



Determination of Total Mercury in Biological and Geological Samples

**A presentation for the 2004 Teledyne Leeman Labs
"Seminar on Low-Level Mercury Data and Analyses"**

By James G. Crock

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This report has not been reviewed for stratigraphic nomenclature.

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Determination of Total Mercury in Biological and Geological Samples

*A presentation for the 2004 Teledyne Leeman Labs: "Seminar on Low-Level Mercury Data and Analyses" ***

James. G. Crock

**This invited presentation was given by the author on September 23, 2004, in Boulder, Colorado, at a seminar/workshop on low-level mercury that was sponsored by Teledyne Leeman Laboratories, Hudson, NH 03051 (603-886-8400; www.LeemanLabs.com).

ABSTRACT

The analytical chemist is faced with several challenges when determining mercury in biological and geological materials. These challenges include widespread mercury contamination, both in the laboratory and the environment, possible losses of mercury during sample preparation and digestion, the wide range of mercury values commonly observed, ranging from the low nanogram per gram or per liter for background areas to hundreds of milligrams per kilogram in contaminated or ore-bearing areas, great matrix diversity, and sample heterogeneity¹. These factors can be naturally occurring or anthropogenic, but must be addressed to provide a precise and accurate analysis.

Although there are many instrumental methods available for the successful determination of mercury, no one technique will address all problems or all samples all of the time. The approach for the determination of mercury used at the U.S. Geological Survey, Crustal Imaging and Characterization Team, Denver Laboratories, utilizes a suite of complementary instrumental methods when approaching a study requiring mercury analyses. Typically, a study could require the analysis of waters, leachates or selective digestions of solids, vegetation, and biological materials such as tissue, bone, or shell, soils, rocks, sediments, coals, sludges, and(or) ashes. No one digestion or sample preparation method will be suitable for all of these matrices. The digestions typically employed at our laboratories include: (i) a closed-vessel microwave method using nitric acid and hydrogen peroxide, followed by digestion/dilution with a nitric acid/sodium dichromate solution, (ii) a robotic open test-tube digestion with nitric acid

and sodium dichromate, (iii) a sealed Teflon® vessel with nitric acid and sodium dichromate, (iv) a sealed glass bottle with nitric acid and sodium dichromate, or (v) open test tube digestion with nitric and sulfuric acids and vanadium pentoxide. The common factor in all these digestions is that they are very oxidative to ensure the conversion of all mercury forms into Hg (II). Each method of digestion has its advantages and limitations.

The method of detection used in our laboratories involves a combination of an in-house, custom, classic continuous-flow cold-vapor atomic absorption spectrometry (CV-AAS), a commercially available, automated, flow-injection and a continuous flow cold-vapor atomic fluorescence spectrometry (CV-AFS) systems, and a relatively new, automated and integrated approach where solid or liquid samples are thermally decomposed under an oxygen atmosphere (a nitrogen atmosphere is used for coals) and the released mercury vapor trapped onto a gold gauze and then thermally released into an AAS system. Other less frequently used instrumental methods available for the determination of mercury include inductively coupled plasma – optical emission spectrometry (ICP-OES), inductively couple plasma – mass spectrometry (ICP-MS) (both solution nebulization and laser ablation), and instrumental neutron activation analysis (INAA).

Results from two case studies involving the determination of mercury in the challenging matrices of biological materials will be presented. These will include fillet, liver and stomach-content samples from grayling for a baseline/background study in Alaska, and samples of meat tissue and shell material from Tanner crabs from Glacier Bay, Alaska. These studies show that the method of digestion is more important than a very sensitive detection limit for mercury.

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Determination of Total Mercury in Biological and Geological Samples

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CICT, GD, USGS

- Analytical Chemistry and Geochemistry – methods development and application
- Geophysics, methods development, and applications
- <http://crustal.usgs.gov>



Analytical Results can be

NO

better than the sample
submitted.

What? Why? When? How?

- What medium to sample—you must define the target population (part or the whole)
- What are the reasons for sampling
- Question(s) to be answered by this sampling
- Desired degree of confidence in the answer(s)
- When to sample—temporal issues

You'll never know the true answer, but you can make a reasonable estimate of the truth!!

Mercury Remains a Major Environmental Problem

- Industrial and Military Sites
- Fossil fuel combustion (power plants and transportation)
- Food chain enhancement (biota pathways to humans)
- Hg is still a growing problem in many areas as is demonstrated by the large increase of advisories for fish consumption

Sources of Hg in the Environment

- Estimated anthropogenic input estimated at $>6 \times 10^6$ Kg/year
- Fossil fuel burning and volcanic activity
- Industrial input (including waste incineration)
- Weathering of geological materials, especially Hg ore deposits and organic-rich sediments
- Agriculture (pesticides), mining activities, especially historic precious metal recovery and sulfide ore development
- Mining and precious metal recovery

Mercury General Geochemistry

- Crustal abundance of 0.08 ppm
Soil (0.06 ppm), Limestone (0.04 ppm),
Shale (0.02-0.4 ppm), Water (0.07 ppb – but this
is an old estimate from the late 1970's and may
overestimate the real water abundance)
- Chalcophile associations (Sulfur-Loving)
(As, Sb, Se, Ag, Fe, Zn, Cu, Pb, Cd)
- High vapor pressure and liquid phase at ambient
temperatures and pressures
- Commonly associated with organic matter and
adsorbed onto clays, oxides, etc.

Soil Mercury Generalizations

- Concentrates in the surface horizons
- Correlates well with organic carbon content
- Mobile in the subsurface as organic compounds
- Forms precipitates as sulfide, hydroxides, phosphates, or carbonates
- Methylated aerobically or anaerobically
- Adsorbed onto clays, hydroxides, oxides

Analytical Challenges

- Widespread anthropogenic Hg contamination.
- High volatility of Hg and most of its compounds (losses in sample preparation are very possible).
- Wide range of values possible (low ng/L in aqueous samples) to 100's of mg/Kg in solids.
- Solid sample heterogeneity (density, phase associations).
- Blanks!!! Use double-distilled (sub-boiling point methods) acids and water, stored in Teflon[®]. Methods are usually blank limited because of reagents.
- Clean all bottles with 10% nitric acid by soaking at least overnight.

