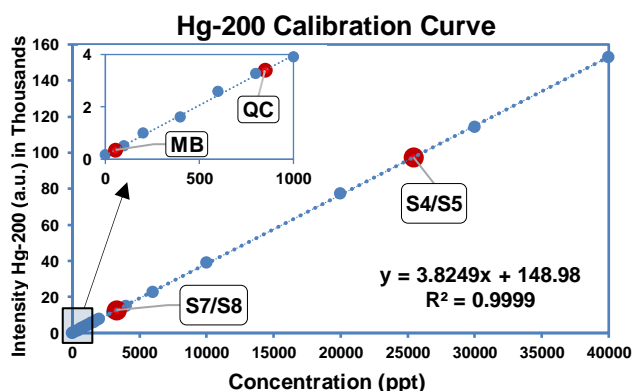


Total Mercury Concentration of Wild Caught Fish Purchased from Grocery Stores: A Potential Public Health Concern

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ABSTRACT: Methylmercury is a highly toxic organic compound that bioaccumulates and biomagnifies in the human body when absorbed by the gastrointestinal tract after ingestion.¹ Thus, monitoring methylmercury levels in fish is crucial for protecting public health and preventing dramatic scenarios such as the Minamata disease crisis in Japan. In this study, three different species of wild caught fish – Sockeye Salmon (*Oncorhynchus nerka*) from Alaska, USA; tuna imported from Vietnam; and swordfish (*Xiphias gladius*) imported from Indonesia – were collected from a Harris Teeter grocery store in Washington D.C. Total mercury concentration was measured by ICP-MS and the analyzed samples' concentrations were 19.8 ± 2.9 ppb, 2.67 ± 0.01 ppm, and 380 ± 9 ppb for Sockeye Salmon, swordfish, and tuna, respectively.



KEYWORDS: Methylmercury, tuna, salmon, swordfish, ICP-MS, FDA, environmental pollution, environmental monitoring, action level, import, Indonesia, and Vietnam.

SYNOPSIS: Consumption of swordfish is likely to pose a public health risk, as its total mercury concentration (ppm wet-weight) is close to three times higher than the Food and Drug Administration's action level.

INTRODUCTION

Fish are widely considered a healthy, low-fat source of high quality dietary protein, calcium, phosphorus, and minerals, such as iron, zinc, iodine, magnesium and potassium.² Due to low cholesterol and high omega-3 fatty acids levels, several studies show that long-term fish consumption reduces risk of coronary heart disease, decreases mild hypertension and incidence of diabetes, prevents cardiac arrhythmias, alleviates chronic inflammatory conditions like rheumatoid arthritis, and stimulates the immune system.²⁻⁵ Indeed, health organizations such as the American Heart Association and the United Kingdom's National Health Service recommend that a healthy diet must contain at least two portions of fish per week.^{6,7} Nevertheless, fish consumption has also been found to be a source of various toxins, mercury being the greatest concern. Thus, monitoring mercury levels in fish is crucial to raise awareness and help people balance the relative benefits and drawbacks of fish consumption.²

Mercury (Hg) is a heavy metal that, when released into the environment, bioaccumulates and biomagnifies in living organisms as methylmercury (MeHg)¹, which is highly toxic to the central nervous system. Particularly, exposure to MeHg among pregnant women leads to increased risk of deformities and cognitive dysfunction of newborn babies, known as the Minamata disease.⁸ In addition, the positive relationship between fish size and Hg (bioaccumulation) puts consumers who eat larger fish at higher risk of Hg exposure than those who eat smaller fish.² In this context, we aim to evaluate consumer exposure to Hg as a function of fish size, species, and region of origin. Results from this study will be of value to those involved in risk communication and public health protection, as well as to provide consumers with the scientific evidence needed to make informed decisions about eating fish.

