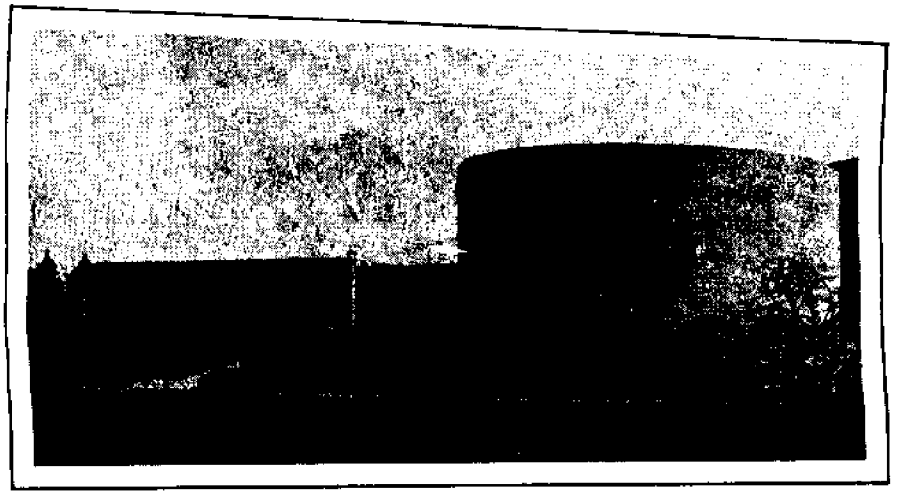
Western New York Nuclear Research Center, Inc.

New York State Sea Grant Program
Project 015-8101-A

FINAL REPORT

October 1, 1971 - September 30, 1972



State University of New York at Buffalo

New York State Sea Grant Program

Project 015-8101-A

ELEMENTAL POLLUTANT DISTRIBUTION IN LAKE ERIE AND LAKE ONTARIO ECOSYSTEMS

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INTRODUCTION

The program, as originally proposed, was intended to extend the Lake Erie ecosystem mercury study (carried out under a Bureau of Sport Fisheries and Wildlife grant) to other pollutants and to Lake Ontario[1]. In view of the limited funding available, it was decided to place the program emphasis on fish from Lake Ontario. However, procedure development work was carried out using Lake Erie fish. A limited investigation of methodology for the analysis of mercury in water samples and for differentiating between inorganic and organic mercury in water was conducted.

The elements of interest in the study were chromium, copper, mercury, cadmium, zinc, arsenic, and selenium. Fish samples were collected at two locations in Lake Ontario. The fish, smelt (Osmerus mordax) and slimy sculpin (Cottus bardii), were collected in September 1971 at Youngstown and Prince Edwards Bay.

Water samples from several areas in Lake Ontario were collected for mercury analysis in early August 1972. However, an extended shutdown of the reactor prevented sample analysis prior to the end of the grant period.

ANALYTICAL PROCEDURES

I. Biological Tissues (Fish)*

A. Sample Preparation

Unless otherwise noted in the specific elemental analysis procedure, sample preparation was as described in this section. The

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*The development of analytical procedures was carried out under U. S. Bureau of Sport Fisheries Contract 14-16-0008-623.

samples of fish and other biological tissues were homogenized using a blender and/or a grinder made of stainless steel or pyrex glass. A convenient analytical sample (about 1 to 3 gm) of the homogenized tissue was carefully weighed into a small polyethylene bag (4 x 12 cm size) made of 0.1 mm thick sheets. The air from the bag was squeezed out and the bag was heat-sealed, allowing a void space equivalent to at least twice the volume of the wet tissue sample, allowing room for the gaseous radiation products produced during reactor irradiation. Wet tissue weights of these samples were used for calculating the results.

B. Simultaneous Determination of Copper and Mercury in Fish Tissues

Copper is one of the elements that is highly sensitive to neutron activation analysis. Copper-63, with a natural abundance of 69.17% and a neutron absorption cross section of 4.7 barns to form 12.8 hour ^{64}Cu, is ideally suited for the determination of copper in parts per billion levels. From the analyses of several species of fish, we have found that the copper level in fish (at least those from Lake Erie) range from a few hundred to a few thousand parts per billion. Because some of the chemical properties of copper are analogous to those of mercury, we have made use of these similarities in separating both copper and mercury simultaneously from the tissue digests. The procedures developed have been found to be highly reliable and involve the following:

1. Raw fish tissues (homogenized) are irradiated for two hours at a thermal neutron flux of about 5×10^{12} neutrons cm^{-2} sec^{-1} with copper and mercury standards.

2. After about a two to three hour decay period, the samples are wet ashed with accurately known amounts of copper and mercury carriers (~ 25 mg of each).
3. The fish tissue digest is then treated with H_2S gas in acid medium to precipitate HgS and CuS along with traces of As, Sb, Sn, Mo, Au, Ag, Pt, Pd, Se, etc.
4. The sulfides of copper and mercury are washed free of As, Sb, and Sn using a mixture of strong alkali and ammonium polysulfide at an elevated temperature.
5. The purified copper and mercury sulfides are then separated into copper and mercury components by treatment with potassium cyanide solution, while HgS remains undissolved.
6. The HgS is washed free of copper and cyanide residues and is used to prepare the electrolytic bath for the deposition of mercury.
7. The mercury deposited on tared gold foils is weighed to determine the chemical recovery of each analysis and appropriate corrections are made in the final numbers to account for all the mercury that was present initially in the sample.

