EVALUATION OF FISH CONSUMPTION ADVISORY FOR LAKE ALAN HENRY, TEXAS AND COMPARISON OF THE ADVISORY METHODS OF THE STATES OF TEXAS, CALIFORNIA, AND ALASKA.

by

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ABSTRACT

In 2008, the Seafood and Aquatic Life Group (SALG) at Texas Department of State Health Services (DSHS) issued a fish consumption advisory for two predatory fish species (Largemouth and Alabama Spotted Bass) harvested from Lake Alan Reservoir, Texas. This study was conducted to examine the Mercury (Hg) concentration in those fish species from the same lake to determine whether the existing fish consumption advisory should be revisited. In addition, the methods used in Texas were compared to those used in the states of California and Alaska.

Eight Largemouth Bass and five Alabama Spotted Bass fish tissue samples were collected in accordance to SALG Standard Operating Procedures and QC/QA manual, and analyzed in the Geochemical and Environmental Research Group (GERG) laboratory for total Hg concentrations using the Cold Vapor Atomic Absorption Spectrometry. Risk characterization was done by comparing the fish tissue Hg concentrations to comparison values derived from the ASTDR's Minimal Risk Level, USEPA Reference Dose and Alaska-Specific Acceptable Daily Intake for the States of Texas, California and Alaska, respectively.

This study showed no significant difference ($p=0.13$, $p>0.05$) in Largemouth Bass Hg concentrations when compared with the previous study. In contrast, Hg concentration in Alabama Spotted Bass did not exceed the comparison value and therefore disagrees with the SALG's previous study. Therefore a revisit of the site by the DSHS is required. However, the State of California's methods suggest a likelihood of adverse human health effects when both fish species are consumed, whereas an absence of risk from consumption of both fish species is suggested using the State of Alaska's method.

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CHAPTER I

INTRODUCTION

Fish consumption has significantly enhanced people's diets around the world. In 2013, fish accounted for about 17% of the global population's animal protein intake and 6.7% of all protein consumed (FAO. 2016). Moreover, fish provided more than 3.1 billion people with almost 20% of their average per capita intake of animal protein. Fish provides a rich source of easily digested, quality protein meat containing essential amino acids, fats (e.g. long chain omega-3 fatty acids), vitamins (D, A and B) and minerals (including calcium, iodine, zinc, iron and selenium). The beneficial effects of regular fish consumption are supported by numerous studies that have repeatedly linked fish consumption with skin protection from ultraviolet injury through a range of mechanisms (Pilkington et. al. 2011). Kris-Etherton et al. (2002) reported that omega-3 polyunsaturated fatty acids obtained from fish consumption lowered triglycerides and reduced cardiovascular disease among aging populations, thereby resulting in healthier hearts.

Despite the tremendous benefits of fish consumption, it is one of the exposure pathways for methylmercury (MeHg) to both ecological and human receptors. MeHg is an organic and neurotoxic form of mercury. It biomagnifies in the aquatic food chain and beyond to higher trophic levels, including man. Over 90% of total mercury in fish tissue is in the form of MeHg that typically accumulates in fish tissue protein rather than fat tissues (Hong et. al. 2012). MeHg

poses an important public health concern due primarily to fish consumption. MeHg's ability to penetrate the blood brain barrier and placenta makes developing fetuses and young children more vulnerable. Developing fetuses' vulnerability to MeHg was observed in Minamata, Japan. Pregnant women and young children consumed contaminated fish high in MeHg, resulting in abnormalities in fetuses and neurotoxicity among children born to mothers with insignificant symptoms (Karagas et. al. 2012). Exposed children suffered from mental retardation, cerebellar ataxia, physical growth disorder, dysarthria, and limb deformities. Other reported symptoms included hyperkinesis, hypersalivation, seizures, and strabismus (Hong et. al. 2012). This incident serves as evidence that MeHg exposure primarily targets the brain and nervous system.

In the U.S., many states have responded to the potential risks of consuming MeHg-contaminated fish by issuing fish consumption advisories. These advisories are provided to advise the public of possible adverse health effects from MeHg contamination and consumption of locally and commercially caught fish from specific bodies of water. The awareness of risks and benefits from fish consumption enlightens individual choices to balance the risk of MeHg and health benefits (Stern and Korn 2011, Oken et. al. 2012, Burger and Gochfeld 2009).

Advisories in the United States differ from state to state. These differences are based on several variables. Scherer et al. in 2008 reported that most advisories target pregnant women, childbearing-age women, and children, while a few states provide advisories for the general population. Most states in the U.S base their advisories on the EPA's reference dose (RfD) for MeHg (0.1µg/kg/day) (USEPA 2013), but a few such as Alaska are based on the FDA's action level (0.5µg/kg/day) (Hamade 2014). Advisories typically consider cultural practices in fish preparation and consumption along with the identification of species that pose the greatest risks. Advisories can address fillet or whole body consumption and the frequency and amount of fish

consumed per day. Typically, a recreational exposure assumes 17.5 grams per day or a one-week 8-oz serving, while a subsistence exposure assumes 142.5 grams per day.

This study was conducted to assess concentrations of MeHg in two predatory sport fish (Largemouth and Alabama Spotted Bass) from Lake Alan Henry Reservoir, Texas and to determine whether the standing fish consumption advisory should be revisited. The Texas Department of Health posted the advisory in 2008 based on data collected in Lake Alan Henry Reservoir. In addition, the methods used in Texas were compared with those used in the states of California and Alaska.

The specific objectives of this study are

- To determine the concentrations of MeHg present in fish obtained from Lake Alan Henry, Texas.
- To compare obtained metal concentrations with a similar study conducted by the Department of State Health Services, Austin Texas in 2008.
- To conduct a Human-health based Assessment for Systemic (noncancerous) effects of consuming MeHg-contaminated fish using assessment protocols from the 3 U.S Coastal States (Texas, California, and Alaska).

