Metal Concentrations in Wild Rice Roots and Seeds, Mollusks, Crayfish, and Fish Collected from Various Wisconsin Water Bodies in the Autumn of 2003

by

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Introduction

Wild rice (*Zizania palustris*) is important to the culture and economies of Lake Superior Chippewa Indian tribes. This important food crop is found in some areas of Wisconsin that are located near sites of potential copper, zinc, gold, silver, and lead mines. The Great Lakes Indian Fish and Wildlife Commission (GLIFWC) initiated this study to determine the metal content of wild rice tissues from eight water bodies (Table 1 and Figure 1) in the treaty territories of Wisconsin ceded in 1837 and 1842. Plants were collected, roots and seeds removed. The samples were immediately frozen and stored until transported to the analytical laboratory at the University of Wisconsin - Superior (UW-S), Superior, WI. This was a repeat sampling and analysis to compare with the results of measurements from the previous years (2000, 2001 and 2002). In addition, muscle tissue from snails, clams, and crayfish were analyzed for concentrations of nine metals. Fish from the same area were analyzed for mercury content.

Table 1. Sampling Sites, Locations, and Coding for Wild Rice Samples Analyzed for Metal Content.

Sample Site	Location	Sample Codes*
Chequamegon Waters Flowage	Taylor County; 90º42'E - 45º12'N	CF101 - CF148
Mondeaux Flowage	Taylor County; 90º25'E - 45º17'N	MF201 - MF248
Fish Lake	Oneida County; 89º15'E - 45º37'N	FL301 - FL348
Spur Lake	Oneida County; 89º9'E - 45º42'N	SL401 - SL448
Rat River	Forest County; 88º42'E - 45º33'N	RR501 - RR548
Swamp Creek	Forest County; 88º57'E - 45º29'N	SC601 - SC648
Rocky Run Flowage	Oneida County; 89º44'E - 45º42'N	RF701 - RF748
Lake Alice	Lincoln County; 89º36'E - 45º29'N	LA801 - LA848

* Sample numbers X01 - X12 = composite 1; X13 - X24 = composite 2; X25 - X36 = composite 3; X37 - X48 = composite 4.

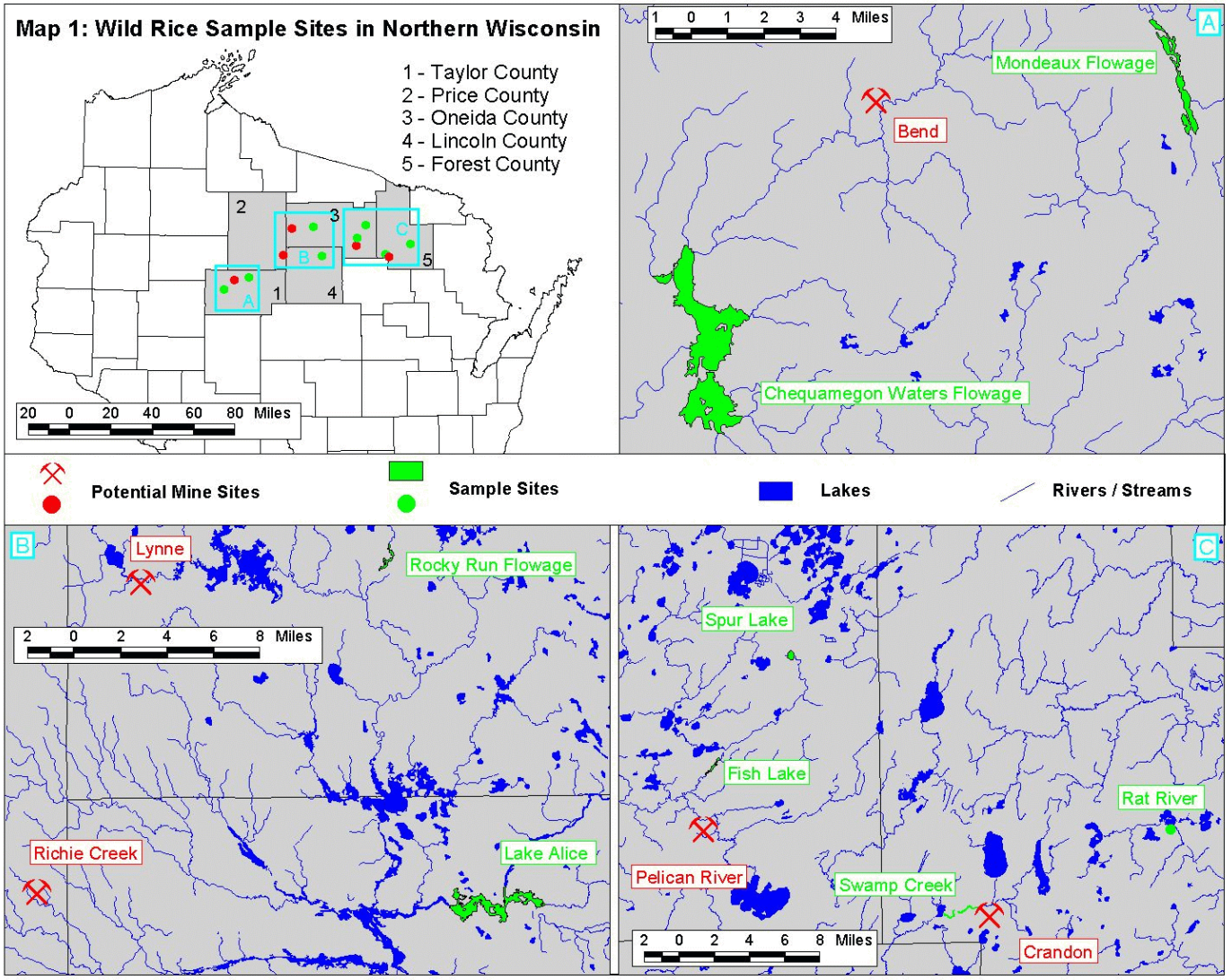

Figure1. Maps of the locations of wild rice sampling sites for samples collected in the autumn of 2003

Methods

Wild Rice – Samples of wild rice roots and seeds with the hull attached were collected during August 2003 from eight water bodies in northeastern Wisconsin. Members of the GLIFWC biological staff collected samples from a canoe. Gatherers of the plants wore surgical gloves when collecting the root and seed samples. Forty-eight plants were collected from each body of water. Within each rice bed, samples were collected from four locations (twelve plants from each of four sites) that were within 7.5 to 15 meters of each other. Water depth was measured from the water surface to the top of the root mass. Individual plants were pulled from the substrate and loose sediment clinging to the roots was rinsed in lake water to remove the majority of the sediment. The plant was labeled and placed in a critically cleaned 20 L plastic container. Seeds were removed from each individual plant by pulling single seeds (15 or more seeds were desired) from the panicle and placing them in a critically cleaned high density polyethylene (HDPE) sample container which was then capped and labeled. An extra jar of seeds was collected from each of the composite locations in the immediate vicinity of the collected plants to be used as "moisture seed." After the seeds had been removed from all plants, the collector changed to a new pair of gloves and removed a portion of the roots without tools and placed the root sample in a critically cleaned HDPE sample container. The procedure was repeated for twelve plants at each of four sampling sites in each of eight bodies of water. The samples were taken to the GLIFWC laboratory in Odanah, WI and frozen at about -18 C. A chain-of-custody record was started.

The samples were transferred to the Environmental Health Laboratory at the UW-S and placed in a freezer. Processing of the samples for analysis began in September of 2003. Before beginning the processing of the root and seed samples, in preparation for metals analysis, the equipment was cleaned using the method described in Appendix A (SA/8). The same cleaning process was repeated after each sample was ground so that each sample was processed with critically cleaned equipment.

The twelve individual root samples from each sampling site were composited into a single sample by combining portions of each plant into a common sample used for analysis of metals. Roots were removed from the freezer and thawed. An entire root sample was removed from its sample container. A 5.5 g subsample was weighed from each individual sample and placed back in its original container. The extra root tissue was discarded. If 5.5 g of tissue was not available, the entire root mass was used. The weight of root tissue used from each root container was recorded on the sample compositing form. Each original container was half-filled with deionized (DI) water. The container was placed into a sonicator (Cavitator Ultrasonic Cleaner, Model ME 11, 200 watts; Mettler Electronics Corporation, Anaheim, CA) and the roots were cleaned by ultrasound for three minutes. After ultrasonic cleaning, the DI water was decanted from the roots, and the roots were rinsed with clean DI water two or more times until no visible soil was present. When all twelve root samples were cleaned, they were removed from the sample containers and placed on multiple layers of white paper towels (Kimwipes EX-L; Kimberly-Clark. Corporation, Roswell, GA). A layer of towels was placed over the roots and

pressed by hand on the roots to remove the excess water on the outside of the root masses. This constituted the composite sample for one site in one water body (Appendix B, SA/40). This procedure was repeated for each of the four sites from each water body and for each of the eight water bodies.

Grinding the root composite samples was accomplished using a food blender (Hamilton Beach/Proctor-Silex, Inc., Model 919; Washington, NC) with a one-liter stainless steel blending cup. The root sample was placed in the blending cup and liquid nitrogen was poured on the root sample (Appendix C, SA/38). When the roots were frozen and the cup was at freezing temperature, the tissues were ground for about one minute. This produced a homogeneous mixture of the tissues that was of flour-like consistency. The sample was poured out of the blending cup and into a clean two-ounce HDPE bottle (Quality Environmental Containers, Beaver, WV) via a cooled plastic funnel. The vial was capped, labeled, and immediately frozen.

Wild rice seeds were also made into a composite sample of seeds from twelve plants from the same plants that root samples were collected. Seeds were processed by placing seeds from a sample bottle on a clean Kimwipe and removing excess moisture (Appendix B, SA/40). Seeds were then examined and sorted to remove hollow, chewed or shriveled seed casings. The fullest, ripest seeds were desired for compositing. Seed beards were removed by trimming with a scissors. This was done to provide a more homogenous final composite, because beards do not grind and do not represent the edible portion of the seed. All acceptable seeds for that sample were then placed in a clean, large weighing pan. This process was repeated for each of the twelve samples comprising a composite and weighed in total. In order to reach the 16 g minimum amount of seeds required for analysis, seeds were also taken from the extra "moisture seed" sample jar as needed. Two lakes (Rocky Run Flowage and Fish Lake) received additional seeds from the "moisture seed" jar in all four replicate sampling sites. The amount of seed weight contributed from the "moisture seed" varied from 3.7 to 74.0 percent. The remainder of the lakes had additional seeds in three or fewer replicate sample sites. The composite weight was recorded on the sample composite sheet. Afterwards, the mixture of the twelve individual seed samples, and "moisture seed" were placed in a stainless steel blender cup and frozen using liquid nitrogen (Appendix C, SA/38). The mixture was blended for one minute, using a food blender (same blender that was used for the root tissue), to produce a homogenous sample. The ground sample was placed in a two-ounce HDPE container and frozen.

Moisture analyses were conducted on all wild rice root samples in duplicate at the time of sample digestion for metals analysis. Moisture analysis on seeds was measured in duplicate on all samples prior to grinding. Two seeds from each individual plant sample were placed into each of two aluminum weighing pans (24 seeds per pan per composite). Moisture was determined by measuring the difference between sample mass before and after drying in an oven at 60 degrees Celsius for more than 24 hours (Appendix D, NT/15). Percentage moisture can be used to compute metal concentrations in the roots and seeds on a dry weight basis.

Mollusks – Samples of clams and snails were collected from sample sites near the Crandon ore body. Clams and snails were received at the UW-S frozen and stored in a freezer until processing. Processing began in February 2003 with samples sorted into composites or left as individuals according to instructions received from GLIFWC. Mollusks were placed on a critically cleaned glass cutting board, and a scalpel was used to open the shell and remove the soft tissue from the shell. The digestate and intestine were removed from the visceral mass, and the remaining sample was rinsed with deionized water. Each individual sample was weighed and the weight was recorded on the sample composite form. Once all individuals that comprised a composite had been cleaned and weighed, they were placed in a stainless steel blender cup and frozen with liquid nitrogen (Appendix C, SA/38). They were ground (same blender that was used for the wild rice root tissue) for approximately one minute or until a homogenous sample was achieved. The sample was then placed in a labeled two ounce HDPE bottle, which was then capped and frozen until analysis. Snails were processed in a similar manner, but no attempt was made to remove digestate or intestine. Juvenile snails were discarded and not used for analysis of metals.

Crayfish – Samples of crayfish of unknown species had intestine contents removed and all of the animal was ground, including the exoskeleton. Moisture analysis was conducted on all clam, snail, and crayfish samples at the time of digestion for metals analysis (Appendix D, NT/15).

Fish – Samples of northern pike (*Esox lucius*), and largemouth black bass (*Micropterus salmoides*) were collected from three lakes (Deephole, Little Sand, and Mole Lakes) in northeastern Wisconsin during October 2003. Frozen whole fish were transferred to the EHL at UWS and placed in a freezer. Processing of the samples occurred in February and March 2004. Fish were measured, weighed and sexed and either one or two filets were removed depending on the size of the filet. Skin was removed from the filets. The filets were ground using liquid nitrogen to freeze the samples. They were then placed in a blender and ground to a homogenous mixture (Appendix C, SA/38). Fish samples were analyzed for mercury on the FIMS-100 mercury analyzer (PerkinElmer Instruments, Shelton, CT) (Appendix E, SA/13). Moisture analysis was conducted on a subsample of fish samples at the time of grinding (Appendix D, NT/15).

Analysis of Metals – Nine metals [arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), lead (Pb), magnesium (Mg), mercury (Hg), selenium (Se), and zinc (Zn)] were analyzed in each composite sample of wild rice roots and seeds. In addition, iron (Fe) was analyzed in wild rice root composites. Mollusk, snail and crayfish samples were analyzed for the same nine metals as wild rice seeds, while fish samples were analyzed for mercury only. Metals were analyzed (Table 2) by flame or cold vapor Atomic Absorption Spectroscopy (AAS; Appendix F, SA/34) or by Inductively Coupled Plasma-Mass Spectroscopy (ICP-MS; Appendix G).

All metals except mercury were prepared for analysis by digesting tissues (5 g tissue or less) with concentrated nitric acid and 30% hydrogen peroxide combined with heating the samples on a hot plate (Appendix H, SA/33). Tissues for mercury analysis (0.5 g or less) were digested with

concentrated sulfuric and nitric acids in a hot block (Environmental Express, Mt. Pleasant, SC). Potassium permanganate and potassium persulfate were used to convert organic mercury to inorganic mercury and stannous chloride converted inorganic mercury to elemental mercury which is analyzed by cold vapor AAS (Appendix E, SA/13).

Quality Assurance – Quality of analysis was monitored by several methods during this study. Analysis of reagent blanks determined if reagents contained appreciable quantities of metals or if contamination occurred during the sample preparation. Analysis of lab control spikes was employed to measure recovery of spikes in reagents only. This allows differentiation between poor spike recoveries, in general, and matrix interferences in samples. Analysis of similar tissues before and after the tissue grinding process (procedural blanks) measured lab bias. Accuracy is measured by analyzing certified reference standards of rice flour and/or dogfish shark tissue, and/or mussel tissue. Duplicate analysis was conducted on a minimum of ten percent of samples as a measure of precision. Analysis of a minimum of ten percent of samples spiked with known concentrations of metals indicated whether matrix interferences were present.

Copper, magnesium, zinc and iron were analyzed at the Environmental Health Laboratory by flame AAS. Standard solutions of known concentrations were prepared from purchased (Fisher Scientific, Chicago, IL) certified solutions (Appendix F, SA/34). Four to five standard solutions were prepared for each metal in 0.5 % nitric acid (trace metals grade). A standard curve was prepared each day of analysis using the standard solutions. After each group of twenty samples, an intermediate concentration standard solution was used to check and adjust the calibration curve if necessary. A quality control standard (Environmental Research Associates, Arvada, CO) was also analyzed at this frequency of sample analysis to ensure accuracy of standards and calibration curve.