8. The KCN solution containing copper sulfide is acidified to reprecipitate copper sulfide.
9. The copper sulfide, washed free of cyanide, is dissolved in acid and reduced to the cuprous state using sodium bisulfite or sulfite.
10. After complete reduction, copper is precipitated as cuprous thiocyanate using KSCN solution.
11. The CuSCN precipitate is washed free of soluble impurities and then collected on tared filter papers.
12. This precipitate is washed with alcohol and dried at a temperature of about 60-70°C for several hours (or until it attains a constant weight).
13. After cooling the samples in a desiccator, the percentage of copper from each sample is determined from the weights of CuSCN.
14. These samples are then used for gamma ray spectrometry of ^{64}Cu isotope to determine the exact amount of copper that was originally present in the sample.

precision of the simultaneous determination of copper and mercury determined by analyzing several portions (1 to 3 gms) of a fish osite. The results of five such analyses, shown in Table 1, cate that the procedures are highly reliable and reproducible.

Table 1

Precision of the Simultaneous
Determination of Cu and Hg in Fish*

SAMPLE NO.	COPPER		MERCURY	
	Copper Content (µg/gm)	Deviation from the Mean	Mercury Content (µg/gm)	Deviation from the Mean
C50A	2.88	+0.24	0.75	+0.08
C50B	2.75	+0.11	0.65	-0.02
C50C	2.68	+0.04	0.72	+0.05
C50D	2.36	-0.28	0.58	-0.09
C50E	2.55	-0.09	0.65	-0.02
Average:	2.64	-	0.67	-

*The composites of the edible tissues of 25 Freshwater Drum caught during the Fall of 1970 from the Western Basin of Lake Erie were used.

In determining the accuracies of the analyses, we have spiked samples of fish homogenates with known amounts of radioactive copper and mercury and employed the above-mentioned procedures to redetermine their mercury and copper levels. The results of these analyses, shown in Table 2, in a wide range of mercury and copper levels indicate good analytical accuracies. Although this method of measuring the accuracies of radioanalytical procedures is very common and considered very reliable, we have certain reservations about its application in the particular case of mercury in biological tissues. These reservations are based on the highly volatile nature and unknown chemical forms of mercury in the biota. However, the analytical procedures we have employed have been used in several interlaboratory comparison studies. We have shown excellent agreement with "good" cold atomic absorption values when the samples were prepared and handled properly.

C. Simultaneous Determination of Zinc and Cadmium in Fish Tissues

The analyses of both cadmium and zinc in fish tissues offered considerable challenges, because of the extremely low concentrations of cadmium in fish and the poor neutron activation properties of zinc. The procedures developed, therefore, are more elaborate and are designed to give both specificity and accuracy in the determination of zinc and cadmium in fish tissues. The analytical procedures include the following:

Table 2

Accuracies of the Simultaneous Determinations of
Copper and Mercury in Biological Tissues

Sample No.	COPPER			MERCURY		
	Cu added (µg)	Cu determined (µg)	% Error	Hg added (µg)	Hg determined (µg)	% Error
CuHg-1	0.048	0.054	+12.50	0.049	0.056	+14.28
CuHg-2	0.048	0.055	+14.58	0.049	0.046	- 6.12
CuHg-3	0.480	0.487	+ 1.45	0.049	0.053	+ 8.16
CuHg-4	0.480	0.477	- 0.62	0.049	0.043	-12.24
CuHg-5	0.960	0.975	+ 1.56	0.980	0.922	- 5.91
CuHg-6	0.960	0.925	- 3.64	0.980	0.924	- 5.71
CuHg-7	1.920	1.905	- 0.78	0.980	1.075	+ 9.69
CuHg-8	1.920	1.924	+ 0.20	0.980	1.028	+ 4.89

1. Raw fish tissues are irradiated along with zinc and cadmium standards for about 4 hours or more at a thermal neutron flux of 5×10^{12} neutrons cm^{-2} sec^{-1}. The irradiated samples are allowed to decay for at least two to three hours prior to chemical processing.
2. The activated tissues are digested by wet ashing in the presence of accurately known amounts of zinc and cadmium carriers (~ 25 mg of each).
3. The fish tissues are digested in a mixture of nitric, sulfuric, and perchloric acids under reflux conditions.
4. After complete ashing and digestion, the solution is neutralized and made basic with NH_4OH.
5. Zinc and cadmium are then precipitated as sulfides using $(NH_4)_2S_x$ in presence of NH_4Cl. The sulfides of Mn, Fe, Al, Cr, Hg, Cu, Pb, etc., are also precipitated along with zinc and cadmium and are then separated.
6. Copper sulfide is removed by treatment with 10% KCN solution.
7. The remaining residues (containing zinc and cadmium) are dissolved in minimum HCl and brought into solution containing 0.12M HCl and 10% NaCl.

8. This solution containing complexes of zinc and cadmium is loaded on a previously prepared anion exchange column. This procedure retains cadmium and zinc on the column while Mn, Fe, Al, Cr, etc., flow through.
9. From the ion exchange column, zinc and cadmium are separately eluted using 2M NaOH containing 2% NaCl and a 1M HNO_3 solution respectively in that order.
10. The tailings of zinc in cadmium fraction and cadmium in zinc fraction are further separated by reprecipitation as sulfides and using the amphoteric properties of zinc.
11. The separated and purified cadmium and zinc are dissolved to make electrolysis baths to isolate these metals.
12. Zinc electrolysis is done in a highly basic solution in NaOH using platinum anodes and gold cathodes.
13. Cadmium electrolysis is done in a solution of cadmium cyanide complex in slightly basic solution using platinum anodes and gold cathodes.
14. After electrolytic deposition of cadmium and zinc, the recoveries are determined by re-weighing the cathode foils.

