Ballard L.	4	14.1	M	0.422

Ballard L.	5	14.7	M	0.292
Ballard L.	10	14.5	M	0.505
Bass (Patterson) L.	1017	11.1	M	0.184
Bass (Patterson) L.	1018	13.4	M	0.156
Bass (Patterson) L.	1019	12.6	M	0.177
Bass (Patterson) L.	1016	15.2	M	0.397
Bass (Patterson) L.	1015	17.3	M	0.601
Bass (Patterson) L.	1014	15.1	M	0.274
Bearskin L.	1462	16.3	F	0.217
Bearskin L.	1463	23.9	F	0.564
Bearskin L.	1464	22.3	M	0.391
Bearskin L.	1465	23.5*	F	0.486
Bearskin L.	1467	19.8	M	0.161
Bearskin L.	1469	15.7	M	0.295
Bearskin L.	1470	20.2	F	0.442
Bearskin L.	1472	15.8	F	0.377
Bearskin L.	1473	10.8	M	0.127
Bearskin L.	1474	11.1	M	0.052
Bearskin L.	1475	11.3*	M	0.103
Big L.	380	10.5*	M	0.596
Big L.	381	10.9	M	0.487
Big L.	382	12.2	M	0.302
Big L.	383	13.4	M	0.492
Big L.	384	14.6	M	0.814
Big L.	385	14.6*	M	0.393
Chippewa Fl. (West Side)	687	11.8*	M	0.682
Chippewa Fl. (West Side)	688	15.6	M	0.230
Chippewa Fl. (West Side)	689	15.8	M	0.270
Chippewa Fl. (West Side)	698	16.3	M	0.322

Chippewa Fl. (West Side)	699	12.4	M	0.323
Chippewa Fl. (West Side)	700	12.4	M	0.303
Chippewa Fl. (East Side)	679	15.0	M	0.256
Chippewa Fl. (East Side)	680	15.8	M	0.413
Chippewa Fl. (East Side)	681	14.0	M	0.807
Chippewa Fl. (East Side)	682	11.7	M	0.361
Chippewa Fl. (East Side)	683	11.6	M	0.555
Chippewa Fl. (East Side)	684	11.6	M	0.566
Crab L.	833	12.6	M	0.545
Crab L.	834	14.1	F	0.668
Crab L.	835	12.0	M	0.374
Crab L.	836	11.7	M	0.252
Crab L.	837	14.1	M	0.742
Crab L.	838	12.9	M	0.237
Gogebic L.	623	11.8	M	0.112
Gogebic L.	621	11.6	M	0.093
Gogebic L.	620	11.9	M	0.185
Gogebic L.	624	16.6	M	0.515
Gogebic L.	611	15.4	M	0.212
Gogebic L.	622	15.8	M	0.253
Gogebic L.	619	17.8	M	0.389
Gogebic L.	618	17.8	M	0.571
Gogebic L.	617	18.4	M	0.544
Gogebic L.	615	22.5	M	0.430
Gogebic L.	613	31.2	F	1.332
Kentuck L.	2062	28.6	F	1.381

Kentuck L.	1181	22.8	F	0.969
Kentuck L.	2097	26.8	F	0.775
Kentuck L.	1450	25.2	F	1.228
Kentuck L.	1185	22.5	F	0.631
Kentuck L.	1189	20.3	F	0.376
Mille Lacs L.	855	16.6	M	0.059
Mille Lacs L.	856	17.1	M	0.095
Mille Lacs L.	857	17.3	M	0.035
Mille Lacs L.	858	18.4	M	0.092
Mille Lacs L.	859	19.3	M	0.116
Mille Lacs L.	860	18.1	M	0.116
Mille Lacs L.	861	14.3	M	0.039
Mille Lacs L.	862	14.3	M	0.070
Mille Lacs L.	863	13.0	M	0.063
Mille Lacs L.	865	27.6	F	0.206
Mille Lacs L.	867	22.0	M	0.176
Mille Lacs L.	868	22.5	M	0.281
Namekagen L.	1386	12.5	M	0.217
Namekagen L.	1387	13.2	M	0.274
Namekagen L.	1388	15.3	M	0.424
Namekagen L.	1389	14.1*	M	0.354
Namekagen L.	1390	18.1	F	0.306
Namekagen L.	1391	18.6	F	0.265
Namekagen L.	1395	25.0*	F	0.920
Namekagen L.	1396	17.6*	M	0.642
Namekagen L.	1397	12.5	M	0.128
Namekagen L.	1398	15.6	M	0.548
Nelson L.	625	12.3	M	0.141
Nelson L.	626	12.5	M	0.190
Nelson L.	627	12.3	M	0.198
Nelson L.	628	16.1	F	0.277