The present study is aimed at quantifying total Hg (CAS No. 7439-97-6) levels for three different species of wild caught fish – Sockeye Salmon from Alaska, USA; tuna imported from Vietnam; and swordfish imported from Indonesia – that were purchased from a Harris Teeter grocery store in Washington D.C. Total Hg concentration was measured using an Inductively Coupled Plasma Mass Spectrometry (ICP-MS) analytical method derived from the Association of Official Agricultural Chemists (AOAC) Official Method 2015.01 for Heavy Metals in Food⁹ and EPA Method 6020B (SW-846): ICP-MS for Heavy Metals in Digests.¹⁰ Both reference methods are suitable for the characterization of total Hg at trace levels in food samples. Since Hg bioaccumulates mainly ($\approx 100\%$) in the form of MeHg [CH_3Hg]⁺, all organic Hg will be transformed to inorganic Hg (Hg^{2+}) by using acid digestion.¹¹

MATERIALS AND METHODS

Chemicals and Materials

Three certified reference materials suitable for ICP trace analysis were provided by Sigma-Aldrich for $(1,000 \pm 2)$ mg/L mercury in 12% HNO_3 , $(1,000 \pm 2)$ mg/L indium in 2% HNO_3 , and $(1,000 \pm 2)$ mg/L gold in 5% HCl . These certified reference materials are traceable to the SI unit of mass, the kilogram. All working solutions, calibration standards, and samples were acidified to a final concentration of 2% w/w HNO_3 to facilitate sample ionization and intensify the signal obtained by the ICP-MS instrument.

Preparation of the Mercury-Stabilizing Au^{3+} Solution

A 100 ppm gold (Au^{3+}) working solution was prepared by dilution of the Au^{3+} primary standard in 2% w/w HNO_3 . Mercury is relatively volatile at room temperature and extremely volatile when heated. In addition, Hg^{2+} shows a strong memory effect and may remain in the measurement system even after reasonable rinse times. For this reason, a final Au^{3+} concentration of 5 ppm was

used in all calibration standards, blanks, and samples as a mercury-stabilizing agent to minimize memory effects, Hg^{2+} loss throughout the digestion process, and prevent Hg^{2+} from plating out in the sample introduction system.^{9,10,12} Although some authors reported that a final Au^{3+} concentration of 2 ppm ensures mercury stabilization, stability studies showed that for this method a final Au^{3+} concentration of 5 ppm was needed to avoid Hg^{2+} loss.¹²

Preparation of the 1 ppb In^{3+} Internal Standard Solution

For the indium internal standard (IS), an intermediate 1 ppm solution and a final 1 ppb working solution were prepared in 2% w/w HNO_3 . The IS is used for validation of the analytical method and is added into the ICP-MS system separately through a second channel of the peristaltic pump so that the concentration (1 ppb) is consistent for all samples and standards measured. This way, experimental errors arising from pipetting aliquots of IS into the standards and samples are avoided. Indium isotopes measured were ^{115}In and ^{113}In .^{9,10}

Preparation of the Hg^{2+} Calibration Curve

For the analyte (Hg^{2+}), two working solutions – 10 ppm (D1) and 100 ppb (D2) – were prepared in 2% w/w HNO_3 . A 20-point Hg^{2+} calibration curve ranging from 100 ppt to 40 ppb was prepared by dilution of Hg^{2+} working solutions D1 and D2 to a final volume of 15 mL 2% w/w HNO_3 . Then, 750 μL of Au^{3+} 100 ppm were added to all standards for a final concentration of 5 ppm. For safety reasons, samples were prepared under a fume hood with thick chemical-resistant nitrile gloves.

