AN EVALUATION OF A REAGENTLESS METHOD FOR THE DETERMINATION OF TOTAL MERCURY IN AQUATIC LIFE

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Abstract. Multiple treatment (i.e., drying, chemical digestion, and oxidation) steps are often required during preparation of biological matrices for quantitative analysis of mercury; these multiple steps could potentially lead to systematic errors and poor recovery of the analyte. In this study, the Direct Mercury Analyzer (Milestone Inc., Monroe, CT) was utilized to measure total mercury in fish tissue by integrating steps of drying, sample combustion and gold sequestration with successive identification using atomic absorption spectrometry. We also evaluated the differences between the mercury concentrations found in samples that were homogenized and samples with no preparation. These results were confirmed with cold vapor atomic absorbance and fluorescence spectrometric methods of analysis. Finally, total mercury in wild captured largemouth bass ($n = 20$) were assessed using the Direct Mercury Analyzer to examine internal variability between mercury concentrations in muscle, liver and brain organs. Direct analysis of total mercury measured in muscle tissue was strongly correlated with muscle tissue that was homogenized before analysis ($r = 0.81$, $p < 0.0001$). Additionally, results using this integrated method compared favorably ($p < 0.05$) with conventional cold vapor spectrometry with atomic absorbance and fluorescence detection methods. Mercury concentrations in brain were significantly lower than concentrations in muscle ($p < 0.001$) and liver ($p < 0.05$) tissues. This integrated method can measure a wide range of mercury concentrations (0–500 μg) using small sample sizes. Total mercury measurements in this study are comparative to the methods (cold vapor) commonly used for total mercury analysis and are devoid of laborious sample preparation and expensive hazardous waste.

Keywords: biological samples, cold vapor atomic absorption spectrophotometry, direct mercury analyzer (DMA-80), fish, homogenization, total mercury, wet weight analysis

1. Introduction

The great number of physical states and chemical forms of mercury in the environment and its high vapor pressure at ambient temperatures qualify it as a very complex metal that is difficult to quantify. In biological samples $MeHg^+$ is the predominant mercury species that is covalently bound to protein sulfhydryl groups (Clarkson, 2002) and it needs to be liberated prior to identification (Bloom, 1998). Instrumental techniques such as energy dispersive X-ray fluorescence (EDXRF),

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neutron activation analysis (NAA), and atomic emission spectrometry (AES), to name a few, are used for the determination of mercury concentrations in solid samples (Nguyen *et al.*, 1998; Robinson *et al.*, 1994; Gerbermann *et al.*, 1997). However, atomic absorption spectrometry (AAS) and atomic fluorescence spectrometry (AFS) have been used extensively for the analysis of environmental and biological samples (Nguyen *et al.*, 1998; Saouter *et al.*, 1994). The amount of sample preparation for the AAS technique is dependent on the environmental matrix and mercury species to be identified. Using the AAS principle for total mercury analysis, generally 0.5 g of wet homogenous biological samples are denatured with hot nitric and sulfuric acids then oxidized with permanganate (U.S. EPA, 1991). The function of the acids is to liberate Hg from the matrix. Potassium permanganate is used to oxidize the organic-Hg^{+} to Hg^{2+}. Because of the high content of organic carbon in some environmental samples complete oxidation is difficult to obtain. To complete the oxidation process potassium sulfate is often used to keep Hg^{2+} dissolved in the solution resulting in excessive oxidizing agent in the sample. The samples are then treated with a reducing agent such as hydroxylamine to remove excess oxidizing agent and to prevent chromatography interference. Finally, Hg^{2+} is reduced to Hg° vapor before analysis. These procedures described are not easily reproducible for mercury analysis because of the volatility of mercury during digestion which requires adequate precautions to be taken to alleviate low mercury yields in the samples. Also, the use of multiple sample preparation steps before analysis could increase the potential for random error.