Reproducibility of the analyses was measured as the relative standard deviation (coefficient of variation) of the repeat measured values. Copper, magnesium, zinc and iron were measured three times on each sample. The mean relative standard deviations of the repeated measures for copper, magnesium, zinc, and iron were 2.6, 0.50, 0.78 and 1.7 percent, respectively. These values were calculated from 102 analyzed samples for each metal except iron. Iron was calculated from 37 values because only roots were analyzed for iron. The relative standard deviation values for the metals analyzed by En Chem, Inc. were not requested due to cost for this service, but are usually less than 5.0 percent.

Reagent blanks were processed with each digestion set by completing the digestion and analysis procedure on samples containing only reagents. This was done to determine if reagents contribute measurable quantities of the metals in question or if contamination is added during the digestion. Arsenic, cadmium, chromium, copper, lead, magnesium, and selenium were all below the Limit of Detection (LOD) or within the Limit of Quantitation (LOQ) in the reagent blanks (Table 3). LOQ is defined as 10/3 of the LOD. Both reagent blanks for iron had values above the LOQ. Zinc had one of seven blank samples with a concentration above the LOQ. The zinc reagent blank values were subtracted from sample concentrations because they were consistent.

Lab control spikes were also processed with each digestion set. A known quantity of mixed metal spiking solution was added into an empty digestion tube and treated as a sample. Lab control spikes determine if a measurable loss or gain of the metals occurred during the digestion process. The lowest mean recovery of spiked metal was 96% for iron while the highest mean recovery was 110% for selenium (Table 4). The maximum standard deviation for six measurements of each metal was 9.2 percent for Cr.

Wild rice seed that had been processed for commercial sale served as procedural blanks for metals analysis. Comparison of metal concentrations analyzed in ground and not ground samples measured laboratory bias by determining whether metals are lost or gained in the grinding procedure (Table 5). None of the metals analyzed tested significantly different (α=0.05) after grinding of the tissues (Table 6). In analyses of wild rice seeds from previous years, chromium had a tendency to increase after grinding and it was speculated that chromium may have been added from the grinder blades. The tendency of chromium to increase in the ground samples was not evident this year.

A rice flour reference standard was purchased from the National Institute of Standards and Technology, Gaithersburg, MD. The Standard Reference Material® 1568a (Rice Flour) was prepared from 100% long grain rice from the State of Arkansas. The rice flour contains certified concentrations of arsenic, cadmium, copper, iron, magnesium, mercury, selenium, and zinc. Certified values were not provided for chromium and lead. Metal concentrations measured in rice flour following sample digestion procedures were in general agreement with expected values with a low mean agreement of 78.0% for magnesium and a high agreement of 280% for iron (Table 7). Analysis of blanks showed significant amounts of iron compared to the reference material and would account for the high percentage agreements for this metal (data comparison of iron in Tables 3 and 7 adjusted for the unit differences).

Mussel tissue was also purchased to use as a reference standard for determining method accuracy. The mussel tissue was purchased from the National Institute of Standards and Technology, Gaithersburg, MD 20899. The Standard Reference Material® 2976 (Mussel Tissue) was prepared from mussels (*Mytilus galloproincialis*) from the coast of France. The tissue contains certified concentrations of arsenic, cadmium, copper, iron, lead, mercury, selenium and zinc. Certified values were not provided for chromium and magnesium. Again, measured values were in general agreement with certified values with a low agreement of 91.4 % for cadmium and a high agreement of 133 % for selenium (Table 8).

Approximately ten percent of the wild rice seed and root samples were analyzed as duplicate samples to measure precision of the analysis. They were digested as two completely separate samples and concentrations were compared for agreement of analysis. Mean duplicate agreement for wild rice seed and root samples ranged from a low of 74.6 % with lead to a high of 95.2 % for zinc (Table 9). Mean duplicate agreement for mollusks had a low percent agreement for chromium (88.5 %), and a high of 100 % was measured for cadmium (Table 10).

Ten percent of wild rice root and seed samples were spiked before digestion with known concentrations of the metal of interest and compared to the concentration in the non-spiked sample to determine if matrix interferences were present. The average spike recoveries for wild rice samples ranged from 93.1 % for lead to 121 % for arsenic (Table 11). In mollusks, spike recovery ranged from 86.5 % for cadmium to 107.6 % for selenium (Table 12). Copper and magnesium had recovery percentages ranging from -718 to 298.6 % which are both unreasonable and should be ignored. The reason for the widely varying spike recoveries for these two metals is, at the time of spiking, there was limited information on levels of metals in mollusk tissues. As a result, the spiking levels for magnesium and copper were well below the measured values for the parent samples and the spike concentrations were insignificant compared to the amount present before spiking.

Mercury analysis was conducted at the EHL by cold vapor AAS on a FIMS-100 analyzer. Mercury standards were made as sets of five concentrations plus a reagent blank with one set run at the beginning of the analysis and another full set analyzed with each set of thirty to forty tissue samples (Appendix I, SA/42). Three absorbance readings are taken for each sample by the instrument, with the reported concentration being an average of those readings.

Commercially purchased canned tuna fish (*Thunnus* sp.) served as a procedural blank for mercury analysis. After the liquid was removed from the can, one portion was transferred directly into a sample bottle. A second portion was ground in the same manner as other muscle samples. This check was made to determine if contamination or loss of mercury was occurring in the grinding process. Analysis of the procedural blanks processed coincident with sample grinding gave an average of 88.5% agreement for mercury concentration (Table 13).

The DORM-2 sample was analyzed as a quality assurance measure for mercury in tissue. The sample has a known concentration of 4.64 ± 0.26 μg Hg/g of tissue. Agreement with the known concentration was 96.7 ± 4.24 percent for sixteen analyses (Table 14).

Duplicate agreement calculations for mercury in wild rice samples averaged 89.1 ± 9.30 (Table 15). In a mollusk and a crayfish, duplicate measurements resulted in 84.4 ± 19.4 percent agreement for mercury (Table 16). Agreement in duplicate analyses in fish samples was 92.7 ± 6.81 percent (Table 17).

Ten percent of all samples measured for mercury concentrations were also spiked with known concentrations of mercury and analyzed for spike recovery. In wild rice roots and seeds, average spike recovery was 104.2 ± 11.6 percent (Table 18). Mollusks had an average spike recovery of 103.5 ± 5.87 percent (Table 19) and fish had an average of 80.7 ± 20.1% (Table 20). It should be noted that when fish sample 1699 was initially analyzed, it had a spike recovery of 53.8 percent. It was spiked and analyzed a second time resulting in a 67.7% spike recovery. This suggests that there is an interference in that particular sample causing the poor spike recovery

because other spike recoveries done on that day with rice seed samples had good recoveries (Table 18).

Results

Data are reported in one of three categories. Some samples yield concentrations below the Detection Limit (DL) of the method. When this happens, the concentration for that sample is reported as a "less than" numerical value. Some data were measured above the detection limit (DL), but are less than ten-thirds of the detection limit and are marked as data between the DL and the Limit of Quantitation (LOQ). There is a lower confidence with values between the DL and LOQ than those above the LOQ. The third category of data are values above the LOQ.

Wild rice – All ten metals were found in the roots and seeds of wild rice plants from the eight bodies of water sampled for this study with the exception of iron which was not measured in the seeds. Metal concentrations (Table 21, 22, and 23) in the seeds ranked in the following order: magnesium > zinc > copper > chromium > cadmium > lead ≈ selenium ≈ arsenic > mercury. In the roots, the rank order was: iron > magnesium > zinc > arsenic > copper ≈ lead > chromium > selenium > cadmium > mercury. When the analyses of a metal for all water bodies were combined, root tissues contained higher concentrations than seeds of arsenic and lead. Seeds contained higher concentrations of copper, magnesium, mercury, and zinc. Cadmium, chromium, and selenium had similar concentrations in both tissues. The elements that are essential for plant growth (copper, iron, magnesium and zinc) are the most abundant elements measured in the seed and root tissues on a wet weight basis. There were variations in concentrations of the measured metal species between the water bodies (Table 21), but no patterns were observed.

Mercury concentrations ranged from <0.00126 to 0.00688 µg/g Hg in wild rice seeds and from <0.00126 to 0.00439 µg/g Hg in wild rice roots (Table 23). Generally, seed concentrations were higher than root concentrations.

Moisture concentrations were measured in the seeds and roots of wild rice. Percent moisture was determined after drying in a 60 C oven for 24 hrs (Tables 24 and 25). Roots contained the higher moisture of the two tissues with a grand mean of 88.4 ± 1.31 % and a range of 84.8 to 92.1 % with 64 measurements. Seeds varied more between water bodies than roots in moisture percentage. Seeds had a grand mean of 42.0 ± 5.72 % moisture and a range of 28.8 to 56.0 % for 64 measurements. Moisture variation in seed samples was most likely due to variations in seed ripeness at the time of sampling.

Mollusks – Total mercury concentrations were measured on a wet weight basis for sixteen samples of mollusks collected in Northwest Wisconsin (Table 26). Four species of clams (Pig toe clam = *Fusconaia flava*; Floater clam = *Pyganodon grandis;* Fluted shell clam = *Lasmigona costata*; Fat Mucket = *Lampsilis siliquoidea*), one genus (*Viviparus* sp.) of snail, and an

unidentified species of crayfish were analyzed as individual organisms or analyzed as a composite of several animals when tissue mass was small. Samples contained mercury concentrations that ranged from 0.0147 to 0.0818 µg/g of total mercury. Snails had higher concentrations of mercury than clams but snails may have had some digestate present during processing. There was also some sand-like material present in the snail samples after the digestion procedure. In clams the metal concentrations (Table 27) ranked as follows: magnesium > zinc > copper > arsenic > chromium > selenium > cadmium > lead. Metal concentrations in snails were ranked as follows: magnesium > zinc > copper > arsenic > selenium > chromium > lead > cadmium. Moisture was measured in all 13 mollusk samples (Table 28). There is a significant difference in moisture values between crayfish and snails/clams. The mean moisture concentration for snails and mollusks was 87.1 ± 1.45 % for 11 samples with a range of moisture concentrations of 85.1 to 89.9 %. The mean moisture for the two crayfish samples was 61.3%.

Fish – Northern pike and largemouth black bass from three lakes were analyzed for total mercury content (Table 29). Thirty-nine fish were fileted and the skinless muscle tissue analyzed. In all lakes combined, mercury concentrations ranged from 0.172 to 1.28 µg/g with largemouth bass having the highest and lowest values. Tissue moisture was measured in all of the 39 filets at the time of mercury analysis. Moisture in the filets ranged from 77.4 to 83.0 percent with an average of 79.4 ± 1.0 (Table 30). The mercury in fish were compared by regression analysis (Figure 2), but only large mouth black bass were captured in each lake. The largemouth black bass appear to increase in mercury concentration in each lake at the rate of approximately 0.1 to 0.4 µg/g/10cm increase in total length on a wet weight basis. Northern pike in Little Sand Lake do not have enough variation in length to get a valid regression line. (The regression equations for Mole Lake large mouth black bass is $Y = -0.16 + 0.017x$, $r^2 = 0.92$; Deep Hole Lake largemouth black bass is $Y = 0.51 + 0.038x$, $r^2 = 0.80$; $Y = -1.13 + 0.097x$, $r^2 = 0.78$; and for Little Sand Lake largemouth black bass and northern pike are $Y = -0.01 + 0.013x$, $r^2 = 0.27$, $Y = 0.71 - 0.0001x$, $r^2 = 0.000029$.)

Table 2. Method of Analysis, Laboratory for Analysis, and Detection Limits for Analysis of Various Metals in Wild Rice Roots and Seeds.

Metal	Method of Analysis	Laboratory for Analysis	Biota Detection Limit[a]
Arsenic	Inductively Coupled Plasma MS	En Chem, Inc., Green Bay, WI	0.076 mg/kg
Cadmium	Inductively Coupled Plasma MS	En Chem, Inc., Green Bay, WI	0.038 mg/kg
Chromium	Inductively Coupled Plasma MS	En Chem, Inc., Green Bay, WI	0.082 mg/kg
Copper	Atomic Absorption Spectroscopy; flame	Environmental Health Laboratory, UW-Superior	0.386 mg/kg
Iron	Atomic Absorption Spectroscopy; flame	Environmental Health Laboratory, UW-Superior	1.37 mg/kg
Lead	Inductively Coupled Plasma MS	En Chem, Inc., Green Bay, WI	0.048 mg/kg
Magnesium	Atomic Absorption Spectroscopy; flame	Environmental Health Laboratory, UW-Superior	0.714 mg/kg
Mercury	Cold Vapor Atomic Absorption Spectroscopy	Environmental Health Laboratory, UW-Superior	0.0019 mg/kg
Selenium	Inductively Coupled Plasma MS	En Chem, Inc., Green Bay, WI	0.12 mg/kg
Zinc	Atomic Absorption Spectroscopy; flame	Environmental Health Laboratory, UW-Superior	0.118 mg/kg

[a] As, Cd, Cr, Pb and Se Biota Detection Limits are based on 1 g tissue. Cu, Mg, and Zn Biota Detection Limits are based on ~4 g tissue. Fe Biota Detection Limit is based on 4 g tissue. Hg Biota Detection Limits are based on 0.5 g tissue.

Table 3. Concentrations of Various Metals in Reagent Blanks.

Sample	Date Digested	As (µg/L)	Cd (µg/L)	Cr (µg/L)	Cu (mg/L)	Fe (mg/L)	Pb (µg/L)	Mg (mg/L)	Se (µg/L)	Zn (mg/L)
Blank 1	1/12/2004	<0.76	<0.38	<0.82	<0.014	[a]	<0.48	0.09[Q]	<1.2	0.026
Blank 2	1/12/2004	<0.76	<0.38	1.1[Q]	<0.014	[a]	<0.48	0.14[Q]	<1.2	<0.005
Blank 3	1/5/2004	<0.76	<0.38	<0.82	<0.014	0.37	0.850[Q]	<0.042	<1.2	<0.005
Blank 4	1/5/2004	<0.76	<0.38	<0.82	<0.014	0.276	0.670[Q]	<0.042	<1.2	<0.005
Blank 5	2/9/2004	<0.76	<0.38	<0.82	<0.014	[a]	1.300[Q]	<0.042	<1.2	0.007[Q]
Blank 6	2/9/2004	1.00[Q]	<0.38	<0.82	<0.014	[a]	<0.48	<0.042	<1.2	0.007[Q]
Blank 7	2/9/2004	[a]	[a]	[a]	<0.014	[a]	[a]	<0.042	[a]	0.012[Q]

[Q] Analyte has been detected between the Limit of Detection and the Limit of Quantitation. The results are qualified due to the uncertainty of analyte concentrations within this range.

[a] Samples were not analyzed for these metals.

Table 4. Percent Recovery of Analyzed Metals in Laboratory Control Spikes

Sample	Date Digested	As	Cd	Cr	Cu	Fe	Pb	Mg	Se	Zn
LCS 1	1/12/2004	105	110	115	102	[a]	100	102	115	100
LCS 2	1/12/2004	105	100	110	102	[a]	95	97	120	97
LCS3	1/5/2004	100	98	100	94	99	95	97	115	109
LCS4	1/5/2004	95	93	90	96	94	95	92	100	105
LCS5	2/25/2004	100	100	110	94	[a]	100	101	105	97
LCS 6	2/25/2004	100	98	100	100	[a]	100	102	105	94
Mean		101	100	104	98	96	98	99	110	100
Std.Dev.		3.8	5.6	9.2	3.8	3.9	2.7	4.0	7.7	5.7

[a] Samples were not analyzed for Fe because no Fe samples were associated with the digestion set.

Table 5. Metal Concentrations (mg/kg) Measured in Procedural Blank Wild Rice Samples Before and After Grinding.