Nelson L.	629	23.9	F	0.806
Nelson L.	630	19.1	F	0.575
Nelson L.	631	16.5	M	0.738
Nelson L.	632	15.3	F	0.175
Nelson L.	633	20.5	F	0.452
Nelson L.	634	21.6	F	0.561
Nelson L.	635	24.1	F	0.621
Parent L.	3287	13.5	M	0.282
Parent L.	3288	17.5	M	0.598
Parent L.	3289	14.7	M	0.328
Parent L.	3290	12.4	M	0.268
Parent L.	3291	18.1	M	0.802
Parent L.	3292	14.3	M	0.344
Parent L.	3293	15.9	M	0.789
Parent L.	3286	11.7	M	0.159
Pelican L.	2079	20.0	F	0.389
Pelican L.	2080	19.0	F	0.201
Pelican L.	2081	12.5	M	0.245
Pelican L.	2082	16.7	M	0.396
Pelican L.	2083	16.2	M	0.171
Pelican L.	2084	13.2	M	0.162
Pelican L.	2085	17.3	M	0.336
Pelican L.	2086	21.8	M	0.665
Pelican L.	2087	21.8	M	0.282
Pelican L.	2088	21.5	F	0.371
Pelican L.	2091	15.7	M	0.256
Pelican L.	2092	13.7	F	0.152
Sherman L.	841	20.3*	F	0.687
Sherman L.	842	12.2	F	0.277
Sherman L.	843	13.2	M	0.274
Sherman L.	844	22.7	F	0.373

Sherman L.	845	24.1	F	1.072
Sherman L.	846	26.9	F	1.478
Sherman L.	847	17.9	M	0.494
Sherman L.	848	15.2	M	0.491
Sherman L.	849	16.1	M	0.380
Sherman L.	850	20.7	F	0.404
Sherman L.	854	11.0	M	0.258
Sherman L.	851	20.8	F	0.697
Turtle-Flambeau Fl.	819	18.2	M	1.178
Turtle-Flambeau Fl.	820	18.3	M	1.261
Turtle-Flambeau Fl.	812	15.1	M	0.705
Turtle-Flambeau Fl.	813	14.2	M	0.764
Turtle-Flambeau Fl.	814	14.5	M	0.663
Turtle-Flambeau Fl.	815	14.9	M	0.897
Turtle-Flambeau Fl.	816	16.4	M	0.762
Turtle-Flambeau Fl.	817	15.0	M	1.313
Turtle-Flambeau Fl.	818	17.8	M	1.579
Upper Eau Claire L.	655	14.8	M	0.417
Upper Eau Claire L.	656	19.7	M	0.630
Upper Eau Claire L.	657	18.5	M	0.544
Upper Eau Claire L.	658	16.0	M	0.392
Upper Eau Claire L.	659	16.5	M	0.393
Upper Eau Claire L.	660	18.3	M	0.464
Upper Eau Claire L.	661	24.2	F	0.887
Upper Eau Claire L.	662	14.7*	M	0.324
Upper Eau Claire L.	663	10.5	M	0.253
Upper Eau Claire L.	664	12.3	M	0.189
Upper Eau Claire L.	666	27.7	F	0.905
Upper Eau Claire L.	667	25.4	F	0.629
Upper St. Croix L.	1481	16.6	M	0.293
Upper St. Croix L.	1482	10.8	M	0.117

Upper St. Croix L.	1483	17.1	M	0.463
Upper St. Croix L.	1484	14.2	M	0.122
Upper St. Croix L.	1485	14.4	M	0.134
Upper St. Croix L.	1486	18.2	F	0.342
Upper St. Croix L.	1487	17.4	F	0.401
Upper St. Croix L.	1495	18.0	M	0.490
Upper St. Croix L.	1496	17.5	M	0.463

* The lengths recorded in this table are those that were measured in this lab prior to grinding. The lengths marked with an asterisk indicate that the fish tail was frayed which would result in an inaccurate reading.