Because of the wide range of mercury concentrations found in wildlife species a quantitative method of analysis that would accurately measure mercury has been sought. Combustion methods of analysis, such as the Direct Mercury Analyzer (DMA-80®; Milestone Inc., Monroe, CT), is being used to quantify total mercury in water (Sarica *et al.*, 2004), soil (Boylan *et al.*, 1998) and biological (Cizdziel *et al.*, 2002) media using small sample sizes. The initial preparation step in the analytical process of the DMA-80 is removing the moisture through a drying process to concentrate the mercury in the sample. Thermal decomposition is used to ionize mercury in the sample to its elemental form. Elemental mercury is then trapped on a gold amalgamator and eventually liberated by heating amalgamator. Elemental mercury is then transported through two cuvette cells by a stream of oxygen and is then measured by AAS. The unknown total mercury in the sample is deduce from an active calibration curve in which various weights of known mercury concentrations in certified reference standards (CRS) are plotted against its absorbance. Costley (2000) was the first to use solid reference standards to generate a linear calibration curve. He also tested the performance of this method of analysis using CRS ($n = 8$) which showed good recoveries $\approx$10% (relative standard deviation) within the documented certified values of Hg from eight reference materials with a wide range of concentrations (between 0–7.0 ug/g). A statistically significant ($p < 0.5$) correlation ($r = 0.99$) was found between mercury levels in muscle tissues of fish using the DMA-80 method and a conventional method of analysis CV-AAS (Cizdziel

et al., 2002) even after data was normalized. A subsequent study by an independent group found a similar correlation ($r = 0.98$) between the two methods of analysis (Cizdziel *et al.*, 2002) suggesting that values recorded from the DMA-80 method can be compared to mercury values found in the literature. This approach to measuring total mercury in biological tissues saves time, saves money (by eliminating hazardous waste), allows a wide range of concentrations to be determined in smaller sample sizes, and reduces potential errors that occur during the sample preparation process when using conventional methods of analysis. This study will provide an overview of the performance of the DMA-80 instrument by documenting the instruments accuracy and precision during several months of analysis. In addition, the effectiveness of the homogenization process will be determined and compared with conventional methods of analysis.

2. Materials and Methods

2.1. Fish samples

2.1.1. *Farm Raised and Wild Captured Fish*

Twenty farm-raised largemouth bass were purchased from a commercial bass farm and transported to the US Geological Survey (USGS) in Gainesville, FL for use during laboratory validation studies. For field studies, largemouth bass were collected in May 2003 from two locations in the Big Cypress National Preserve which is known to be impacted by mercury contamination (U.S. EPA 2001). Samples were taken from the L-28 canal in the northeast corner of the preserve (UTM zone 17, 2894780N, 510542E) and from the southern end of the Turner River canal adjacent to US Highway 41, in the southwestern portion of Big Cypress (UTM zone 17,2863264N, 473777E).

2.1.2. *Farm-Raised Largemouth Bass*

To investigate the relationship between sample mass and mercury concentration 25 plugs of various weights (0.011–0.090 g) were removed from the axial muscle tissue of an individual largemouth bass and analyzed for mercury. Each plug was obtained after rinsing the fish in deionized water before removing the skin with disposable stainless steel scalpels. Scalpels and the biopsy punch were discarded after each use. The location of each plug was identified by numbers (1–25), omitting plug numbers 17 and 25 as schematically shown in Cizdziel (2002). Based on data from these plugs total mercury concentrations were independent of the mass of sample analyzed therefore, it was not necessary to establish a specific sample mass, for the following analyses. The internal variability of mercury concentration in muscle tissue was also determined from the same 25 plugs.

Often biological matrices undergo grinding or blending to obtain homogeneity before mercury analysis takes place. The DMA-80 method does not require

any pre-treatment of the sample. To compare the DMA-80 method to the homogenization process, 3 muscle biopsy plugs were removed from 10 individual skinless farm-raised largemouth bass and immediately measured for total mercury. Remaining muscle tissue from each largemouth bass was chopped and mixed for 30 minutes in a 10-mL glass beaker with a 115V Biohomogenizer M133/128i (Biospect Products Inc., Bartlesville, Okla.). Mixer and beaker were rinsed in 10% nitric acid and deionized water prior to use and after use. Homogenized samples were placed in 10-mL Teflon PTFE (polyetrafluorethylene) vial capped and frozen in a $-20\,^{\circ}$C freezer until analysis.

2.1.3. *Wild-Caught Largemouth Bass*

Muscle, liver, and brain tissues were removed from wild captured largemouth bass. Whole liver and brains were analyzed immediately. In muscle tissue 3-biopsy plugs were removed from three locations across each fish for total mercury analysis. Whole liver and brain were obtained with disposable scalpels. Gender, length and mass of each sample were recorded. Fish otoliths were also removed for the determination of fish age. All wild largemouth bass caught were determined to be over 1 year old.