Sample	Date Digested	As	Cd	Cr	Cu	Pb	Mg	Se	Zn
Unground 11/17/03	1/5/2004	0.057[Q]	<0.0093	0.250	1.17	0.021[Q]	779	<0.029	37.8
Ground 11/17/03	1/5/2004	0.046[Q]	<0.0091	0.150	1.03	0.017[Q]	648	<0.029	32.5
Unground 11/11/03	2/9/2004	0.044[Q]	<0.0081	0.058[Q]	1.11	0.020[Q]	895	<0.026	33.7
Ground 11/11/03	2/9/2004	0.049[Q]	<0.0076	0.160	1.14	0.019[Q]	827	<0.024	33.3
Unground 12/4/03	2/9/2004	0.052[Q]	<0.0078	0.200	1.42	0.017[Q]	831	<0.025	33.7
Ground 12/4/03	2/9/2004	0.049[Q]	<0.0077	0.074	1.11	0.022[Q]	833	<0.024	37.5

[Q] The analyte has been detected between the Limit of Detection and the Limit of Quantitation. The results are qualified due to the uncertainty of the analyte concentrations within this range.

Table 6. Comparison of Mean Metal Concentrations (mg/kg) Measured in Procedural Blanks Before and After Grinding.

Metal	Before		After		RPD[a]
	Mean	Std.Dev.	Mean	Std.Dev.	
As	0.051	0.0066	0.048	0.0017	6.1
Cd	[b]	[b]	[b]	[b]	-
Cr	0.17	0.010	0.13	0.047	27.6
Cu	1.24	0.16	1.09	0.059	12.3
Pb	0.019	0.0021	0.019	0.0025	0.0
Mg	835	58.0	769	105	8.1
Se	[b]	[b]	[b]	[b]	-
Zn	35.1	2.34	34.4	2.70	2.0

[a] RPD = Relative Percent Difference (| Before - After| /Mean of Before and After) x 100.
[b] Values not determined because all were below detection limit.

Table 7. Comparison of Measured Rice Flour Values (mg/kg) with Certified Concentrations for Seven Metals. (Values in Parentheses are the Percentage Recovery of the Certified Values for Standard Reference Material No. 1568A.[**])

Sample ID	Date Digested	As	Cd	Cu	Fe	Mg	Se	Zn
RF1	1/12/2004	0.41 (141)	0.027 [Q] (123)	2.51 (105)	NA	412 (73.6)	0.48 (126)	17.8 (91.7)
RF2	1/12/2004	0.33 (114)	0.029 [Q] (132)	2.42 (101)	NA	408 (72.8)	0.41 (108)	17.1 (88.4)
RF3	1/5/2004	0.41 (141)	0.023 [Q] (104)	2.09 (87.2)	13.7 (186)	431 (77.0)	0.55 (145)	17.7 (91.7)
RF4	1/5/2004	0.35 (121)	0.024 [Q] (109)	2.25 (94.0)	27.7 (374)	429 (76.6)	0.45 (118)	17.9 (92.3)
RF5	2/29/2004	NA	NA	2.44 (102)	NA	504 (90.0)	NA	19.2 (99.2)
Certified Value		0.29±0.03	0.022±0.002	2.4±0.3	7.4±0.9	560±22	0.38±0.04	19.4±0.5
Mean Percent		129	117	97.7	280	78.0	124	92.7
Std.Dev.		14	12	7.0	130	7.0	16	4.0

** No certified values were available for Cr or Pb.
NA = Not analyzed for this metal.

Table 8. Comparison of Measured Mussel Tissue Values (mg/kg) with Certified Concentrations for Seven Metals (Values in Parentheses are the Percentage Recovery of the Certified Values for Standard Reference Material No. 2979.[**])

Sample ID	Date Digested	As	Cd	Cu	Pb	Se	Zn
Mussel-2976-1	2/29/2004	14 (105)	0.76 (92.7)	3.66 (91.1)	1.2 (101)	2.4 (133)	128 (93.2)
Mussel-2976-2	2/29/2004	14 (105)	0.74 (90.2)	3.97 (98.9)	1.3 (109)	2.4 (133)	124 (90.9)
Certified Value		13.3±1.8	0.82±0.16	4.02±0.033	1.19±0.18	1.80±0.15	137±13
Mean Percent		105	91.4	95.0	105	133	92.0
Std.Dev.		0.0	1.7	5.5	5.9	0.0	1.6

** No certified values were available for Cr or Mg.

Table 9. Percent Duplicate Agreement for Wild Rice Seed and Root Samples Collected from Wisconsin Lakes during August and September 2003. (See Table 21 for Measured Values)

Composite ID	Date Digested	As	Cd	Cr	Cu	Fe	Pb	Mg	Se	Zn
MFR103	1/5/04	89.7	89.9	96.7	91.8	89.4	93.0	85.8	98.4	90.8
FLR303	1/5/04	85.0	NC	94.4	99.7	87.5	93.5	86.3	50.0 NC	85.5
RRR203	1/5/04	96.0	NC	100	93.3	98.3	96.7	89.5	96.3	98.3
CFS203	1/12/04	93.6	NC	74.1	97.2	NA	90.0	93.7	NC	99.5
MFS404	1/12/04	95.2	NC	80.0	86.7	NA	32.6	93.8	NC	98.3
SCS103	1/12/04	91.2	NC	79.2	82.2	NA	36.4	98.9	NC	94.3
LAS403	1/12/04	NC	NC	95.0	89.4	NA	80.0	97.2	NC	99.7
Mean		91.8	89.9	88.5	91.5	91.7	74.6	92.2	81.6	95.2
Std.Dev.		4.1	-	10.3	6.0	5.8	27.9	5.1	27.4	5.4

NC Indicates that one or more of the values were below the LOD. Half of the detection limit was used in these calculations unless both values were below the LOD.
NA No iron samples were analyzed coincident with these samples.

Table 10. Percent Duplicate Agreement for Mollusks Collected from Wisconsin Lakes during August and September 2003. (See Table 23 for Measured Values)

Composite ID	Date Digested	As	Cd	Cr	Cu	Pb	Mg	Se	Zn
SC3-A-5	2/25/04	90.6	100	94.6	99.5	96.6	97.9	89.6	97.4
HWY 55 A-20	2/25/04	95.8	NC	82.4	95.8	82.4	97.3	95.2	96.9
Mean		93.2	100	88.5	97.7	89.5	97.6	92.4	97.2
Std. Dev.		3.7	0	8.6	2.6	10.0	0.4	4.0	0.4

NC Indicates that one or more of the values were below the LOD. Half of the detection limit was used in these calculations unless both values were below the LOD.

Table 11. Percent Recovery of Various Metals Spiked into Wild Rice Seed and Root Samples.

Composite ID	Date Digested	As	Cd	Cr	Cu	Fe	Pb	Mg	Se	Zn
MFR103	1/5/04	167	86.9	92.4	96.8	107	100.2	81.9	112.8	111.7
FLR303	1/5/04	83.0	101[NC]	108	111	86.9	95.0	88.2	113.1	95.2
RRR203	1/5/04	185	94.3[NC]	105	97.3	104	110.1	87.9	108.4	110.4
CFS203	1/12/04	75.0	78.1[NC]	75.0	65.4	75.1	72.5	79.0	99.3	77.3
MFS404	1/12/04	114	103[NC]	120	97.4	[NA]	98.8	102.4	105.6[NC]	95.7
SCS103	1/12/04	108	110[NC]	113	93.9	[NA]	77.8	119.4	114.5[NC]	97.2
LAS403	1/12/04	116[NC]	97.6[NC]	115	97.8	[NA]	97.2	99.4	118.2[NC]	98.4
Mean		121	95.9	104	94.3	93.3	93.1	94.0	110.3	98.0
Std.Dev.		40.9	10.7	15.5	13.9	15.0	13.2	14.1	6.3	11.4

[NC] Indicates that one or more of the values used in calculating the spike recovery was below the LOD. Half of the detection limit was used in these calculations.
[NA] No iron samples were analyzed coincident with these samples.

Table 12. Percent Recovery of Various Metals Spiked into Mollusk Samples.

Composite ID	Date Digested	As	Cd	Cr	Cu*	Pb	Mg*	Se	Zn
SC1-D-5	2/25/04	106	82.6[NC]	94.7	-718	102	42.5	111	83.0
ML-C-6	2/25/04	104	90.3	89.2	299	98.9	-106	104	101
Mean		105	86.5	92.0	-210	101	-32.0	108	92.1
Std.Dev.		1.9	5.4	3.9	719	2.5	105	5.4	12.9

[NC] Indicates that one or more of the values used in calculating the spike recovery was below the LOD. Half of the detection limit was used in these calculations.
* The spiking solution used had too low a concentration resulting in poor spike recovery.

Table 13. Percent Agreement of Procedural Blank Samples of Tuna Before and After Grinding for Total Mercury.

Date of Analysis	Before Grinding (µg Hg/g tissue)	After Grinding (µg Hg/g tissue)	Percent Agreement
2/10/2004	0.109	0.107	98.1
3/12/2004	0.102	0.130	78.9
		Mean	88.5

Table 14. Mercury Concentrations (µg Hg/g, Dry Weight) of Dogfish Shark Tissue Supplied by the National Research Council Canada (DORM-2) that were Measured Coincident with the Analysis of Wild Rice, Mollusks, and Fish. The Tissue has a Known Mercury Concentration of 4.64 ± 0.26 µg Hg/g tissue.

Date	#1	#2	Mean	Std. Dev.	Percent of
12/16/2003	4.82	4.76	4.79	0.04	103.3
12/16/2003	4.62	4.44	4.53	0.13	97.7
2/10/2004	4.09	4.22	4.16	0.09	89.6
2/10/2004	4.43	4.50	4.46	0.05	96.2
3/12/2004	4.31	4.50	4.40	0.14	94.9
3/12/2004	4.47	4.70	4.58	0.17	98.8
3/17/2004	4.45	4.82	4.63	0.26	99.9
3/17/2004	4.32	4.32	4.32	0.002	93.1
				Mean and Std. Dev.	96.7 ± 4.24

Table 15. Percent Agreement Between Duplicate Analysis for Total Mercury (µg Hg/g Wet Weight) Content in Wild Rice Collected and Composited during 2003.

Date of Analysis	Composite ID	Sample 1	Sample 2	Percent Agreement
12/16/2003	LAR403	0.0013[Q]	0.0014[Q]	97.0
12/16/2003	MFR203	0.0036[Q]	0.0040[Q]	89.2
12/16/2003	FLR103	<0.0013	0.0013[Q]	[NC]
12/16/2003	SLR303	<0.0013	<0.0013	[NC]
3/17/2004	CFS103	0.0030[Q]	0.0030[Q]	99.2
3/17/2004	SCS403	0.0030[Q]	0.0038[Q]	77.0
3/17/2004	MFS403	0.0014[Q]	0.0017[Q]	83.2
			Mean and Std.Dev	89.1 ± 9.30

[NC] Not Calculable because both values are below the detection limit.

[Q] The analyte has been detected between the Limit of Detection and the Limit of Quantitation. The results are qualified due to the uncertainty of analyte concentrations within this range.

Table 16. Percent Agreement Between Duplicate Analysis for Total Mercury Content in A Mollusk and A Crayfish Collected in 2003.

Date of Analysis	Composite ID	Sample 1 (µg Hg/g)	Sample 2 (µg Hg/g)	Percent Agreement
2/10/2004	SC1-A-4 (mollusk)	0.0312	0.0221	70.7
2/10/2004	SC1-D-5 (crayfish)	0.0183	0.0180	98.1
			Mean and Std. Dev.	84.4 ± 19.4

Table 17. Percent Agreement Between Duplicate Analysis for Total Mercury Content in Fish Collected in 2003.

Date of Analysis	Sample ID	Sample 1 (µg Hg/g)	Sample 2 (µg Hg/g)	Percent Agreement
3/12/2004	1687	0.194	0.2300	84.5
3/12/2004	1677	0.248	0.241	97.3
3/12/2004	1699	0.790	0.784	99.2
3/12/2004	1663	0.358	0.322	89.8
			Mean and Std. Dev.	92.7 ± 6.81

Table 18. Percent of Mercury Recovered from Wild Rice Roots and Seeds Spiked with a Known Quantity of Mercury Coincident with the Analysis of Wild Rice Samples (2003)

Date of Analysis	Composite ID	Spike #1	Spike #2	Mean	Std. Dev.
12/16/2003	LAR403	127	128	127	0.9
12/16/2003	MFR203	104	109	106	3.7
12/16/2003	FLR103	103	104	103 [NC]	0.5
12/16/2003	SLR303	101	102	102 [NC]	0.9
3/17/2004	CFS103	109	100	105	6.1
3/17/2004	SCS403	92.1	89.2	90.6	2.0
3/17/2004	MFS403	98.1	93.3	95.7	3.4
			Mean	104.2	2.5

[NC] Indicates that sample was below the LOD so half of the limit of detection was used to calculate spike recoveries.

Table 19. Percent of Mercury Recovered from Mollusk Samples Spiked with a Known Quantity of Mercury Coincident with the Analysis of Mollusk Samples.

Date of Analysis	Composite ID	Spike #1	Spike #2	Mean	Std. Dev.
2/10/2004	SC1-A-4	99.2	99.5	99.3	0.2
2/10/2004	SC1-D-5	109	106	108	2.0
			Mean	103.5	1.1

Table 20. Percent of Mercury Recovered from Fish Samples Spiked with a Known Amount of Mercury Coincident with the Analysis of Fish Samples

Date of Analysis	Sample ID	Spike #1	Spike #2	Mean	Std. Dev.
3/12/2004	1687	116	93.3	105	16.4
3/12/2004	1677	91.2	90.9	91.0	0.3
3/12/2004	1699[R]	50.1	57.5	53.8	5.2
3/12/2004	1663	85.0	87.5	86.2	1.7
3/17/2004	1699[R]	71.8	63.6	67.7	5.8
			Mean	80.7	5.9

[R] Sample Rerun due to low spike recovery.

Table 21. Measured Concentrations (mg/kg Wet Weight) of Various Metals in Wild Rice Seeds and Roots for Four Individual Composite Samples from Each Lake.