Walleye captured from Lake Superior during October 1998 were analyzed for total mercury and selenium in the skinless fillets. Mercury concentrations ranged from 0.146 to 1.39 µg/g (parts per million) in the fillets (Table 13). Selenium concentration ranged from 0.486 to 0.713 µg/g in the fillets (Table 13).

Table 13. Mercury and Selenium Concentrations (Parts per Million) in Walleye Captured fromLake Superior (Michigan Fish Management Unit MI-4) during the Fall of 1998.

Monel Tag Number	Fish Length (inches)	Sex	Date Collected	Concentration (µg Se/g)	Concentration (µg Hg/g)
3001	25.5	F	10/17/98	0.662	0.754
3002	24.5	F	10/17/98	0.652	0.700
3003	28.2	F	10/17/98	0.634	1.39
3004	23.4	F	10/17/98	0.708	0.415
3005	19.4	F	10/17/98	0.687	0.247
3006	18.9	M	10/17/98	0.701	0.185
3010	23.2	F	10/17/98	0.628	0.476
3011	21.6	F	10/17/98	0.713	0.373
3012	21.8	M	10/17/98	0.554	0.283
3013	21.3	M	10/17/98	0.609	0.774
3014	20.4	F	10/17/98	0.698	0.308
3015	19.1	F	10/17/98	0.573	0.416
3016	17.6	M	10/17/98	0.653	0.146
3017	18.2	M	10/17/98	0.602	0.159

3018	16.4	M	10/17/98	0.541	0.218
3019	22.2	M	10/17/98	0.599	0.296
3024	27.5	F	10/22/98	0.544	0.791
3025	26.2	F	10/22/98	0.658	0.779
3026	25.9	F	10/22/98	0.500	0.763
3027	25.7	F	10/27/98	0.486	0.889
3029	27.2	F	10/23/98	0.624	0.918
3030	27.6	F	10/23/98	0.631	0.806
3031	27.3	F	10/26/98	0.604	0.972
3032	27.3	F	10/28/98	0.584	0.612

An attempt was made to measure total mercury in walleye eggs and sperm (milt) collected from fish captured from Kentuck Lake, Wisconsin (Table 14). It was possible to measure mercury concentrations in eggs with concentrations ranging from <0.005 to 0.019 µg/g (parts per million). The eggs have a much lower concentration of mercury than the muscle tissue which may be reflective of the difference in the protein concentrations of the two tissues. Mercury in the sperm was not of sufficient quantity to achieve the minimum detectable quantity of mercury (7 ng); therefore, measurements could not be detected.

Table 14. Mercury Concentrations (Parts per Million) in Eggs and Sperm from Walleye Captured from Kentuck Lake, Wisconsin during Spring 1998. Sperm Volumes were too Low to Achieve Measurable Quantities for Mercury.

Lake and Sample Identification	g Hg/g
Eggs	
Kentuck 1181 E	0.011
Kentuck 1185 E	0.007
Kentuck 1189 E	<0.005
Kentuck 1450 E	0.015
Kentuck 2062 E	0.019
Kentuck 2097 E	0.010
Sperm (Milt)	
Kentuck 2097 M	<0.024
Kentuck 2062 M	<0.025
Kentuck 1182 M	<0.035
Kentck 1185 M	<0.034

Kentuck 1189 M		<0.034

Total mercury concentrations were measured in lake sturgeon captured from the Bad River, Ashland County, Wisconsin. Eleven fish were analyzed with mercury concentrations (Table 15) in skinless fillet tissues ranging from <0.028 to 0.133 µg/g (parts per million). There is a tendency for the concentration of mercury to increase in the muscle tissue with size and age.

Table 15. Mercury Concentrations (Parts per Million) in Lake Sturgeon from the Bad River Juvenile Lake Sturgeon Assessment.

Sample Identification	Fish Size (mm)	Age (years)	Date Collected	Concentration (µg Hg/g)
01	529	III	6/26/98	0.065
02	747	V	6/26/98	0.072
03	764	VII	6/26/98	0.091
04	639	V	6/26/98	0.083
05	803	VI	6/26/98	0.086
06	994	IX	6/26/98	0.133
07	670	VI	6/26/98	0.096
08	631	IV	6/26/98	<0.029
09	593	IV	6/26/98	0.040
10	524	V	6/26/98	<0.028
11	494	III	6/26/98	0.032

APPENDIX A

PROCEDURES FOR COLLECTING, PREPARING AND TRANSPORTING FISH SAMPLES

INTRODUCTION

This SOP includes general guidelines for the collection of fish samples at the study sites, preparing the specimens as samples, wrapping and labeling samples, preservation, and transportation to the laboratory for further studies. Species of fish collected may vary, and the preparation of each species may vary slightly, depending on the needs for the analysis to be performed. The objective of this SOP is to provide to the analytical laboratory samples of fish tissue that is properly identified, labeled, wrapped, preserved, and comparable from one sample to the next.