Methods of Mercury Analyses

A method should be able to detect Hg in the sub-part per billion range (ng/g) for solids and pg/L for waters

- ICP-AES, ICP-AFS, ICP-MS*, INAA
- GF-AAS, F-AAS, CV-AAS*, CV-AFS*
- Thermal decomposition – AAS (TD-AAS)*

*methods available to CICT, USGS (summarized in CICT methods manual USGS Open-File Report 02–0223)

Hg Method Requirements

- Inorganic, organic, and adsorbed species of Hg are possibly present in the sample
- Rigorous digestion, but it must limit Hg volatilization problems
- Minimize Hg contamination from other samples, reagents, labware, and laboratory
- Large dynamic range

Standard Reference Materials

- Define and monitor accuracy and precision – quantitate the quality of the results
- Establish effective working range
- Must be analyzed routinely
- Extensive list of SRMs available ranging from low ppb to over 100 ppm Hg
- Should try to match the sample matrix, if possible

SRM's for Fish Tissue, Hg (ppm)

- NRC Can DOLT 2 (dog fish liver) 2.14 ± 0.28
- NRC Can DORM 2 (dog fish muscle) 4.64 ± 0.26
- NRC Can TORT-1 (lobster) 0.33 ± 0.06
- NIST 1641d (Hg in 2% HNO₃) 1.590 ± 0.018
- NIST 1566b (oyster tissue) 0.037 ± 0.001
- NIST 1575a (pine needles) 0.0399 ± 0.0007
- NIST 1573a (tomato leaves) 0.034 ± 0.004
- NIST 1577b (bovine liver) 0.003

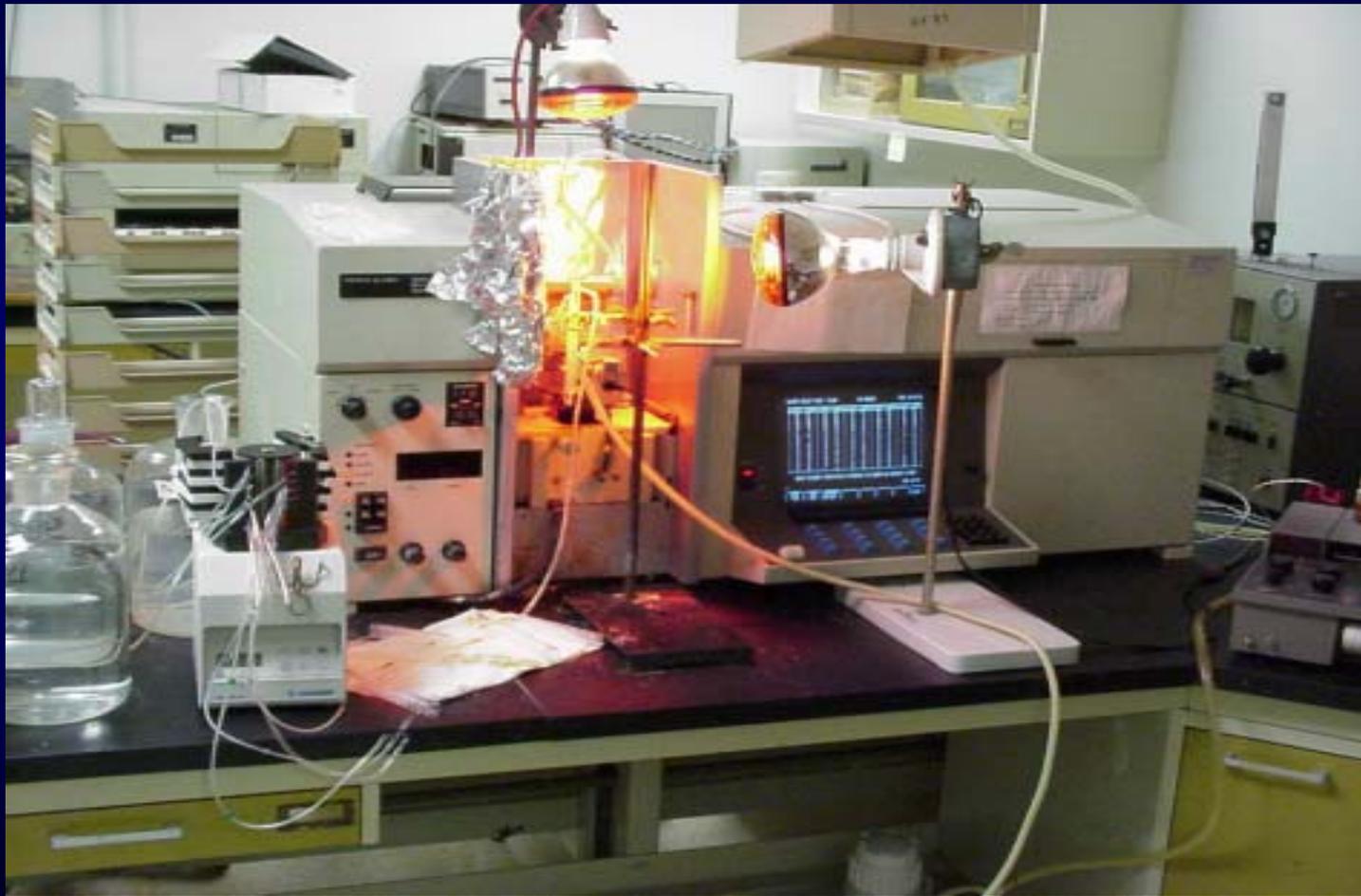
Selected SRM's for Geological Materials, Hg (ppm)