Composite ID[a]	Date Digested	As	Cd	Cr	Cu	Fe	Pb	Mg	Se	Zn
					Seeds					
CFS103	1/12/04	0.036[Q]	<0.0093	0.42	0.805	[b]	0.03[Q]	521	<0.029	19.6
CFS203	1/12/04	0.047[Q]	<0.0085	0.27	0.693	[b]	0.03[Q]	566	<0.13[E]	12.9
CFS203 DUP	1/12/04	0.044[Q]	<0.0080	0.2	0.713	[b]	0.027[Q]	530	<0.13[E]	12.8
CFS303	1/12/04	0.035[Q]	<0.0094	0.33	0.937	[b]	0.032[Q]	473	<0.030	11.1
CFS403	1/12/04	0.019[Q]	<0.0088	0.27	0.507	[b]	0.026[Q]	586	<0.028	8.6
MFS103	1/12/04	0.047[Q]	<0.0099	0.25	1.07	[b]	0.017[Q]	668	<0.031	16.0

MFS203	1/12/04	0.036[Q]	<0.0093	0.18	0.465	[b]	0.016[Q]	486	<0.029	11.7
MFS303	1/12/04	0.042[Q]	<0.0078	0.17	0.347	[b]	0.02[Q]	633	0.038[Q]	16.9
MFS404	1/12/04	0.02[Q]	<0.0083	0.15	0.612	[b]	0.043	544	<0.026	6.0
MFS403 DUP	1/12/04	0.021[Q]	<0.0092	0.12	0.531	[b]	0.014[Q]	510	<0.029	5.9
FLS103	1/12/04	0.048[Q]	<0.0092	0.15	1.81	[b]	0.028[Q]	692	<0.029	24.2
FLS203	1/12/04	0.020[Q]	0.025[Q]	0.12	0.921	[b]	0.035[Q]	624	<0.028	11.2
FLS303	1/12/04	0.041[Q]	<0.0094	0.095	1.73	[b]	0.045	652	<0.030	20.3
FLS403	1/12/04	0.022[Q]	<0.0089	0.22	0.801	[b]	0.016[Q]	496	<0.028	11.5
SLS103	1/12/04	0.029[Q]	<0.0091	0.27	1.01	[b]	0.037[Q]	443	<0.029	16.7
SLS203	1/12/04	0.041[Q]	<0.0093	0.25	1.00	[b]	0.033[Q]	508	<0.029	17.6
SLS303	1/12/04	0.023[Q]	<0.0081	0.27	0.793	[b]	0.022[Q]	428	<0.026	17.0
SLS403	1/12/04	0.047[Q]	<0.0088	0.38	1.72	[b]	0.024[Q]	594	<0.028	16.4
RRS103	1/12/04	0.024[Q]	<0.0094	0.15	0.546	[b]	0.022[Q]	473	<0.030	13.0
RRS203	1/12/04	0.038[Q]	<0.0084	0.42	0.644	[b]	0.026[Q]	483	<0.027	10.9
RRS303	1/12/04	0.024[Q]	<0.0095	0.35	1.17	[b]	0.038[Q]	513	<0.030	15.9
RRS403	1/12/04	0.03[Q]	<0.0087	0.22	0.776	[b]	0.041	463	<0.027	12.1
SCS103	1/12/04	0.031[Q]	<0.0092	0.19	1.66	[b]	0.14	449	<0.058[E]	12.6
SCS103 DUP	1/12/04	0.034[Q]	<0.0088	0.24	1.37	[b]	0.051	444	<0.056[E]	11.8
SCS203	1/12/04	0.026[Q]	<0.0095	0.26	1.50	[b]	0.043	441	<0.030	13.5
SCS303	1/12/04	0.027[Q]	<0.0096	0.12	1.58	[b]	0.04[Q]	576	<0.030	11.9
SCS403	1/12/04	0.036[Q]	<0.0091	0.18	1.31	[b]	0.029[Q]	444	<0.029	12.3
RFS103	1/12/04	0.086	<0.0076	0.3	1.31	[b]	0.082	543	<0.024	16.9
RFS203	1/12/04	<0.018	<0.0090	0.26	0.613	[b]	0.03[Q]	426	<0.028	10.8
RFS303	1/12/04	<0.019	<0.0095	0.32	1.15	[b]	0.065	482	<0.030	14.8
RFS403	1/12/04	<0.019	<0.0095	0.19	0.677	[b]	0.037[Q]	486	<0.030	12.5
LAS103	1/12/04	<0.019	<0.0097	0.2	0.661	[b]	0.038[Q]	518	<0.030	8.0
LAS203	1/12/04	<0.018	<0.0090	0.13	0.781	[b]	0.031[Q]	452	<0.028	6.6
	1/12/04	0.047[Q]	<0.0095	0.33	0.373	[b]	0.033[Q]	483	<0.030	8.3
LAS403	1/12/04	<0.019	<0.0097	0.2	1.07	[b]	0.028[Q]	470	<0.061[E]	10.6
LAS403 DUP	1/12/04	<0.020	<0.0098	0.19	0.957	[b]	0.035[Q]	457	<0.062[E]	10.6
Mean		<0.0323	<0.096	0.238	0.971	[b]	0.0368	519	<0.0341	13.4
Std.Dev.		<0.006	<0.0028	0.0856	0.421	[b]	0.0231	74.3	<0.0192	4.08

					Roots					
CFR103	1/5/04	1.800	0.0140[Q]	0.200	0.347	7460	0.44	115	0.062[Q]	2.66
CFR203	1/5/04	2.600	0.0110[Q]	0.130	0.286	4760	0.44	105	0.038[Q]	2.13
CFR303	1/5/04	1.400	0.0130[Q]	0.160	0.470	5720	0.35	92.7	0.046[Q]	2.57
CFR403	1/5/04	0.650	<0.0078	0.096	0.287	4650	0.28	85.9	0.032[Q]	1.39
MFR103	1/5/04	2.600	0.0710	0.290	0.551	5050	0.53	87.3	0.064[Q]	9.24
MFR103 DUP	1/5/04	2.900	0.0790	0.300	0.600	5650	0.57	102	0.063[Q]	10.17
MFR203	1/5/04	2.500	0.0390	0.260	0.562	6070	0.44	122	0.062[Q]	8.76
MFR303	1/5/04	1.300	0.0230[Q]	0.200	0.335	5180	0.36	102	0.048[Q]	6.09
MFR403	1/5/04	0.620	0.0190[Q]	0.250	0.391	2840	0.39	135	0.053[Q]	7.76
FLR103	1/5/04	2.100	<0.0082	0.200	0.281	5080	0.41	80.8	0.041[Q]	2.57
FLR203	1/5/04	0.290	0.0083[Q]	0.140	0.961	1900	0.44	89.2	0.032[Q]	1.20
FLR303	1/5/04	1.700	<0.012	0.170	0.961	4730	0.43	72.5	<0.037	2.29
FLR303 DUP	1/5/04	2.000	<0.010	0.180	0.958	5410	0.46	84.0	0.037[Q]	2.68
FLR403	1/5/04	0.240	<0.0082	0.086	0.279	2850	0.38	66.3	0.030[Q]	2.08
SLR103	1/5/04	0.590	<0.0086	0.120	0.563	4300	0.59	95.6	0.029[Q]	1.87
SLR203	1/5/04	1.300	0.0079[Q]	0.041[Q]	0.121	5380	0.62	122	<0.024	1.19
SLR303	1/5/04	0.410	<0.0076	0.059	0.099	2860	0.42	92.4	<0.024	1.13
SLR403	1/5/04	1.300	<0.0089	0.100	0.140	5080	0.86	102	<0.028	1.35
RRR103	1/5/04	2.200	<0.0084	0.160	0.133	3720	0.29	178	0.051[Q]	0.91
RRR203	1/5/04	2.400	<0.0078	0.190	0.184	3520	0.29	158	0.054[Q]	1.13
RRR203 DUP	1/5/04	2.500	<0.0075	0.190	0.198	3580	0.3	176	0.052[Q]	1.15
RRR303	1/5/04	0.770	<0.0082	0.160	0.193	2300	0.21	174	0.050[Q]	1.03
RRR403	1/5/04	2.800	<0.0086	0.170	0.136	6160	0.38	198	0.063[Q]	1.09
SCR103	1/5/04	2.900	<0.0081	0.210	0.339	5880	0.24	212	0.070[Q]	1.91
SCR203	1/5/04	2.600	<0.0080	0.280	0.782	7980	0.33	222	0.095	1.33
SCR303	1/5/04	3.400	<0.0086	0.160	0.204	5510	0.31	216	0.058[Q]	1.11
SCR403	1/5/04	11.000	<0.0079	0.190	0.228	6850	0.3	218	0.094	1.14
RFR103	1/5/04	0.130	0.0160[Q]	0.076	0.174	2170	0.23	125	0.024[Q]	1.20
RFR203	1/5/04	0.190	<0.0078	0.098	0.103	2830	0.32	114	0.025[Q]	1.01
RFR303	1/5/04	0.130	<0.0075	0.078	0.079	2270	0.28	106	<0.024	1.12
RFR403	1/5/04	0.160	<0.0077	0.180	0.726	2040	0.37	108	0.033[Q]	1.55
LAR103	1/5/04	0.130	<0.0081	0.058[Q]	0.256	791	0.15	74.5	0.026[Q]	1.20
LAR203	1/5/04	0.180	<0.0076	0.110	0.341	807	0.15	79.0	0.033[Q]	1.66
LAR303	1/5/04	0.310	<0.0079	0.140	0.376	1740	0.33	82.7	0.038[Q]	1.73

LAR403	1/5/04	0.490	<0.0080	0.100	0.296	2970	0.3	92.9	0.030[Q]	1.42
Mean		1.60	<0.0126	0.152	0.390	4110	0.341	142	0.0438	2.94
Std.Dev.		2.00	<0.0123	0.0655	0.277	1900	0.166	99.6	0.0192	3.16

[a] First two letters identify sample location (see Table 1);third letter identifies sample type (S=seed; R=root); first number indicates composite number; 03 indicates sample was collected in 2003.
[b] Iron was only contracted to be analyzed in root samples.
[Q] Analyte has been detected between the Limit of Detection and the Limit of Quantitation. The results are qualified due to the uncertainty of analyte concentrations within this range.
[E] Indicates elevated Limit of Detection.

Table 22. Mean and Standard Deviation of Concentrations (mg/kg Wet Weight) of Various Metal Measured in Roots and Seeds from the Combined Composite Samples.

Composite ID		As	Cd	Cr	Cu	Fe	Pb	Mg	Se	Zn
					Seeds					
CF	Mean	0.034	[a]	0.314	0.738	[b]	0.029	532	[a]	13.1
CF	Std. Dev.	0.011	[a]	0.081	0.181	[b]	0.003	47.4	[a]	4.72
MF	Mean	0.036	[a]	0.184	0.614	[b]	0.020	579	[a]	12.6
MF	Std. Dev.	0.011	[a]	0.048	0.319	[b]	0.006	85.9	[a]	5.00
FL	Mean	0.033	0.025	0.146	1.32	[b]	0.031	616	[a]	16.8
FL	Std. Dev.	0.014	[a]	0.054	0.530	[b]	0.012	84.7	[a]	6.48
SL	Mean	0.035	[a]	0.293	1.13	[b]	0.029	493	[a]	16.9
SL	Std. Dev.	0.011	[a]	0.059	0.406	[b]	0.007	75.5	[a]	0.519
RR	Mean	0.029	[a]	0.285	0.785	[b]	0.032	483	[a]	13.0
RR	Std. Dev.	0.007	[a]	0.122	0.276	[b]	0.009	21.5	[a]	2.12
SC	Mean	0.030	[a]	0.194	1.48	[b]	0.052	477	[a]	12.5
SC	Std. Dev.	0.005	[a]	0.059	0.118	[b]	0.030	66.4	[a]	0.707
RF	Mean	<0.036	[a]	0.268	0.936	[b]	0.054	484	[a]	13.8
RF	Std. Dev.	<0.033	[a]	0.057	0.344	[b]	0.024	48.1	[a]	2.65
LA	Mean	<0.026	[a]	0.214	0.707	[b]	0.033	479	[a]	8.39
LA	Std. Dev.	<0.014	[a]	0.084	0.267	[b]	0.003	28.8	[a]	1.65
					Roots					
CF	Mean	1.61	0.013	0.147	0.348	5650	0.378	99.7	0.045	2.190
CF	Std. Dev.	0.813	0.002	0.044	0.086	1300	0.078	12.9	0.013	0.580
MF	Mean	1.79	0.039	0.251	0.466	4860	0.435	113	0.057	8.08
MF	Std. Dev.	1.01	0.026	0.039	0.121	1400	0.083	18.5	0.007	1.54
FL	Mean	1.12	[a]	0.150	0.620	3720	0.419	78.7	0.035	2.08
FL	Std. Dev.	0.993	[a]	0.049	0.393	1609	0.030	9.46	0.005	0.630
SL	Mean	0.900	0.008	0.080	0.231	4410	0.623	103	0.029	1.39
SL	Std. Dev.	0.468	[a]	0.036	0.222	1130	0.181	13.4	[a]	0.334
RR	Mean	2.06	[a]	0.170	0.163	3940	0.294	179	0.054	1.04

RR	Std. Dev.	0.891	[a]	0.014	0.033	1620	0.069	13.2	0.006	0.097
SC	Mean	4.98	[a]	0.210	0.388	6550	0.295	217	0.079	1.37
SC	Std. Dev.	4.03	[a]	0.051	0.269	1110	0.039	4.47	0.018	0.371
RF	Mean	0.153	0.016	0.108	0.271	2330	0.300	113	0.027	1.22
RF	Std. Dev.	0.029	[a]	0.049	0.306	345	0.059	8.37	0.005	0.234
LA	Mean	0.278	[a]	0.102	0.317	1580	0.233	82.3	0.032	1.50
LA	Std. Dev.	0.161	[a]	0.034	0.052	1030	0.096	7.85	0.005	0.245

[a] Not calculable because one or more of the values is below the detection limit.
[b] Iron was only contracted to be analyzed in root samples.

Table 23. Total Mercury Concentrations (Wet Weight) in Wild Rice Composites Collected During 2003.

Water Body	Composite Number (Sample ID)	Seed (µg Hg/g)	Root (µg Hg/g)
Chequamegon Flowage	1 (101-112)	0.00296[Q]	0.00260[Q]
Chequamegon Flowage	2 (113-124)	0.00296[Q]	0.00258[Q]
Chequamegon Flowage	3 (125-136)	0.00214[Q]	0.00255[Q]
Chequamegon Flowage	4 (137-148)	<0.00126	0.00175[Q]
Fish Lake	1 (101-112)	0.00170[Q]	<0.00126
Fish Lake	2 (113-124)	0.00172[Q]	<0.00126
Fish Lake	3 (125-136)	0.00213[Q]	0.00305[Q]
Fish Lake	4 (137-148)	0.00131[Q]	<0.00126
Spirit River	1 (101-112)	0.00169[Q]	<0.00126
Spirit River	2 (113-124)	0.00262[Q]	0.00179[Q]
Spirit River	3 (125-136)	0.00434	0.00181[Q]
Spirit River	4 (137-148)	0.00168[Q]	0.00134[Q]
Mondeaux Flowage	1 (101-112)	0.00246[Q]	0.00266[Q]
Mondeaux Flowage	2 (113-124)	0.00294[Q]	0.00379[Q]
Mondeaux Flowage	3 (125-136)	0.00304[Q]	0.00394[Q]
Mondeaux Flowage	4 (137-148)	0.00154[Q]	0.00439
Rocky Run Flowage	1 (101-112)	<0.00126	<0.00126
Rocky Run Flowage	2 (113-124)	0.00128[Q]	0.00132[Q]
Rocky Run Flowage	3 (125-136)	0.00213[Q]	0.00218[Q]
Rocky Run Flowage	4 (137-148)	0.00253[Q]	0.00134[Q]
Rat River	1 (101-112)	0.00424	<0.00126
Rat River	2 (113-124)	0.00173[Q]	0.00134[Q]
Rat River	3 (125-136)	0.00129[Q]	<0.00126
Rat River	4 (137-148)	0.00211[Q]	0.00132[Q]
Swamp Creek	1 (101-112)	0.00389[Q]	0.00219[Q]
Swamp Creek	2 (113-124)	0.00671	0.00269[Q]

Swamp Creek	3 (125-136)	0.00688	0.00312[Q]
Swamp Creek	4 (137-148)	0.00340[Q]	0.00176[Q]
Spur Lake	1 (101-112)	<0.00126	0.00174[Q]
Spur Lake	2 (113-124)	<0.00126	<0.00126
Spur Lake	3 (125-136)	<0.00126	<0.00126
Spur Lake	4 (137-148)	<0.00126	<0.00126

[Q] Analyte has been detected between the Limit of Detection and the Limit of Quantitation. The results are qualified due to the uncertainty of analyte concentrations within this range.

Table 24. Wild Rice Root Moisture Measured at the Time of Analysis After 24 Hours of Drying.