EQUIPMENT LIST

- Permanent Ink Marker
- Solvent Rinsed Aluminum Foil
- Gallon-Size Freezer Bags
- Knives Sufficient to Fillet Fish
- Freezer Space for Storage of Samples
- Coolers for Shipment
- Ice for Coolers
- Log Sheet to Record Data
- Label Tape
- Pencil

PROCEDURE

1. Collect fish samples in a manner appropriate for the study.
2. Identify the species of fish for sampling.
3. Prepare a waterproof label to identify each sample (use pencils or indelible ink only).
   a. Label the species.
   b. Label the date of capture.
   c. Label the place (lake) of capture.
   d. Total length and weight of whole fish.
   e. Sex of fish (when necessary or possible).
   f. Other data as required.
4. Prepare the fish as a sample (i.e., whole animal, entrails removed, fillet with skin or without skin, etc.).
5. Place sample in acetone- or hexane-rinsed aluminum foil if the sample is to be analyzed for organic materials. Place sample in a plastic bag if the sample is to be analyzed for metals.
6. Dual labels are recommended. Place a waterproof label in the package with the sample and another label on the outside of the package.
7. Place the sample on ice in the field as soon as possible (within two hours) and deliver to a freezer within the same 24-hour period.
8. Record on a separate log (sheet of paper or log book) the data that was included on the labels with the fish samples.
9. Transport sample to the laboratory in frozen condition (do not let samples thaw until ready for analysis).

Example of Label

Name of Study:	Date:
Species:	Location of Capture:
Total Length (units):	Weight (units):
Sex:	Name of Investigator:
Other Information:	

APPENDIX B

STANDARD OPERATING PROCEDURE

COLD VAPOR MERCURY ANALYSIS - ROUTINE LABWARE CLEANING

INTRODUCTION

This cleaning procedure is used for the routine cleaning of labware being used during any cold vapor mercury analysis procedures. The proper safety equipment must be worn during the entire cleaning procedure. This includes gloves, goggles, and lab coat.

EQUIPMENT LIST

- Deionized Water
- Gloves
- Lab Coat
- Micro or Liquinox Detergent
- Various Labware Washing Brushes
- Plastic Dish Rack
- Plastic 14"x10"x10" HPDE tank with cover
- Ammonium Hydroxide, 30% (reagent grade)
- Nitric Acid, Concentrated (Reagent grade)
- Dish Pan
- Goggles
- Labware to be Washed
- pH Indicator Strips
- Wash Bottle

PROCEDURE: LABWARE CLEANING

1. Scrub the labware thoroughly in hot water containing Micro or Liquinox detergent.
2. Rinse the labware with hot water until there is no presence of soap.
3. Rinse the labware once with deionized water.
4. Place the labware in the plastic tank containing 10% nitric acid. Be sure the labware is completely filled with acid. Allow the labware to soak for a minimum of 60 minutes.
5. Remove the labware from the tank, emptying the acid back into the tank.
6. Rinse the labware three times with deionized water.
7. Place the clean labware in a plastic rack to air dry. When the labware is dry, cover the labware with a lid, stopper, or aluminum foil. Place the labware in a proper storage location until used.

PROCEDURE: PLASTIC TANK CONTAINING 10% (V/V) NITRIC ACID

1. Fill the tank with 14.4 liters of deionized water. Then add 1.6 liters of concentrated nitric acid and stir. The tank is now ready to be used to soak labware.
2. Every few months change the acid in the tank. Neutralize the acid with ammonium hydroxide until a pH of between 6 and 10 is achieved. Measure the pH in the tank with pH indicator strips.
3. Pour the neutralized acid down the drain with running cold water. Run the cold water for an additional 10 minutes.
4. Rinse the tank with warm tap water and then with deionized water. Fill the tank with 10% nitric acid as in step 1.