Three types of blanks were prepared, calibration blank, method blank, and rinse blank. The calibration blank was prepared by acidifying water using the same combination and concentrations of acids used in the preparation of the matrix-matched calibration standards, 2% HNO_3 and 5 ppm of Au^{3+} . Calibration blanks help determine signals produced at the detector that do not arise from the presence of the intentionally added analyte to the calibration standards. Method blanks contain

neither the analyte nor the sample but are prepared following all steps of the sample preparation procedure (digestion, dilution, filtering, etc.) and are used to monitor potential contamination resulting from the sample preparation procedure.⁶ The rinse blank is used to flush the system between all samples and standards, and it was prepared as a 2% HNO₃ solution.¹⁰

Sampling and Preparation of Fish Muscle Samples

Three different fish species – Sockeye Salmon from Alaska, USA; tuna imported from Vietnam, and swordfish imported from Indonesia – that were advertised as wild caught were collected from a Harris Teeter Super Market at 1631 Kalorama Rd NW, Washington, DC, 20009. After homogenization in a blender, each specimen yielded three samples (n=9). For each species, one sample was spiked with known concentrations of Hg²⁺ to calculate recoveries. AOAC Official Method 2015.01 for Heavy Metals in Food was followed using a Multiwave Go Plus microwave-assisted digestion system provided by Anton Paar, in order to digest the fish muscle samples.⁹

First, the edible portion was blended and homogenized and approximately 1.0 g wet weight (wet wt) were placed into microwave digestion vessels. Then, 6 mL of 70% HNO₃ ICP-grade and 2 mL of distilled water (DI H₂O) were added into each vessel. Finally, 320 µL of the 100 ppm Au³⁺ working solution were added to achieve a final gold concentration of 5 ppm in each sample. One method blank and one Hg²⁺ spiked sample for each species were prepared per batch of samples digested. Samples were spiked with varying amounts of Hg²⁺ to account for different Hg concentrations expected for each species after exploratory tests were conducted. Thus, salmon, tuna, and swordfish samples were spiked with a final concentration of 2 ppb, 35 ppb, and 300 ppb of Hg²⁺, respectively. Spiked samples are used in method validation to identify matrix effects and determine the recovery of the analytical method for a given analyte in a given matrix.

The temperature ramp used for microwave-assisted digestion is shown in **Table 1**. Digestion was achieved and no residues were observed.

Step	Temp., °C	Ramp, min	Hold, min
1	100	20	10
2	180	20	10
3	Cool down	NA	10

Table 1 Temperature Ramp for Fish Muscle Tissue Microwave-Assisted Digestion.

After digestion, 1 mL of the acidic samples were diluted with DI H₂O to a final volume of 10 mL (Dilution factor = 10). **Table 2** shows the most abundant and recommended Hg isotopes for analysis and their potential interferences. **Hg²⁰⁰** was the isotope used for quantification due to lower potential interferences.

Element	Isotope (amu)	Isotope Abundance (%)	Potential Interferences
Hg	200	23	WO ⁺
	202	30	WO ⁺

Table 2 Recommended Mercury Isotopes for Analysis and their Potential Interferences.

Measurement Instrumentation

Instrumental measurement was performed with an Agilent ICP-MS consisting of a 7800 ICP and a quadrupole MS with an autosampler. The system has a mass range from 2-260 amu and detection limits of 0.1 ppt for ²⁰⁹Bi. It also includes a mass-flow controller for the nebulizer Argon and a peristaltic pump for sample solutions. ICP-MS MassHunter software was used for analyte quantification. The software allows correction for isobaric interferences and the application of IS validation technique. The ICP-MS was set to measure in Helium mode, which reduces polyatomic interferences, improves detection limits, and increases dynamic range.^{13,14} Isotopes ¹⁹⁹Hg through ²⁰²Hg were measured, but only ²⁰⁰Hg was used for quantification as it shows lower interferences.