Composite ID	Water Body	Percent Moisture	Duplicate Agreement	Mean Percent Moisture	Std. Dev.
CFR103	Chequamegon Flowage	87.47	99.89	-	-
CFR103 DUP	Chequamegon Flowage	87.57	-	-	-
CFR203	Chequamegon Flowage	87.93	99.35	-	-
CFR203 DUP	Chequamegon Flowage	88.51	-	-	-
CFR303	Chequamegon Flowage	88.58	99.43	-	-
CFR303 DUP	Chequamegon Flowage	89.08	-	-	-
CFR403	Chequamegon Flowage	87.89	99.72	-	-
CFR403 DUP	Chequamegon Flowage	88.14	-	88.1	0.55
FLR103	Fish Lake	89.86	99.37	-	-
FLR103 DUP	Fish Lake	89.30	-	-	-
FLR203	Fish Lake	89.01	99.80	-	-
FLR203 DUP	Fish Lake	89.18	-	-	-
FLR303	Fish Lake	88.23	98.05	-	-
FLR303 DUP	Fish Lake	89.98	-	-	-
FLR403	Fish Lake	89.37	99.74	-	-
FLR403 DUP	Fish Lake	89.14	-	89.3	0.54
LAR103	Lake Alice	90.11	99.68	-	-
LAR103 DUP	Lake Alice	89.81	-	-	-
LAR203	Lake Alice	88.87	99.88	-	-
LAR203 DUP	Lake Alice	88.76	-	-	-
LAR303	Lake Alice	88.84	99.79	-	-
LAR303 DUP	Lake Alice	88.65	-	-	-
LAR403	Lake Alice	88.44	99.74	-	-
LAR403 DUP	Lake Alice	88.21	-	89.0	0.66
MFR103	Mondeaux Flowage	86.52	99.95	-	-
MFR103 DUP	Mondeaux Flowage	86.48	-	-	-
MFR203	Mondeaux Flowage	84.81	99.96	-	-
MFR203 DUP	Mondeaux Flowage	84.84	-	-	-

MFR303	Mondeaux Flowage	87.56	99.60	-	-
MFR303 DUP	Mondeaux Flowage	87.21	-	-	-
MFR403	Mondeaux Flowage	85.50	95.12	-	-
MFR403 DUP	Mondeaux Flowage	85.47	-	86.0	1.05
RFR103	Rocky Run Flowage	88.07	99.45	-	-
RFR103 DUP	Rocky Run Flowage	88.55	-	-	-
RFR203	Rocky Run Flowage	90.37	99.97	-	-
RFR203 DUP	Rocky Run Flowage	90.34	-	-	-
RFR303	Rocky Run Flowage	89.12	99.03	-	-
RFR303 DUP	Rocky Run Flowage	89.99	-	-	-
RFR403	Rocky Run Flowage	88.81	99.41	-	-
RFR403 DUP	Rocky Run Flowage	89.34	-	89.3	0.85
RRR103	Rat River	88.18	99.95	-	-
RRR103 DUP	Rat River	88.13	-	-	-
RRR203	Rat River	88.59	99.67	-	-
RRR203 DUP	Rat River	88.89	-	-	-
RRR303	Rat River	87.03	99.88	-	-
RRR303 DUP	Rat River	87.14	-	-	-
RRR403	Rat River	88.73	99.49	-	-
RRR403 DUP	Rat River	88.28	-	88.1	0.69
SCR103	Swamp Creek	88.20	99.53	-	-
SCR103 DUP	Swamp Creek	88.61	-	-	-
SCR203	Swamp Creek	89.11	99.66	-	-
SCR203 DUP	Swamp Creek	88.80	-	-	-
SCR303	Swamp Creek	88.86	99.64	-	-
SCR303 DUP	Swamp Creek	88.54	-	-	-
SCR403	Swamp Creek	89.09	99.57	-	-
SCR403 DUP	Swamp Creek	89.47	-	88.8	0.39
SLR103	Spur Lake	87.19	99.52	-	-
SLR103 DUP	Spur Lake	87.61	-	-	-
SLR203	Spur Lake	87.51	99.59	-	-
SLR203 DUP	Spur Lake	87.15	-	-	-
SLR303	Spur Lake	92.05	96.40	-	-
SLR303 DUP	Spur Lake	88.74	-	-	-
SLR403	Spur Lake	89.53	99.75	-	-
SLR403 DUP	Spur Lake	89.75	-	88.7	1.70

Table 25. Wild Rice Seed Moisture Measured at the Time of Analysis After 24 Hours Drying.

Composite ID	Lake	Percent Moisture	Duplicate Agreement	Mean % Moisture	Std. Dev.
CFS103	Chequamegon Flowage	38.64	99.43	-	-
CFS103 DUP	Chequamegon Flowage	38.86	-	-	-
CFS203	Chequamegon Flowage	58.48	13.15	-	-
CFS203 DUP	Chequamegon Flowage	7.69	-	-	-
CFS303	Chequamegon Flowage	36.25	98.62	-	-
CFS303 DUP	Chequamegon Flowage	36.76	-	-	-
CFS403	Chequamegon Flowage	41.44	94.65	-	-
CFS403 DUP	Chequamegon Flowage	43.78	-	37.7	14.1
FLS103	Fish Lake	32.89	92.96	-	-
FLS103 DUP	Fish Lake	29.13	-	-	-
FLS203	Fish Lake	31.34	100.0	-	-
FLS203 DUP	Fish Lake	31.33	-	-	-
FLS303	Fish Lake	28.74	89.41	-	-
FLS303 DUP	Fish Lake	32.14	-	-	-
FLS403	Fish Lake	41.13	86.96	-	-
FLS403 DUP	Fish Lake	35.76	-	32.8	4.02
LAS103	Lake Alice	36.33	99.14	-	-
LAS103 DUP	Lake Alice	36.02	-	-	-
LAS203	Lake Alice	31.98	69.39	-	-
LAS203 DUP	Lake Alice	46.09	-	-	-
LAS303	Lake Alice	39.10	99.55	-	-
LAS303 DUP	Lake Alice	38.93	-	-	-
LAS403	Lake Alice	43.34	87.94	-	-
LAS403 DUP	Lake Alice	38.12	-	38.7	4.39
MFS103	Mondeaux Flowage	31.16	99.44		
MFS103 DUP	Mondeaux Flowage	30.98	-		
MFS203	Mondeaux Flowage	42.16	90.96		
MFS203 DUP	Mondeaux Flowage	38.35	-		
MFS303	Mondeaux Flowage	33.33	95.94		
MFS303 DUP	Mondeaux Flowage	31.98	-		
MFS403	Mondeaux Flowage	47.90	89.54		
MFS403 DUP	Mondeaux Flowage	42.89	-	37.3	6.44
RFS103	Rocky Run Flowage	34.90	86.20	-	-
RFS103 DUP	Rocky Run Flowage	40.48	-	-	-
RFS203	Rocky Run Flowage	38.67	87.01	-	-
RFS203 DUP	Rocky Run Flowage	44.44	-	-	-
RFS303	Rocky Run Flowage	38.11	99.35	-	-
RFS303 DUP	Rocky Run Flowage	37.86	-	-	-

RFS403	Rocky Run Flowage	41.75	89.39	-	-
RFS403 DUP	Rocky Run Flowage	46.70	-	40.4	3.83
RRS103	Rat River	44.49	98.68	-	-
RRS103 DUP	Rat River	45.08	-	-	-
RRS203	Rat River	46.78	99.74	-	-
RRS203 DUP	Rat River	46.90	-	-	-
RRS303	Rat River	50.21	94.14	-	-
RRS303 DUP	Rat River	53.33	-	-	-
RRS403	Rat River	43.16	96.09	-	-
RRS403 DUP	Rat River	44.92	-	46.9	3.37
SCS103	Swamp Creek	50.23	88.28	-	-
SCS103 DUP	Swamp Creek	56.90	-	-	-
SCS203	Swamp Creek	44.53	92.89	-	-
SCS203 DUP	Swamp Creek	41.37	-	-	-
SCS303	Swamp Creek	50.00	100.0	-	-
SCS303 DUP	Swamp Creek	50.00	-	-	-
SCS403	Swamp Creek	55.90	98.13	-	-
SCS403 DUP	Swamp Creek	54.86	-	50.5	5.46
SLS103	Spur Lake	45.33	97.87	-	-
SLS103 DUP	Spur Lake	46.32	-	-	-
SLS203	Spur Lake	41.06	92.07	-	-
SLS203 DUP	Spur Lake	37.80	-	-	-
SLS303	Spur Lake	46.88	98.10	-	-
SLS303 DUP	Spur Lake	45.99	-	-	-
SLS403	Spur Lake	44.81	88.76	-	-
SLS403 DUP	Spur Lake	39.77	-	43.5	3.44

Table 26. Total Mercury Concentrations (Wet Weight) for Crayfish, Clams, and Snail Samples collected in 2003.

Sample/Composite number	Water Body Name	Sample Numbers in composite	µg Hg/g
	Crayfish		
SC1-D-5	Swamp Creek-Swampy Lane	composite	0.0182
SC3-A-5	Swamp Creek-HWY.M	composite	0.0232
	Clams		
SC1-A-4	Swamp Creek-Swampy Lane	101-104	0.0266
SC1-B-4	Swamp Creek-Swampy Lane	105-108	0.0252
SC1-C-4	Swamp Creek-Swampy Lane	109-112	0.0286
ML-D-1	Mole Lake	404	0.0185
SC3-B-2	Swamp Creek-HWY.M	201-202	0.0147
	Snails		
HWY55-A-20	Swamp Creek-HWY.55	composite	0.0241
HWY55-B-20	Swamp Creek-HWY.55	composite	0.0376
HWY55-C-20	Swamp Creek-HWY.55	composite	0.0365
ML-A-7	Mole Lake	composite	0.0575
ML-B-6	Mole Lake	composite	0.0818
ML-C-6	Mole Lake	composite	0.0800

[a] Fluted Shell = Lasmigona costata; Fat Mucket = Lampsilis siliquoidea; Pig toe clam = Fusconaia flava; Floater clam = Pyganodon grandis; Snail = Viviparus sp.; Crayfish = Unknown.

Table 27. Concentration (mg/kg Wet Weight) of Various Metals Measured in Crayfish, clams, and Snails Collected during 2003.

Sample ID	Water Body Name	Sample Numbers in Composite	Date Digested	As	Cd	Cr	Cu	Pb	Mg	Se	Zn
					Crayfish						
SC1-D-5	Swamp Creek-Swampy Lane	composite	2/25/04	0.48	<0.0075	0.12[A]	15.6	0.016[Q]	929	0.95	22.4
SC3-A-5	Swamp Creek-HWY M	composite	2/25/04	0.53	0.010[Q]	0.09	13.1	0.050	707	0.96	24.2
SC3-A-5 DUP	Swamp Creek-HWY M	composite	2/25/04	0.48	0.010[Q]	0.09	13.0	0.054	692	0.86	23.6
					Clams						
SC1-A-4	Swamp Creek-Swampy Lane	101-104	2/25/04	0.88	0.059	0.78	1.01	0.056	192	0.37	39.1
SC3-B-2	Swamp Creek-HWY M	201-202	2/25/04	0.77	0.017[Q]	0.47	0.958	0.037	284	0.39	85.8
SC1-B-4	Swamp Creek-Swampy Lane	105-108	2/25/04	0.95	0.029	0.55	1.28	0.036	271	0.47	68.6
SC1-C-4	Swamp Creek-Swampy Lane	109-112	2/25/04	0.89	0.064	0.71	0.920	0.039	187	0.38	39.2
ML-D-1	Mole Lake	404	2/25/04	0.37	0.310	0.170	2.14	0.150	103	0.23	25.4
					Snails						
ML-A-7	Mole Lake	composite	2/25/04	0.50	0.037	0.052[Q,A]	14.0	0.065	833	0.26	49.5
ML-B-6	Mole Lake	composite	2/25/04	0.46	0.035	0.091[A]	10.3	0.100	671	0.21	38.3
ML-C-6	Mole Lake	composite	2/25/04	0.52	0.036	0.042[Q]	15.4	0.059	981	0.28	42.9
HWY 55 A-20	Swamp Creek-HWY 55	composite	2/25/04	0.46	0.0077[Q]	0.170[A]	5.31	0.034	432	0.20	26.5
HWY 55 A-20 DUP	Swamp Creek-HWY 55	composite	2/25/04	0.48	<0.0077	0.140[A]	5.54	0.028[Q]	444	0.21	27.3
HWY 55 B-20	Swamp Creek-	composite	2/25/04	0.52	0.0076[Q]	0.084[A]	7.32	0.062	511	0.27	47.3

HWY 55 C-20	HWY 55 Swamp Creek-HWY 55	composite	2/25/04	0.57	0.0076[Q]	0.058[A]	5.81	0.031[Q]	558	0.26	43.2

[Q] Analyte has been detected between the Limit of Detection and the Limit of Quantitation. The results are qualified due to the uncertainty of analyte concentrations within this range.
[A] Analyte detected in method blank.

Table 28. Moisture Measured in Crayfish, Clam, and Snail Tissues at time of Metals Analysis.

Composite ID	Water Body	Percent Moisture
	Crayfish	
SC1-D-5	Swamp Creek-Swampy Lane	61.13
SC3-A-5	Swamp Creek-HWY.M	61.51
	Clams	
SC1-A-4	Swamp Creek-Swampy Lane	88.34
SC1-B-4	Swamp Creek-Swampy Lane	85.87
SC1-C-4	Swamp Creek-Swampy Lane	85.08
ML-D-1	Mole Lake	86.22
SC3-B-2	Swamp Creek-HWY.M	85.45
	Snails	
HWY55-A-20	Swamp Creek-HWY.55	89.87
HWY55-B-20	Swamp Creek-HWY.55	88.39
HWY55-C-20	Swamp Creek-HWY.55	86.82
ML-A-7	Mole Lake	87.91
ML-B-6	Mole Lake	86.52
ML-C-6	Mole Lake	87.18

Table 29. Total Mercury Concentrations (Wet Weight) for fish samples collected in 2003. (No weights available.)

Sample ID	Lake	Species	Length (in)	Length (cm)	Sex	µg/g
1664	Deep Hole	Large Mouth Bass	9.5	24.1	F	0.375
1665	Deep Hole	Large Mouth Bass	10.2	25.9	M	0.397
1666	Deep Hole	Large Mouth Bass	8.2	20.8	M	0.300
1667	Deep Hole	Large Mouth Bass	10.3	26.2	M	0.414
1673	Deep Hole	Large Mouth Bass	9.9	25.1	F	0.441
1678	Deep Hole	Large Mouth Bass	8.8	22.4	M	0.442
1681	Deep Hole	Large Mouth Bass	6.8	17.3	U (immature)	0.251
1683	Deep Hole	Large Mouth Bass	9.4	23.9	M	0.374
1686	Deep Hole	Large Mouth Bass	9.9	25.1	F	0.345
1688	Deep Hole	Large Mouth Bass	16.1	40.9	F	1.28
1689	Deep Hole	Large Mouth Bass	12.3	31.2	M	0.468
1693	Deep Hole	Large Mouth Bass	14.0	35.6	M	0.697
1699	Deep Hole	Large Mouth Bass	9.5	24.1	F	0.446
1662	Little Sand Lake	Large Mouth Bass	6.7	17.0	F	0.227
1663	Little Sand Lake	Large Mouth Bass	8.4	21.3	M	0.340
1668	Little Sand Lake	Large Mouth Bass	10.3	26.2	M	0.279
1671	Little Sand Lake	Large Mouth Bass	8.8	22.4	M	0.414
1672	Little Sand Lake	Large Mouth Bass	6.6	16.8	M	0.172
1677	Little Sand Lake	Large Mouth Bass	8.5	21.6	M	0.244
1680	Little Sand Lake	Large Mouth Bass	8.2	20.8	M	0.202
1669	Little Sand Lake	Northern Pike	21.8	55.4	M	0.790
1670	Little Sand Lake	Northern Pike	17.9	45.5	M	0.683
1674	Little Sand Lake	Northern Pike	20.4	51.8	M	0.784
1675	Little Sand Lake	Northern Pike	25.1	63.8	F	0.865
1676	Little Sand Lake	Northern Pike	23.2	58.9	F	0.549
1679	Little Sand Lake	Northern Pike	18.5	47.0	M	0.710
1682	Little Sand Lake	Northern Pike	23.6	59.9	M	0.547
1684	Mole Lake	Large Mouth Bass	15.2	38.6	F	0.523
1685	Mole Lake	Large Mouth Bass	15.8	40.1	F	0.624
1687	Mole Lake	Large Mouth Bass	9.0	22.9	M	0.212
1690	Mole Lake	Large Mouth Bass	11.2	28.4	M	0.316
1691	Mole Lake	Large Mouth Bass	18.4	46.7	F	0.606
1692	Mole Lake	Large Mouth Bass	12.7	32.3	M	0.302
1694	Mole Lake	Large Mouth Bass	9.6	24.4	M	0.230
1695	Mole Lake	Large Mouth Bass	11.5	29.2	F	0.336
1696	Mole Lake	Large Mouth Bass	7.8	19.8	F	0.194

1697	Mole Lake	Large Mouth Bass	8.5	21.6	M	0.221
1698	Mole Lake	Large Mouth Bass	7.5	19.1	F	0.188
1700	Mole Lake	Large Mouth Bass	15.6	39.6	F	0.474

Table 30. Fish Tissue Moisture Measured at time of Mercury Analysis.