2.2. INSTRUMENTAL ANALYSIS

2.2.1. *Nickel-Coated Boat Preparation for DMA-80 Analysis*

Newly purchased nickel coated boats appear to contain elevated levels of mercury (0.011–0.021 μg) indicating possible contamination by oil used during the manufacturing process (personal communication, Mikhail Mensch, Milestone Application Specialist). This could be problematic during the analysis process causing erroneous calculations in measured total mercury concentrations. Because of this nickel boats must go through the entire combustion process at least twice to obtain a low mercury background level. The complete combustion analysis time is < 7 minute per sample which is equivalent to 4 hours and 40 minutes per batch run. This could postpone sample analysis for more than a day. To circumvent the extra time it takes to reduce background mercury levels, sample boats were stacked in a muffle furnace. The furnace was programmed to reach a temperature of 1000 °C in 1 hour and was turned off. Boats remained in the furnace throughout the cool down period (3 hours) and were ready for use within the same day. Empty nickel boats were run periodically to determine background levels of mercury and to identify mercury carryover between sample analysis and at the start and end of sample batch analysis. After the cleaning process, blank readings were typically below 0.003 μg of mercury.

Total mercury (inorganic and organic) in tissue was analyzed using the Direct Mercury Analyzer (Milestone Inc., Monroe, CT) as described by EPA Method 7473 (EPA, 1998). A 4-mm biopsy punch-sampling tool as described by Cizdziel *et al.* (2002) was used to obtain triplicate portions of fish muscle for total mercury

analysis while other tissue analyses were performed in duplicate. Bulk fillet was removed from the freezer and allowed to thaw. Plug samples were excised, placed in pre-treated nickel coated boats then weighed to the nearest 0.0001 g on an analytical balance. All samples weighed < 0.2-g and were analyzed within 30 days of fish capture.

2.2.2. *Complete Combustion Atomic Absorption Spectrometry*

The best instrumental conditions for the determination of total mercury concentrations in tissues were determined by investigating and evaluating various heating rates and temperatures needed to obtain the desired decomposition of tissue samples. The DMA-80 total mercury analyzer was set to operate in autosampler mode at all times. The drying temperature and time was chosen based on the published method (Cizdziel *et al.*, 2002). However, the decomposition time was increased because replicate analysis of certified reference material resulted in calculated relative standard deviation (RSD) $> 5\%$. Therefore, it was determined that total sample decomposition would be best achieved by using the following optimized conditions. After weighing, the sample is automatically introduced into the combustion chamber on a nickel coated sample boat. Inside the instrument, high purity oxygen used as a carrier gas flows continuously. The sample is initially dried at a temperature of 300 °C and then is decomposed (combusted) as the temperature is ramped to 850 °C and held for 180 s. The vaporous byproducts are released from the tissue sample and carried through a Mn_3O_4/CaO-based catalyst to complete reduction of the mercury and oxidants that could potentially degrade the amalgamator. Elemental mercury vapor and other by-products are carried to an atomizer chamber containing a gold-coated amalgamator. In this chamber, elemental mercury is selectively trapped on the gold amalgamator and the other products are flushed out of the system. Finally, the amalgamator is heated for 12 seconds to release the mercury vapor, which is transported to the spectrophotometer. The DMA-80 chromatograms of each sample were electronically acquired using a data acquisition system (DMA-80 software, Rev. 4.00) on a PC based system. The absorbance of the sample is measured at 253.7 nm. The value of total Hg (THg) can be calculated from the amount of Hg measured (ng) and the mass of the sample (g) according to

$$\text{THg in } \mu\text{g/kg} = |\text{result (ng Hg)}|/\text{m (g)} \tag{1}$$

In this study, all total mercury values are reported in μg/g on a wet-mass basis.

2.2.3. *Cold Vapor Atomic Absorption Spectroscopy*

A subset of remaining muscle tissue homogenate were randomly selected and sent to an independent laboratory for confirmation of the direct combustion method of analysis. Total mercury analysis using conventional cold vapor AAS was performed at the Florida Department of Environmental Protection Central Laboratory using

revised EPA Method 245.6 (DEP, 2003). Quality control certified reference material, matrix spikes and laboratory fortified blanks were run along with samples to ensure precision and accuracy of analysis. The detection limit for this method is 0.125 μg/L based on 0.25 g wet weight in 40 mL of water. Recovery of QC samples were $>90\%$ of its expected value.

2.2.4. *Purge and Trap With Atomic Fluorescence Detection*

Methylmercury analysis was conducted at FL DEP Innovation Park Laboratory utilizing cold vapor purge and trap with atomic fluorescence detection. Tissue samples were analyzed using revised EPA Draft Method 1630 (DEP, 2003). Using this method, methylmercury concentrations in tissue samples can be measured at 0.001 μg MeHg /g as Hg°. Quality control samples analyzed included a rinse (to insure that the instrument is clean before sample analysis); method blank; 1.0 μg/mL standard (check machine and reagents); 0.38 μg/mL Dorm-2 (check the calibration curve); matrix spike (checks the efficacy of the digestion and ethylating procedures) and matrix spike duplicate; MDL (0.02 μg/mL); and laboratory fortified blanks (duplicate analysis). Recovery for all QC samples was between 80–120% of its expected value.