RESULTS

Sockeye Salmon

In order to determine the total Hg concentration for Sockeye Salmon, only calibration points 1 through 11 – ranging from 0 to 1800 ppt – were used (**Figure 1**). Sockeye Salmon showed the lowest concentration of Hg and, therefore, the calibration curve was trimmed at low Hg levels to provide more reliable results. No IS normalization was needed as indium showed great stability during the measurement process. Good linearity was achieved with a correlation coefficient (R^2) of 0.9982, which signifies high confidence in the correlation between the independent and dependent variable.²⁹ In addition, the statistical F-test was used to determine any outliers in the calibration curve, as described by Andriamahanina et al.¹⁵ No outliers were detected using a 95% confidence interval. The detection limit was 91.4 ppt, which was calculated using the method described by Skoog et al., where the average of 25 blank measurements is added to three times the standard deviation of the blank measurements.¹⁶

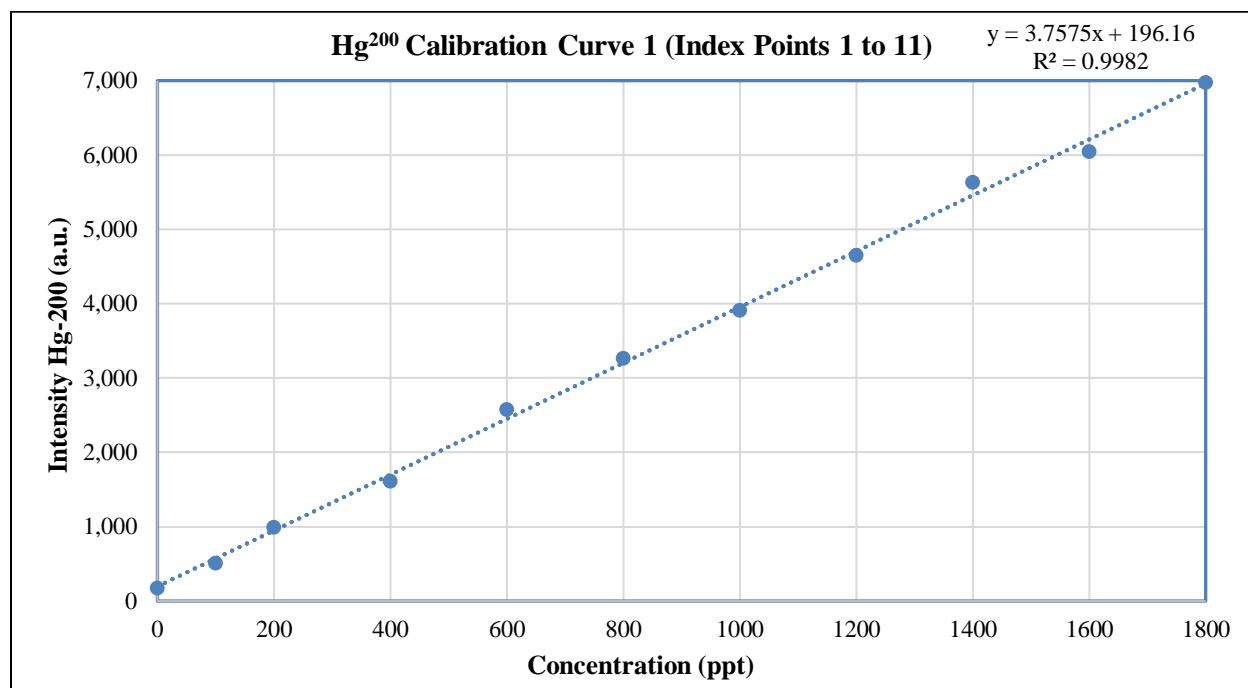


Figure 1 Calibration Curve 1 with Index Points 1 through 11.

A method blank and three salmon fish samples were measured, one of which was spiked. **Table 3** shows the values obtained for the method blank and salmon samples. The concentration of the sample was converted to represent the total Hg concentration in the edible portion. The average total Hg concentration was **19.8 ± 2.9 ppb wet wt**, and the concentration for S3-Spike was **31.3 ppb wet wt**. Error analysis was performed by calculating the standard deviation in concentration (S_c), as described by Skoog et al., which was then transformed to account for the weight of each sample.¹⁶ The percent recovery was **59.3%**.