15. The radioactivities from ^{115}Cd (major activity) and ^{69m}Zn (major activity) are measured independently using gamma ray spectrometry, and the initial concentrations of zinc and cadmium in the fish tissues are calculated.

The results of experiments performed to determine the precision and accuracy of these analytical procedures are presented in Tables 3 and 4. The precision of the measurements of cadmium in several portions of a composite of freshwater drum shown in Table 3 are not as good as the results of the zinc analyses values for the same fish. The major factor in this difference is the extremely low levels of cadmium and the relatively higher levels of zinc in these samples.

D. Determination of Chromium in Fish Tissues

The nuclear properties of chromium are such that its detection and quantitation at μg/gm levels in biological tissues is extremely difficult. The ^{50}Cr isotope, with a low natural abundance of 4.35%, has a neutron absorption cross section of 16 barns to produce 27.8 day ^{51}Cr isotope. The combination of these properties and its relatively lower levels in fish has necessitated (i) extremely long irradiations of the samples in the reactor to produce sufficient radioactive chromium and (ii) a systematic chemical isolation of chromium from all other interfering elements. The procedures outlined briefly below seem satisfactory and are capable of determining chromium in fish tissues at less than 0.1 ppm levels.

Table 3

Precision of the Simultaneous
Determination of Cd and Zn in Fish*

SAMPLE NO.	CADMIUM		ZINC	
	Cadmium Content (μg/gm)	Deviation from the Mean	Zinc Content (μg/gm)	Deviation from the Mean
C5-B	0.094	+0.017	9.18	-0.34
C5-C	0.085	+0.008	9.47	-0.05
C5-D	0.069	-0.008	9.65	+0.13
C5-E	0.062	-0.015	9.77	+0.22
C5-F	0.094	+0.017	9.37	-0.15
C5-G	0.060	-0.017	9.67	+0.05
Average:	0.077	-	9.52	-

*The composites of the edible tissues of 25 Freshwater drum from the Western Basin of Lake Erie caught during Fall 1970 were used for these analyses.

Table 4

Accuracies of the Simultaneous Determination of
Zinc and Cadmium in Biological Tissues

	ZINC			CADMIUM		
Sample No.	Zn added (μg)	Zn determined (μg)	% Error	Cd added (μg)	Cd determined (μg)	% Error
ZnCd-1	0.970	0.922	-4.94	0.050	0.059	+18.0
ZnCd-2	0.970	0.895	-7.73	0.050	0.052	+ 4.0
ZnCd-3	9.700	10.430	+7.52	0.025	0.029	+16.0
ZnCd-4	9.700	10.000	+3.09	0.025	0.023	- 8.0
ZnCd-5	38.800	37.340	-3.76	0.100	0.097	- 3.0
ZnCd-6	38.800	39.290	+1.26	0.100	0.089	-11.0

In irradiating biological tissues for long periods of time in a nuclear reactor, one of the immediate problems is the moisture contents of these samples, which, due to radiation decomposition, can produce explosive mixtures of hydrogen and oxygen within the irradiation containers. Therefore, a series of experiments were conducted to determine whether freeze-drying the tissues would cause any loss of chromium from the tissues. The results of these experiments are given in Table 5. In irradiating raw tissues, several quartz tubes containing fish tissues ($\sim$ 0.2 gms each) were carefully heat-sealed. These samples, after reactor irradiation for 24 hours or more, were frozen in dry ice and were opened within a stainless steel encapsulation. Several such samples were combined together to make the sample sizes required for the analysis. The remainder of the analytical procedures for the determination of chromium in both freeze-dried samples and raw tissues were similar. These procedures include the following:

1. The fish tissues are freeze-dried at -50°C and at a pressure of 30 to 50 microns for 20 hours or more. The weight loss of the tissues is accurately determined to account the final results in terms of the raw tissue weights.
2. Sixteen samples ($\sim$ 0.4 gms of dry tissue in each) contained in sealed quartz tubes along with two chromium standards are irradiated for a period of 24 hours or more in a thermal neutron flux of 3×10^{13} neutrons cm^{-2} sec^{-1}

Table 5

Comparison of the Results of Chromium Determinations
Using Freeze-dried Tissues and Raw Tissues

Sample No.*	Chromium Content (μg/gm)	Deviations from the Mean
FREEZE-DRIED		
C5-22	0.792	+0.022
C5-23	0.755	-0.015
C5-24	0.849	+0.079
C5-25	0.830	+0.060
C5-26	0.815	+0.045
C5-27	0.791	+0.021
Average (of six)	0.805	-
RAW TISSUES		
C5-301	0.718	-0.052
C5-311	0.766	-0.004
C5-323	0.758	-0.012
C5-331	0.812	+0.042
Average (of four)	0.764	-
Average (of ten)	0.770	-

*All the samples used in these determinations are from the composites of edible tissues of 25 Freshwater Drum from the Western Basin of Lake Erie (1970 Fall catch).

using a rotating sample irradiation facility called "merry-go-round".