CHAPTER II

LITERATURE REVIEW

2.1 Mercury

Mercury is a naturally occurring element in the earth's crust. It is a highly reactive heavy metal seldom found as a free element in nature. In its elemental form, mercury is released into the environment through human activities such as fossil-fuel combustion, mining, and industrial production (Streets et. al. 2011). When released into the air, mercury can be deposited through wet or dry deposition that enters surface water where it accumulates in streams, lakes and oceans. Microbial action of bacteria in the water and sediments can transform the inorganic forms of mercury into the organic form; methylmercury (MeHg). MeHg can be bioconcentrated by fish, both through contact with the water they live in, and through the food chain through Biomagnification.

2.2 Sources of Mercury

Mercury is released into the atmosphere from various natural and anthropogenic sources (fig 2.1 and 2.2). Mercury emissions from natural sources consist in the contribution from primary natural sources and re-emission processes of historically deposited mercury over the surfaces of land and sea. The mercury released from volcanoes, geothermal sources and earth are from primary natural sources, while those released from land use changes, biomass burning, and meteorological conditions are from re-emission of previously deposited mercury on land,

vegetation, and water surface. Anthropogenic sources of mercury released into the atmosphere include fossil-fuel fired power plants (810 Mg/yr), artisanal small-scale gold mining (400 Mg/yr), non-ferrous metals manufacturing (310 Mg/yr), cement production (236 Mg/yr), waste disposal (187 Mg/yr) and caustic soda production (163 Mg/yr) (Pirrone et. al. 2010).

At present, the largest anthropogenic emission sources include fuel combustion, mainly from coal, and "artisanal and small-scale gold mining" (ASGM). Mercury emissions from natural sources are estimated at 45–66% of the global emissions, with the largest part of emissions originating from the oceans while anthropogenic processes contribute 34-55% to mercury emissions (Gworek et. al. 2017). Although there are no established methods used to analyze a water sample for mercury and then determine the difference between mercury from human pollution and that which comes from a natural source, a recent study suggests that there is a way to at least separate the bulk contributions of human and natural sources over time using the ratio of phosphate to mercury in water deeper than 1000 meters for the former and carbon dioxide as a tracer for the latter (Carl et. al 2014).

According to the United Nation's update of "Modelling Results in the Global Mercury Assessment 2013", the total global mercury emission from anthropogenic sources is estimated at 1,875 tons/year and differs greatly by geographical regions and level of industrialization. For example, industrial regions such as Central Europe, East and South Asia, and the eastern part of North America are highly noted for weighty mercury emissions. Central and South America, Sub- Saharan Africa and Southeast Asia, also have high emission fluxes which are due to ASGM, but others like the Arctic and Antarctic regions have almost no emissions. Wilson et al. (2012) broke down mercury emissions from anthropogenic sources into continents and regions

and noted that the highest emission of 45% is from East and South-East Asia, followed by South Africa's 10.9%, South America's 10.4% and South Asia's 9.5%.

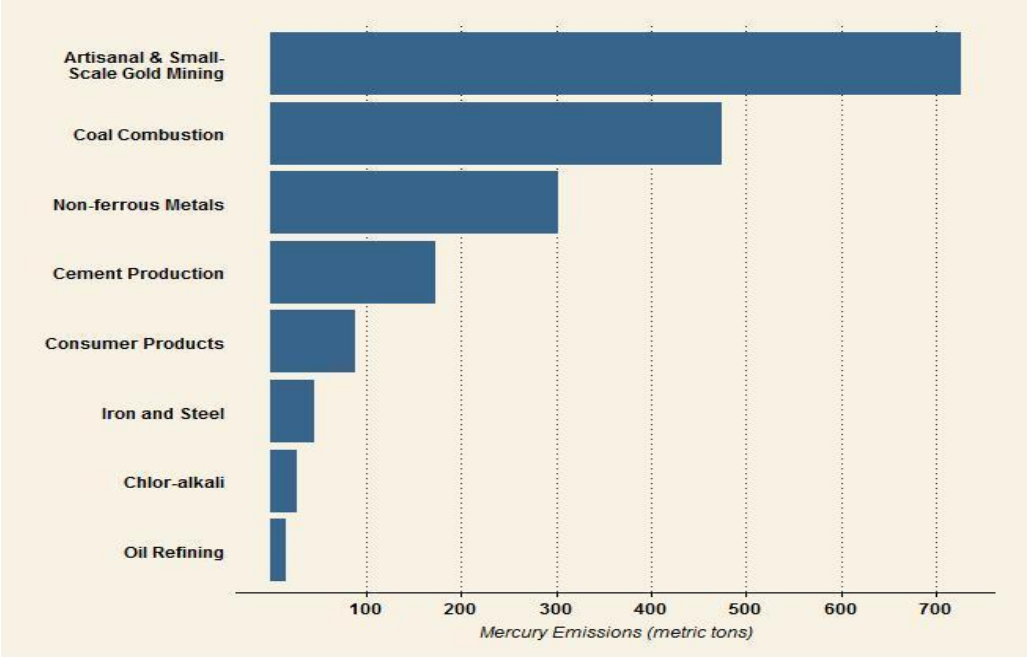

Fig 2.1: Mercury emissions from the eight highest emitting industry sectors. Data for 2010 from the 2013 UNEP Global Mercury Assessment. Total estimated global anthropogenic mercury emissions are 1960 metric tons.