Sample	Weight	Concentration Diluted (ppt)	Concentration Digested (ppt)	²⁰⁰ Hg/gram (ng/g or ppb)	Error Analysis (ng/g or ppb)
Method Blank	NA	54.4	544	NA	NA
S1	0.8111	160.9	1610	19.8	19.8 ± 2.92
S2	0.8480	167.2	1672	19.7	
S3-Spike	0.9033	282.6	2826	31.3	31.3

Table 3 Salmon Hg²⁺ Concentration and Error Analysis.

Swordfish and Tuna

To determine the total Hg concentration for swordfish and tuna, calibration points 1 through 20 – ranging from 0 to 40,000 ppt – were used. The Hg²⁺ internal spike concentration for swordfish and tuna were 300 ppb and 35 ppb before dilution, respectively. Again, the F-test was used to determine any outliers in the calibration curve based on a 95% confidence interval, and no outliers were detected. Good linearity is demonstrated in **Figure 2**, with a R² value of 0.9999. The limit of detection was 91.4 ppt.

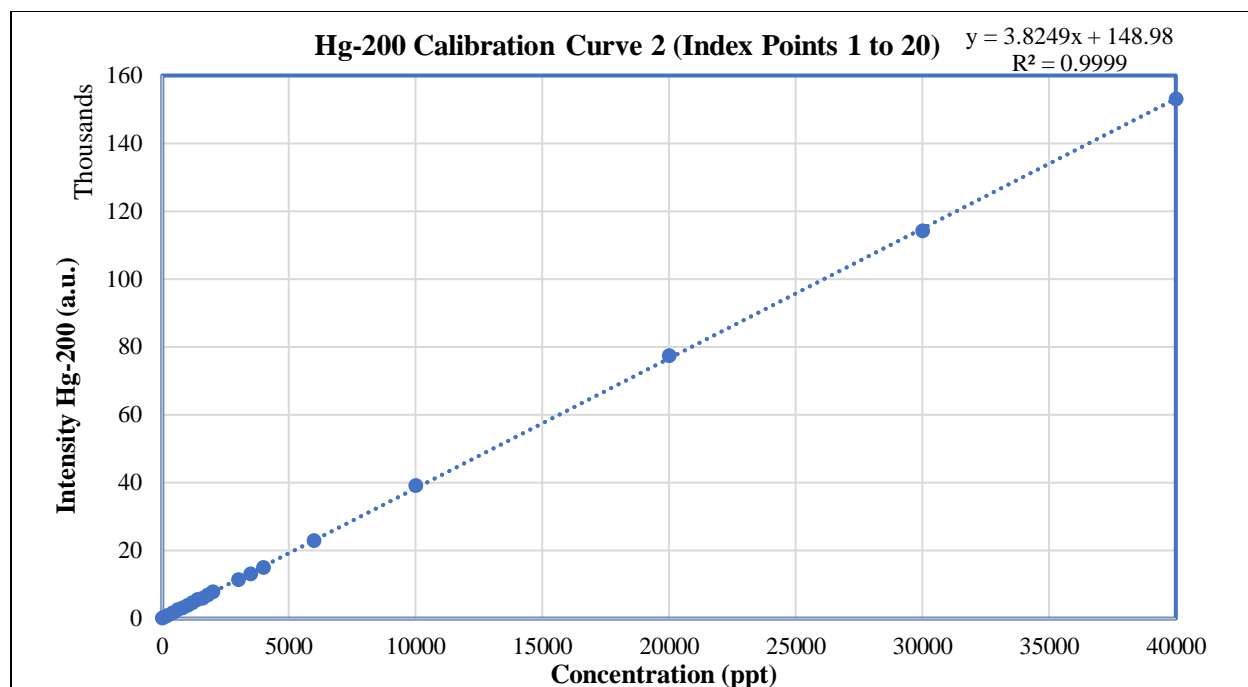


Figure 2 Calibration Curve 2 with Index Points 1 through 20.