3. The samples are allowed to decay for 5 to 7 days in the reactor pool prior to handling.
4. The radioactive samples in quartz containers are removed and frozen in dry ice and crushed into a digestion flask with about 10 ml of HNO_3.
5. An accurately known amount of chromium carrier (~ 25 mg) is added to the flask containing the irradiated tissues. After adding a mixture of 5 mls of conc. H_2SO_4 and 5 mls of $HClO_4$ (70%), the tissues are digested under reflux conditions.
6. After all the tissues have gone into solution, the refluxing column and the condenser are removed and the contents of the flask heated to about 160°C to volatilize some of the volatile elements (including mercury).
7. An additional 10 mls of a 1:1 mixture of conc. H_2SO_4 and $HClO_4$ are added to the flask and heated to 200°C to oxidize all the chromium to CrO_4^{--} and/or $Cr_2O_7^{--}$ forms.
8. After cooling the flask, it is fitted with a small air-cooled condenser and provision is made to bubble dry hydrogen chloride gas through the solution.

9. The solution in the flask is heated to above 160°C and HCl gas is bubbled through the solution when chromium is converted into volatile CrO_2Cl_2. The vapors of chromyl chloride distilled over are condensed and collected in a beaker containing water.

10. The chromium salts formed are carefully neutralized with NH_4OH and $Cr(OH)_3$ is precipitated using minimum amount of NH_4OH and $(NH_4)_2S_x$.

11. The chromic hydroxide is washed free of sulfate and dissolved in minimum HCl.

12. This solution is diluted and scavenged with freshly prepared $BaSO_4$ precipitate (Ba^{++} and SO_4^{--} ion free) to remove S and Se compounds.

13. After removing the $BaSO_4$, the chromic salt is oxidized to chromate using Na_2O_2.

14. This chromate solution is neutralized with acetic acid and its pH adjusted to about 6.0 using ammonium acetate.

15. Chromium is now precipitated as $BaCrO_4$ using a few drops of Barium Acetate solution.

16. The $BaCrO_4$ precipitate is washed and collected on tared filter papers to determine the chemical yields from each sample.

17. The samples are then used to determine the ^{51}Cr activities from the fish tissues using scintillation gamma ray spectrometry.

The average loss of weight due to freeze-drying of the fish tissues was about 70 to 75%. The results shown in Table 5 indicate that there is no observable loss of chromium from the fish tissues during freeze-drying. Also, these results indicate that good precision can be obtained using this chromium procedure at less than 1 ppm level in fish tissues.

A set of fish tissues spiked with radioactive chromium (^{51}Cr) containing accurately known amounts of chromium was used to determine the accuracies of chromium analyses in the range of 0.3 to 3.0 μg of chromium in about 1 to 3 gms of raw fish tissue. The results shown in Table 6 indicate that the method can provide very satisfactory results at less than 1 μg/gm level in fish tissues.

E. Determination of Arsenic in Fish Tissue

Arsenic is one of the elements which is highly sensitive to thermal neutron activation analysis. In an ideal matrix, about 1×10^{-9} gm of arsenic can be readily detected by neutron activation analysis. However, fish tissue is one of the most undesirable matrices in which to detect and quantitate arsenic at less than one ppm, unless accompanied by adequate chemical isolation. The procedures

Table 6

Accuracies of the Determination of Chromium in Biological Tissues (as measured by ^{51}Cr tracers)

Sample No.	Cr Added (μg)	Cr Determined (μg)	% Error
Cr-S	0.292	0.258	-11.64
Cr-S	0.292	0.264	- 9.58
Cr-S2	0.584	0.606	+ 3.76
Cr-S2	0.584	0.537	- 8.04
Cr-S2	0.584	0.527	- 9.76
Cr-S2	0.584	0.547	- 6.33
Cr-S10	2.920	3.040	+ 4.10
Cr-S10	2.920	2.730	- 7.53

we have developed involve:

1. the thermal neutron irradiation of the samples for two hours at a thermal neutron flux of 5×10^{12} neutrons cm^{-2} sec^{-1};
2. the processing of the samples after a decay period of two to three hours to allow the short-lived activities to decay at least partially;
3. the wet-digestion of the samples with non-radioactive arsenic using a mixture of nitric and sulfuric acids and with hydrogen peroxide;
4. the destructive distillation of arsenic from the mixture with a combination of conc. HCl and HBr;
5. the hydrolysis of the distillation products to arsenous acid and the eventual reduction to elemental arsenic using ammonium hypophosphite; and
6. the measurement of ^{76}As (26.4 hours) radioactivity using scintillation gamma-ray spectrometry to quantitate the arsenic levels of the fish tissues used.

The accuracy of arsenic determinations, using the above-mentioned procedures, were determined using radioactive tracers (^{76}As) added to raw fish tissues and isolating and quantitatively determining the arsenic by these procedures. The results of several analyses of arsenic ranging from 0.08 μg to 1.44 μg in about 5 gm of fish tissues are shown in Table 7. Because of some of the very desirable nuclear properties of arsenic (neutron capture cross section of 4.3 barns and an isotope of abundance of 100% for ^{75}As), such

Table 7

Accuracy of Arsenic Determinations
of Raw Fish Tissues Spiked with ^{78}As

Sample No.	Arsenic Added	Arsenic Determined	Absolute Error
A	0.08 µg	0.08 µg	0.00
B	0.08 µg	0.07 µg	-0.01
C	0.24 µg	0.25 µg	+0.01
D	0.24 µg	0.28 µg	+0.03
E	1.44 µg	1.43 µg	-0.01
F	1.44 µg	1.51 µg	+0.07

accuracies are attainable in practice using the procedures described.