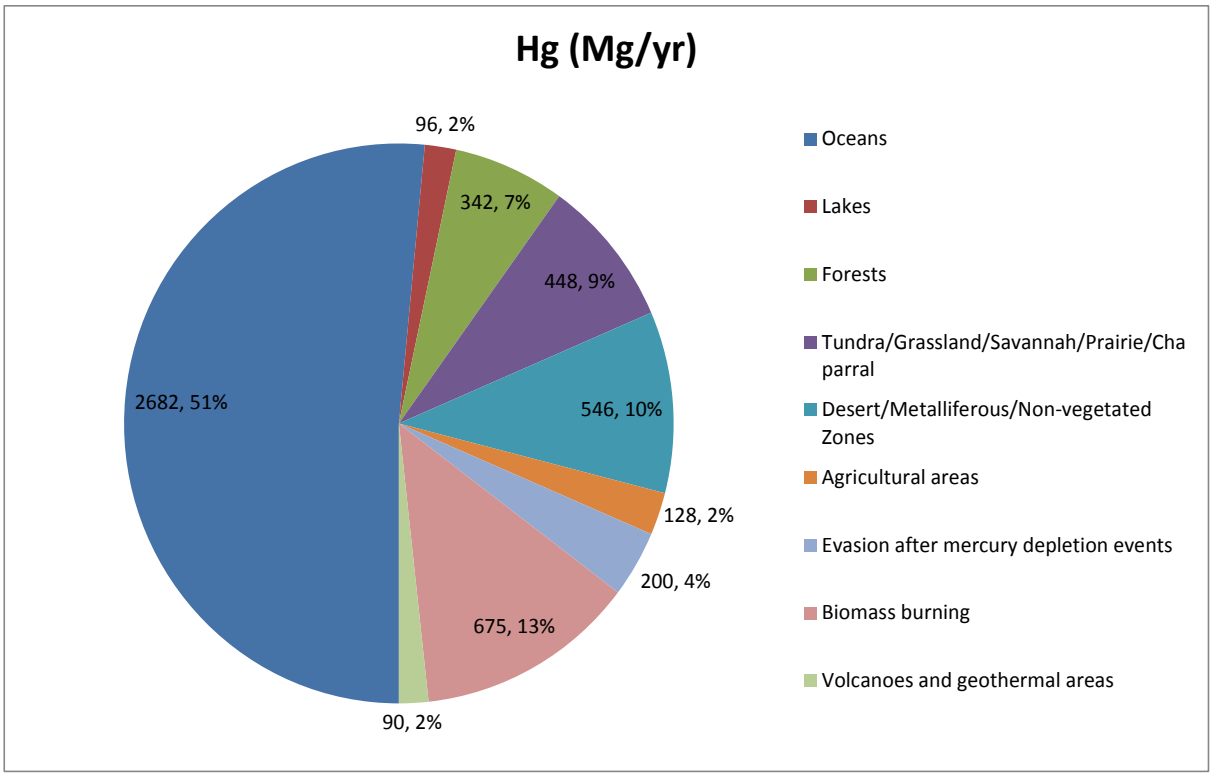

Fig 2.2: Global mercury emissions by natural sources estimated for 2008. (Original data from (Pirrone et. al. 2010))

2.3 Physical and Chemical Properties of Mercury

Mercury (Hg) is a naturally occurring metal that exists in three forms. These forms are elemental (or metallic), inorganic and organic mercury (Park and Zheng 2012). Metallic Hg is a shiny, silver-white odorless liquid that combines with other elements such as sulfur, chlorine and oxygen to form inorganic mercury compounds or salts, which typically are powdered or crystals. Organic mercury is formed by the combination of metallic Hg with carbon, and when microscopic anaerobic organisms in water or soil react with elemental and inorganic Hg. Of all known pure metals, Hg has the lowest melting point (−39°C) and it is the only pure metal that is liquid at room temperature. It is a very important material in many industrial products due to its

several physical and chemical advantages such as its low boiling point (357°C) and easy vaporization (Chang et. al. 2009).

Metallic Hg in its ground state (Hg^0) is important in global mercury cycling and volatilizes readily to mercury vapor that is fairly stable and resides in the atmosphere for a period of 0.5 to 2 years (Xu et. al. 2013). Inorganic mercury compounds exist in two major oxidative states: (1) mercurous (H^+) and (2) mercuric (Hg^{2+}). The former, mostly unstable and found in the form of calomel or mercurous chloride, is formed when Hg^0 losses one electron. The latter, which is the most common and second oxidation state, is formed when two electrons have been removed from Hg^0 resulting in the creation of almost all the inorganic chemical compounds of Hg. Hg^{2+} is a product of the breakdown of inhaled mercury vapor, organic mercury compounds and discharge from mercurous ion. Common organic forms of mercury are methylmercury and ethyl mercury. Methylmercury (MeHg) is formed from methylation of inorganic mercury by microorganisms in the environment (Park and Zheng 2012). It is a stable white crystallized salt with a slightly sweet odor (Lewis 2005), and it is commonly found as methyl mercuric chloride. Mercuric Chloride is used in experiments to investigate the effects of methyl chloride. The physical and chemical properties of some mercury compounds are shown in Table 2.1.

Compounds of inorganic mercury such as $HgCl_2$ are very soluble in water while those of organic mercury, such as MeHg chloride and dimethyl mercury --a super toxic derivative of chemical synthesis of MeHg -- are lipophilic and less soluble in water. It is therefore important to note that the solubility of mercuric compounds plays a significant role in their different toxicity (NRC 2000).

Clarkson and Magos (2006) reported that mercuric mercury chemistry is controlled by its high attraction to thiol (sulfhydryl) groups, precisely the thiol anion $R\text{-}S^-$. This is usually a reversible reaction that occurs rapidly in such a way that mercury quickly migrates between thiol groups. Mercury's high affinity for sulfhydryl groups is a common motif for enzyme modification. These permanent covalent modifications deactivate the enzyme, thereby inducing devastating effects on an organism's metabolic functions (Ynalvez, Guitierrez and Gonzalez-Cantu 2016).

