An Evaluation of Mercury and Selenium Fish Tissue Monitoring Alternatives: Fish Biopsy Plug Samples Versus Homogenized Whole Fillets

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Background

The U.S. Environmental Protection Agency (EPA) and some states have collected and analyzed fish fillet plugs (i.e., biopsy punch samples) as a more cost-effective alternative for monitoring mercury levels in fish for human health applications than the routine approach of removing entire fillets from each whole fish sample and analyzing homogenized fillet tissue. In 2013, EPA's Office of Water added fillet plug sampling from fish collected at river and stream sites to its whole fillet sampling approach during the 2013–2014 National Rivers and Streams Assessment (NRSA). EPA has continued to use fillet plug sampling and whole fillet sampling for monitoring mercury concentrations in fish during the 2018-2019 NRSA and the 2015 and 2020 National Coastal Condition Assessments (NCCAs). In addition, EPA received comments about including fillet plug sampling with respect to implementing the Agency's 2016 Selenium Criterion.

Study Objectives

More states have been introducing fillet plug sampling into their fish monitoring programs since 2013 when EPA began applying this technique in the NRSA and NCCA. However, the question remained about whether fish fillet plug sampling and analysis can serve as a reliable alternative for homogenizing and analyzing fillet tissue from each whole fish sample to monitor mercury and selenium concentrations. To answer this question, EPA designed and conducted the Fish Plug Evaluation Study to assess the comparability of mercury and selenium concentrations in fish fillet plugs vs. homogenized whole fillet tissue samples in order to meet the following objectives: (1) to test whether collecting and analyzing fish fillet plug samples can serve as a reliable alternative for homogenizing and analyzing whole fillet tissue to monitor mercury concentrations in fish (mercury phase) and (2) to investigate if it is technically feasible to collect fillet plug samples and analyze them for monitoring selenium levels in fish to support implementation of EPA's tissue-based water quality criterion for selenium (selenium phase).

Fish Plug Evaluation Study Design Summary

Design Element	Mercury Phase	Selenium Phase	Description
Waterbody Types	2	2	Great Lakes and East Coast Rivers
Sampling Sites and Fish Species Collected	6	6	Lake Erie, Walleye; Lake Michigan, Lake Trout; Lake Ontario, Chinook Salmon; Anacostia River, Blue Catfish; Potomac River, Largemouth Bass; St. Lawrence River, Smallmouth Bass
Fish Collected per Site	10	5	Each fish sample consisted of a single specimen
Fish Tissue Sample Types	2	2	Fillet plug samples (2 plugs per sample) and Homogenized fillet tissue samples
Replicates per Sample Type	5	4	Number applies to each individual fish sample
Total Fillet Plug Samples Analyzed	300	120	Sampling sites (6) x Fish collected per site x Replicates per sample type
Total Homogenized Fillet Tissue Samples Analyzed	300	120	Sampling sites (6) x Fish collected per site x Replicates per sample type
Total Fillet Samples Analyzed	600	240*	Sampling sites (6) x Fish collected per site x Sample types (2) x Replicates per sample type

*An additional 120 single-plug fillet samples were analyzed for percent solids to convert wet-weight concentrations to dry-weight concentrations.

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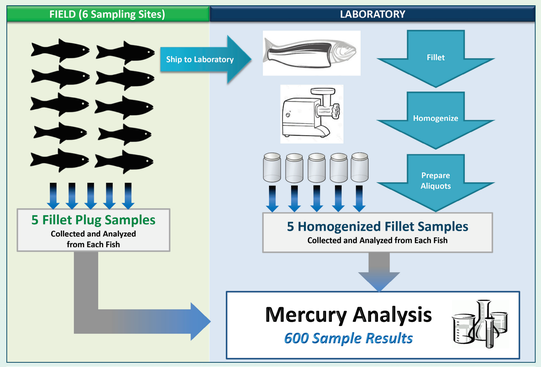