- USGS SDO-1 (marine sediment) 0.190
- NRC Can BCSS-1 (marine sediment) 0.129
- NRC Can MESS-1 (marine sediment) 0.171
- NIST 1645 (river sediment) 1.1
- NIST 1646 (sediment) 1.44
- NIST 8408 (river sediment) 107 ± 2
- NIST 2781 (domestic sludge) 3.64 ± 0.25
- NIST 2711 (soil) 6.25 ± 0.19
- NIST 2710 (soil) 32.6 ± 1.8
- NIST 2709 (soil) 1.40 ± 0.08

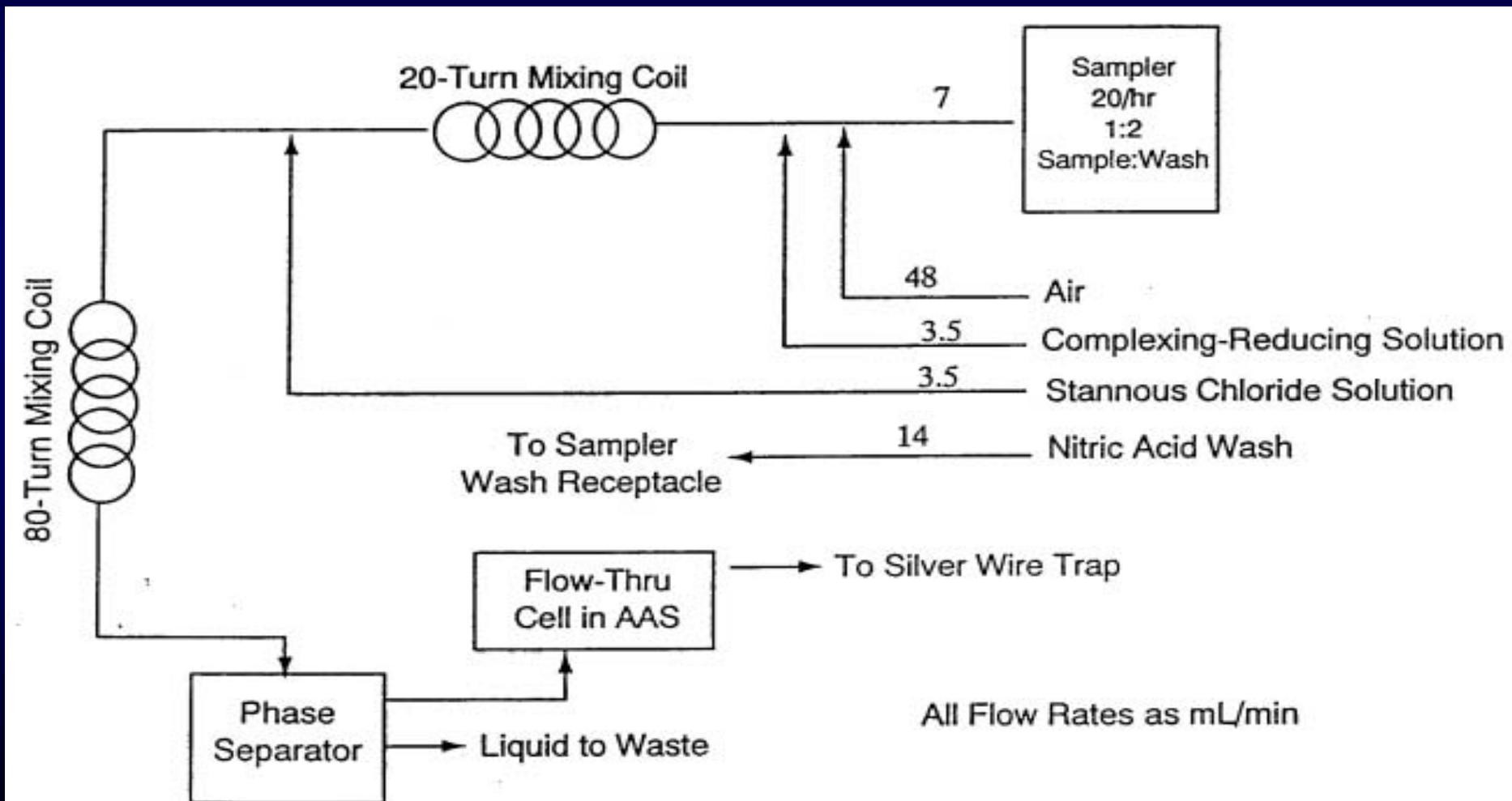
Leeman Hydra[®] AFS Instrumentation



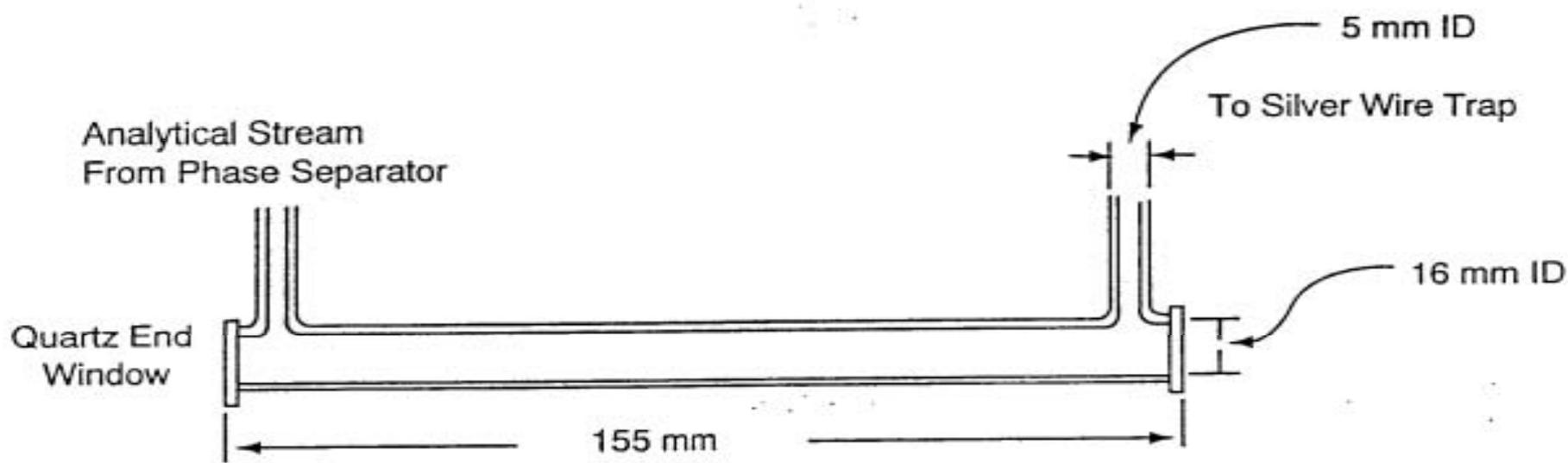
Continuous-Flow-Cold-Vapor-Atomic Absorption Spectrometry



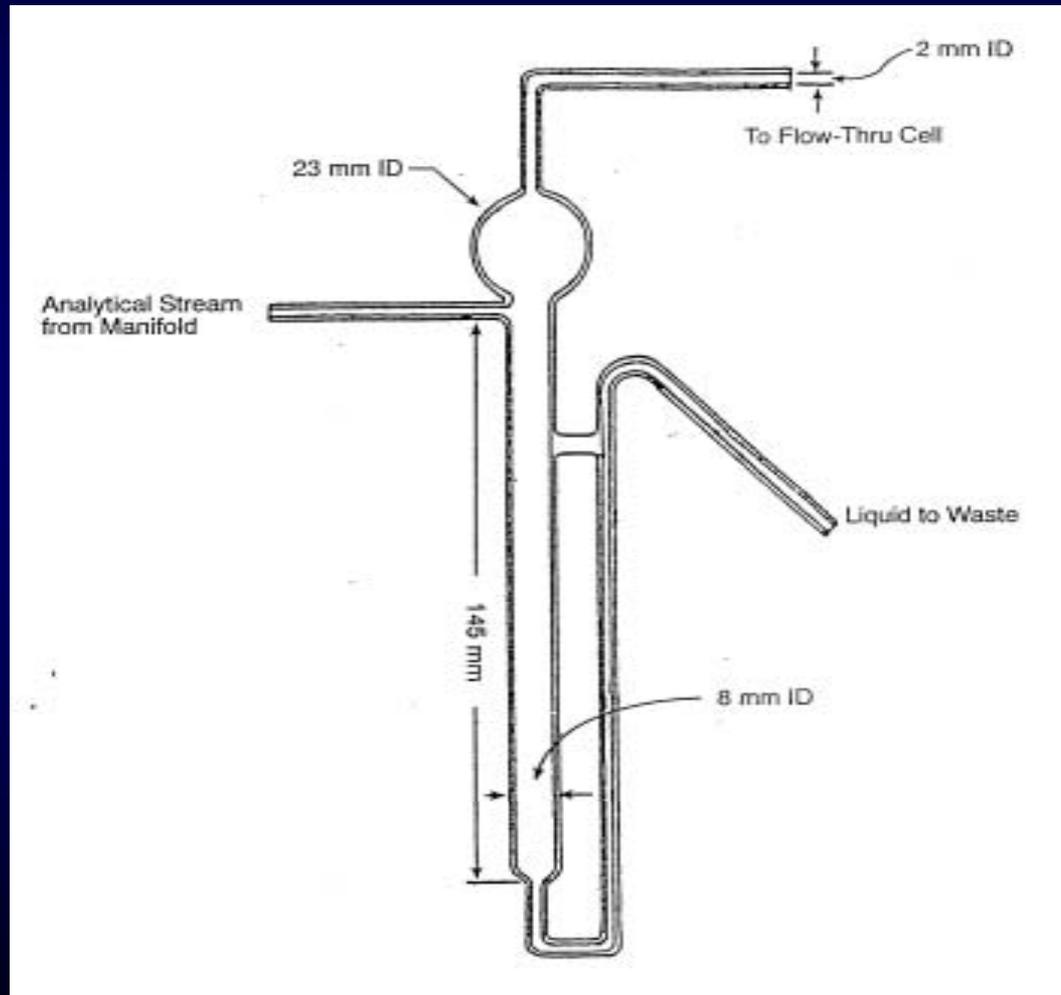
Manifold used for the automated generation of Hg vapor for CF-CV-AAS