APPENDIX C

STANDARD OPERATING PROCEDURE

COLD VAPOR MERCURY ANALYSIS - MEAT GRINDER CLEANING

INTRODUCTION

This cleaning procedure is only required for meat grinder and labware being used for grinding of fish samples for cold vapor mercury analysis. The proper safety equipment must be worn during the entire cleaning procedure. This includes gloves, goggles, and lab coat.

EQUIPMENT LIST

- Plastic Pan
- Dish Pan
- Goggles
- Liquinox Detergent
- Various Labware Washing Brushes
- Meat Grinder
- Ammonium Hydroxide, 30% (Reagent grade)
- Hydrochloric Acid, Concentrated (Reagent grade)
- Deionized Water
- Gloves
- Lab Coat
- pH Indicator Strips
- Wash Bottle
- Labware to be Washed

PROCEDURE: MEAT GRINDER AND LABWARE CLEANING

1. Dismantle the meat grinder before washing.
2. Scrub the meat grinder components and labware thoroughly in hot water containing Liquinox detergent.
3. Rinse the meat grinder components and labware with hot water until there is no presence of soap.
4. Rinse the meat grinder components and labware with deionized water.
5. Place the meat grinder components and labware in a plastic pan containing 0.1 M HCl. Be sure that the meat grinder components and labware are completely immersed in the acid. Allow the meat grinder components and labware to soak for 30 seconds.
6. Rinse the meat grinder components and labware with deionized water.
7. Assemble the meat grinder which is ready to be used.

PROCEDURE: PLASTIC PAN CONTAINING 0.1 M HYDROCHLORIC ACID

1. Fill the plastic pan with 4 liters of deionized water. Then add 33 mL of concentrated hydrochloric acid and stir. The pan is now ready to be used to soak.
2. Periodically change the acid in the plastic pan. Neutralize the acid with ammonium hydroxide until a pH of between 6 and 10 is achieved. Measure the pH in the plastic pan with pH indicator sticks.
3. Pour the neutralized waste down the drain with running cold water. Run the cold water for an additional five minutes.
4. Rinse the plastic pan with warm tap water and then with deionized water. Fill the plastic pan with 0.1 M hydrochloric acid as in step 1.

APPENDIX D

STANDARD OPERATING PROCEDURE

COLD VAPOR MERCURY ANALYSIS - FISH GRINDING

INTRODUCTION

This procedure is for the grinding of fish fillets into homogeneous samples. The meat grinder and labware used to grind the fish is cleaned by the "Cold Vapor Mercury Analysis - Meat Grinder Cleaning (SA/9)" procedure. The jars the ground fish samples are placed in are cleaned by the "Cold Vapor Mercury Analysis - New Labware Cleaning (SA/15)" procedure. The proper safety equipment must be worn during the entire grinding procedure. This includes gloves, goggles, and lab coat.

EQUIPMENT LIST

- Fish Fillets Samples
- Gloves
- Lab Coat
- Spatula
- Aluminum Foil
- Tuna fish
- Food Processor with Grinding Attachments
- Fillet Knife
- Goggles
- Grinder
- Beaker
- Scintillation Vials

PROCEDURE: GRINDING FISH FILLET SAMPLES

1. Cut the fish fillets into small pieces that will fit through the grinder feed tube or food processor with grinding attachments.
2. Pass the fish through the grinder or food processor, discarding the first few grams of tissue that come through. Collect the fish tissue in a beaker.
3. Mix the fish tissue with a spatula.
4. Repeat steps 2 and 3 an additional two times.
5. Place the fish in a previously acid-cleaned container. Seal securely with the screw top lid. Label the vial with the appropriate information and place in a freezer until analyzed.
6. Wash the grinder (or food processor) and labware by the "Cold Vapor Mercury Analysis - Meat Grinder Cleaning " procedure before grinding the next fish sample.
7. Continue to grind each fish sample by steps 1 - 7.

PROCEDURE: PREPARING THE PROCEDURAL BLANK

1. Drain a can of tuna fish to be used as the procedural blank. Grind half the tuna fish as a procedural blank by use of steps 2 - 7. Label the tuna fish as "ground" and include with the analysis set.
2. The other half of the tuna is left unground and handled like a sample by use of steps 5 + 6. Label the tuna fish as "unground" and include with the analysis set.