2.3. Data quality assessment

2.3.1. *Linear Range*

In accordance with the quality assurance to validate the performance of an instrument for accurate detection of mercury in a sample, a linearity study was performed. This study will verify the instruments ability to measure an amount of mercury in a sample within a certain range of concentrations in which the response is linearly proportional to concentration. Certified reference standards (CRS) Tort-2 (lobster liver) and Dorm-2 (dogfish muscle) were chosen to create the calibration curve because of their range in mercury concentration. Tort-2 with 0.27 ± 0.06 ppm mercury concentration and Dorm-2 with 4.6 ± 0.26 ppm mercury concentration were measured for total mercury at 5 or more concentration levels. Each CRS (Tort-2 and Dorm-2) concentration level was analyzed a minimum of three times and evaluated using the optimum conditions determined during the accuracy and precision studies. Linearity range was established by evaluating the CRS using a series of masses (0.0037, 0.0185, 0.0371, 0.074, 0.0975, 0.1109 g) for the low calibration curve and (0.1294, 0.0216, 0.0436, 0.0651, 0.0871, 0.1080, 0.1169 g) for the high calibration curve and plotting the mercury response (absorbance) versus the measured concentration. A correlation coefficient of 0.99 or greater was considered an acceptable fit of data to the regression line. The CRS Dolt-2 (dogfish liver) with 2.14 ± 0.28 ppm of mercury was used to check the efficiency of the calibration curve during batch sample analysis.

2.3.2. *Statistical Analysis*

Trends in the data were analyzed using Prism 3.03 (GraphPad Software, Inc.) statistical programs. Parametric statistical student t-tests were applied to test differences between concentrations of total mercury measured with homogenized samples and those measured directly. Using the Kolmogorov –Smirnov test data appeared to be normal ($p > 0.10$) therefore a one-way analysis of variance was used to determine significance in variability of mean mercury concentrations between the various instrumental methods and between muscle, liver, and brain organs from largemouth bass. When significant differences in mean mercury concentrations were observed, a posteriori test (Tukey's Multiple Comparison Test) was applied.

3. Results and Discussion

3.1. DMA-80 METHOD VALIDATION

3.1.1. *Linearity and Range*

DMA-80 analysis using certified reference standards to establish a calibration curve was first performed by Costley *et al.* (2000). The DMA-80 analyzer contains two cells that were both utilized in this study. The first cell is a long cell (10 cm) that detects lower levels of mercury ($< 0.035\ \mu$g), the combined use with the shorter cell (1 cm) allows for higher levels of mercury to be identified. Linear regression analysis of total mercury analysis was determined using a first-order fit incorporating the equation $y = mx + b$. The R^2 value or coefficient of determination is the simplest statistic used to measure goodness of fit (linearity) of the regression line, see Figure 1 and 2. Both curves show good linearity for the ranges 0.0010–0.030 μg ($n = 6$) and 0.035–0.55 μg ($n = 7$) as indicated by the R^2 values of 0.99. This means that 99% of the variation in concentration is explained by the DMA-80 analysis. All concentration versus absorbance data are close to the regression line with a RSD $< 0.02\ \mu$g.

3.1.2. *Accuracy and Precision*

Accuracy was assessed by analyzing certified reference standards (CRS) of known concentrations and comparing the measured value to the true value. In addition, recoveries were determined by spiking the matrix with a known amount of mercury using CRS. In the latter approach two biopsy punches of tissue was analyzed separately for mercury. A known amount of mercury in CRS was added to each punch of tissue to determine mercury recoveries. The data found in Tables I and II consist of the statistical means, standard deviation, 95 percent confidence intervals for the means, along with approximately 140 samples from which the data were obtained. CRS percent recoveries of the spiked tissues yield results that are quite respectable for the recovery of spikes under normal analytical conditions. DMA-80 method demonstrates little change in mercury concentrations over time. Table I

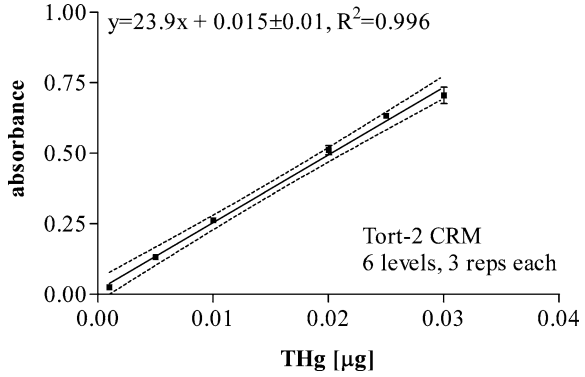

Figure 1. Low Calibration Curve Range 0.0010–0.030 μg Hg at the 95% CI.