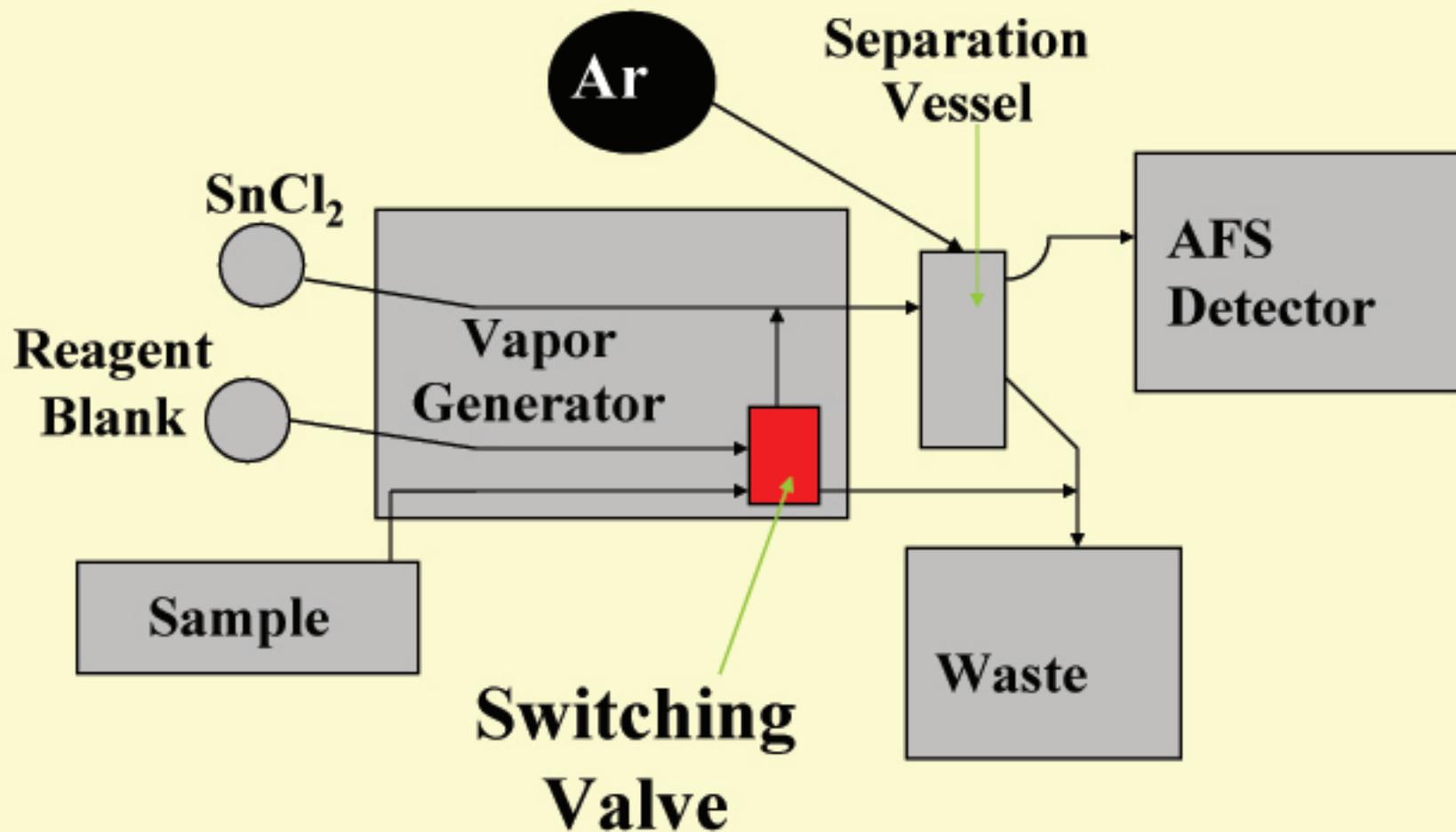
Flow-through Pyrex® glass cell for CF-CV-AAS