APPENDIX E

COLD VAPOR MERCURY ANALYSIS - FISH SAMPLE WEIGHING

INTRODUCTION

This procedure is for the weighing of ground fish tissue for cold vapor mercury analysis. The fish should be ground by use of the "Cold Vapor Mercury Analysis - Fish Grinding" procedure. The labware used in this procedure should be cleaned by the "Cold Vapor Mercury Analysis - Routine Labware Cleaning" procedure. The proper safety equipment must be worn during this entire procedure. This includes gloves, safety glasses or goggles, and lab coat.

EQUIPMENT LIST

- Ground Fish Samples
- Goggles or Safety Glasses
- Nitric Acid (10%)
- Glass Bottles with Ground Glass Stoppers
- Balance Capable of Reading to the Nearest 0.001 g
- Gloves
- Lab Coat
- Spatula
- Kimwipes

PROCEDURE

1. Remove the fish to be analyzed from the freezer and allow to partially thaw.
2. Check the level of the balance and adjust if necessary. Clean the top of the balance of any foreign materials with a soft brush.
3. Zero the balance with the zero adjustment to read 0.000 g.
4. Place a clean glass bottle on the balance and measure weight. Tare the balance.
5. Weigh approximately 0.2 g - 0.3 g of fish tissue into the glass bottle.
6. Weigh and record the total weight of the glass bottle and fish tissue.
7. Rinse the spatula with water, 10% nitric acid and deionized water. Wipe the spatula clean with a Kimwipe.
8. Label and record each glass bottle and fish sample. Be sure that none of the fish tissue adheres to the side of the glass bottle.

APPENDIX F

COLD VAPOR MERCURY ANALYSIS - STOCK, STANDARD AND SPIKE PREPARATION

INTRODUCTION

This procedure is used for the preparation of the stock, analytical standards, blanks and spikes for cold vapor mercury analysis. The fish used for the spike should be weighed by use of the "Cold Vapor Mercury Analysis - Fish Sample Weighing (SA/11)" procedure. The labware used in this procedure should be cleaned by the "Cold Vapor Mercury Analysis - Routine Labware Cleaning" (SA/8) procedure.

EQUIPMENT LIST

- Ground Fish Samples for Spikes
- Class "A" Pipets
- Wash Bottle
- Pipet Bulb
- Mercuric Chloride, Reagent Grade
- Nitric Acid, Concentrated (TraceMetal Grade)
- Deionized Water
- Mercury Waste Container
- 1,000 mL Plastic Graduated Cylinder
- Kimwipes
- Glass Bottles with Ground Glass Stoppers

PROCEDURE: STOCK PREPARATION

1. Weigh out 0.1355 g ± 0.0050 g of mercuric chloride into a 100-mL volumetric flask.
2. Add 10 mL of concentrated nitric acid (trace metals grade).
3. Dilute to volume with deionized water.
4. Calculate concentration of the mercury stock solution. Use the following calculation:

$$\frac{\text{mass of } HgCl_2 \text{ (g)}}{271.50 \text{ g/mol } HgCl_2} \times \frac{200.59 \text{ g mol Hg}}{100 \text{ mL}} \times \frac{\text{purity (\%)}}{100\%} \times$$

$$\frac{10^6 \text{ µg}}{\text{g}} = \text{concentration (µg Hg/mL)}$$

PROCEDURE: STANDARD AND SPIKE PREPARATION

1. Pipet 10 mL of the ~1000 µg/mL mercuric chloride stock solution into a 100-mL volumetric flask containing 10 ml HNO_3 and diluting to 100 mL with deionized water to prepare a ~100 µg/mL mercury sub-stock.
2. Pipet 5.0 mL of a ~100 µg/mL mercuric chloride stock solution into a 100-mL volumetric flask containing 0.5 mL of concentrated nitric acid and dilute to volume with deionized water to prepare a ~5000 ng/mL Hg sub-stock.
3. Pipet 1.0 mL of the ~5000 ng/mL mercuric chloride stock solution into a 100-mL volumetric flask containing 0.5 mL of concentrated nitric acid and dilute to volume with deionized water to prepare a ~50 ng/mL Hg sub-stock.
4. Calculate the concentration of the mercury sub-stocks using the following equation:
   $C_1 V_1 = C_2 V_2$ where: C_1 = conc. of Hg stock solution; C_2 = conc. of diluted solution;
   V_1 = volume of stock solution; V_2 = volume of diluted solution.
5. Prepare standards with the approximate concentrations: 25, 50, 100, 200, and 300 ng of mercury by pipetting 0.5, 1.0, 2.0, 4.0, and 6.0 mL of the ~50 ng/mL Hg sub-stock into separate bottles. Determine the amount of Hg added to each bottle in ng. Use the following calculation:
   ng of Hg = conc. of Hg sub-stock (ng/mL) X mL of sub-stock used.
6. Add deionized water to the bottles with mercury standards so that each bottle has an equivalent volume of liquid (i.e., pipet 5.5 mL of deionized water into the 25 ng mercury standard bottle).
6. Each standard should be prepared in triplicate.
7. Label and record the bottle and concentration of mercury added for each of the standards prepared.
8. Additional standards can be prepared if necessary, as mercury has a linear response curve up to 2000 ng.
9. Three to five reagent blanks (containing 6 mL of deionized water) should be prepared with each analysis set.