Species	Lake	Sample ID	Percent Moisture
Large Mouth Bass	Deep Hole	1664	78.22
Large Mouth Bass	Deep Hole	1665	79.03
Large Mouth Bass	Deep Hole	1666	78.71
Large Mouth Bass	Deep Hole	1667	79.45
Large Mouth Bass	Deep Hole	1673	78.00
Large Mouth Bass	Deep Hole	1678	78.78
Large Mouth Bass	Deep Hole	1681	81.65
Large Mouth Bass	Deep Hole	1683	78.75
Large Mouth Bass	Deep Hole	1686	80.56
Large Mouth Bass	Deep Hole	1688	78.80
Large Mouth Bass	Deep Hole	1689	77.41
Large Mouth Bass	Deep Hole	1693	79.18
Large Mouth Bass	Deep Hole	1699	78.71
Large Mouth Bass	Little Sand Lake	1662	79.07
Large Mouth Bass	Little Sand Lake	1663	79.05
Large Mouth Bass	Little Sand Lake	1668	79.06
Large Mouth Bass	Little Sand Lake	1671	78.70
Large Mouth Bass	Little Sand Lake	1672	80.10
Large Mouth Bass	Little Sand Lake	1677	78.28
Large Mouth Bass	Little Sand Lake	1680	80.27
Northern Pike	Little Sand Lake	1669	83.04
Northern Pike	Little Sand Lake	1670	80.02
Northern Pike	Little Sand Lake	1674	78.67
Northern Pike	Little Sand Lake	1675	79.00
Northern Pike	Little Sand Lake	1676	79.47
Northern Pike	Little Sand Lake	1679	78.51
Northern Pike	Little Sand Lake	1682	79.57
Large Mouth Bass	Mole Lake	1684	78.89
Large Mouth Bass	Mole Lake	1685	78.36
Large Mouth Bass	Mole Lake	1687	80.26
Large Mouth Bass	Mole Lake	1690	80.56

Large Mouth Bass	Mole Lake	1691	78.81
Large Mouth Bass	Mole Lake	1692	80.49
Large Mouth Bass	Mole Lake	1694	79.78
Large Mouth Bass	Mole Lake	1695	80.26
Large Mouth Bass	Mole Lake	1696	80.24
Large Mouth Bass	Mole Lake	1697	79.70
Large Mouth Bass	Mole Lake	1698	80.03
Large Mouth Bass	Mole Lake	1700	78.84

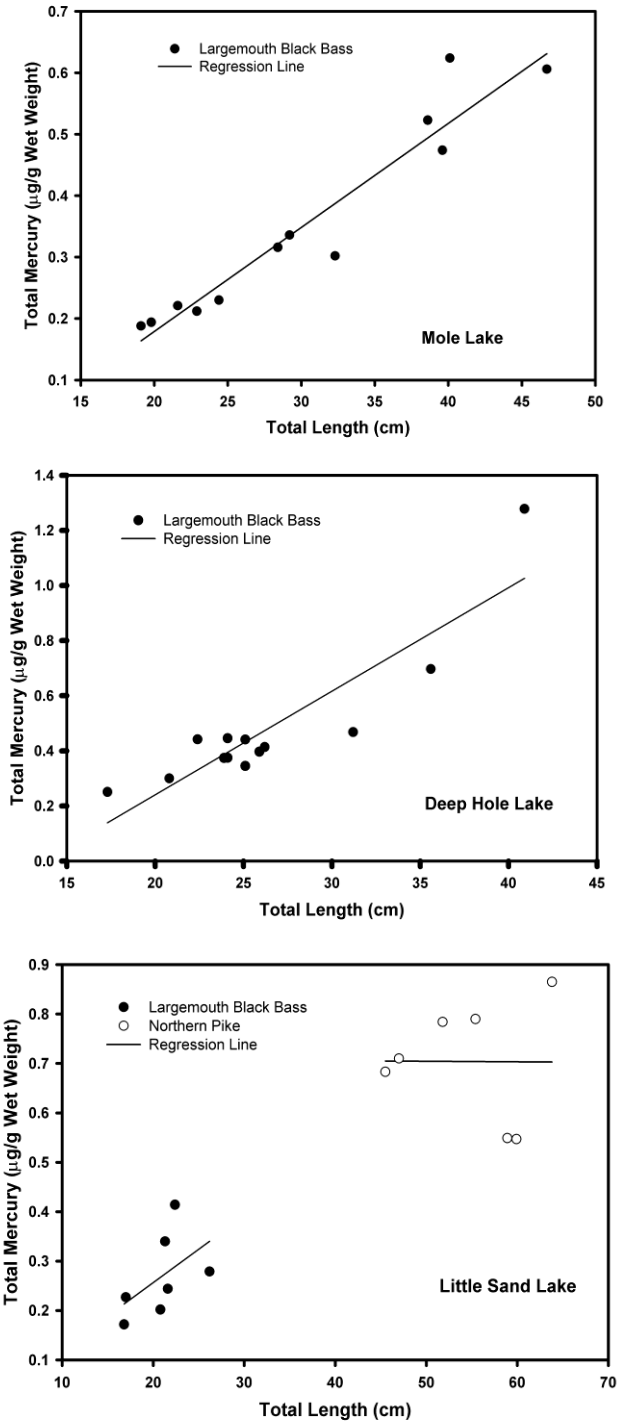

Figure 2. Linear regression relationship of total mercury in muscle tissue of fish sampled from Mole Lake, Deep Hole Lake, and Little Sand Lake during 2003.

APPENDIX A

ROUTINE LABWARE CLEANING FOR METALS ANALYSIS (SA/8)

INTRODUCTION

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

EQUIPMENT LIST

- Deionized Water
- Hydrochloric Acid, Conc. (12 M)
- Dish Pan
- Gloves
- Lab Coat
- Micro or Liquinox Detergent
- Ammonium Hydroxide, 30% (VWR reagent)
- Nitric Acid, Concentrated (Fisher Reagent)
- Plastic tank with cover
- Grinder
- Goggles
- pH Indicator Strips

PROCEDURE: CLEANING EQUIPMENT USED FOR FISH GRINDING

1. Scrub equipment in water containing Liquinox detergent.
2. Rinse equipment with tap water.
3. Rinse equipment once with deionized water.
4. Soak equipment in 0.1 M HCl for 30 seconds.
5. Rinse equipment three times with deionized water.
6. Upon drying, cover equipment with aluminum foil to store until used.

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 5 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 B

PROCESSING PLANT TISSUES FOR METAL ANALYSIS (SA/40)

INTRODUCTION

Metals analysis can be performed on various plant tissues when the tissues are fine enough for processing with acids and other chemicals necessary to provide a solution containing the dissolved metals. Processing of the plant components involves reducing them to a homogeneous mixture of ground tissues. This procedure allows for the processing of several plants into one sample (composite sample) for analyzing a group of associated plants.

EQUIPMENT LIST

- Analytical Balance (0.001 g)
- Sonicator
- Deionized Water
- Forceps
- Paper Towels (Kimwipes)
- Wash Bottle
- Food Blender
- Liquid Nitrogen
- 3-Dram Vials
- Labels
- Funnels
- Spatula

PROCEDURE

Roots

1. Remove roots from storage container and place in clean beaker (can use the storage bottle if it fits in the sonicator).
2. Add deionized water to the sample containing the roots and place in sonicator for three minutes.
3. Rinse the roots with squeeze bottle filled with deionized water until no soil is noticed.
4. Place all roots used to make a composite sample on several layers of paper towels and pat dry with a layer of towels.
5. Select the smallest group of roots and weigh in a clean and tared beaker. The weight of this group of roots should be adequate to contribute to its share of the total composite sample (i.e., one tenth of the total if ten plants are used to compose the composite sample).
6. Add similar weights of each plant to the composite sample.
7. Place the composited root tissue into a stainless steel blender cup and add liquid nitrogen.
8. Grind the tissues until they appear homogeneous (about) one minute.
9. Label the sample bottle.
10. Place ground tissue into a clean 3-dram vial using a funnel or a clean HDPE 2-oz bottle. (Caution: Do not tighten the cap on the vial until all liquid nitrogen has effervesced; otherwise an explosion may result.)
11. Keep the sample frozen until analyzed.

Seeds

1. Remove seeds from storage container and place on a clean paper towel, roll seeds with paper

towel on top to remove excess moisture, pick out unusable seeds, and trim off beards. (Place all the seeds on the towel keeping them separate to determine the maximum number of seeds the smallest sample can contribute to the composite sample.)

2. Place the composite sample in the stainless steel blender cup and add liquid nitrogen.
3. Grind the sample until homogeneous (about one minute).
4. Label the sample bottle.
5. Place the sample into a properly labeled 3-dram vial using a funnel or a 2-oz HDPE bottle. (Caution: Do not tighten the cap on the vial until all liquid nitrogen has effervesced; otherwise an explosion may result.)
6. Keep the sample frozen until analyzed.

APPENDIX C

PREPARATION OF TISSUES FOR ANALYTICAL DETERMINATIONS USING LIQUID NITROGEN (SA/38)

INTRODUCTION

This SOP describes the method for grinding tissue samples into homogeneous samples. Liquid nitrogen is used to obtain a more homogenous sample than obtained with the grinder. Samples that may be treated this way include clams, snails, fish fatty tissue, fish muscle, and fish skin. The blender and labware used in this procedure are cleaned by the Cold Vapor Mercury Analysis-Meat Grinder Cleaning (SA/9) procedure. The sample vials the samples are placed into 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.

REFERENCES

Preparation of Tissues for Analytical Determinations in the Laboratory, EnChem, Inc., 525 Science Drive, Madison, WI. 53711. May 2, 1997.

EQUIPMENT LIST

- Samples
- Gloves
- Spatula
- Stainless Steel Bowls
- Tuna Fish
- Glass Cutting Boards
- Fillet Knives
- Goggles
- Scintillation Vials (previously acid-cleaned)
- Liquid Nitrogen
- Industrial Strength Blender (with stainless steel pitcher)

PROCEDURE: PREPARING THE PROCEDURAL BLANK

1. Drain the can of tuna to be used as the procedural blank.
2. Fill one scintillation vial with tuna. Label the tuna fish as not ground with the date, and include with the analysis set.
3. Place the remainder of the tuna sample in a stainless steel bowl. Pour liquid nitrogen over the sample until the tuna is frozen solid. Transfer the frozen tuna to the pitcher of the blender and pulse the blender until the tuna sample is homogenized. Place the tuna in a scintillation vial using a spatula and label the tuna fish as ground with the date, and include in the analysis set.

PROCEDURE: GRINDING SAMPLES

1. Remove the sample from the freezer and partially thaw. Cut the sample into 1/2" cubes on a glass cutting board using a sharp knife.
2. Place the cubed sample into a stainless steel bowl. Pour liquid nitrogen over the sample until it is frozen solid. Transfer the frozen sample into the pre-cooled pitcher of the blender and pulse the blender until the sample is homogenized. Place the homogenized sample into a previously cleaned scintillation vial that is labeled appropriately. Place the sample in the freezer until analyzed.
3. Wash the equipment and labware following SOP SA/9 - Cold Vapor Mercury Analysis-Meat Grinder Cleaning before homogenizing the next sample.
4. Continue to homogenize samples following steps 1-3.

APPENDIX D

PROCEDURES FOR DETERMINING PERCENT MOISTURE IN TISSUE SAMPLES (NT/15)

INTRODUCTION

This SOP includes general guidelines for the analysis of tissue samples for moisture content. It is a gravimetric technique requiring careful weighing techniques.

EQUIPMENT LIST

- Analytical Balance (i.e., Mettlers AG245, AB204, PB303, PB303-S, H34, H72 and H80)
- Aluminum Weighing Pans
- Drying Oven (60^{o} C)
- Desiccation Container
- Spatula
- Forceps

PROCEDURE

1. Calibrate analytical balance using Class 1 weights. Label the aluminum weighing pans and dry at 60^{o} C for 16 hours.
2. Place dried weighing pans in desiccator, using forceps, until cool.
3. Weigh the dried and cooled weighing pans on an analytical balance to the 0.0001 g.
4. Weigh approximately 1.0 g of thawed tissue and place in the labelled weighing pan.
5. Weigh the pan and the tissue on an analytical balance to the nearest 0.0001 g.
6. Dry pan and tissue in drying oven at 60^{o} C for 16 hours or until constant dry weight is achieved.
7. Remove dried pans and tissue from the oven and place in desiccator until cool.
8. Weigh the pan with the tissue on an analytical balance to the nearest 0.0001 g.
9. It may be necessary to dry the pan and tissue a second time when the tissue is a large mass, desiccate, and reweigh to prove than an equilibrium dry weight has been achieved.

10. Calculations:

    Dry Aluminum Pan - Aluminum pan with wet tissue = Wet weight of tissue

    (Aluminum pan and wet tissue weight - Aluminum pan and dry tissue / Wet tissue weight) X 100 = Percent moisture of tissue

APPENDIX E

COLD VAPOR MERCURY DETERMINATION IN BIOTA (SA/13)

INTRODUCTION

This procedure is used for the determination of total mercury in fish, hair 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
- Hydrochloric Acid, Trace Metals Grade
- Nitric Acid, Fisher, Trace Metals Grade
- Mercury Cold Vapor Analyzer
- Hollow Cathode Mercury Lamp
- Variable Autotransformer
- Neptune Dyna-Pump Model 4K
- Hot Block (Environmental Express)
- Varian SpectrAA 200 Spectrophotometer
- FIMS-100 (Perkin Elmer) Mercury Analyzer
- Labindustries Repipet II Dispenser, 3 - 10 mL and 1 - 5 mL
- Wheaton Instruments Socorex Dispenser Model 511, 10 mL
- Polypropylene Digestion Cups and Covers
- Pipets/Pipettors
- Beakers
- Spatulas
- 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 for Spectra AA Analysis
- 0.05M Potassium Permanganate-5% (v/v) Sulfuric Acid
- 1000 ug/mL Mercuric Nitrate Stock
- 5 ug/mL Mercuric Nitrate Substock for Spectra AA Analysis
- 50 ng/mL Mercuric Nitrate Substock for Spectra AA Analysis
- 10 mg/L Mercuric Nitrate Substock for FIMS-100 Analysis
- 100 ug/L Mercuric Nitrate Substock for FIMS-100 Analysis
- Silicon Defoaming Agent (Perkin Elmer)
- Deionized Water in Teflon Squirt Bottle

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.
2. Place the digestion cups in Hot Block at 110^{o}C and allow to digest for approximately 15 minutes or until all the fish tissue is dissolved.
3. Turn off the Hot Block and allow the digestion cups to cool to room temperature.
4. Add 5.0 mL of 5% potassium permanganate to each bottle in 1.0 mL increments swirling the digestion cups after each addition.
5. Add 10.0 mL of 5% potassium permanganate to each digestion cup in 5.0 mL increments, swirling the digestion cup after each addition. Additional 5% potassium permanganate solution (maximum of 5 mL) or solid potassium permanganate should be added to the samples if necessary so that the samples remain purple in color for at least 15 minutes. If extra potassium permanganate is added to a sample, an equal amount should be added to one set of standards and a blank.
6. Add 8 mL of 5% potassium persulfate to each digestion cup, and cover and swirl.
7. Allow the digestion cup to set overnight to oxidize organic mercury compounds to inorganic mercury ions.
8. The samples will remain stable for several days before analysis.