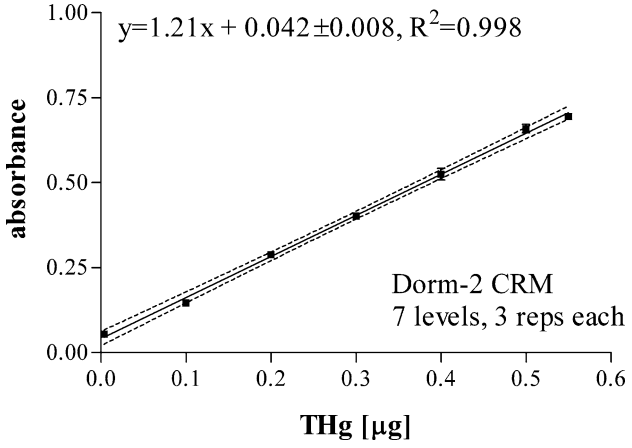

Figure 2. High Calibration Range 0.035–0.55 μg Hg at the 95% CI.

and II show the accuracy and precision of the DMA-80 for total mercury in tissue. Percent recovery of the CRS yielded results respectable for the recovery mercury spiked samples. The limit of detection for the DMA-80, based on the mean of the blank sample plus 3 times the pooled standard deviation of the blank ($n = 165$) was 0.001 μg/g.

3.2. Plug method for fish tissue analysis

3.2.1. *Mercury Differentiation Within Fish Muscle*

A description of the procedure to compare mercury concentrations within the muscle tissue of the largemouth bass can be found in the literature (Cizdziel *et al.*, 2002). Difference in mercury concentration measured along the dorsal muscle (top of the fillet) was minimal (Figure 3); the relative standard deviation was approximately

TABLE I

Data from DMA-80 analysis of total mercury in CRS to evaluate accuracy and precision during 12-weeks of testing

	Certified reference standard											
	Tort-2			Dolt-2			Dorm-2					
Known value, μg	0.001	0.005	0.010	0.030	0.050	0.050	0.10	0.15	0.20	0.35	0.40	0.50
No. of samples	10	6	29	8	10	3	6	10	9	8	16	6
Measured value, μg	0.001	0.0047	0.010	0.029	0.049	0.046	0.099	0.14	0.20	0.37	0.38	0.50
Standard deviation	0.00003	0.0003	0.001	0.0006	0.001	0.00007	0.001	0.009	0.002	0.007	0.014	0.0008
Lower C.I. - Upper C.I.	0.00098–0.0010	0.0043–0.0050	0.0097–0.011	0.029–0.030	0.049–0.050	0.046–0.046	0.097–0.100	0.13–0.14	0.19–0.20	0.37–0.38	0.38–0.39	0.49–0.50
Coefficient of Variation, %	2.8	6.8	10.0	1.9	2.1	0.15	1.4	6.7	1.1	1.8	3.7	0.16
% Recovery	100	94	100	97	98	92	99	93	100	106	95	100

[a]Tort-2 is lobster liver and Dolt-2 and Dorm-2 are dogfish muscle and liver, respectively.

TABLE II

Data from total mercury spiked matrix sample for the evaluation of accuracy of the DMA-80 during 12-weeks of testing

Certified reference standard	THg in sample, μg	Spiked amount, μg	Measured value, μg	Recovery %	Av. Recovery[a] %	Standard deviation	Lower 95% C.I.-Upper 95% C.I.
Tort-2	0.0010	0.011	0.012	107	103	0.00024	0.012–0.013
	0.0015	0.011	0.012	100			
	0.0012	0.011	0.012	103			
	0.0012	0.011	0.012	103			
	0.0012	0.011	0.013	106			
	0.0013	0.011	0.012	98			
					86	0.016	0.047–0.055
Dorm-2	0.0020	0.051	0.055	105			
	0.0017	0.051	0.051	97			
	0.0010	0.057	0.059	102			
	0.0012	0.048	0.050	102			
	0.048	0.048	0.082	73			
	0.045	0.045	0.073	60			
	0.048	0.048	0.094	97			
	0.051	0.051	0.076	48			