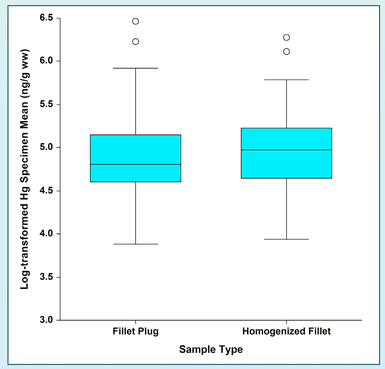
Analytical and Statistical Methods

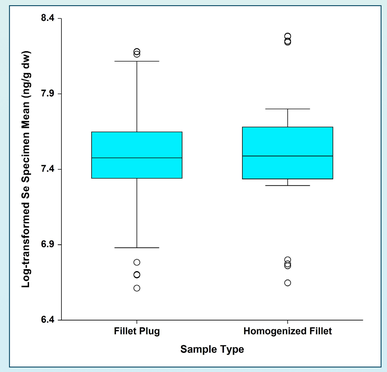
- 300 fillet plug samples and 300 homogenized fillet samples were prepared and analyzed for mercury using *Appendix to Method 1631, Total Mercury in Tissue, Sludge, Sediment, and Soil by Acid Digestion and BrCl Oxidation* from Method 1631 Revision B and Revision E, respectively (USEPA 2001 and 2002).
- 120 fillet plug samples and 120 homogenized fillet samples were prepared and analyzed for selenium using a modification to *Method 200.8, Determination of Trace Elements in Waters and Wastes by Inductively Coupled Plasma-Mass Spectrometry (USEPA 1994).*
- Null hypotheses (H_0) for mercury and selenium: both methods of collecting samples (fillet plugs vs. homogenized fillets) would yield equivalent mean concentrations of mercury and of selenium, respectively, for any given specimen.
- An analysis of variance (ANOVA) model on log-transformed data averaged across specimens was used to determine whether there are any significant differences across the two sampling methods, for each analyte (mercury and selenium), and an alpha value of 0.05 was used to assess significance.
- The statistical methods evaluated the potential impact of factors that could affect results, including waterbody type (lake vs. river), specific waterbody (6 locations), and fish species (6 species).

Summary Statistics by Sample Type

	Mercury (wet weight)		Selenium (dry weight)	
	Fillet Plug Concentration, ng/g (n=300)	Homogenized Fillet Concentration, ng/g (n=300)	Fillet Plug Concentration, ng/g (n=120)	Homogenized Fillet Concentration, ng/g (n=120)
Minimum	44.2	23.0	711.0	750.0
10th percentile	82.4	91.0	859.0	874.5
25th percentile	101.0	105.0	1544.5	1542.0
Median	121.0	143.0	1762.0	1781.5
Mean	155.2	161.4	1922.0	1996.0
75th percentile	171.3	185.3	2101.0	2090.0
90th percentile	271.0	262.0	3402.0	3829.5
Maximum	649.0	556.0	3814.0	4084.0
Standard Deviation	101.6	86.5	810.1	936.2
Relative Standard Deviation	65.5%	53.6%	42.2%	46.9%

Log-transformed Mercury (Hg) and Selenium (Se) Results by Sample Type





Study Findings

- The ANOVA main effects models indicated that for mercury (p=0.4048) and for selenium (p=0.3786), **there was no significant difference between fish fillet plugs and homogenized whole fillets**.
- The mercury data and the selenium data were log-normally distributed. For each analyte, there was a large overlap across the different fillet tissue sampling methods, and large variability across waterbodies.
- Since there was a large variance across waterbodies, waterbody was included as a blocking factor in the ANOVA model equation used for each analyte.
- The interaction term (Method:Waterbody) was not significant (p=0.9728 for mercury, p=0.6740 for selenium), indicating the effect of sample types was not impacted by site- or species-specific factors.

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