Sample Analysis Using Varian SpectraAA 200

Instrument Conditions

Current = 3.0 mA
Wavelength = 253.7 nm
Atomic Absorption Mode (AA)
Double Beam Mode (DB)
Statistics = 99
Integration = 1.0 seconds
D_2 Background Correction with diffraction grating filter
Circulating Pump autotransformer = 70% power

Instrument Conditions for Varian SpectrAA 200

Sampling Mode = AutoMix
Calibration Mode = Scale Expansion
Measurement Mode = Integrate
Replicates Standard = 20
Replicates Sample = 20
Expansion Factor 1.0
Minimum Reading = Disabled
Smoothing = 9 pt
Conc. Units = ng
Conc. Decimal places = 2

Wavelength = 253.7 nm
Slit Width = 1.0 nm
Lamp Current = 3.0 mA
Background Correction = BC on
Cal. Zero Rate = 0
Measurement Time = 4.5 s
Pre-Read Delay = 0 s
Vapor Type = Cold Vapor
Burner Height = 16.0 mm

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. Autozero the AA by aerating deionized water through the cold vapor mercury analyzer.
3. Transfer the sample from the digestion cup to a glass bottle. Add 10 mL of hydroxylamine hydrochloride/10% sodium chloride to the digestion cup, then transfer to the glass bottle with the sample. Swirl sample until no purple or brown color remains. Rinse the digestion cup with three portions of deionized water, adding the rinse to the sample in the glass bottle each time. Be careful not to end up with the bottle more than two-thirds full.
4. Add 5.0 mL of 10% stannous chloride/0.5 M sulfuric acid 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 and record the maximum absorbance as the response.
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. Record the volume of waste neutralized in the Acid/Base Waste Log.
10. Collect the exhausted stocks and standards in a glass bottle identified as "Hazardous Waste - Mercuric Nitrate in % acid solutions. Corrosive Toxic." Note the start date. Each waste bottle will require an analysis before it will be accepted for disposal.

Sample Analysis Using Perkin Elmer FIMS-100 Flow Injection Mercury Analysis System

♦ Prepare the following:
Carrier Solution (3% HCl)

Reductant Solution (5% $SnCl_2$, 1% Silicon Defoaming Agent, in 3% HCl)

Weigh 50g $SnCl_2$ and add to 990 mL 3% HCl. Add 10 mL Silicon Defoaming Agent using 5 mL micropipettor.

- Turn on computer and printer.
- Turn on Nitrogen (400 psi).
- Turn on FIMS 100 mercury analyzer and allow to warm up for 10 minutes minimum.
- Press Ctrl+Alt+Del (on computer).
- Username: administrator.
- Leave password field blank. Click on "OK".
- Open appropriate project Excel file prepared from Hg Calculations-Master and minimize the Excel window.
- Double click on AA Winlab Analyst icon.
- Choose "Use a custom designed workspace".
- Choose "Hg.fms" > "file" > "open" > "method" > "Hg Analysis".
- Click on "Browse" in Results Data Set window and enter a new data set name (DateProject). Be sure that the save data and print log boxes are both checked.
- Turn clamps on the peristaltic pump rollers in order to allow pump to work.
- Check filter compartment cover to see that it has been tightened.
- Attach tubing from filter compartment to cell.
- Click on Manual button (on top toolbar).
- Click on FIAS button (on top toolbar). Run FIAS once using clean deionized water (Click on the "FIAS on/off" button). Place collection tubes into appropriate solution bottles (Red = Reductant solution, Yellow = Carrier Solution) and run FIAS two more times checking the flow of the instrument and the lines for bubbles while it is running. Remember while running a sample set to periodically check carrier and reductant volumes, so they do not deplete.
- Just prior to analysis of all blanks, standards and samples (steps 19-22), add 10 mL of 10% (w/v) Hydroxylamine Hydrochloride - 10% (w/v) Sodium Chloride in two 5 mL aliquots, mix sample until no purple or brown color remains. Dilute to 50 mL with deionized water using the correct line on the digestion cup.
- Rinse the collection tube with deionized water and place in the blank solution. Click on "analyze blank" and allow instrument time to complete triplicate analysis.
- Rinse the collection tube with deionized water and place in the lowest standard. Choose appropriate standard concentration and click on "analyze standard" and allow instrument time to complete triplicate analysis. In the appropriate Excel file for that project, enter 0.000 for the blank absorbance and enter the mean Blank Corrected Signal value for the standard. Repeat this step for each of the five standards to be run in order of lowest to highest to develop the standard curve.
- Rinse the collection tube with deionized water and place in appropriate sample. Enter sample ID code into the appropriate field. Rinse the collection tube with DI water and place in appropriate sample. Click on "analyze sample" and allow instrument time to complete triplicate analysis. Enter the mean Blank Corrected Signal value into the appropriate Excel file for that project. Repeat this step for each of the samples to be analyzed.

- The second Blank, second set of standards, and Dorm-2 samples should be run as they were above, sometime in between samples, to check the precision of the instrument. For example, if the sample set contains 52 samples, including duplicates and spikes, run the first set of standards (~13 samples), the Blank and the lowest standard (50 ng/L), Dorm 2-1 (1) and (2) (~13 samples), the next two standards (100 ng/L and 500 ng/L), Dorm 2-2 (1) (~13 samples), the last two standards (1000 ng/L and 6000 ng/L) and finally Dorm 2-2 (2). It is best to try to analyze the duplicates and spikes without interruption, so more or less than 13 samples may be analyzed between standards in order to keep the samples together and in order.

WHEN ANALYSIS OF ALL SAMPLES AND STANDARDS IS COMPLETE:

- Place sample collection tube, and lines from reductant and carrier solutions into beaker of deionized water.
- Flush/clean tubing with deionized water by running FIAS two times.
- Lift collection tubing out of deionized water and run FIAS one more time to allow air to pass through all tubing. When FIAS is finished running, place collection tubing back into beaker of DI water for storage.
- Raise waste lines out of liquid in waste container so liquid does not back up.
- Release the peristaltic pump rollers so that tubing is not compressed.
- Detach line from cell.
- Unscrew the filter compartment cover and, using forceps to handle filter, dry filter with a Kimwipe.
- Print report. Choose "file" > "utilities" > "reporter" . "Open Design"
  Choose "WR01 Mussel" (double-click), then double-click on the number 1 under result name and choose the data set for that day. Click "OK" > "Print Report" and close the reporter window.
- Save Excel file to floppy disk.
- Turn off FIMS instrument, computer, nitrogen, gas and printer.
- Record the date, project, analyst, number of injections, and time run in FIMS-100 usage record book located on top of instrument.

APPENDIX F

STANDARD AND SPIKE PREPARATION AND ANALYSIS OF SAMPLES FOR METALS BY FLAME ATOMIC ABSORPTION (SA/34)

INTRODUCTION

This procedure is used for the preparation of analytical standards in the analyses of metals other than mercury.

EQUIPMENT LIST

- Appropriate Metal Reference Solution
- Nitric Acid (Trace Metal Grade)
- 100 mL Volumetric Flask
- Deionized Water
- Atomic Absorption Spectrophotometer
- Class "A" Pipets

PROCEDURE

1. Pipette 1 mL of 1000 mg/L Metal Reference solution plus 0.5 mL Trace Metal Grade HNO_3 into a 100 mL volumetric flask and dilute to volume with deionized water. This constitutes the 10 mg/L substock.
2. Using the 10 mg/L substock, make the following suggested dilutions: (actual dilutions made depend on the metal being analyzed)

mL of 10 mg/L Substock	mL Conc. HNO_3	Final Vol. (mL)	Conc. Metal (mg/L)
0.5	0.5	100	0.050
1	0.5	100	0.100
3	0.5	100	0.300
5	0.5	100	0.500

3. Analysis of Samples

   Analysis of metals are conducted on the Varian SpectAA200 atomic absorption spectrophotometer. The conditions described below are an example. They are the conditions recommended for the analysis of copper. The instrument is set up for the analysis of each metal following the conditions recommended in the instrument operation manual. The conditions are optimized before initiating analysis.

   Lamp Current: 4.0 mA Slit Width: 0.5 nm
   Wavelength: 324.8 nm Air Flow: 13.50 L/min

Background Correction Deuterium	Acetylene Flow: 2.00 L/min
Replicates Std: 3	Measurement Time: 4.00 s
Replicates Sample: 3	Flame Height: 13.5 mm

4. Run standard curve, followed by a QC standard to ensure the accuracy of the standard curve.
5. Analyze the samples with a standard run every 10 samples to check for instrument drift.

APPENDIX G

Inductively Coupled Plasma Mass Spectroscopy

APPLICATION: Digested sample matrices including water, soils, biota, industrial wastes, sludges, sediments, solid wastes and leaching extracts for the determination of metals.

DEPARTMENT: Inorganic - Metals

REFERENCES: EPA Manual SW846,3rd Edition, 6020, Sept. 1994
EPA Manual SW846,3rd Edition, 6020A, Jan. 1998
EPA Method 200.8, EPA-600/4-79-020, 1994

Scope and Application

- Inductively coupled plasma-mass spectrometry (ICP-MS) is applicable to the determination of sub-ppb (μg/L) concentrations of a large number of elements in water samples and in waste extracts or digests. When dissolved constituents are required, samples must be filtered and acid-preserved prior to analysis. No digestion is required prior to analysis for dissolved elements in water samples. Acid digestion prior to filtration and analysis is required for groundwater, aqueous samples, industrial wastes, soils, sludges, sediments, and other solid wastes for which total (acid-leachable) elements are required.

- ICP-MS has been applied to the determination of over 60 elements in various matrices. Analytes for which EPA has demonstrated the acceptability Method 6020 in a multi-laboratory study on solid wastes are listed in Table 1. Instrument detection limits, sensitivities, and linear ranges will vary with the matrices, and operating conditions.

- If Method 6020 is used to determine any analyte not listed in Table 1, it is the responsibility of the analyst to demonstrate the accuracy and precision of the method in the waste to be analyzed. The analyst is always required to monitor potential sources of interferences and take appropriate action to ensure data of known quality.

Use of this method is restricted to spectroscopists who are knowledgeable in the recognition and in the correction of spectral, chemical, and physical interferences in ICP-MS.

Table 1: Method SW846 6020 is applicable to these listed elements. Additional elements (see Table 2) may be determined pending demonstration of accuracy and precision in the waste being analyzed.

	Chemical Abstracts

Element		Services registry Number (CASRN)
Aluminum	Al	7429-90-5
Antimony	Sb	7440-36-0
Arsenic	As	7440-38-2
Barium	Ba	7440-39-3
Beryllium	Be	7440-41-7
Cadmium	Cd	7440-43-9
Chromium	Cr	7440-47-3
Cobalt	Co	7440-48-4
Copper	Cu	7440-50-8
Lead	Pb	7439-92-1
Manganese	Mn	7439-96-5
Nickel	Ni	7440-02-0
Silver	Ag	7440-22-4
Thallium	Tl	7440-28-0
Zinc	Zn	7440-66-6

Table 2: Method SW846 6020A is applicable to these listed elements.

Element		Chemical Abstracts Services registry Number (CASRN)
Aluminum	Al	7429-90-5
Antimony	Sb	7440-36-0
Arsenic	As	7440-38-2
Barium	Ba	7440-39-3
Beryllium	Be	7440-41-7
Cadmium	Cd	7440-43-9
Calcium	Ca	7440-70-29
Chromium	Cr	7440-47-3
Cobalt	Co	7440-48-4
Copper	Cu	7440-50-8
Iron	Fe	7440-89-6
Lead	Pb	7439-92-1
Magnesium	Mg	7439-95.4
Manganese	Mn	7439-96-5
Mercury	Hg	7439-97-6
Nickel	Ni	7440-02-0
Potassium	K	7440-09-7
Selenium	Se	7782-49-2

Silver	Ag	7440-22-4
Sodium	Na	7440-23-5
Thallium	Tl	7440-28-0
Vanadium	V	7440-62-2
Zinc	Zn	7440-66-6

Summary of Method

- Prior to analysis, samples which require total ("acid-leachable") values must be digested using appropriate sample preparation methods (such as Methods 3005 - 3051).

- Method 6020 describes the multi-elemental determination of analytes by ICP-MS. The method measures ions produced by a radio-frequency inductively coupled argon plasma. Analyte species originating in a liquid are nebulized and the resulting aerosol transported by argon gas into the plasma torch. The ions produced are entrained in the plasma gas and introduced, by means of an interface, into a mass spectrometer. The ions produced in the plasma are sorted according to their mass-to-charge ratios and quantified with a channel electron multiplier. Interferences must be assessed and valid corrections applied or thedata flagged to indicate problems. Interference correction must include compensation for background ions contributed by the plasma gas, reagents, and constituents of the sample matrix.

Interferences

- Isobaric elemental interferences in ICP-MS are caused by isotopes of different elements forming atomic ions with the same nominal mass-to-charge ratio (m/z). The HP-4500 ChemStation data system is be used to correct for these interferences. This involves determining the signal for another isotope of the interfering element and subtracting the appropriate signal from the analyte isotope signal. Isobaric molecular and doubly-charged ion interferences in ICP-MS are caused by ions consisting of more than one atom or charge, respectively. Most isobaric interferences which could affect ICP-MS determinations have been identified. Examples include $ArCl^+$ ions on the As signal and MoO^+ ions on the cadmium isotopes. While the approach used to correct for molecular isobaric interferences is demonstrated below using the natural isotopic abundances from the literature, the most precise coefficients for an instrument must be determined from the ratio of the net isotope signals observed for a standard solution at a concentration providing suitable (<1 percent) counting statistics. Because the ^{35}Cl natural abundance of 75.77 percent is 3.13 times the ^{37}Cl abundance

of 24.23 percent, the chloride correction for arsenic can be calculated (approximately) as follows (where the $^{38}Ar^{37}Cl^{+}$ contribution at m/z 75 is a negligible 0.06 percent of the $^{40}Ar\ ^{35}Cl^{+}$ signal): Corrected arsenic signal (using natural isotopes abundances for coefficient approximations) =

(m/z 75 signal) - (3.13) (m/z 77 signal) + (2.73) (m/z 82 signal),

where the final term adjusts for any selenium contribution at 77 m/z.

NOTE: Arsenic values can be biased high by this type of equation when the net signal at m/z 82 is caused by ions other than $^{82}Se^{+}$, (e.g., $^{81}BrH^{+}$ from bromine wastes [6] or ^{82}Kr from krypton contamination in the Ar).

Similarly, the corrected cadmium signal (using natural isotopes abundances for coefficient approximations) =
(m/z 114 signal) - (0.027)(m/z 118 signal) - (1.63)(m/z 108 signal),

(where last 2 terms adjust for any tin or MoO^{+} contributions at m/z 114).

NOTE: Cadmium values will be biased low by this type of equation, $^{92}ZrO^{+}$ ions contribute at m/z 108, but use of m/z 111 for Cd is even subject to direct ($^{92}ZrOH^{+}$) ions and indirect ($^{90}ZrO^{+}$) additive interferences when Zr is present.

NOTE: As for the arsenic equation above, the coefficients in the Cd equation are **ONLY** illustrative. The most appropriate coefficients for an instrument can be determined from the ratio of the net isotope signals observed for a standard solution at a concentration providing suitable (<l percent) counting precision.

♦ The accuracy of these types of equations is based upon the constancy of the OBSERVED isotopic ratios for the interfering species. Corrections that presume a constant fraction of a molecular ion relative to the "parent" ion have not been found to be reliable, e.g., oxide levels can vary. If a correction for an oxide ion is based upon the ratio of parent-to-oxide ion intensities, the correction must be adjusted for the degree of oxide formation by the use of an appropriate oxide internal standard previously demonstrated to form a similar level of oxide as the interferent. This type of correction has been reported for oxide-ion corrections using ThO^{+}/Th^{+} for the determination of rare earth elements. The use of aerosol desolvation and/or mixed plasmas have been shown to greatly reduce molecular interferences. These techniques can be used provided that method detection limit, accuracy, and precision requirements for analysis of the samples can be met.