Table 2.1: Physical and Chemical Properties of Some Toxicologically Relevant Mercury Compounds (Data from ASTDR's *Toxicological Profile for Mercury* (ATSDR 2015))

Chemical Name	Elemental Mercury[a]	Mercuric Chloride[b]	Mercurous Chloride[c]	Methyl mercuric Chloride[d]	Dimethyl mercury
Molecular formula	Hg^0	$HgCl_2$	Hg_2Cl_2	CH_3HgCl	C_2H_6Hg
Molecular structure	Hg	Cl-Hg-Cl	Cl-Hg-Hg-Cl	CH_3-Hg-Cl	CH_3-Hg-CH_3
Molecular weight	200.59	271.52	472.09	251.1	230.66
Solubility	5.6×10^{-5} g/L at 25°C	69 g/L at 20°C	2.0×10^{-3} g/L at 25°C	0.100 g/L at 21°C	1 g/L at 21°C
Density	13.534 g/cm^3 at 25°C	5.4 g/cm^3 at 25°C	7.15 g/cm^3 at 19°C	4.06 g/cm$_3$ at 20°C	3.1874 g/cm^3 at 20°C
Oxidation state	+1, +2	2	1	2	2
Color	Silver-white (liquid metal);tin-white(solid mercury)	White	White	White	Colorless
Melting Point	-38.87°C	277°C	Sublimes: 400-500°C without melting	170°C	No data
Boiling Point	356.72°C	302°C	354°C	92°C	No data

[a]Also known as metallic mercury. [b]Also known as Calomel. [c]Methylmercuric is used experimentally to investigate the effects of methylmercury.

2.4 Toxicology of Mercury

The primary target organ of mercury exposure is the brain, but pathological symptoms on lungs, kidney and heart have also been described (Jan et. al. 2015). All the forms of mercury affect normal cellular functions by altering the tertiary and quaternary structure of proteins and by binding with sulfhydryl and selenohydryl groups (Bernhoft 2012). The inorganic mercury form consists of the metallic mercury vapor (H^o), mercurous and mercuric mercury (usually $HgCl_2$). Acute exposures to more than 1-2 mg/m3 of Ho for a few hours have been found to cause bronchitis, bronchiolitis and pneumonitis. While exposure after two hours results in the lungs appearing as hyaline membrane formation, and eventually to extensive pulmonary fibrosis (Asano et. al. 2000). Mercurous and mercuric mercury acute poisoning generally targets the gastrointestinal tract and the kidneys resulting in an extensive precipitation of enterocyte proteins. Signs and symptoms such as abdominal pain, vomiting, and bloody diarrhea with potential necrosis of the gut mucosa have been reported. Although chronic poisoning of mercuric mercury is rare, cases of anxiety have been noted. Toxicity effects on the kidney involve either renal tubular necrosis, or autoimmune glomerulonephritis, or both. Hypersensitivity reactions including asthma and dermatitis, various kinds of autoimmunity, and suppression of natural killer cells and disruption of various other lymphocyte subpopulations are other results of exposure to inorganic mercury. Atrophy and capillary damage to the thigh muscle have been reported (Bernhoft 2012, Chehimi et. al. 2012, and Nadorfy-lopez 2000).

Organic mercury, typically methylmercury (MeHg), primarily stems from biological sources. It has been established to be highly toxic because it is easily absorbed, bioaccumulated and biomagnified, particularly in animals at the top of the food chain. MeHg reacts with sulfhydryl groups in the body, hence disrupting the function of any cellular or subcellular

structure. It can also inhibit DNA transcription, repair and protein synthesis in the developing brain, which results in the destruction of endoplasmic reticulum and loss of ribosomes. Its disruptive effects on some subcellular elements in the central nervous system and other organs and in mitochondria have been described. Adverse effects resulting in damage to many parts of the brain and peripheral nervous system have similarly been reported for cell membrane integrity, generation neurotransmitter disruption, heme synthesis, and stimulation of neural excitoxins. MeHg has been reported to reduce the activity of Natural Killer cells, in addition to imbalance between the T helper cell 1 and 2 ratios (Th1/Th2). This may eventually lead to decreased autoimmunity. MeHg's affinity for sulfhydryl groups of the mitochondrial oxidative phosphorylation complex associated with the destruction of mitochondrial membranes may contribute to chronic fatigue syndrome (Bernhoft 2012 and O'Hara et. al. 2002).

2.5 Biogeochemical cycling of Mercury

The biogeochemical cycle of mercury (Figure 2.3) begins with the evaporation of elemental mercury (Hg) from natural and anthropogenic sources. The natural biogeochemical cycle of mercury involves atmospheric transport, deposition to land and ocean, and re-volatilization. The natural sources include volcanic eruptions and emissions from the ocean while the anthropogenic (human-caused) emissions include mercury that is released from fuels or raw materials, or from uses in products or industrial processes. Globally, ASGM is the largest source of anthropogenic mercury emissions (37%), followed closely by coal combustion (24%) (UNEP 2008). Anthropogenic sources also emit two different mercury forms which are the divalent form (Hg(II)) and mercury associated with particulate matter (Hg_p). The most abundant form of mercury in the atmosphere is Hg(0), which is present globally at a concentration of about 1.6 ng/m^3 in surface air. Hg has a lifetime of about 0.5 -1 y in the atmosphere and thus is

globally well-mixed in the atmosphere. However, Hg(II) and Hg_P are more soluble in water than Hg, and thus are the predominant forms of mercury deposited to ecosystems (Noelle 2009). Most mercury inputs to ocean regions are from wet and dry atmospheric deposition. Other inputs are ocean margins (rivers, estuaries), groundwater, benthic sediments, and hydrothermal vents. This mercury, typically the inorganic divalent form Hg(II), can be transported laterally and vertically by ocean circulation and settling of suspended particulate matter, or may be reduced to dissolved gaseous elemental mercury and evaded to the atmosphere. Obviously, most of the Hg deposited in the ocean is lost through gas evasion and possible cycles between the surface ocean and the marine boundary layer (Mason and Sheu 2002 and Mason et. al. 2012).