Several aliquots of the edible tissues of a composite of gizzard shad from the Western Basin of Lake Erie were used to determine the precision of this analytical procedure. The results of eight analyses are presented in Table 8. These fish tissues were found to contain about 0.3 ppm arsenic and they are determinable with excellent precision.

F. Determination of Selenium in Fish Tissues

Selenium lends itself to neutron activation analysis up to a few parts per billion in matrices that are usually considered complex for trace element analysis. The nuclear properties of selenium isotopes listed in Table 9 are relevant to thermal neutron activation analysis of selenium. Three of these nuclides (^{75}Se, ^{77m}Se, and ^{81m}Se) can be produced in high specific activities. Of these, the most desirable one, ^{77m}Se has an exceedingly short half-life. ^{81m}Se, on the other hand, has a 57 minute half-life and is a desirable isotope for activation analysis. When processing large numbers of samples economically and efficiently, this isotope (^{81m}Se) is not an ideal choice. Therefore, this investigation attempted the utilization of the long-lived ^{75}Se isotope. An immediate disadvantage that becomes apparent in using ^{75}Se is the long radiation times required to produce sufficient radioactivity and the consequent desirability of using samples containing minimum amounts of moisture for reactor irradiation. Therefore, a series of experiments were conducted using some of the fish from Lake Erie to determine the effect of freeze-drying

Table 8

Precision of Arsenic Analysis Using
Neutron Activation Analysis of Raw Fish Tissues

Sample No.	Arsenic (ppm)	Deviation from the Mean
09A	0.35	+0.01
09B	0.35	+0.01
09C	0.33	-0.01
09D	0.32	-0.02
09E	0.37	+0.03
09F	0.36	+0.02
09G	0.32	-0.02
09H	0.32	-0.02

Average and Standard Deviation: 0.34 ± 0.02

Table 9

Radionuclides Produced by Thermal Neutron Flux of 3×10^{13} Neutrons cm^{-2} sec^{-1} on Selenium

Isotopes Produced	Activity of Se-isotope after Activation for one Half-life (Microcuries/μg)	Half-life	Gamma Energies (Mev)
^{75}Se	7.5×10^{-1}	120.4 days	0.27, 0.12, 0.40
^{77m}Se	2.9×10^{0}	17.5 secs	0.16
^{79m}Se	8.7×10^{-2}	3.9 mins	0.096
^{81m}Se	4.5×10^{-2}	57.0 mins	0.103
^{81}Se	7.5×10^{-1}	18.6 mins	none
^{83m}Se	1.4×10^{-2}	69.0 secs	0.65, 1.01, 2.02
^{83}Se	1.2×10^{-3}	25.0 mins	0.36, 2.34

Note: The thermal neutron flux at one of the sample irradiation positions of PULSTAR is 3×10^{13} neutrons cm^{-2} sec^{-1}.

on the selenium contents of fish tissues. Parallel studies were conducted using these two different approaches. The results presented in Table 10 show that there is no significant loss of selenium from the fish tissues during freeze-drying. The raw tissues were analyzed employing short irradiation (2 hours) and ^{81m}Se isotope, while the freeze-dried tissues were analyzed using long irradiation (24 hours) and ^{75}Se isotope. The use of ^{75}Se has the advantage for batch-processing of large numbers of samples, while the use of ^{81m}Se (because of its short half-life) restricts the analysis to one or two samples at a time.

The analytical procedures used in the determination of selenium in the fish tissues is a modification of the procedures developed earlier in our laboratory for the determination of selenium in fossil fuels.[2] Briefly, the procedures used are as follows:

1. Sixteen freeze-dried samples contained in quartz vials are mounted on a rotating sample irradiation facility (merry-go-round) and exposed to a thermal neutron flux of 3×10^{13} neutrons cm^{-2} sec^{-1} for a period of 24 hours.
2. After five to seven days of cooling, the samples are frozen in dry ice and the vials are opened and all the materials, including the quartz vials, are transferred to a digestion apparatus containing a non-radioactive selenium carrier.
3. The samples are wet-ashed with a mixture of nitric, sulfuric and perchloric acids.

Table 10

Comparison of the Results of Selenium Determination Involving Two Sample Preparation Procedures Prior to Neutron Activation

Identification (No., Species, Lake Erie Basin)		Selenium Content (ppm)* Determined using Freeze-dried Fish Tissues and ^{75}Se radioactivity	Selenium Content (ppm)* Determined using Raw Fish Tissue and Short-lived Isotopes of Selenium
03, White Bass	(Western)	0.54	0.62
05, Freshwater Drum	(Western)	0.64	0.66
16, Freshwater Drum	(Central)	0.78	0.79
17, Carp	(Central)	0.52	0.50
23, Yellow Perch	(Eastern)	0.51	0.55
25, Carp	(Eastern)	0.58	0.58

*Averages of two or more determination. All the results are expressed as μg of Se per gram of raw fish tissue.

4. Selenium is converted from the non-volatile hexavalent form to volatile quadrivalent halides and it is distilled out and hydrolyzed prior to reduction as elemental selenium using sulfur dioxide.

5. The radioactivities of ^{75}Se (120 days) are measured by gamma-ray spectrometry using a large (4" x 4" well-type) NaI(Tl) scintillation detector to quantitate the selenium levels in the fish tissues.

The precision and accuracy of the selenium analysis were determined by repeat analyses of the composites of freshwater drum from the Western Basin of Lake Erie (see Table 11) and by the use of radioactive tracers (^{75}Se) added to fish tissues as described earlier for arsenic (Table 12).

