Mercury, Arsenic and Selenium in Catfish Caught in the Allegheny River; Implications for Identification of Pollution Sources.

Submitted by

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As part of the requirements for a tutorial in Biology

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Problem Statement:

Power plant emissions are a major source of mercury, selenium and arsenic in aquatic environments. Upon entering the rivers, these water contaminants enter the food chain and begin to move between trophic levels. If they are not directly introduced into the food chain, those contaminants can be deposited in river sediment. As a result, plants and animals that are low on the food chain take up the contaminants as well as nutrients via bio-concentration and bio-accumulation. There is a lack of data regarding the level of metals and metalloids, especially, methylmercury, arsenic and selenium in fish along major portions of the Allegheny River however; fish can be used as bioindicators of human exposure to these metal and metalloid toxins. Concentrations of arsenic, mercury, and selenium from the flesh tissue of catfish caught from different locations on the Allegheny River which are correlated with proximity to industrial facility effluents, power plant fallout or fly ash pile leechate, can be compared to help determine sources of these toxins. It is hypothesized that higher levels of mercury, selenium, and arsenic will be found in catfish in the areas near coal fired power plants and areas adjacent to wastewater release. Furthermore, it is hypothesized that store-bought catfish will have lower Hg, As, and Se fillet concentration than fish caught within the sites of the Allegheny River because of the lack of industrial and municipal waste in waters breeding farm raised catfish.

Introduction:

The Allegheny River was at the focus of the industrial revolution in the United States and has had many historical anthropogenic establishments on its banks, especially from the iron and steel, foundry and power plant operations. Although the land and water uses have changed many times, the legacy of past activities is evident and the contaminants of concern in this area are well known.

Arsenic, selenium, and mercury occur widely in nature, though at low levels, and are associated with sulfide ores, elemental sulfur, and in crude oil and coal deposits. These metals are used in the manufacture of numerous industrial products, such as semiconductors, fuel cells, photographic processes, agricultural products, preservatives and pharmaceuticals (Kanluen and Amer, 2006). However, the largest source of mercury pollution is coal-fired power plants. Airborne mercury emitted by these facilities is deposited anywhere from within a few hundred kilometers of the smokestacks to across continents, far from its source. Mercury from coal-fired power plants is released into the air through the exhaust system when coal is burned.

Solid wastes from coal-fired power plants also contain toxic elements like arsenic, selenium, chromium, and cadmium; carcinogenic organic compounds; and radioactive elements (Larsen, 2004). These toxins can leach into streams and groundwater supplies, compromising people's health. After entry into the aquatic environment, mercury is transformed to organic forms by bacteria. Organic forms of mercury will bioconcentrate and bioaccumulate in aquatic organisms. Metallic mercury is converted to methylmercury

by the methylating action of bacteria. Methylmercury is highly toxic and can build up in fish, shellfish and animals that eat fish including birds, mammals and humans.

The primary exposure for Americans occurs when mercury falls to the earth and runs into our lakes, rivers, and streams and contaminates the fish. This becomes a significant issue of public health. In 2004, 47 states and territories had fish consumption advisories for mercury for at least some of their waters (EPA, 2005). Fish and shellfish consumption are the main sources of methylmercury exposure to humans. One study showed that a family from Wisconsin consumed a diet that included three to four fish meals per week, an analysis of these fish found that only one species from their diet, imported sea bass from Lake Superior, contained significant mercury levels (Knobeloch, 1995)**.** The levels of methylmercury in fish and shellfish depend on what they eat, how long they live, how high they are in the food chain and the concentration of methylmercury in water and sediment.

Epidemics of mercury poisoning following high-dose exposures to methylmercury in Japan and Iraq demonstrated that neurotoxicity is the health effect of greatest concern. In the fishing village of Minamata, Japan where a plastics factory had been discharging mercury wastes into Minamata Bay, 700 people died and 9,000 were left with degrees of paralysis and brain damage (Nadakavukaren, 2000). In Iraq, where mass poisonings occurred from the consumption of rice treated with mercurial fungicide, over 6000 people were hospitalized and over 500 deaths occurred, largely due to CNS failure. In both episodes, neurotoxicity was the most common adverse health effect noted (Nadakavukaren, 2000).