PROCEDURE: 1% (V/V) NITRIC ACID PIPET SOAKING SOLUTION

1. Place enough glass wool in the bottom of a previously cleaned 1,000-mL plastic graduated cylinder to cover the bottom.
2. Fill the graduated cylinder with approximately 800 mL of deionized water.
3. Add 8 mL of concentrated nitric acid to the graduated cylinder and stir.
4. Pipets used for mercury analysis should be soaked in this solution when not in use.

APPENDIX G

STANDARD OPERATING PROCEDURE

COLD VAPOR MERCURY DETERMINATION

INTRODUCTION

This procedure is used for the determination of total mercury in hair, fish, and other tissue samples. Do not use this procedure for analyzing human blood.

REFERENCES

"Determination of Mercury in Tissues by Cold Vapor Atomic Absorption Spectrometry", Environmental Monitoring Systems Laboratory, Office of Research and Development, U.S. Environmental Protection Agency, Cincinnati, Ohio 45268, April 1991.

EQUIPMENT LIST

- Stannous Chloride, Analytical Reagent
- Magnesium Perchlorate, Anhydrous for Elemental Analysis
- Potassium Persulfate, Reagent Suitable for Mercury Determination
- Hydroxylamine Hydrochloride, Reagent Suitable for Mercury Determination
- Potassium Permanganate, Certified A.C.S.
- Sodium Chloride, Certified A.C.S.
- Sulfuric Acid, A.C.S. Reagent, Suitable for Mercury Determination
- Nitric Acid, Fisher, Trace Metals Grade
- Mercury Cold Vapor Analyzer
- Hollow Cathode Mercury Lamp
- Variable Autotransformer
- Neptune Dyna-Pump Model 4K
- Hot Plate
- Instrumentation Laboratory Video 12 aa/ae Spectrophotometer
- Electric Meat Grinder
- Labindustries Repipet II Dispenser, 3 - 10 mL and 1 - 5 mL
- Wheaton Instruments Socorex Dispenser Model 511, 10 mL
- Glass Bottles with Ground Glass Stoppers
- Pipets/Pipettors
- Beakers
- Volumetric Flasks
- Spatulas
- Water Bath 18"x30"
- 5% (w/v) Potassium Permanganate
- 5% (w/v) Potassium Persulfate
- 10% (w/v) Hydroxylamine Hydrochloride-10%(w/v) Sodium Chloride
- 10% (w/v) Stannous Chloride-0.5M Sulfuric Acid
- 0.05M Potassium Permanganate-5% (v/v) Sulfuric Acid
- 1000 µg/mL Mercuric Chloride Stock
- 5 µg/mL Mercuric Chloride Sub-stock
- 50 ng/mL Mercuric Chloride Sub-stock

PROCEDURE

Digestion

1. Add 4.0 mL of concentrated sulfuric acid and 1.0 mL of concentrated nitric acid to each sample, standard, spike, duplicate and blank and stopper.

2. Place the bottles in hot water bath at 80-90°C and allow to digest for approximately 15 minutes or until all the fish tissue is dissolved.
3. Vent the bottles occasionally during the heating process.
4. Turn off the hot plate and allow the bottles to cool to room temperature.
5. Add 5.0 mL of 5% potassium permanganate to each bottle in 1.0 mL increments swirling the bottles after each addition.
6. Add 10.0 mL of 5% potassium permanganate to each bottle in 5.0 mL increments, swirling the bottles after each addition. Additional 5% potassium permanganate solution should be added to the samples if necessary to that the samples remain purple in color for at least 15 min.
7. Add 8 mL of 5% potassium persulfate to each bottle, and stopper and swirl.
8. Allow the bottles to set overnight to oxidize organic mercury compounds to inorganic mercury ions.
9. The samples will remain stable for several days before analysis.