Pyrex® glass Phase Separator for CF-CV-AAS



Schematic for the CV-AFS Generator



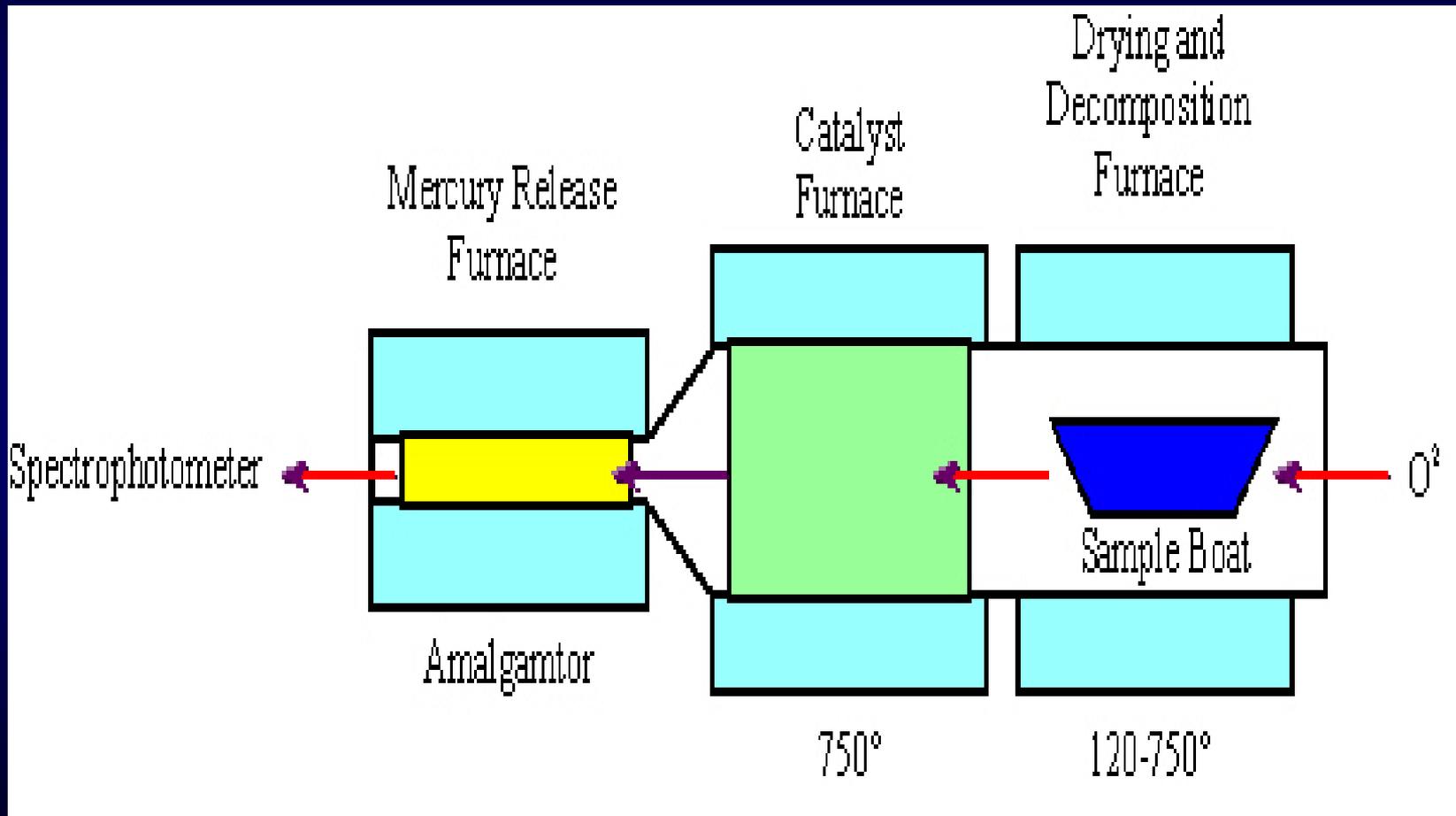
Thermal Decomposition – AAS



Thermal Decomposition – AAS Methodology Outline



Schematic of Hg Thermal Decomposition Method



Methods of Determining Mercury by ICP-MS

- Can be coupled with vapor generation and amalgamation for increased sensitivity
- Direct comparison with aqueous or solid (laser ablation) standards
- Standard addition of standard solutions
- Simultaneous determination of multiple isotopes with addition of a known amount of an enriched-isotope standard (Isotope Dilution)
- Laser ablation of solids – applicable to tree cores, rock core profiles, glasses, pressed pellets of powdered materials, fused samples

Role of ICP-MS in the Analysis of Mercury in Natural Waters

- Accurately determine mercury concentrations in secondary reference standards used to validate mercury concentrations in unknown water samples.
- Determine mercury concentrations in critically important water samples with difficult sample matrices.

ICP-MS vs. CV-AFS for Routine Monitoring of Mercury in Natural Waters

- **ICP-MS**

- **PRO**

- extremely accurate
- no matrix effects

- **CON**

- long analysis time
- costly
- instrumentation difficult to setup and maintain
- major memory effects

- **CV-AFS**

- **PRO**

- superior detection limit
- easy to maintain
- matrices are controlled
- memory effect corrected
- fast analysis time
- inexpensive to operate

- **CON**

- standardization required
- needs check by SRWS

CV-AFS AND CV-AAS METHODOLOGY

Four Basic Steps

- Sample decomposition {all Hg to Hg(II)}
- Quantitative Reduction of Hg(II) to Hg⁰
- Phase separation
- Hg measurement/quantification

Digestions

- Must be vigorous and highly oxidative
- Use strong mineral acids (HF not required), auxiliary oxidants, high temperatures and(or) pressure
- Direct and(or) indirect, microwave heating
- Open or closed vessels with “hotplate methods”
- For most biological samples, freeze drying is essential! (It preconcentrates elemental compositions by up to a factor of 10 by eliminating water while digestion reagents are not diluted.)

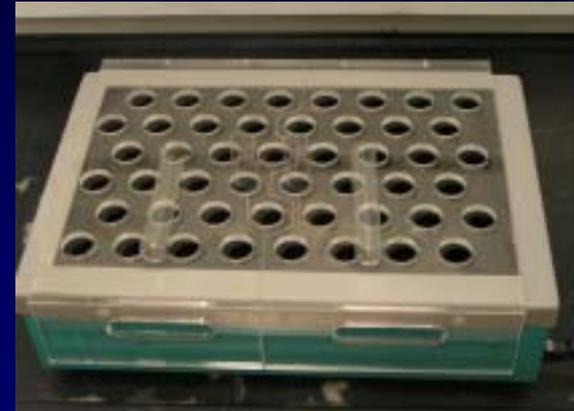
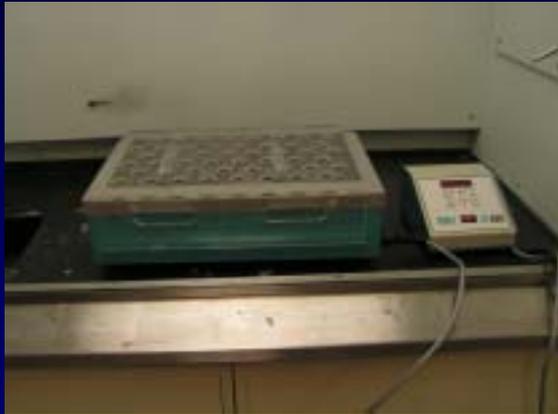
Freeze Drying the Biological Tissues



Microwave Digestion Oven and Digestion Vessel



Hotplate Digestion for Biological Matrices



Microwave Digestion for Fish Tissues and Sludges

- Weigh 0.5 g (sludge) or 0.25 g (fish) into Teflon® vessel. Add 2 mL water and 5.0 mL nitric acid.
- Step 1: Ramp to 200°C over a 30 min program. Cool to room temperature (~45 min).
- Step 2: Add 1.5 mL hydrogen peroxide.
- Step 3: Ramp to 200°C over a 20 min program and cool.
- Step 4: Pipette an aliquot of digest (<2 mL) into a 16 mm test tube, add 1.5 mL of the dichromate reagent, dilute with water to volume and analyze by CV-AAS.

Digestion Method for Coals and Biological Samples

- Weigh 0.15 g coal or dry tissue (0.75 to 1.5 g wet tissue) into a 16*150 mm test tube.
- Add ~0.1 g vanadium pentoxide, 3.5 mL nitric acid, and 1.5 mL sulfuric acid. Mix well.
- Cover with watch glass, ramp slowly (2 hours) to 150°C in an Al heating block. Maintain temperature overnight.
- Remove from heating block and cool. Dilute to 15 mL with water. Mix well and centrifuge. Analyze with CV-AAS or ICP-MS. Matrix match the standards.