By using catfish as bioindicators, the ecological health of aquatic environments; and sources of pollution as well as, human fish consumption safety can be monitored. Catfish make a good bio- monitoring model because they are piscivorous fish, meaning they eat other fish. In the complex food chain catfish fall at the middle tropical levels in aquatic environments, and they can bioaccumulate the contaminants mercury and selenium (Burger, 2004). Since Arsenic is soluble, it does not bioconcentrate, as a result, the levels of arsenic found in the flesh tissue is representative of the recent relative concentration of arsenic in the water. Other characteristics of the catfish that makes it a useful indicator are that they are widespread, numerous, and they are a popular sport fish commonly eaten by anglers. The EPA determined that it is more appropriate to base the methylmercury criterion on a fish tissue residue concentration than on ambient water concentration because fish tissue concentrations directly measure human exposure to mercury. Mercury very strongly accumulates in the food chain therefore; diet rather than water is the greater source of exposure. Additional considerations for using a methylmercury criterion in fish residue were the difficulties in measuring methylmercury in the water column and relating the levels to concentrations in aquatic organisms.

Methylmercury is recognized as one of the most hazardous environmental pollutants, largely due to endemic disasters such as Minamata disease in Japan and methylmercury poisoning in Iraq, as well as industrial accidents involving methylmercury compounds. Once methylmercury is consumed by humans about ninety-five percent of it is taken up by red blood cells, and then distributed throughout the body with the brain being its primary target organ (Myers and Davidson, 1998). In pregnant women, methylmercury readily crosses the placenta and has a high affinity for fetal

hemoglobin. Prenatal methylmercury poisoning, or Minamata disease, clinically presents as mental retardation, cerebral palsy, microcephaly, and seizures (Myers and Davidson, 1998).

Like mercury, most but not all of the arsenic in fish tissue is in organic forms. The arsenic criteria recommended by the EPA are specific to inorganic arsenic. This is because unlike mercury, the more toxic form of arsenic is believed to be the inorganic form. Arsenic occurs naturally in rocks and soil, water, air, and plants and animals and is odorless and tasteless. It enters drinking water supplies from natural deposits in the earth or from agricultural and industrial practices. Approximately 90 percent of industrial arsenic in the U.S. is currently used as a wood preservative but arsenic is also used in paints, dyes, metals, drugs, soaps and semi-conductors (EPA, March, 2007). Industry practices such as copper smelting, mining and coal burning also contribute to arsenic in our environment (Hughes, 2006).

Since arsenic can be found in several different valence states and many different inorganic and organic compounds, the analysis of the toxic effects of arsenic is complicated. Arsenic can target a number of metabolic processes because of this it affects nearly all organ systems of the body by different exposure pathways. For ingestion and dermal routes of exposure, adverse effects are most often manifested in the skin resulting in discoloration and lesions and in the gastrointestinal tract nausea, diarrhea, and abdominal pain occur (EPA, 2007). Ingestion exposure has also been linked to cancer of the skin, bladder, liver, and lungs.

Like arsenic and mercury, selenium also occurs naturally in the environment and can be released by both natural and manufacturing processes. People working in or living

near industries where selenium is produced, processed, or converted into commercial products may be exposed to higher levels of selenium in the air. People living in the vicinity of hazardous waste sites or coal burning plants may also be exposed to higher levels of selenium. Selenium dust can enter the air from burning coal and oil. This selenium dust will eventually settle over the land and water and also enters water from rocks and soil, and from agricultural and industrial waste. Some selenium compounds will dissolve in water, and some will settle to the bottom as particles. Insoluble forms of selenium will remain in soil, but soluble forms are very mobile and may enter surface water from soils. Once introduced into the water, Selenium may accumulate up the food chain however; selenium has both beneficial and harmful effects in regards to human consumption. Low doses of selenium are needed to maintain good health. On the contrary, when exposure levels are too high, adverse health effects are seen. Epidemiological studies of humans chronically exposed to high levels of selenium in food and water have reported discoloration of the skin, pathological deformation and loss of nails, loss of hair, excessive tooth decay and discoloration, lack of mental alertness, and listlessness. Exposure to high selenium concentrations is believed to cause teratogenic deformities in fish, and massive reproductive failure (Lemly1993; Lemly, 1997).

Data from the Pittsburgh Fish Consumption Project (2005-2007) has shown significantly higher levels of mercury, as well as selenium in channel catfish caught in the Kittanning area of the Allegheny River as opposed to those caught in the Pittsburgh Pool. Additionally, white bass caught in Lake Erie and marketed at Wholey's market in the Strip District are significantly higher in arsenic, mercury, and

selenium than white bass caught in the Pittsburgh Pool (University of Pittsburgh Center for Healthy Environments and Communities, 2007). This study was the preliminary study of the Allegheny River Stewardship Project that I will be participating in for my tutorial project.