II. Procedure for Analysis of Mercury in Water

The water analysis procedure is a modification of that described by Becknell, Marsh and Allie[3], and is described below:

1. Samples are collected in acid cleaned glass bottles and are frozen as soon as possible. A two to four liter sample is required.

2. If possible, the sample should be made 0.1 N in hydrochloric acid before freezing. If this is not possible, thaw the sample, make 0.1 N in HCl and allow to stand 48 hours.

3. Without mixing, transfer aliquots (500 ml minimum) to clean bottles and freeze until ready to carry out the analysis.

Table 11

Precision of Selenium Determination
Using Freeze-dried Samples and Neutron
Activation Analysis

Sample No.	Selenium (ppm)	Deviation from the Mean
05A	0.64	-0.03
05B	0.66	-0.01
05C	0.69	+0.03
05D	0.67	0.00
05E	0.63	-0.04
05F	0.72	+0.05

Average and Standard Deviation: 0.67 ± 0.03

Table 12

Accuracy of Selenium Determinations as Determined by Fish Samples Spiked with ^{75}Se

Sample No.	Selenium Added	Selenium Determined	Absolute Error
A	0.03 µg	0.03 µg	0.00
B	0.03 µg	0.04 µg	+0.01
C	0.45 µg	0.46 µg	+0.01
D	0.45 µg	0.42 µg	-0.03
E	0.90 µg	0.90 µg	0.00
F	0.90 µg	0.84 µg	-0.06

4. Thaw sample and transfer 500 ml to a Greenberg-Smith impinger bottle. Connect bottle to Cl_2 generator and bubble Cl_2 through samples for 30 to 60 minutes. This will destroy organic mercury compounds and produces a HgCl anionic complex.
5. Filter through Reeve-Angel SB-2 anion exchange paper. Repeat 3 to 4 times and air dry the filters.
6. Seal filters in heavy polyethylene bags labeled as done for fish samples.
7. Standards containing approximately 50 micrograms are spotted on the resin paper, air dried and packaged as above. A resin paper blank is similarly packaged.
8. Irradiate at a flux of 5×10^{12} n/cm^2 sec for 1 to 4 hours. After irradiation, remove the package label and place sample (including bag) in an unirradiated bag. Label the new bag.
9. Count samples with either a thin NaI(Tl) or a Ge(Li) detector. Calculate the total mercury content in each sample and report as nonograms per ml. Counting is done within 24 to 48 hours after irradiation.

The pre-irradiation steps (Steps 4 and 5) have been checked with mercury-203 labeled phenyl mercuric acetate, methyl mercury chloride and mercuric nitrate to determine the recoveries that can be obtained. Solutions of the labeled compounds were made up in tap water (adjusted to 0.1 N in HCl) with concentrations ranging from 0.01 to 12 ppb. The results obtained with mercuric nitrate are in agreement with those reported by Becknell and his

co-workers, in that for concentrations below about 10 ppb, 100% of the mercury present can be obtained. Becknell, et al also obtained 100% recovery of the mercury present in methyl-mercuriguanidine at a concentration of 2.7 ppm. Our study has shown that for concentrations below about 10 ppb the recovery of organic mercurials is essentially quantitative.

The balance of the procedure is in essence, identical with the irradiation and counting technique used for analysis of biological samples (neglecting the chemical separation). In addition, identical irradiation and counting techniques have been used in this laboratory for the past ten years for analysis of mercury at levels similar to those anticipated in the water samples. For these reasons, it was not felt necessary to test this section of the procedure with simulated samples.

As indicated previously, water samples were collected from several areas in Lake Ontario. However, due to the extended reactor shutdown in August and September, it was not possible to irradiate the samples prior to the program completion date.

III. Sample Collection

Smelt and slimy sculpin samples were collected from the Youngstown area and Prince Edwards Bay during September 1971. Each sample was a composite of a number of individual fish of about the same size. The collection and composite data are shown in Table 13.

A composite walleye pike sample was divided into two subsamples which were submitted for analysis as two separate samples. These samples provided a blind specimen check on the analysis.

Table 13

Fish Sample Data

Species	Sample Number	Location and Collection Date		Number of Fish in Composite
Smelt	513	Youngstown	9/19/71	27
Smelt	515	Youngstown	9/19/71	16
Slimy Sculpin	514	Youngstown	9/19/71	11
Slimy Sculpin	516	Prince Edwards Bay	9/14 - 9/17/71	48
Slimy Sculpin	519	Prince Edwards Bay	9/14 - 9/17/71	34
Smelt	517	Prince Edwards Bay	9/14 - 9/17/71	16
Smelt	518	Prince Edwards Bay	9/14 - 9/17/71	20

In addition to the analysis of the smelt and slimy sculpin samples from Lake Ontario, a number of fish from the Western Basin of Lake Erie were analyzed. These specimens were collected during the fall of 1970 for the Lake Erie ecosystem mercury study[1,4].

IV. Counting and Data Processing

The gamma and/or x-ray emissions from the radioisotopes of interest were counted with sodium iodide detectors and a 400-channel pulse height analyzer. Samples were counted in special sample mounts which assured a reproducible geometry. The pulse height analyzer data were used to calculate the amount of the element in the original sample. The data from large numbers of samples were processed using Schonfeld's ALPHA-M computer program[5].