10%. Average mercury concentration along the lateral line was 0.036 ± 0.02 μg/g with an even higher RSD (60%). For all plugs measured, the RSD was 54%. Although these findings suggest less mercury differentiation across the dorsal line which is in agreement with the Cizdziel (2002) results, our variability was much higher for all plugs. This is expected because the mercury levels reported in this study are much smaller than those reported by Cizdziel and this caused an increase in the variability of mercury concentrations. It is clear from other data that the concentration of mercury in tissue can depend on lipid content, although this effect does not appear to be large for most fish. Cizdziel found slightly higher muscle mercury concentrations in the ventral area of the fish, which tend to be higher in lipid therefore he suggests sampling plugs from the same location of the dorsal portion of the muscle tissue. Although this is ideal it is not always possible to achieve. It is not uncommon for investigators to share biological tissues for contaminant analysis. In this instance using the DMA-80 3-plug method will suffice. As seen in Figure 4, mercury concentrations (0.016–0.078 μg/g) measured was independent of sample mass (0.011–0.091 g). RSD for sample mass was high, 54%. The high variability is possibly a result of error produced when weighing small samples because of the potential for atmospheric moisture to accumulate on the surface of the sample and thus producing a bias in the analytical measurement.

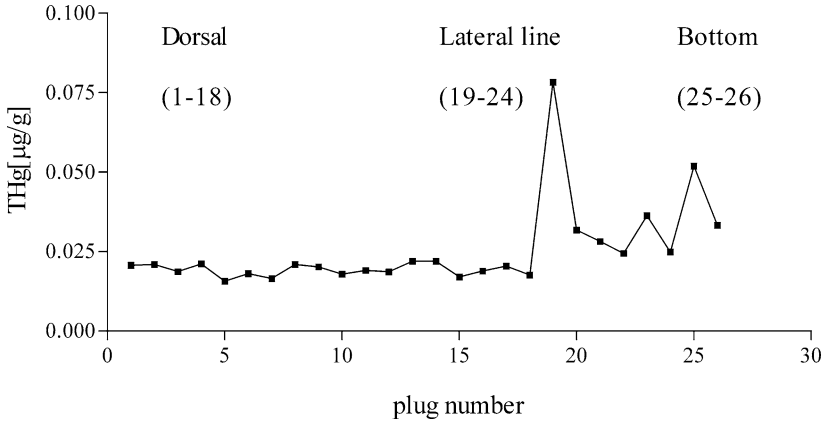

Figure 3. Location differentiation of total Hg in muscle plugs from a largemouth bass. Total Hg concentrations are reported in μg/g (wet-mass basis).

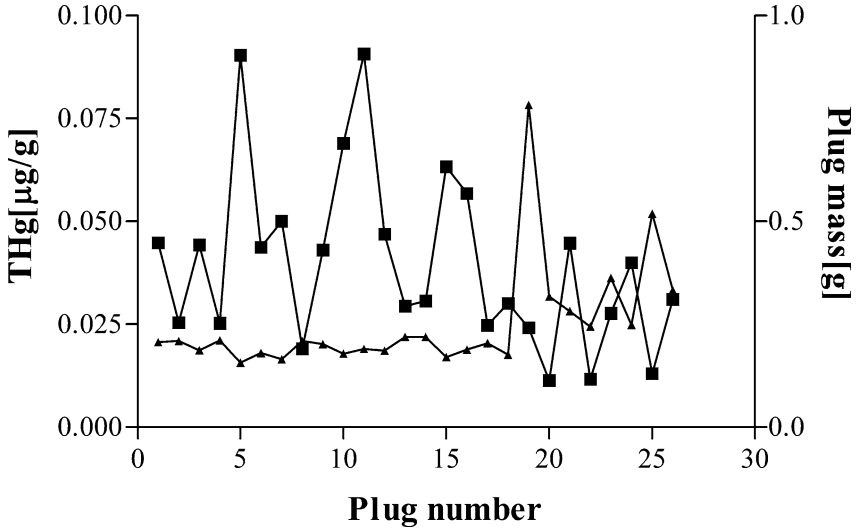

Figure 4. Effects of sample mass [g] on total mercury concentration [ug/g-wet mass] in largemouth bass.