Materials and Methods:

All materials for this project will be provided for by the Allegheny River Stewardship Project. Study methods and detailed protocols for this project can be found in Appendix I.

Budget/Ethics:

Participation in the Allegheny River Stewardship Project eliminates a budget for this project. A major ethical consideration for this study is that fish will be sacrificed for the project. It is standard practice to submit a proposal to an Internal Review Board for this kind of practice associated with a research study. In this case a full and detailed proposal was submitted to the University of Pittsburgh IRB and Institutional Animal Use and Care Committee (IACUC). IRB approval and approval number can be found in Appendix II.

Timeline:

May 2008- Select tutorial examination board. Hold initial meeting with the tutorial examination board.

May 10th 2008- Attend community fishing day for ARSP- fish collection, sampling, and dissection. (Ford City)

May 31st 2008- Attend community fishing day for ARSP- fish collection, sampling, and dissection. (Cheswick/Springdale)

June 7th 2008- Attend community fishing day for ARSP- fish collection, sampling, and dissection. (Freeport)

June 14th 2008 - Attend community fishing day for ARSP- fish collection, sampling, and dissection. (Allegheny Head Waters)

June-August 2008- Collect and analyze data.

August-October 2008- Edit and finish final draft of the tutorial proposal. Continue to analyze data.

October 2008- Write a primary outline of this project for the tutorial examination board.

October 2008- First meeting of tutorial examining board, continue to analyze data.

November 2008- Completion of analyzing the data, focus on literature review.

December 2008- Continue to work on literature review and write the first draft of tutorial.

January 2009- First draft of the tutorial completed and submitted to tutor.

March 2009- Complete final draft of tutorial.

April 2009- Hold final tutorial board meeting.

Appendices

Appendix I: Study Methods and Detailed Protocols

Method of Catch, Anglers, Compliance with PA Fish and Boat Regulations and IUCAC Protocols-

Fish will be caught using rod and reel from shore and from boats by community members, researchers, fisherman, interns and all other volunteers for the project. Each person fishing, as part of the study, will be required to follow the regulations of the Pennsylvania Fish and Boat Commission, including having valid fishing licenses. The principal investigator (PI), project manager (PM) and all academic assistants will assure that all fishing is done in accordance with scientific collection permits issued by the Pennsylvania Fish and Boat Commission and that fish are sacrificed using the IACUC protocol show as Appendix A to this set of procedures. John Lucadamo and assistants from the partner organization Venture-Outdoors will also assist anglers, as will RiverQuest personnel aboard the Explorer.

River Procedure Following Fish Catch

It is envisioned that each river location will be divided into smaller river segments each to be serviced by either a shore or river retriever. It is also anticipated that retrievers will be in radio contact with key anglers or with groups of anglers at various locations. These retrievers will be responsible for completing the initial documentation of the fish catch, sacrifice of the fish, wrapping the fish and documentation in aluminum foil (Al), placing the fish on ice and bringing the fish to the Dissection Center as soon as possible (the retriever must follow this procedure exactly as stated in the order stated-fish are to be kept alive until the initial documentation is completed). Figure A shows the Initial Fish Documentation Form; this form must be filled out completely for the sample to be valid and must include-the river location, month/day and year of catch, the time of catch, the GPS coordinates of the catch, the name of the angler catching the fish, the retrievers name and an initial categorization of the fish species, along with specifics of the river reach or segment the fish was caught in. This form will be copied onto write on rain paper and must be filled out with a permanent marker such as a sharpie. Fish sacrifice will be performed immediately after the

documentation is finished. The method of sacrifice is by pithing (See Attachment A), which is the almost instantaneous severing of the spinal cord and dislocation and scrambling of the brain. This is done by insertion of a blade or wire very close to where the spinal column leaves the brain stem, severing the spinal cord and continuing to insert the device forward into the cavity of the brain, while twisting and turning the device to insure that the brain is mixed-much like an egg is scrambled. Once this operation is performed the fish with initial documentation should be immediately wrapped in Al foil (acid washed-shiny side out), put on ice and transported to the Dissection Center.