Samples were, in general, analyzed in duplicate. Rejection criteria, based on percent error (standard deviation), were established for each element being determined. If the standard deviation of a replicate analysis exceeded this limit, a third analysis was performed. The rejection criteria were as follows:

Cadmium	± 20%	Copper	± 10%
Zinc	± 10%	Mercury	± 15%
Chromium	± 20%	Selenium	± 20%
Arsenic	± 10%		

In the majority of the cases, the error in duplicate analysis was well below the rejection limit.

RESULTS AND DISCUSSION

The results of the blind sample analysis are presented in Table 14. As can be seen, except for chromium, the results obtained on these samples are in excellent agreement. The chromium results are not in agreement within one standard deviation, but are in agreement within two standard deviations. The chromium results are in agreement within the 20 percent rejection criteria. The reasons for this problem with the chromium determination are not apparent at the present time. Duplicate analysis (run on different days) on the individual samples indicates a reproducibility of about 10 to 15 percent (one standard deviation). The results obtained in the chromium procedure development work (Table 5) also indicate a reproducibility of the order of 10 percent. This would suggest a lack of homogeneity in the samples with respect to chromium. However, in view of the excellent agreement obtained for the other elements, this does not appear likely. Thus, it would appear that in comparison of chromium levels obtained in this study, one must apply a standard deviation of 20 percent.

A series of experiments were carried out testing labeled organic and inorganic mercury compounds to determine the feasibility of differentiating between inorganic and organic mercurials in water. Mercury-203 labeled methyl mercuric chloride, phenyl mercuric acetate, and mercuric nitrate were used as representative compounds. Tap water solutions of each compound were prepared such that the mercury concentration was 10 ppb. One hundred ml aliquots were extracted with 50 mls of either chloroform or benzene. The extractions were repeated three times. The amount

Table 14

Heavy Element Concentrations in Blind Samples

	CONCENTRATION (ppm)*	
Element	Sample 501	Sample 509
Cadmium	0.052 ± 0.006	0.050 ± 0.005
Zinc	11.8 ± 0.95	11.5 ± 0.73
Chromium	0.12 ± 0.017	0.083 ± 0.008
Arsenic	0.43 ± 0.026	0.45 ± 0.020
Copper	0.53 ± 0.046	0.53 ± 0.080
Mercury	0.59 ± 0.063	0.65 ± 0.034
Selenium	0.74 ± 0.047	0.74 ± 0.042

* Errors are standard deviation

removed from the aqueous phase was determined after each extraction. Results of this investigation are shown in Table 15. It is apparent that neither solvent is suitable for preferentially removing organic mercurials from water. Therefore, the designation of chloroform extractable mercurials as organic and the non-extractable mercurials as inorganic, which some workers have used, is open to question.

The results of the analysis of Lake Erie fish samples are presented in Table 16. A variety of species from the Western Basin were analyzed. Walleyes of different ages collected in the Central Basin were also analyzed. Analysis of specimens from the Eastern Basin was not carried out, in part, due to sample limitation. The results presented are probably representative of the highs and lows in Lake Erie fish samples. It should be noted that our previous work[1,4] has shown that mercury concentrations in adult fish of the same species tended to be highest in the Western Basin and lowest in the Eastern Basin. The notable exception to this rule was Coho salmon which did not vary appreciably from basin to basin, probably as a result of their migratory habits.

The data obtained on walleye from the Central Basin indicate that the accumulation and sequestration of the heavy elements with age is a highly pronounced phenomenon in the case of mercury. A similar trend is not readily discernible with the other elements studied. In fact, the concentrations of copper and zinc appear to decrease somewhat with age, while the others remain essentially constant. The mercury accumulation

Table 15

Solvent Extraction of Mercurials from Water

Compound	Extractant	PERCENT EXTRACTED		
		1 Extraction	2 Extractions	3 Extractions
Methyl Mercuric Chloride	Chloroform	40.2	46.0	49.9
Phenyl Mercuric Acetate	Chloroform	51.9	49.6	54.7
Mercuric Nitrate	Chloroform	4.5	8.1	13.1
Methyl Mercuric Chloride	Benzene	54.6	79.4	83.6
Phenyl Mercuric Acetate	Benzene	34.0	56.8	66.8
Mercuric Nitrate	Benzene	15.6	59.7	64.9

Table 16

Heavy Metallic Pollutants in Lake Erie Fish Samples*
(1970 Fall Catch)

Species	Cr	Hg	As	Se	Cu	Cd	Zn
(WESTERN BASIN)							
Walleye	0.12	0.79	0.11	0.48	0.52	0.053	8.01
Yellow Perch	0.29	0.61	0.04	0.38	1.46	0.057	14.93
White Bass	0.16	0.60	0.28	0.62	0.87	0.060	12.87
Channel Catfish	-	0.36	0.07	0.29	0.53	0.057	11.41
Freshwater Drum	0.77	0.67	0.09	0.64	2.64	0.077	9.52
Carp	0.29	0.23	0.20	0.50	0.96	0.048	42.95
Coho Salmon	0.10	0.69	0.09	0.42	0.93	0.039	8.08
White Sucker	0.14	0.55	0.11	0.44	0.89	0.049	12.24
Gizzard Shad	0.12	0.20	0.34	0.35	0.91	0.024	8.59
(CENTRAL BASIN)							
Walleyes (YOY)	0.18	0.39	0.10	0.34	1.73	0.039	11.24
Walleyes (YLG)	0.54	0.59	0.12	0.33	0.76	0.030	10.23
Walleyes (II+)	0.26	0.75	0.12	0.34	0.81	0.032	9.96

*All the results reported are as μg of element per gram of raw fish tissue composites. The composites used were prepared from the edible tissues of about 25 individual specimens.

would indicate that this element (presumably in the form of methylmercury) has a long biological half-life. This observation is consistent with results obtained by Dr. E. J. Massaro and his co-workers in pulse feeding studies with rainbow trout. They have found that the biological half-life of methylmercury is of the order of 1,000 days[6].