♦ Physical interferences are associated with the sample nebulization and

transport processes as well as with ion-transmission efficiencies. Nebulization and transport processes can be affected if a matrix component causes a change in surface tension or viscosity. Changes in matrix composition can cause significant signal suppression or enhancement. Dissolved solids can deposit on the nebulizer tip of a pneumatic nebulizer and on the interface skimmers (reducing the orifice size and the instrument performance). Total solid levels below 0.2% (2,000 mg/L) have been currently recommended to minimize solid deposition. An internal standard can be used to correct for physical interferences, if it is carefully matched to the analyte so that the two elements are similarly affected by matrix changes. When the intensity level of an internal standard is less than 30 percent or greater than 170 percent of the intensity of the first standard used during calibration, the sample must be reanalyzed after a fivefold (1+4) or greater dilution has been performed.

- Memory interferences can occur when there are large concentration differences between samples or standards which are analyzed sequentially. Sample deposition on the sampler and skimmer cones, spray chamber design, and the type of nebulizer affect the extent of the memory interferences which are observed. The rinse period between samples must be long enough to eliminate significant memory interference.

Safety

- The use of laboratory equipment and chemicals exposes the analyst to several potential hazards. Good laboratory technique and safety practices should be practiced at all times.

- Safety glasses and acid resistant gloves should be worn at all times when handling samples or reagents, or when in the vicinity of others handling these items.

- Liquid argon represents a potential cryogenic and suffocation hazard and safe handling procedures should be employed at all times when handling liquid argon tanks and fittings.

- The HP 4500 is fully interlocked to prevent user exposure to harmful electrical voltages, radio frequency emissions, ultraviolet radiation, high temperatures and other hazards. At no time should the operator attempt to disable these interlocks or operate the instrument if any safety interlock is suspected to be disabled.

- Spilled samples and reagents should be cleaned up from instrument and

laboratory surfaces immediately. Acid spills should be neutralized with sodium bicarbonate solution before cleanup.

- All additional company safety practices and procedures should be followed at all times.

Apparatus and Equipment

- Hewlett-Packard HP 4500 ICP-MS system which is capable of providing resolution better than or equal to unit resolution at 10% peak height. The HP-4500 mass range of 2-260 AMU exceeds the method requirement of 2-240 AMU. The HP-4500 ChemStation allows automatic corrections for isobaric interferences and correction for internal standard responses as required by the method. All critical argon flows including nebulizer argon are under mass flow controller control and a peristaltic pump is used for sample introduction.

  Includes HP 4500 ICP-MS Instrument, ChemStation, HP LaserJet printer and Cetac ASX-500 Autosampler

- 15 mL polypropylene test tubes for samples (Fisher Scientific #14-956-7E) and 50 mL polypropylene centrifuge tubes for standards (Fisher Scientific part # 14-375-150)

- Calibrated mechanical pipetters in the following ranges:

  100 mL

  100-1000 mL

  1000-5000 mL

- Trace metal grade pipette tips.

- Talc free gloves.

- Argon gas supply (high purity grade gas or liquid, 99.99%).

  For the determination of trace levels of elements, contamination and loss are of prime consideration. Potential contamination sources include improperly cleaned laboratory apparatus and general contamination within the laboratory environment from dust etc. A clean laboratory work area, designed for trace element sample handling must be used. Standards,

samples and blanks should be exposed to the laboratory environment as little as possible. The use of preparation blanks and spikes should be used to verify the absence of sources of contamination and loss. If necessary, polypropylene sample tubes should be rinsed and stored in dilute acid prior to use.

NOTE: Chromic acid must not be used for cleaning glassware for trace metals analysis.Sample preparation apparatus and equipment is detailed in the various preparation methods.

Standards and Reagents

- Acids used in the preparation of standards and for sample processing must be of high purity. Redistilled acids are recommended because of the high sensitivity of ICP-MS. Nitric acid should be used exclusively wherever possible to avoid polyatomic interferences caused by other acids. Many more molecular-ion interferences are observed on the analytes when hydrochloric and sulfuric acids are used. 1-2% nitric acid is the ideal concentration for all standards blanks and samples. It is important to match the acid concentration in standards and samples. Concentrations of antimony and silver between 50-500 µg/L require 1% (v/v) HCl for stability; for concentrations above 500 µg/L additional HCl will be needed. For this reason, it is recommended that antimony and silver concentrations in samples and standards be maintained below 500 ppb wherever possible.

- Calibration stock solutions may be prepared in the laboratory or purchased from commercial suppliers. Mixed calibration standard solutions are prepared by diluting the stock-standard solutions to levels in the linear range for the instrument in a solvent consisting of 1-2 percent (v/v) HNO_3 in reagent water. The calibration standard solutions must contain a suitable concentration of an appropriate internal standard for each analyte. On-line addition of internal standards is recommended using the second channel of the sample inlet peristaltic pump. Generally, an internal standard should be no more than 50 amu removed from the analyte. Internal standard elements should also match the ionization potential and other chemical and physical properties of the associated analyte elements as much as possible. Recommended internal standards include ^{6}Li, ^{45}Sc, Ge, ^{89}Y, ^{115}In, and ^{209}Bi. Prior to preparing the mixed standards, each stock solution must be analyzed separately to determine possible spectral interferences or the presence of impurities. Care must be taken when preparing the mixed standards that the elements are compatible and stable.Transfer the mixed standard solutions to freshly acid-cleaned Teflon or low density polyethylene bottles for storage. Fresh mixed standards must be prepared daily. Calibration standards must

be initially verified using a quality control standard (ICV) and monitored weekly for stability.

- Nitric Acid, concentrated "Trace Metal" Fisher Scientific 500 mL in glass, catalog # A509-500 or "INSTRA-ANALYZED" Mallinkrodt-Baker, 500 mL in poly-coated glass, catalog # 9598-00

- Reagent water equivalent to ASTM Type 1 (ASTM D 1193) >18 Megohm/centimeter resistivity.

- 1:1 (vol/vol) nitric acid

  Prepare by adding 50 mL concentrated nitric acid to 50 mL ASTM Type 1 water in a clean 125 mL HDPE bottle.

- 1:50, e.g. 2% (vol/vol) nitric acid

  Prepare by adding 2mL concentrated nitric acid to 98 mL ASTM Type 1 water in a clean 125 mL HDPE bottle.

- Tuning Solution: 10 mg/L Li, Ce, Y, Tl in 1% HNO_3. Available from Inorganic Ventures, catalog # HP4500B-TS

- Tune Check Solution stock: 10 mg/L Li, Co, In, Tl

  Prepare by pipetting 1 mL of each 1,000 mg/L single element stock solution into a 100 mL volumetric flask. Add 1 mL concentrated nitric acid and dilute to 100 mL with reagent water.

- Tune Check Solution: 50 mg/L Li, Co, In, Tl.

  Prepare by pipetting 0.5 mL of Tune Check Stock solution into a 100 mL volumetric flask, add 2 mL concentrated nitric acid and bring to 100 mL total volume.

- Internal Standard Stock Solution: 10 mg/L ^{6}Li, Sc, Y, In, Tb, Ho, and Bi (available from SPEX, catalog # CLISS-1).

- Internal Standard Working Solution for on-line addition of internal standards, (1 mg/L each element).

  Prepare by pipetting 5mL of Internal Standard Stock into a 50 mL volumetric flask. Add 1 mL concentrated nitric acid and bring to 50 mL total volume.

- Purchased Multi-Element Stock Solutions: Multi-element stock solutions may also be purchased, pre-prepared and certified from reputable vendors.

Such vendors include for example, High Purity Standards, Spex, and Inorganic Ventures.

- The following mixes are recommended:

  Instrument Calibration Standard 1: 100 ppm of Sb, As, Be, Ca, Cd, Cr, Co, Cu, Fe, Pb, Mg, Mn, Mo, Ni, Se ,Ti, Tl, V, Zn. (Use for calibration standards.)
  Instrument Calibration Standard 2: 100 ppm of Al, Ag, B, Ba, Na, 1000 ppm K. (Use for calibration standards.)

  Instrument Check Standard 1: 100 ppm of Sb, As, Be, Ca, Cd, Cr, Co, Cu, Fe, Pb, Mg, Mn, Mo, Ni, Se, Ti, Tl, V, Zn.

- Blanks: Three types of blanks are required for the analysis. The calibration blank is used in establishing the calibration curve. The preparation blank is used to monitor for possible contamination resulting from the sample preparation procedure. The rinse blank is used to flush the system between all samples and standards.

  - The calibration blank consists of the same concentrations of the same acid(s) used to prepare the final dilution of the analyte calibration solutions (2 percent HNO_3, (v/v) in reagent water). Use of HCl for antimony and silver is cited in Section 7.1.

  - The preparation (or reagent) blank must be carried through the complete preparation procedure and contain the same volumes of reagents as the sample solutions.

  - The rinse blank consists of 2-4 percent HNO_3 (v/v) in reagent water. Prepare a sufficient quantity to flush the system between standards and samples.

  - Quality Control Standard or Initial Calibration Verification (ICV): The quality control standard is the initial calibration verification solution (ICV), which must be prepared in the same acid matrix as the calibration standards. This solution must be an independent standard near the midpoint of the linear range at a concentration other than that used for instrument calibration. An independent standard is defined as a standard composed of the analytes from a source different from those used in the standards for instrument calibration. A suitable concentration for the ICV is 110 ppb for the trace elements and 11000 ppb for the major (optional) elements.

  - The interference check solution (ICS) is prepared to contain known

concentrations of interfering elements that will demonstrate the magnitude of interferences and provide an adequate test of any corrections. Chloride in the ICS provides a means to evaluate software corrections for chloride-related interferences such as $^{35}CI\ ^{16}O$ on ^{51}V, and $^{40}Ar\ ^{35}Cl$ on $^{75}As^{+}$. Iron is used to demonstrate adequate resolution of the spectrometer for the determination of manganese. Molybdenum serves to indicate oxide effects on cadmium isotopes. The other components are present to evaluate the ability of the measurement system to correct for various polyatomic isobaric interferences. The ICS is used to verify that the interference levels are corrected by the data system to within quality control limits.

- ICS-A: Dilute Interferents A, (Cat # CL-INT-A2) 1 to 10 by adding 10 mL to a 100 mL plastic centrifuge tube, add 1 mL HNO3. Bring to 100 mL.

- ICS-AB Dilute Analytes B, (Cat # CL-INT-B2) 1 to 100 by adding 1000 µL to a 100 mL plastic centrifuge tube containing 1 mL HNO3 and 10 mL Interferents A stock solution. Bring to 100 mL.

APPENDIX H

SAMPLE PREPARATION PROCEDURE FOR SPECTROCHEMICAL DETERMINATION OF TOTAL RECOVERABLE ELEMENTS IN BIOLOGICAL TISSUES (SA/33)

INTRODUCTION

This method provides sample preparation procedures for the determination of total recoverable metals in biological tissue samples.

REFERENCES

William McDaniel. 1991. Sample Preparation Procedure for Spectrochemical Determination of Total Recoverable Elements in Biological Tissues. Method 200.3. EPA/600/4-91/010. Office of Research and Development, Cincinnati, Ohio.

EQUIPMENT LIST

- Erlenmeyer Flask (125 mL or 250 mL Beaker)
- Erlenmeyer Flask (100 mL)
- Hot Plate
- Analytical Balance (0.001 g)
- Nitric Acid (Trace Metals Grade)
- Hydrogen Peroxide (30%)
- Hydrochloric Acid (Trace Metals Grade)
- Deionized Water
- Cups
- Filtermates

PROCEDURE

1. Place up to a 5 g sub-sample of frozen tissue into a 125-mL Erlenmeyer flask or 250 mL beaker. 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 5-10 mL. 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. NOTE: If the sample is to be analyzed by graphite furnace atomic absorption, omit this step as the hydrochloric acid interferes with the analyses.

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.

8. Filter samples using FilterMate filters (purchased from Environmental Express). Half of the sample (50ml) is poured into a polypropylene digestion vessel (purchased from Environmental Express) and a FilterMate filter was pushed through the liquid to the bottom of the vessel, filtering the sample. The detachable plunger of the FilterMate was then removed and discarded, leaving the filter in the vessel. A screw cap was then put on the labeled vessel to store the sample until time of analysis. The other half of the sample was filtered like the first half but then poured into a clean labeled bottle and capped for analysis by another laboratory if necessary.

APPENDIX I

FIMS MERCURY ANALYSIS - STOCK, STANDARDAND SPIKE PREPARATION (SA/42)

INTRODUCTION

This procedure is used for the preparation of the stock, analytical standards, blanks and spikes for analysis using the Perkin Elmer FIMS-100 Mercury Analyzer. The fish/tissue used for the spikes should be weighed by the use of the "Sample Weighing for Metals Analysis (SA/11)" procedure. The labware used in this procedure should be cleaned by the "Routine Labware Cleaning for Metals Analysis" (SA/8) procedure.

EQUIPMENT LIST

- Ground Tissue Samples for Spikes
- Class A Pipettes (1 mL and 3 mL)
- Deionized Water
- Pipette Bulb
- 1000 mg/L Mercuric Nitrate Stock/Reference Solution
- Concentrated Hydrochloric Acid (Trace Metal Grade)
- 5% (w/v) Potassium Permanganate ($KMnO_4$)
- Micropipettes and Tips
- Teflon Beakers for Making Substocks
- Mercury Waste Container
- 2 Volumetric Flasks (100 mL)
- Polypropylene Digestion Cups (Environmental Express)

PROCEDURE

1. Pipet 1 mL of a 1000 mg/L mercuric nitrate stock solution into a 100 mL volumetric flask containing ~60 mL of deionized water, 1 mL trace metal grade concentrated HCl, and 100 µL 5% $KMnO_4$. Dilute to 100 mL with deionized water to prepare a 10 mg/L Hg substock. Label this solution with the concentration, date and initials as it must be remade once a month.
2. Pipet 1 mL of the 10 mg/L Hg substock solution into a 100 mL volumetric flask containing ~60 mL of deionized water, 0.5 mL trace metal grade concentrated HCl, and 100 µL 5% $KMnO_4$. Dilute to 100 mL with deionzed water to prepare a 100 µg/L Hg substock. Label this solution with the concentration, date and initials as it must be remade once a week.
3. Pipet the following volumes of deionized water and 100 µg/L Hg substock into digestion cups labeled with the appropriate concentrations which are based on the final volume (50 mL) of standard at time of analysis. Use a micropipette to deliver all water volumes and stock Hg volumes less than 1 mL. Use a class A pipet to deliver 3 mL 100 µg Hg/L

substock.

Concentration (ng/L)	Amount of 100 µg/L substock	Amount of DI water
Blank	0	3 mL
50	25 µL	2975 µL
100	50 µL	2950 µL
500	250 µL	2750 µL
1000	500 µL	2500 µL
6000	3 mL	0 mL

4. Each blank and standard should be prepared in duplicate.
5. A total of 10% of samples analyzed for mercury should be spiked in duplicate. Spiking is accomplished by pipetting a known volume of the 100 µg/L Hg substock into a digestion cup containing a known weight of fish tissue. A micropipette may be used to deliver two 750 µL aliquots onto pre-weighed tissue to give a total spiking volume of 1.5 mL.
6. All mercury waste from rinsing pipettes, beakers, etc. should be disposed of in mercury waste container. Volume and concentration placed in waste container should be recorded on the hazardous waste container inventory form for that bottle.
7. Ten percent of the samples should be analyzed in duplicate and spiked. An example spike would involve adding 1.0 mL of 10 mg/L metal standard to a preweighed sample. Spiking should be done at least 30 minutes prior to the addition of nitric acid for the digestion.