Anglers volunteering to fish for this study, will be told to keep fish alive until a retriever is notified and takes responsibility for the catch. If the fish is caught from a boat the catch can be stored in live wells. But boats not equipped with GPS units, under the direction of the PI, should not bring fish back to the central dissection area-these boats must drop anchor and wait for a retriever to register the fish.

Procedure to be Followed at Dissection Center

a) **Storage of Fish**- Retrievers will bring fish to the Dissection Center and will place the fish in coolers stored near the centers entrance. Ice chests will be marked A, B, C etc. The retriever will insure that no more than 8 wrapped fish are put into any one container. Fish will be dissected in approximately the same order that they are brought in and entered on the ice chest log. Retrievers should insure that once a cooler is full that the next cooler in the sequence should be used to store the fish. Retrievers should report the preliminary fish species and catch location to the PI or alternate person in charge of keeping the project logbook.

b) **Unwrapping of Fish and Physical Measurements**- No fish will be unwrapped until the logbook coordinator/PI has asked for the next fish to be unwrapped. Once the fish is unwrapped it will be placed on a high-density cutting surface for identification verification by the PI or designated assistant. The fish will at that time be given an exact project identification number, the weight, sex, standard, fin and total length, head, snout and post-orbital length, body depth and girth of all fish species caught will be noted. The GPS coordinates of all fish catch will be recorded. Each fish will have a unique specimen number and will include. ARSP (Location number, 00) - Study Number (001-999) – Date Caught (month-day-year as 030608 is the 6th day of March, 2008) – Fish Type Code (00) – River Section (00)}.

c) **The heart, gonads, liver, gills, and kidneys of each fish will be archived for analysis in subsequent studies (See Labeling Below).** Dissections will be performed in the field, in a specifically designated tent, where possible. Other species of interest, caught by researchers, which can be legally taken will be sampled and archived for analysis in subsequent studies. Samples in excess of those that will be analyzed as part of this study are being taken so that the project has adequate voucher

specimens and alternate samples if mistakes are made in the sample preparation or analytical sequence.

d) **Otolith Removal**-All specimens will have their otoliths removed according to procedures to be taught the research team on March 14th, 2008 at the Somerset PA Fish and Boat Commission facility (procedures will be incorporated into this document). All otoliths will be stored in envelopes for drying and marked with the complete sample number of the specimen. As a precaution the sample number will also be copied on a separate form and included inside the envelope with the otoliths.

e) Important Aspects of Initial Fish Documentation

i. **Correct identification of species -** All personnel involved in collection of any biological samples will be under the direction of someone who is expert in the identification of the species, and has been involved in collections previously.

ii. **Voucher specimens-** A number of voucher specimen from each species will be collected whole, frozen entirely.

iii. **Photographic voucher-** A digital photograph will be taken not only of each species type but also of each specific specimen for purposes of future verification if necessary.

iv. **Collection location physically-** The locations of all collections will be recorded in the field and in the laboratory notebooks. All physical locations will be noted using GPS in digital format as well as on documentation forms.

Sample preparation

a) General

Dissection of specimens was/will be done as soon as possible but within 48 days after freezing or immediately if specimen is not frozen. Gross dissection for fillets, heart, liver, kidney and gonads, gills and otoliths as well as skin and stomach contents will be done in the field or at the PI's Gibsonia storage facility or in Dr Talal El Hafnawy's laboratory in the UPCI, Shadyside-Pittsburgh, PA. Nitrile gloves will be changed between each sample, and aluminum foil (used to wrap frozen fish) will be discarded between each sample. Dissection tools will be washed with deionized water as a group between samples. All surfaces and instruments will be washed/treated with isopropyl alcohol after each specimen dissection. Instruments will be air-dried following wiping off of isopropyl alcohol with Kimwipes.

b) **Fish Sample Body Measurements and Tissues to be Taken** - the weight, sex, standard, fin and total length, head, snout and post-orbital length, body depth and girth of all fish species caught will be noted in the logbook after the fish is identified and given a complete sample number.. The GPS coordinates of all fish samples will be recorded in the project

logbook and also will be recorded in an excel spreadsheet. **All numbers and data in the logbook are the primary numbers and data for all samples and any mistake of entry of data into the project logbook requires that the person in charge put a line through the faulty data, insert the new data and put their initials and date-along with any explanatory note necessary to understand this change.** Each fish will have a unique specimen number. The gills, heart, gonads, liver and kidneys, stomach contents and otoliths of each fish will be archived for analysis in subsequent studies- a numbering system using abbreviations for these tissues is presented below.