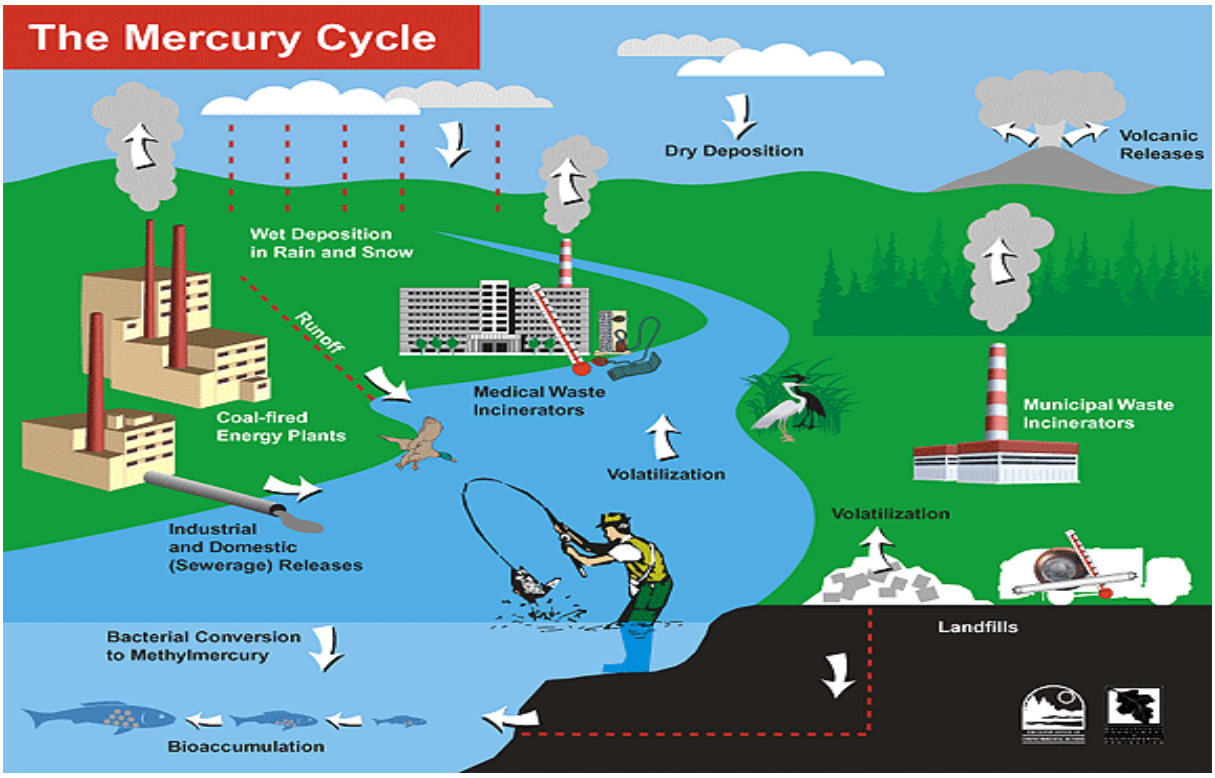

Figure 2.3: Biogeochemical cycle of mercury from natural and anthropogenic sources: (Picture use authorized by NEWMOA 2018)

Fitzgerald and Mason (1997) noted that about 90% of ocean mercury is released to the atmosphere and below 10% to the deep ocean sediments. About 2% is methylated and accumulated in the food chain but also a small portion of the methylated form, typically dimethyl mercury, volatilizes into the atmosphere.

2.6 Methylation of Mercury

Mercury is transformed into a toxic form that can be accumulated in muscle and fatty tissue of fish in a process called Methylation. Methylation of inorganic mercury (Hg(II)) in the aquatic environment has been largely the result of biological processes, primarily by sulfate-reducing bacteria (SRB) such as strains of *Desulfobulbus propionicus* and *Desulfovibrio vulgaris*. However, some reports suggest that chemical reactions involving methyl donors such as methylcobalamin, methyltin compounds and methyl iodide, with appropriate environmental factors such as pH, temperature, and complexing agents, is another promising pathway for mercury methylation in the aquatic environment (Celo, Lean and Scott 2006, Shao, Kang and Wong 2012). Anoxic zones in wetlands and lakes make appropriate conditions available for methylation. Numerous studies suggest that biotic sediments are the major sites for methylation of Hg(II) and that SRBs contribute considerably to MeHg production. Nevertheless, reports on MeHg formation from abiotic methylation of Hg(II) are also available (Paranjape and Hall 2017, Celo, Lean and Scott 2006, and Weber 1993).

SRB in anoxic sediments are the primary methylators of mercury in the aquatic environment. Although researchers have recognized mechanisms that positively link Hg(II) methylation to sulfate reduction and have suggested operative enzyme pathways, the exact mechanisms and limitations used to establish this link remain elusive (King, Kostka, Frischer and Saunders 2000). The suggested biochemical pathways are:

1. The acetyl-coenzyme A (CoA) pathway: The acetyl-CoA pathway is a carbon metabolism pathway that has serine as the most effective methyl donor to mercury. It is important to note that serine is not directly involved in the acetyl-CoA pathway, but instead it provides a methylene group to tetrahydrofolate which is a methyl-transferring coenzyme found in numerous pathways.

2. Methionine Synthase: This pathway has been hypothesized as a possible alternative site for mercury methylation. It transfers methyl groups to homocysteine.

There is a need for adequate understanding of methylation in all SRBs because one pathway alone does not satisfactorily describe the methylation. There are at least two distinct pathways for mercury methylation in SRB, one of which is independent of the acetyl-CoA pathway and possibly does not involve vitamin B_{12} (Ekstrom, Morel and Benoit 2003).

The rates of methylation and sulfate reduction in aquatic systems are usually higher in the summer due to a lower rate of bacterial growth and a lower microbial activity in the winter (Hines et. al. 2012). The mechanism of the pH effects on methylation is unknown. But research on "lake water" and on "water and sediment-water interface" suggests that an increased methylation at low pH might be as a result of reduced binding of Hg(II) to Dissolved Organic Compounds (DOC), making the Hg(II) more accessible for methylation. It is a possibility that low pH favors the activity of certain SRBs, or the operation of biochemical enzyme pathways that are effective at methylating mercury (Miskimmin, Rudd and Kelly 1992). The rate of SRB methylation of mercury in freshwater and saltwater environments is not the same. There is a higher rate of methylmercury formation in freshwaters than there is in marine water (Compeau and Bartha 1987).