The mercury data also indicates that the highest concentrations are in those species which are higher in the food chain. A clear differentiation on the basis of food chain position is not apparent for any of the other elements studied. There are indications of possible preferential accumulation of certain elements by individual species, eg., cadmium and copper by drum, and zinc by carp.

The results of the analysis of Lake Ontario smelt and slimy sculpin are presented in Tables 17 and 18.

If one assumes that the concentration of a given element in two samples of a given species of fish collected in the same area and at the same time should be in reasonable agreement, the results obtained for copper in smelt are surprising. The cadmium values also show a larger discrepancy than might be expected on the basis of the blind sample results. Several explanations are possible. First, sample contamination could account for the observed discrepancy; however, since a similar phenomenon is not apparent in the sculpin data, sample contamination does not appear likely. A second possibility is that with respect to these elements, a larger number of fish are required to provide a representative composite sample. Thirdly, these differences could be real. At the present time, it is not possible to draw a firm conclusion as to the

Table 17

Heavy Element Concentrations in

Lake Ontario Smelt

Element	CONCENTRATION (ppm) Youngstown (513)	Youngstown (515)	Prince Edwards Bay (517)	Prince Edwards Bay (518)
Cadmium	0.048 ± 0.006	0.060 ± 0.007	0.047 ± 0.006	0.069 ± 0.008
Zinc	31.6 ± 1.96	29.8 ± 1.85	25.9 ± 1.61	24.0 ± 1.49
Chromium	0.35 ± 0.024	0.26 ± 0.025	0.13 ± 0.003	0.11 ± 0.006
Arsenic	0.57 ± 0.022	0.49 ± 0.019	0.39 ± 0.031	0.43 ± 0.028
Copper	1.60 ± 0.16	2.46 ± 0.16	1.23 ± 0.10	0.90 ± 0.075
Mercury	0.24 ± 0.008	0.26 ± 0.015	0.17 ± 0.023	0.16 ± 0.010
Selenium	0.39 ± 0.008	0.42 ± 0.003	0.50 ± 0.005	0.48 ± 0.011

Table 18

Heavy Element Concentrations in
Lake Ontario Slimy Sculpin

	CONCENTRATION ppm		
Element	Youngstown (514)	Prince Edwards Bay (516)	Prince Edwards Bay (519)
Cadmium	0.096 ± 0.012	0.14 ± 0.017	0.15 ± 0.018
Zinc	19.8 ± 0.5	16.6 ± 1.03	17.6 ± 1.09
Chromium	0.19 ± 0.022	0.094 ± 0.015	0.13 ± 0.015
Arsenic	0.23 ± 0.010	0.33 ± 0.019	0.33 ± 0.013
Copper	2.00 ± 0.14	1.80 ± 0.14	1.92 ± 0.16
Mercury	0.51 ± 0.063	0.23 ± 0.014	0.30 ± 0.010
Selenium	0.66 ± 0.023	0.71 ± 0.043	0.73 ± 0.044

reason for the discrepancies in the smelt data.

The mercury levels in both smelt and sculpin indicate a higher level in fish collected at the western end of Lake Ontario. This is as might be expected since the major source of mercury input to the Lake is from the Niagara River. Zinc shows a similar trend although not as marked. There does not appear to be any clear-cut geographic trend with any of the other elements. It should be noted that the mercury levels in Youngstown smelt are similar to the levels we found in Eastern Basin Lake Erie smelt (0.3 ppm)!

A maximum permissible level for mercury in fish has been established by the Food and Drug Administration (FDA). Except for the sculpin sample from Youngstown, the mercury levels found in smelt and sculpin were below the FDA action level (0.5 ppm). This, however, does not imply that mercury levels in Lake Ontario fish are, in general, low. The phenomenon of higher concentrations in fish higher up in the food chain exhibited in Lake Erie fish can be expected to be found in Lake Ontario fish. This has been demonstrated by other workers. Furthermore, we have found mercury levels as high as 1.3 ppm in single specimens of Great North Pike caught in the St. Lawrence River region. Permissible levels of other heavy elements in food products have not been established. The relatively high levels of arsenic and selenium found in this work strongly suggests the need for investigations of the public health aspects of chronic ingestion of heavy elements.

CONCLUSIONS AND RECOMMENDATIONS

Several conclusions can be drawn from the work described in this report.

1. The use of chloroform or benzene extraction techniques to differentiate between inorganic and organic mercurials in water samples is not valid.
2. Mercury accumulates in fish muscle with age.
3. Mercury concentrations are higher in species higher in the food chain.
4. The data indicates higher levels of mercury in fish from the western end of Lake Ontario. A similar pattern is found in Lake Erie.
5. The levels of several of the heavy metals appear to be relatively high. However, ther is insufficient information available to determine the significance of these elemental concentrations.

The results of this study lead us to recommend:

1. An in-depth study of the levels of the heavy elements in Lake Ontario aquatic life.
2. Investigation of the public health aspects of chronic ingestion of heavy elements in food products.
3. An in-depth study of the sources of heavy elements in Lake Ontario.

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