c) **Specific Fish Dissection Standard Operating Procedure Protocols**

1.0 Objective

This SOP is to ensure uniformity in the preparation of fish to secure tissue for experimental analyses. Generally fish tissue will go into one of two general analytical streams, either analysis for metals, metalloids and toxic elements or estrogenicity (MCF-7 cell proliferation assay) with LC/GC/MS identification of selected specific pharmaceutical estrogens and xenoestrogens (primarily the alkylphenoxylates like nonylphenol). As a general rule all tissues for metals-metalloids and elemental analysis can be stored in plastic type bottles and bags including twirl bags. All tissues destined for estrogenic analysis or analysis of specific estrogenic substances will be stored in only in glass vessels, vial and bottles. These tissues should not come into contact with plastic at any time in the tissue preparation process. If the tissue does come into contact even incidentally with plastic the sample must be discarded and a note that effect entered into the project log as well as the electronic database.

2.0 Apparatus

Cutting board-high density molecular weight substance, filleting knives, dissection instruments, Kimwipes, paper towels, isopropyl alcohol, deionized water, fish tape measures, gravimetric weight devices-500mg and 2kg scales, analytical balances, logbook, digital camera and laptop computer in excel program.

3.0 **Procedure**-To be performed by two person teams working with PI/prime assistant entering data in logbook.

- 3.1 Lay fish on a cutting board with preliminary project label and with specific project sample number completed on this form (cutting board must be cleaned with alcohol and fish is only laid after board is completely dry).
- 3.2 Take a picture using digital camera of fish before dissection-with documentation form. Insure that both sides of fish are photographed, including any lesions or other distinguishing marks.
- 3.3 Sharpen the cutting/filet knife and insure all dissection instruments are cleaned and are dry.

3.4 Measure the fin length, standard and total length, head, snout and post-orbital length, body depth and girth on all fish as well as lower jaw length on channel catfish and record all in logbook and in excel program

3.5 Weigh the fish and record the weight in logbook

3.6 Remove, store and label otoliths.

3.7 All completed samples will be stored on dry ice with movement to –80 degree Centigrade freezers at the conclusion of each day.

3.8 Take the complete gill structure from one side of the fish. Set aside for archiving, gill sample must be put into the appropriate glass vial that has the sample number of the specimen followed by LL.

3.9 Remove scales (if present), fins and tail-remove skin of channel catfish. Fish are to be prepared for dissection as they are generally prepared for the table by anglers.

3.10 Filet fish and set aside first fillet for metals analysis stream.

3.11 Remove the filet exposing the posterior gut area.

3.12 If all gut is not exposed then cut very carefully along the side of gut with scissors in order to expose the gut.

3.13 Identify the gonads and record the fish's sex in the logbook and excel spreadsheet.

3.14 Remove the gonads and weigh, put in a glass vial that has the sample number of the specimen followed by a GD. Record the gonad's weight in the logbook and in the Excel spreadsheet

3.15 Harvest internal organs (designated below) of the fish including the stomach contents and label appropriately using the unique labeling of the first and last letter of the organ e.g., LR for liver HT for Heart, KY for Kidney, SC for stomach contents. All tissue will be put into glass vials and labeled with the specimen's unique number followed by the tissue code. This number will be typed on prepared project labels.

3.16 Cut approximately a 200g sample of the filet (that was set aside) right about center of the filet, weigh and record weight. Place in a small zip lock sandwich bag and label with the fish number and weight. Record the weight in the logbook

3.17 Label this portion for metal analysis=M.

3.18 Place the remaining portion of this filet in a small zip lock sandwich bag and label. Set this aside in an internal cooler for possible compositing of fillet tissue with other specimens of the same species/location and length-weight.

3.19 Turn the fish over on its other side and cut a filet from this other side. Do not remove any fat from this sample.

3.20 Cut approximately a 200g sample of the filet right about center of the filet and weigh and record weight. Label this portion for estrogen analysis. Put this sample in a **GLASS VIAL only**.

3.21 Remove a 2-gram piece of fat tissue from the remaining portion of this filet. Put this sample in a **GLASS VIAL only**. Label this with the sample number followed by FT for Fat.

3.22 Place the remaining portion of this filet in aluminum foil with appropriate external labeling. Set this aside in an internal cooler for possible compositing of fillet tissue with other specimens of the same species/location and length-weight.