147 In addition to a method blank, three samples of swordfish and three samples of tuna were
 148 prepared, of which two were spiked. **Table 4** demonstrates the concentration and error analysis for
 149 Hg^{2+} in the edible portion. For swordfish, the average concentration was **2.67 ± 0.01 ppm wet wt**,
 150 with an S6-Spike concentration of **5.32 ppm wet wt**. The percent recovery was **81.4%**. For tuna,
 151 the average concentration was **380 ± 9 ppb wet wt**, with an S9-Spike concentration of **852 ppb**
 152 **wet wt**. The percent recovery was **148.9%**.

Species	Sample	Weight	Concentration Diluted (ppt)	Concentration Digested (ppt)	^{200}Hg /gram ($\mu\text{g/g}$ or ppm)	Error Analysis ($\mu\text{g/g}$ or ppm)
NA	Method Blank	NA	54.4	544.04	NA	NA
Swordfish	S4	0.9484	25012.5	250125	2.64	2.67 ± 0.00769
	S5	0.9591	25858.9	258589	2.70	
	S6-Spike	0.9370	49850.9	498509	5.32	5.32
Tuna	S7	0.9042	3123.5	31235	0.35	0.380 ± 0.00853
	S8	0.8341	3457.1	34571	0.41	
	S9-Spike	0.9983	8501.1	85011	0.85	0.852

Table 4 Swordfish and Tuna Hg^{2+} Concentration and Error Analysis.

DISCUSSION

The present analytical method is validated for quantification of Hg in fish muscle samples. Accuracy was demonstrated by calculating recoveries. For Sockeye Salmon (59%) and for tuna (149%), recoveries were very close to, but did not lie within the ideal 70-130% range. Sufficient recoveries were achieved for swordfish (81%). In addition, a low limit of detections of 91.4 ppt was achieved and the method blank did not show significant Hg contamination. Good linearity was demonstrated for the calibration curve ($R^2 = 0.9999$), and a quality control with a 6% error confirmed precision of the method.

Results from this study are consistent with bioaccumulation effects and show that total Hg concentration is dependent on the fish species and fish size. Mercury concentration in Sockeye Salmon from Alaska (USA) was determined to be 20 ppb, significantly below the Food and Drug Administration's (FDA) action level of 1 ppm (wet wt) in the edible portion.¹⁷ In the case of tuna imported from Vietnam, Hg concentration was found to be 380 ppb (wet wt), also significantly below FDA's action level. In contrast, Hg concentration in swordfish imported from Indonesia was estimated to be 2.7 ppm (wet wt), which is close to three times FDA's action level and poses a risk to human health. FDA's recorded mean concentration of swordfish from over 600 samples is 0.995 ppm. A frequency distribution and a scatter plot of this data is provided in **Figure 3** and **Figure 4**, respectfully.¹⁸ Further analysis of imported swordfish samples should be conducted in the future to determine if samples imported from Indonesia present an additional risk compared to those from other regions, as our sample is more than three standard deviations away from the mean. It is also important to note that the FDA level is less strict than the European Union's action level of 0.5 ppm.¹⁹