2.7 Human Exposure pathways to Mercury

Human exposure pathways to mercury depend on the form of mercury. Exposure to Inorganic elemental mercury (H^0) is primarily by inhalation (of mercury vapor, mist, dust), ingestion, absorption (through skin, mucus membrane or eyes), injection (of ethyl mercury in the form of thimerosal in vaccines), and to MeHg primarily by ingestion of fish. The exposure to H^0 is low due to its deliberate uses (e.g., occupational, dental, and possibly cultural practices), although there exists a controversy on the health consequences of widespread mercury exposure from dental amalgams. However, fish consumption remains the most significant route of exposure to MeHg (Park and Zheng 2012, Gochfeld 2003). The various pathways of mercury exposure are given in Table 2.2.

Table 2.2: The different Sources of mercury, routes of exposure and elimination, and main effects. (Kim and Zoh 2012)

Mercury	Elemental	Inorganic	Organic Mercury
Sources of	Environmental	Environmental	Environmental Conversion
exposure	Volcanic explosions, rock weathering, degassing	None	Fish and shellfish
	Anthropogenic inadvertent	Industrial Products	(e.g., methylmercury)
	Combustion: fossil fuels (coal), waste incineration	Disinfectants, antimicrobials	Industrial Production
	Industrial: gold/silver mining, chloralkali plants, batteries,	Alternative medicines, cosmetics	Fungicides, bactericides
	switches, fluorescent lights, thermometers,	Vapor lamps, embalming	(e.g., phenylmercury
	sphyngomanometers	Photography	Vaccine preservstives
	Anthropogenic intentional	Latex paint (pre 1990s)	(e.g., thiomersal)
	Dental amalgams	Example: mercuric chloride	
	Ritual and folk medicine use		
Routes of Exposure	Inhalation (volatile at room temp): 75% to 85% absorption Ingestion and skin: almost no absorption	Ingestion: 10% absorbed Skin: can be high and deadly	Gastrointestinal: rapid & complete absorption Parenteral: 100% absorbed Transplacental (concentrated in cord blood)
Elimination	Urine and Feces	Renal	Feces:T1/2 45 to 70 days in adults
Toxicity	Lungs, eyes, gingival, skin Also: central nervous system, kidneys, immune system	Primary: kidneys, gastrointestinal t Secondary: central nervous system	Primary: central nervous system Secondary: cardiovascular

Fish Consumption has significantly enhanced people's diets around the world, being a rich source of easily digested and high-quality proteins containing all essential amino acids, fats (e.g.

long chain omega-3 fatty acids), vitamins (D, A and B) and minerals (including calcium, iodine, zinc, iron and selenium) (FAO 2016). MeHg bioaccumulation in the marine environment and predominantly in fish tissue is a common concern, as upper-level consumers in the food web such as wildlife and humans can be harmfully affected (Scheuhammer et. al. 2007).

MeHg represents between 75-90% of the total Hg in fish (Gochfeld 2003). A number of studies investigated how different ways of cooking seafood relative to raw samples of the same seafood affected Hg bioaccessibility. In general, cooking tends to increase the wet weight concentration of Hg in seafood, though this is almost certainly due to loss of moisture during cooking rather than any change in the amount of Hg in the seafood itself. Cooking also tends to decrease Hg bioaccessibility from seafood, relative to raw seafood; the more intense the cooking process, the less Hg bioaccessibility there is after cooking (i.e., cooking treatments in order of most to least bioaccessible would be raw > steamed/boiled (40%) > grilled (53%) > fried (60%). These studies on seafood show that cooking methods have an effect on MeHg bioaccessibility. However, no studies have investigated MeHg bioavailability from fish after different cooking methods (Bradley, Barst, and Basu 2017).

Exposure to MeHg, a known human neurotoxin, is of concern because of its adverse effects on the sensitive developing fetus. The USEPA/IRIS has classified MeHg as a possible human carcinogen. There are ranges of adverse human health effects of exposure to MeHg based on the dose and exposure duration. The central nervous system is usually the primary target when humans are exposed to methyl mercury (Fernandes et. al. 2012 and Health Canada 2007). As the levels of methylmercury increase, the earliest symptom, paresthesia (a tingling sensation), appears. Ataxia is the next adverse effect to appear, followed by difficulty in

pronouncing words (dysarthria), deafness, and extremely high concentration may result in coma and death (Health Canada 2007 and Weiss, Clarkson, and Simon 2002).

There are several papers that confirm MeHg neurotoxicity on the developing fetus (Health Canada 2007). The neurotoxin can transfer to the embryo through the placenta and, because the embryo is highly susceptible to neurotoxicity, the damage to the developing nervous system in utero can be severe, even at substantially lower doses (Mandana 2016).

Studies have reported associations between cardiovascular disease and MeHg exposure. It was reported that exposure to mercury compounds, caused by frequent consumption of fish by the population of the Amazon basin (Brazil), has a strong correlation with increased arterial blood pressure (BP). Studies regarding BP and heart rate variability (HRV) among aboriginal populations from Quebec indirectly exposed to mercury and methylmercury have also been reported. These studies suggest a deleterious impact of MeHg on BP and HRV in adults, while MeHg exposure during childhood affects HRV among children (Genchi et. al. 2017).

2.8 Mercury in Fish

Fish and shellfish are an important part of a healthy diet. They contain high-quality protein and other essential nutrients, low saturated fat, and contain omega-3 fatty acids. However, virtually all fish and shellfish contain traces of MeHg. Yet, some contain higher levels of MeHg that may harm the human nervous system if consumed in sufficient quantities. Larger fish such as sharks, swordfish, king mackerel, and tilefish that have lived longer have the highest MeHg levels. Maximum allowable thresholds for fish tissue MeHg range between 0.3 and 1.0 μg/g. The highest concentrations that exceed these values have been observed in predatory, long-lived fish. This is because top predators acquire greater body burdens of MeHg than the fish they consume and they have had more time to bioaccumulate - up to a million

times - in the surrounding water or ecosystem. Therefore, women of childbearing age and young children are advised to avoid these larger fish (USFDA and USEPA 2014, GOC 2013).