3.2.2. *Effects of Homogenization*

The effectiveness of the homogenization process was tested by comparing the results of total mercury determined by taking 3-plugs from an individual fillet of fish and comparing the average total mercury results to the values measured from the remaining homogenized fillet. Figure 5 illustrates the differences in total mercury concentrations in ten fillets of skinless farm-raised fish. DMA-80 muscle plug concentration (0.022 ± 0.01, expressed as mean $\pm$ standard deviation) was equivalent to its homogenized muscle tissue concentration (0.023 ± 0.01). The aver-

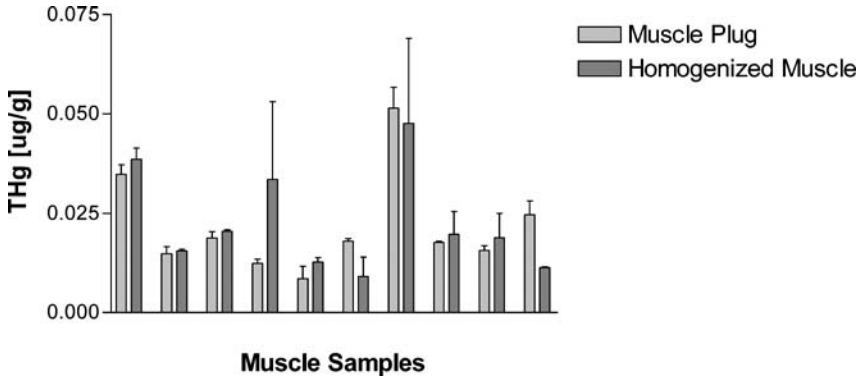

Figure 5. Comparison of DMA-80 Values from Plug Muscle and Homogenized Muscle Tissues.

age plug mercury concentration showed no statistical difference ($p = 0.726$) when homogenized and a high correlation value ($r = 0.806$) was determined.

3.2.3. *Measurement Comparison for Complete DMA-80, Cold Vapor-AAS and Purge and Trap-AFD*

It was important to compare total mercury data measured by DMA-80 with conventional methods of analysis. Methylmercury is the most significant species of mercury as an environmental contaminant because it is highly toxic and bioaccumulates through the food chain. Furthermore it is documented that methylmercury is the dominant species in biological tissues, however, the analysis of methylmercury in biological tissues is costly and time consuming and therefore; total mercury is the method of choice. Shown in Table III below is the total and methylmercury data for homogenized and plug (DMA-80) fresh fish samples analyzed in multiple laboratories. All values are reported in μg/g-wet wt. (ppm). The three different methods of analyses; DMA-80 (homogenized and plug), CVAA, and CVAF of homogenized tissue, showed no significant difference ($p = 0.83$) in reported average mercury concentration. Homogenized muscle/DMA-80 values were on average 13% higher than CVAA and 10% higher than CVAF data. Results obtained from muscle plug/DMA-80 values were on average 0.5% higher than CVAA and 1.7% lower than CVAF data. Muscle plug/DMA-80 mercury data shows a better correlation with CVAA and CVAF than did homogenized muscle/DMA-80. The DMA-80 3-plug method offers a better recovery of mercury from muscle tissue and provides a better representation of mercury burden in whole fillet.

3.2.4. *Field Verification Study*

The direct mercury analysis procedures described above were successfully used to measure total mercury levels in muscle, liver, and brain tissues of largemouth bass of Big Cypress National Preserve. Recoveries from spiked specimen during this analysis ranged from 85% to 103%. For quality control purposes all blanks

TABLE III

Comparison of DMA-80, Cold vapor-AAS and Cold vapor-AFS Values of Homogenized Largemouth bass Muscle Tissue

Muscle sample no.	DMA-80 Av. THg (μg/g) $\pm$ Stdev.	DMA-80[a] Av. THg (μg/g) $\pm$ Stdev.	CVAAS[b] THg (μg/g)	CVAFS[b] THg (μg/g)
1	0.014 $\pm$0.001	0.012 $\pm$0.002	0.016	0.019
2	0.013 $\pm$0.002	0.014 $\pm$ 0.005	0.016	0.016
3	0.014 $\pm$ 0.006	0.018 $\pm$0.001	0.018	0.020
4	0.011 $\pm$0.001	0.025 $\pm$ 0.006	0.021	0.018
5	0.019 $\pm$0.001	0.018 $\pm$0.0006	0.018	0.019
6	0.019 $\pm$0.001	0.016 $\pm$0.002	0.018	0.016
7	0.048 $\pm$ 0.004	0.051 $\pm$ 0.009	0.017	0.016

[a]3-plug muscle tissue was analyzed for total Hg. [b]Samples analyzed using CV techniques were performed using a single muscle sample. Concentrations are reported as μg THg/g muscle-wet mass.

that were measured during the analysis were below the calculated detection limit. Periodic calibration checks performed during batch runs using CRS-Dolt-2 gave mercury values within the accepted $\pm$ 10% of the certified concentration.