Sample Analysis

Instrument Conditions

Current = 3.0 mA Wavelength = 253.7 nm
Atomic Absorption Mode (AA) Double Beam Mode (DB)
Statistics = 90 Integration = 1.0 seconds
D_2 Background Correction with diffraction grating filter
Circulating Pump autotransformer = 70% power

1. Set the AA to the instrument conditions listed above and allow instrument warm-up time. Prepare the 10% stannous chloride/0.5 M sulfuric acid solution and the magnesium perchlorate drying tube. Attach the drying tube in the cold vapor mercury analyzer.
2. Auto-zero the AA by aerating deionized water through the cold vapor mercury analyzer.
3. Add 10.0 mL of 10% hydroxylamine hydrochloride/10% sodium chloride solution and deionized water to each sample so that all samples contain the same volume (this is to adjust for any additional 5% potassium permanganate added to samples). Swirl the sample until no purple or brown color from the potassium permanganate remains.
4. Add 5.0 mL of 10% stannous chloride to a sample and immediately attach to the mercury analyzer.
5. Measure the absorbance of the sample until the maximum absorbance is reached and begins to decline.
6. Change the valves of the mercury analyzer to draw the mercury into a 0.05 M potassium permanganate/5% sulfuric acid trap. Purge the mercury analyzer of mercury until the absorbance reaches a minimum similar to the background absorbance.
7. Return the valves to the "analyze" position and rinse the aerator with deionized water before analyzing the next sample. Dispose of the analyzed and purged sample into an Acid Waste container.
8. Alternate analyzing the samples, standards and blanks by use of steps 3-7.
9. Neutralize the "Acid Waste" in a fume hood with ammonium hydroxide until the pH is between 6 and 10. Pour the neutralized waste down the drain with running cold water.
10. Dispose of the unused stocks and standards in a glass bottle identified as "Hazardous Waste - Mercuric Chloride in % acid solutions. Corrosive Toxic." The start date. Each waste bottle will require an analysis before it will be accepted for disposal.

Appendix H

SAMPLE PREPARATION PROCEDURE FOR SPECTROCHEMICAL DETERMINATION OF TOTAL RECOVERABLE ELEMENTS IN BIOLOGICAL TISSUES[1/]

INTRODUCTION

This method of tissue sample preparation was used to analyze fish for concentrations of copper, lead, and selenium.

EQUIPMENT

- Erlenmeyer Flask (125 mL)
- Erlenmeyer Flask (100 mL)
- Hot Plate
- Analytical Balance (0.001 g)
- Nitric Acid (reagent grade)
- Hydrogen Peroxide (30%)
- Hydrochloric Acid (reagent grade)
- Deionized Water

PROCEDURE

1. Place up to a 5 g sub-sample of frozen tissue into a 125-mL Erlenmeyer flask. Any sample spiking solutions should be added at this time and allowed to be in contact with the sample prior to addition of acid.
2. Add 10 mL of concentrated nitric acid and warm on a hot plate until the tissue is solubilized. Gentle swirling the samples or use of an oscillating hot plate will aid in this process.
3. Increase temperature to near boiling until the solution begins to turn brown. Cool sample, add an additional 5 mL of concentrated nitric acid and return to the hot plate until the solution once again begins to turn brown.
4. Cool sample, add an additional 2 mL of concentrated nitric acid, return to the hot plate and reduce the volume to 5-10mL. Cool sample, add 2 mL of 30%hydrogen peroxide, return sample to the hot plate and reduce the volume to 5-10 mL.
5. Repeat Procedure 4 until the solution is clear or until a total of 10 mL of peroxide has been added. **Note:** A laboratory reagent blank is especially critical in this procedure because the procedure concentrates any reagent contaminants.
6. Cool the sample, add 2 mL of concentrated hydrochloric acid, return to the hot plate and reduce the volume to 5 mL.
7. Allow the sample to cool and quantitatively transfer to a 100-mL volumetric flask. Dilute to volume with deionized water, mix, and allow any insoluble material to separate. The sample is now ready for analysis.

[1/] Taken from EPA/600/4-91/010 “Methods for Determination of Metals in Environmental Samples.”

ATTACHMENT 3

Raw Chlorinated Organic Sturgeon Data