Dichromate Digestion Procedure (Manual or Robotic)

- Weigh 0.1 – 0.5 g –100 mesh sample into a 30 mL Teflon® bottle.
- Add 2.0 mL nitric acid and 0.5 mL sodium dichromate solution.
- Cap, heat at 110°C for 3 hours in an Al block.
- Cool, transfer into flint glass test tube and bring to volume with distilled water. Mix. Let settle.

Robotic Nitric/Dichromate Digestion for Solid Materials



Reduction

- Must be quantitative
- Stannous Chloride (sodium borohydride)
- Source of most chemical interferences (more severe when using sodium borohydride)
- $\text{Hg}^{+2}_{\text{aq}} + \text{Sn}^{+2}_{\text{aq}} \rightarrow \text{Sn}^{+4}_{\text{aq}} + \text{Hg}^0 \text{ (gas)}$

Transportation and Separation

- Minimize surfaces contacted
- Surfaces should be glass or Teflon® and be warmed (prevents Hg⁰ loss by adsorption to the labware)
- Distribution coefficient between phases relatively independent of most variables

Reagents for CV-AFS

- Sodium Dichromate : 25% W/V, in concentrated Nitric Acid
- Stannous Chloride: 2% W/V, in 1.2 M HCl
- Nitric Wash: 0.16 M Nitric Acid (1% V/V)

Standards (traceable to NIST 1641c)

CV-AFS

0, 0.0294, 0.073, 0.147, 0.294, and 0.730 $\mu\text{g/L}$ Hg in
0.5% (W/V) Sodium Dichromate in 3.2 M Nitric Acid

CV-AAS

0, 1, 5, and 10 $\mu\text{g/L}$ Hg in 0.5% (W/V) Sodium
Dichromate in 3.2 M Nitric Acid

(All dichromate will be eliminated from this method in the
near future.)

Elements showing no interference on a 10 µg/L Hg standard determined by CV-AAS and a 0.1 µg/L Hg solution determined by CV-AFS

- 100 ppm Zn Pb Mn
- 50 ppm Cu Fe
- 10 ppm Co V U Mo Ni
- 50 ppm Cd Bi
- 3 ppm As
- 1 ppm Sb

Interfering Elements in CV-AAS and CV-AFS in the reduction step

Se Au Te Pt Ag

**Water vapor and chlorine gas also
interfere in the detection step.**

Applications of Different Mercury Methods at the USGS

- Baseline/Background geochemical studies of soil profiles, vegetation, and rocks (CV-AAS or TD-AAS) and water and soil extracts (CV-AFS) for mining activities in Alaska, Nevada, and Colorado
- Reference material characterization (all methods)
- Environmental studies including the analysis of fish tissues (Yellowstone National Park) (CV-AAS or AFS)
- Sewage sludge monitoring program for agricultural applications, including the wheat (both the grain and the entire plant), soils, water, sludges, and parent rocks (all methods)

Applications (Continued)

- Power plant impact (vegetation, water, soil) (CV-AAS, CV-AFS, and/or TD-AAS)
- Analysis of coal and coal by-products (CV-AAS or TD-AAS)
- Rock and soil extracts (CV-AAS or CV-AFS)
- Ground and spring waters (ICP-MS or CV-AFS)
- Shell, bone fragments, and hair samples (CV-AFS, TD-AAS)



Results of Elemental and Stable Isotopic Measurements, and Dietary Composition of Arctic Grayling (*Thymallus arcticus*) Collected in 2000 and 2001 from the Fortymile River Watershed, Alaska

by J.G. Crock, R.R. Seal II, L.P. Gough, and P. Weber-Scannell

This report is preliminary and has not been reviewed for conformity with U.S. Geological Survey editorial standards or with the North American Stratigraphic Code. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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Arctic Grayling

Important salmonid subsistence and sport fish

Circum-boreal in distribution

Non-anadromous (doesn't possess a sea-run phase)

Migrate up to 100 Km between summer feeding areas and over-wintering ice-free deep pools

Display remarkable inter-year fidelity to summer feeding sites (productive feeding site adaptation)

Some data on over-wintering fidelity as well

Voracious feeders!

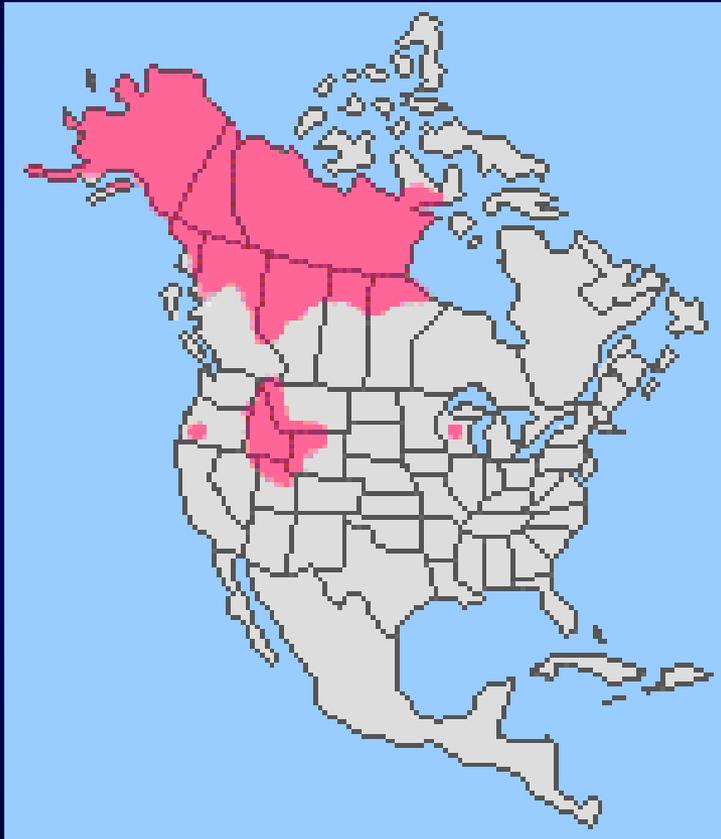


Field Methods:



- Collected by rod and lure – September 2000: period of maximum weight gain and prior to winter migration
- Similar weight (age) and length
- Three each (mix of male/female) from 10 sites on 80-km stretch of main stem plus four from tributary 13-km north (n = 34)
- Dissected in the field (muscle, liver, stomach contents); frozen; shipped to labs
- Standard sampling and laboratory QA/QC

Fortymile Grayling Study



Grayling Range



Field Dissection for fillet,
stomach, and liver

Digestion and Sample Preparation

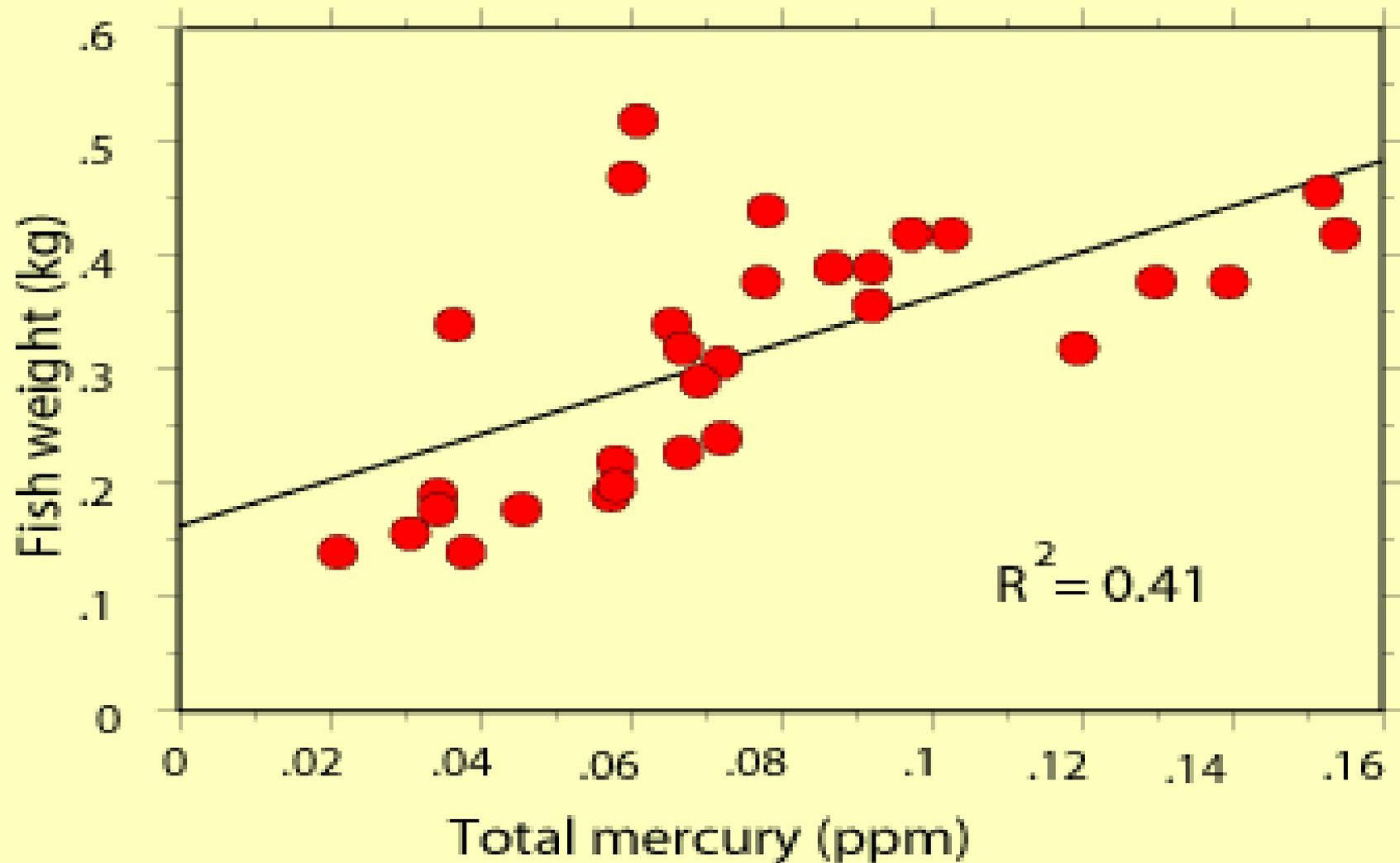


Stomach Contents with HNO_3



Hotplate Digestion

Simple regression of fish weight vs. total Hg in muscle tissue (wet-weight basis)



Summary

Total Hg levels in fish muscle ranged from 0.021–0.15 ppm (wet-weight basis) with a GM of 0.069 ppm; for liver tissue 0.031–0.10 ppm with a GM of 0.062—nearly an order of magnitude below the FDA permissible values for MeHg.

Biogeochemical baseline values and/or ranges were developed for 38 elements in grayling muscle, liver, and stomach contents from a watershed with a mix of lithologic units.

Hg shows a physiological positive correlation with fish weight (age); most other trace elements do not show a physiological association.

Hg in Tanner Crabs, Glacier Bay, Alaska



Preliminary Results for Hg (ppm) in Tanner Crabs, Glacier Bay

Sample Matrix	Range	Mean
Carapace (shell)	0.03 – 0.042	0.013
Leg Sheaves	0.03 – 0.27	0.012
Body Meat	0.09 – 0.68	0.26
Leg Meat	0.13 – 0.62	0.31



Questions?

- Would you really eat this fish?
- Answer – YES!! Grilled with garlic and lime.