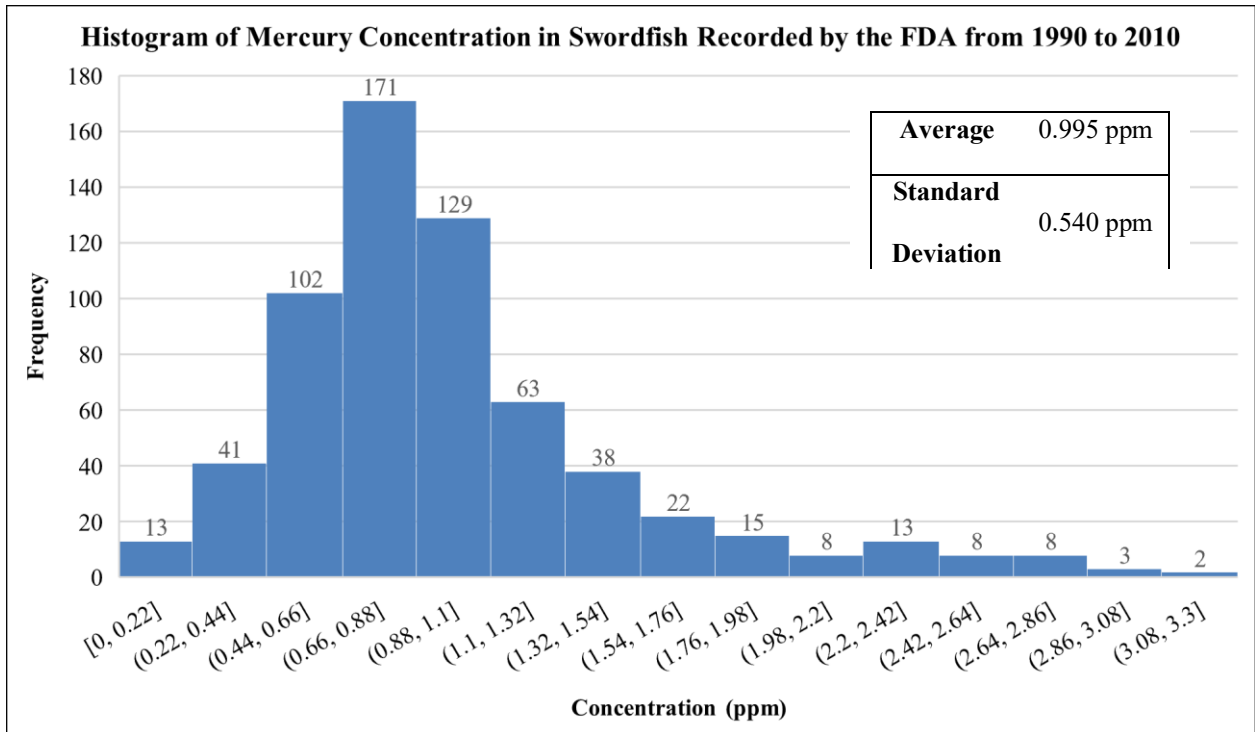


Figure 3 Frequency Distribution of Mercury Concentration in Swordfish Samples

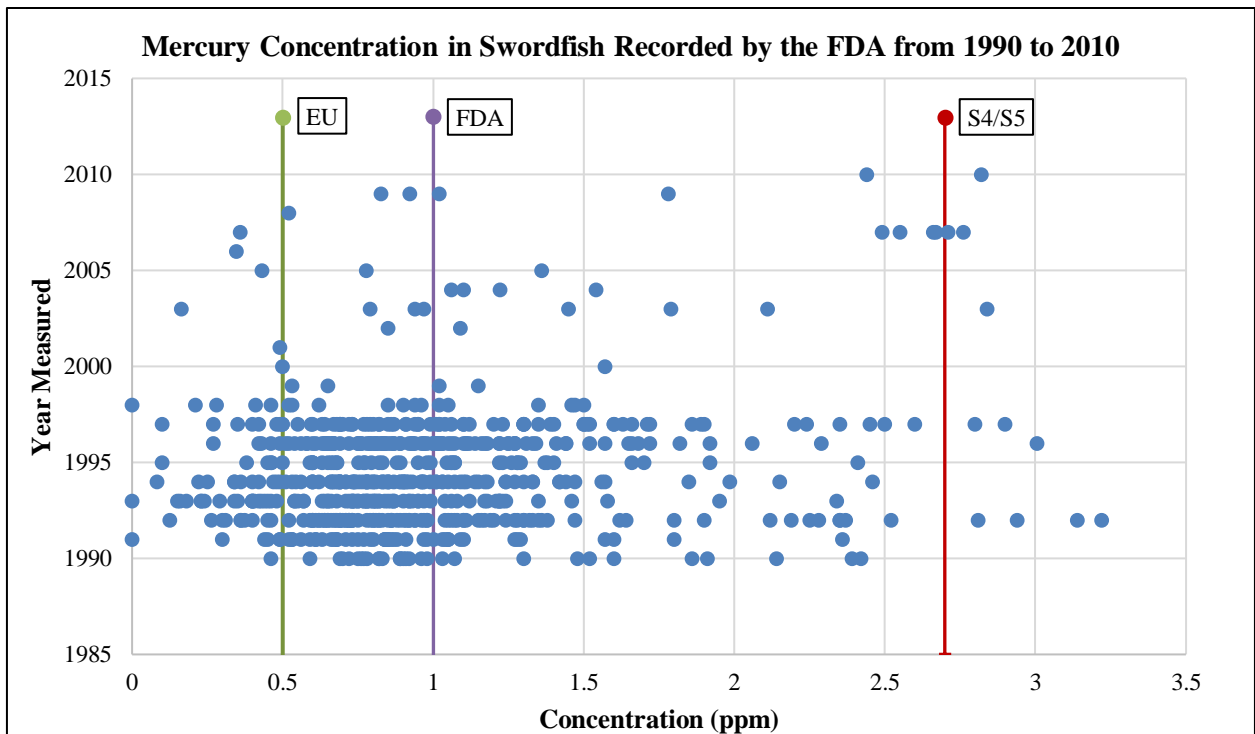


Figure 4 Scatter Plot of Year Measured and Concentration of Swordfish Samples

Given that wild caught fish samples analyzed in the present study were purchased from a Harris Teeter grocery store located in Washington, D.C., no information is available as to the size and weight of the original fish, which is key to understanding bioaccumulation effects. However, there is information on average size and weight of these species that is consistent with the results obtained from this experiment. Sockeye Salmon, which showed the lowest Hg concentration, are estimated to be 1.5 to 2.5 feet in length and 4 to 15 pounds²⁰, whereas swordfish, which showed the highest Hg concentration, are predatory fish that commonly weigh 200 on average, but can reach to 14 feet in length and weigh almost 1,200 pounds.²¹ Although fully mature adult tuna species, like the Atlantic and Southern bluefin tuna, average 6.5 to 8 feet long and weigh around 550 pounds, these larger species are critically endangered.^{22,23} Since no specifications were provided in the grocery store, it is most likely that the tuna sample analyzed belongs to a smaller more commonly sold species, thus explaining significantly lower Hg concentrations in tuna compared to swordfish.