MeHg concentrations in various types of fish, ranging from low to high, are illustrated in Figure 2.4. Several speciation studies have been conducted to determine the concentration of MeHg in fish samples and have shown that MeHg is species-specific. The level of MeHg varies among fish of the same species. For example, a research on various species of tuna showed that the percentage of MeHg in proportion to Total Hg ranged from 61% to 94% (Forsyth et. al 2004).

Another study on a similar fish species showed the range to be 70% to 77% (Yamashita, Omura, and Okazaki 2005). In addition, a study on three Marlin (*Makaira nigricans)* samples and ten Swordfish (*Xiphias gladius)* samples showed MeHg proportion of Total mercury (THg) to range between 51% and 63%, and 43% and 76%, respectively (Forsyth et. al 2004). Another study conducted on seven *M. nigricans* and seven *X. gladius* samples showed 72% and 43%, respectively (Yamashita, Omura, and Okazaki 2005).

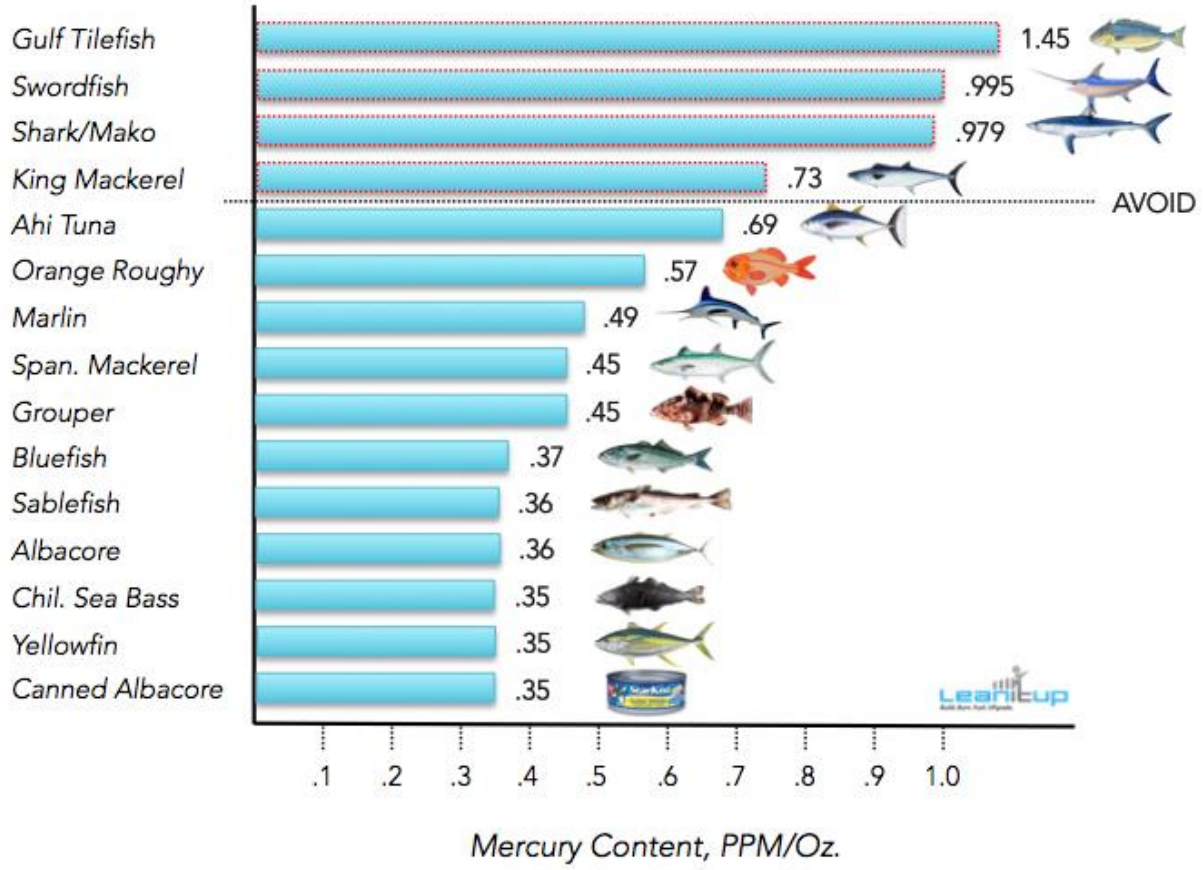

Figure 2.4: 15 Fish with the Highest Mercury Content. (Original data from USFDA (2017a)

2.9 Mercury bioaccumulation in the aquatic food web

Mercury contaminates rivers, streams, and wetlands, but only accumulates in fish after it has been microbially converted to the chemical compound MeHg. Mercury is deposited in the aquatic sediments from both natural and anthropogenic sources. Coal-burning power plants and Artesian Small Gold Mining (ASGM) are the largest anthropogenic source contributors (Gworek et. al 2017). The rate of deposition of Mercury in lake sediments is about 10-20 µg m^{-2} yr^{-1} (Biester et. al 2007). Mercury could bioaccumulate in food webs when there is a seasonal difference in the ratios of MeHg to total mercury (Hg) in invertebrates and MeHg

bioavailability to organisms (Lavoie et. al 2013, Ward, Nislow, and Folt 2010, Muhaya, Leermakers, and Baeyens 1997). A study on sediments and *Nereis diversicolor* (polychaete worm) established that seasonal variations significantly affect Hg concentrations in sediments and MeHg in *N. diversicolor.* Hg concentrations in sediments were significantly ($p<0.05$) higher in autumn and winter, whereas MeHg concentrations were lowest in both sediments and the worm. On the other hand, concentrations of MeHg were significantly ($p<0.01$) higher in spring and summer, whereas Hg in the *N. diversicolor* were lowest in spring. The seasonal changes of MeHg in *N. diversicolor* are similar to the seasonal MeHg behavior in sediments and this is probably due to seasonal variations in temperature and their effects on MeHg production in the environment and on the polychaete worm feeding behavior and metabolic activity (Muhaya, Leermakers, and Baeyens 1997).