Total mercury concentrations were reported in each tissue analyzed. In this study concentrations of mercury in brain in individual fish was significantly lower than other internal organs, muscle ($p < 0.001$) and liver ($p < 0.05$). Sample analysis for the liver and brain was performed in duplicate using sample masses in the range of 0.05 – 0.1 g. Liver and brain of largemouth bass contained an average of 0.556 and 0.234 μg/g Hg, respectively. Reported mercury values were generally highest in the muscle tissue compared to other tissues with concentrations that ranged from 0.198 to 1.20 μg/g [average 0.662 μg/g]. Mercury binds to cysteine or sulfhydryl groups in protein and is distributed throughout the body via the blood to the muscle, gill, liver, kidney and brain tissues of fish (Spry and Weiner, 1991). While the toxic methylated mercury which accounts for 95–99% of mercury in fish accumulates in muscle tissue (Bloom 1992), the remaining mercury undergoes demethylation and is cycled from the endothelial cells of the blood brain barrier, where it is excreted into bile and then is further degraded into a form that is reabsorbed into the bloodstream to be returned to the liver (Clarkson *et al.*, 2002).

The continuous exposure to methylmercury increases the volume accumulated in the fish tissues and the potential toxic effects. However, some researchers suggest that it is the demethylation and oxidation of mercury to its mercuric form which is responsible for the most tissue damage (Dalton 2004; NRC, 2000). The understanding of mercury speciation their relationships to toxic impacts are not yet clear however all forms of mercury are toxic at some level. This remains a concern for subsistence fishermen and other aquatic species that rely upon largemouth bass as a part of their diet. In this study fifteen of the largemouth bass examined (75%) had

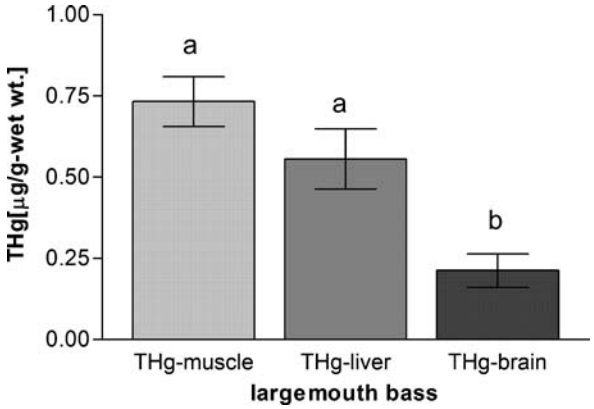

Figure 6. Illustration of tissue mercury concentrations (μg/g) in muscle, liver and brain tissues of largemouth bass ($n = 11$) from Big Cypress National Preserve. Difference in letters represents statisical differences in mercury concentrations among tissues when $p < 0.05$.

muscle concentrations equal or higher than 0.5 μg/g the state level for safe consumption of fish. This is a concern also because these concentrations (0.5 – 1.5 μg/g) fall within those reported to decrease reproductive hormone levels in male and female fish (Arnold 2000).

As illustrated in Figure 6, mean mercury concentrations in muscle and liver tissues were not statistically different ($p > 0.05$). This correlation of mercury in muscle and liver tissues is in agreement with other studies (Wiener *et al.*, 1984; Goldstein *et al.*, 1996). Goldstein *et al.* (1996) also showed a direct correlation of mercury accumulation in muscle tissue with the fish wholebody accumulation. This relationship has led to the use of muscle tissue to estimate wholebody mercury concentrations in fish when estimating the risk of human and wildlife health.

4. Conclusions

In this study the DMA-80 method of analysis illustrated stability by showing precision and accuracy in mercury values during 12 weeks of analysis. Results also demonstrate the determination of a wide range of concentrations even in small sample sizes. The DMA-80 also has the capability of accurately detecting mercury in a variety of biological tissues. Our three plug method proved to be representative of mercury in a homogenized fillet of fish and was comparable to conventional CV-AAS and CV-AFS methods of instrumental analysis. However, this comparative data is based on low levels of mercury in fish tissue. Thus, we are unable to estimate the relationship of mercury values analyzed using the DMA-80 method and conventional CV-AAS when mercury levels in fish are elevated. This gap in our understanding is critical to use of the DMA-80 for monitoring mercury in fish

and comparing historical datasets. The DMA-80 method appears to be more convenient because using this method reduces potential dilution and calculation errors as needed with conventional methods as well as any error that may occur during the thermal decomposition of the sample because all preparation is done within the automated system. .

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