In terms of how the region of origin might affect Hg concentration, it is impossible to draw clear conclusions as no comparison has been conducted between sample species from different locations. Thus, it remains unclear whether the region of origin influences Hg concentration. Given that fish imported from certain countries or regions might pose additional risk than others, future experiments will be geared towards analyzing same fish species, mainly swordfish, from different locations around the globe. The United States Environmental Protection Agency (EPA) publishes the National Listing of Fish Advisories (NFLA), which uses geographic information systems (GIS) to provide a historical listing of fish advisories in the U.S. from 1974 to 2011.²⁴ Individual states usually provide residents with current local advisories, which is often also provided in a GIS format. Currently, there are no listings for fish advisories for fish imported into the U.S. and using

GIS for Hg mapping for imported fish would provide valuable data to those involved in risk communication and public health protection and would help consumers make informed decisions on fish consumption.

Results showed that from the three wild caught fish species analyzed, only swordfish is likely to pose a risk to public health. While the FDA and EPA advise against the consumption of swordfish for pregnant women and children, it is still allowed to be sold in the U.S.²⁵ Thus, further research and environmental monitoring will need to be conducted in the future to ensure that the average Hg concentration of imported swordfish falls below current action levels. In addition, working with local fish market to ensure fish size would enable estimation of bioaccumulation effects in different species.

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