3.23 Place all internal organs in one cooler, all samples for metal analysis in a second cooler and estrogenic analysis in a third cooler. Samples should be bundled so that all specimens from a specific fish stay together.

3.24 Complete the remaining requirements on the prepared label.

Check that all the fish information is correctly entered on the Chain of Custody record for that sampling/dissection round. Wash the cutting board with DI water if necessary dry and wipe thoroughly with alcohol and let dry thoroughly-do the same with all instruments and knives used on that fish.

Numbering Specimens- Also See Figure 1 below.

a) Code Sequence

ARSP (Location number, 00) - Study Number (001-999) – Date Caught (month-day-year as 030608 is the 6th day of March, 2008) – Fish Type Code (00) – River Section (00) =applicable code for internal organ or estrogen sequence or metals sequence.

All composite samples fort metals, fats or estrogen sequence will be denoted with a C prefix to the entire code sequence such as C=ASRP (01)-001-030608-01-02-M

b) ARSP Location Number- All fish caught as part of the Allegheny River Stewardship Project at any of the 4 river locations detailed below will first bear the code ARSP. Location specific information will be coded as follows;

    a) 01 – Fish caught at the Ford City Location.
    b) 02 – Fish caught at the Springdale/Cheswick Power Plant area.
    c) 03 - Fish caught in the Freeport area location.
    d) 04 – Fish caught at the Upper Allegheny Control site.
    e) 05- Fish bought at the Fishmongers

(So the first section of the code will be for instance ARSP01-This indicates a fish caught during the Allegheny River Stewardship Project at the Ford City Location). Fish caught in conjunction with other collaborative initiatives will have a different prefix and further location codes will be introduced. For instance-it is quite possible that ARSP researchers will have an opportunity to work with the United States Geological Survey-PA Department of Environmental Protection Emerging Contaminants Program, the prefix for fish or fish samples that may be had from work on this survey will be USGSDEP. Additional locational codes will be developed depending on agreements that may be reached.)

c) Study Number- Numbered consecutively starting with the first fish processed and ending with the last fish processed. Beginning number is 001.
d) Date Caught – Fish will be caught in the fall of 2005 through the spring of 2006. The date caught consists of first the month caught and than the date of that month caught. So 1029 means that the fish was caught on October 29th of 2005.
e) Fish Type Code
    01 Walleye
    02 Smallmouth bass
    03 Freshwater Drum
    04 Crappie Bass
    05 White Bass
    06 Channel Catfish[
f) River Section- To be determined.
g) Codes-Last to be inserted

M- Metal Analysis Stream
E- Estrogenicity Analysis Stream
FT - Fat
LL – Gills
GD – Gonads
LR – Liver
KY – Kidney
HT – Heart
SC – Stomach Contents
MR – Metals Fillet Remnants
ER - Estrogenicity Remnants

Specimen Handling at UPMC and EOH and Associated Laboratories

a) **Specimen tracking -**A specimen tracking system will been set up by Chuck Christen, who will manage the electronic data base with the help of Volz Group Graduate Students. The database will prepare using SPSS Version 15.0.

b) Specimen selection -Specimens to be prepared each day will be selected by Drs El Hefnawy, Eagon, Peterson and Volz. All steps will be recorded in a laboratory notebook, and later entered into the computer database. Volz Group Graduate Students may assist in this process as needed.

Protocols for Metals Analysis

One fillet of each fish will be used for metals analysis. Amounts over those necessary to perform the analysis will be achieved for latter use. 200gm of tissue will be digested by a nitric acid/hydrogen peroxide method – typically 2 mL 12 M ('metal-free') HNO_3 + 1 mL 30% (w/w) H_2O_2 added to ~200 g tissue, dissolved in 2% HNO_3 after the instrument-

controlled microwave-based digestion cycle. Microwave-based approaches enable us to routinely prevent background contaminants from entering the samples.
Samples will be analyzed using Inductively Coupled Plasma- Atomic Emission Spectroscopy (ICP-AES) for a suite of metals including As, Cd, Cr, Mn, Pb, Se, Co, Cu, Fe, Ni, Zn. Mercury (Hg) will be measured by a Cold Vapor Technique.

Appendix II

Approved Institutional Review Board Number

Approval Number

<<0510031 x Volz app 110705.pdf>>

Dear Dr. Volz:

An electronic version of your IRB approval letter is attached. You may now go forward with your research project. If you have any questions, please don't hesitate to contact the IRB office.

Kathy Yobbi

Institutional Review Board

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