Bioaccumulation is the increase of contaminant concentrations in aquatic organisms following uptake from the ambient environmental medium. It involves, relative to the ambient value, an increased concentration of a metal (e.g. Hg) in a biological organism over time. In a typical aquatic food web, zooplanktons pick up the MeHg as they filter the water and feed on algae. When small fish eat zooplankton, the MeHg builds up in their bodies as the fish grow bigger and older. The MeHg is lipophilic and is stored in the tissue. Small fish are eaten by larger fish, and the MeHg concentration increases at each step in the aquatic food chain (Hosseini, Nabavi, and Parsa 2013). Several factors that contribute to the uptake and accumulation of MeHg in aquatic food webs have been established in research conducted by Weiner et al. (2003), Das et al. (2003), and Ziola-Frankowska et al. (2017). These factors are age, gender, body mass index, dietary preference, trophic position, metabolic rate and geographic diversity. Mercury levels increase with size and age of the ingested fish, and they

tend to be higher in species that occupy higher trophic levels (Hosseini, Nabavi, and Parsa 2013).

However, MeHg levels in fish may be affected by differences in feeding strategies, foraging locations, as well as migratory behaviors (Dorea, Barbosa, and Silva 2006).

2.10 Consumption advisory for Mercury in Fish

Fish consumption is a very important part of a healthy diet. This is a fact that is generally accepted by nutritionist and health professionals. Fish, especially oily fish such as salmon, contain Omega-3 fatty acids. These acids can help in lowering blood pressure, heart rate, and improve other cardiovascular risk factors. Fish consumption reduces the risk of heart disease, the leading cause of death in both men and women. Fish intake has been linked to a lower risk of stroke, depression, and mental decline with age. Fish intake is also important to pregnant women, breastfeeding mothers and women of childbearing age because it supplies docosahexaenoic acid (DHA), a specific omega-3 fatty acid that is beneficial for the brain development of infants (Torpy, Lynm, and Glass 2006). DHA can also help to reduce blood cholesterol, aid in positive pregnancy outcomes as well as improve child development (Oken et. al 2008). A growing body of evidence suggests that MeHg exposure from fish consumption can also lead to an increased risk of adverse cardiovascular impacts by offsetting the beneficial cardiovascular effects of omega-3 fatty acids (Roman et. al 2011, Park and Mozaffarian 2010, Guallar et. al 2002). Therefore, daily fish consumption of fish with high MeHg levels may significantly expose consumers to a high dose of MeHg (His, Hsu, and Chang 2016).

As a result, health agencies monitor concentrations of MeHg in fish tissues and may issue consumption advisories, after a human health assessment is conducted. A consumption

advisory is a recommendation, not a regulation, to limit or avoid eating certain species of fish or shellfish caught from specific water bodies or types of water bodies (e.g., lakes, rivers or coastal waters) due to chemical contamination. They are issued for the general public or for specific groups such as the high fish consumers, the elderly, pregnant mothers, nursing mothers and children, in order to protect public health (USEPA 2018). The U.S. Food and Drug Administration and the U.S. Environmental Protection Agency (USFDA/USEPA) issue general nationwide advice regarding fish consumption. This advice allows the general public and sensitive groups such as women who are pregnant or may become pregnant, breastfeeding mothers and parents of young children to make informed choices regarding fish consumption. This advice refers to fish and shellfish collectively as "fish". They created a reference chart that sorts 62 fish types into 3 categories, as shown in Fig. 2.5. These categories are 1. Best choices (eat 2-3 servings a week), 2. Good choices (eat 1serving a week) and 3. Fish to avoid. It is, however, important to note that the "best choices" category make up nearly 90% of fish eaten in the United States.

Fish consumption data evaluated by the USFDA found that 50% of pregnant women ate <2 ounces a week and 75% of pregnant women ate <4 ounces per week (USEPA/USFDA 2017). They are advising a minimum fish consumption level for people, especially pregnant mothers and young children. The advice recommends 2-3 servings of lower-mercury fish (shrimp, pollock, salmon, canned light tuna, tilapia, catfish and cod) per week, or 8 to 12 ounces. The maximum level of consumption recommended in the final advice is consistent with the previous recommended level of 12 ounces per week. For adults, a typical serving is 4 ounces of fish, measured before cooking. Serving sizes for children should be smaller and adjusted for their age and total calorie needs. It is advised that children eat fish once or twice a week,

selected from a variety of fish types. This advice also cautions parents of young children and certain women to avoid seven types of fish because they have higher mercury levels. These are tilefish from the Gulf of Mexico, shark, swordfish, orange roughy, bigeye tuna, marlin, and king mackerel (USFDA/USEPA 2017). The USFDA and USEPA (Fig 2.5), in an attempt to categorize fish, calculated the highest average amount of mercury that could be in a fish when eaten 1-3 times a week without going over the maximum acceptable mercury intake amount for an average pregnant woman. The maximum acceptable mercury intake is determined by comparing the reference dose (RfD) developed by the EPA to the predicted exposure from the consumption of different fish species. The EPA's recommended MeHg RfD (0.1 µg/kg body weight/day) was drawn from the National Research Council's (NRC) human epidemiological studies in the Seychelles Islands, the Faroe Islands, and New Zealand. The Seychelles study showed no evidence of impairment related to MeHg exposure, while the other studies revealed dose-related adverse effects on several neuropsychological endpoints. The Faroe Islands study, after an extensive peer review, was used for the derivation of the RfD ((USFDA/USEPA 2017, Axelrad et. al 2007, USEPA 2017a). In general, the RfD is an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure of the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (USEPA 2013). However, the RfD for mercury is protective of neurodevelopmental effects for a fetus during pregnancy. A tenfold uncertainty factor (UF) is incorporated by the USEPA to allow for differences in susceptibility, distribution and elimination (USFDA 2017b).

Figure 2.5: Advice chart on Fish eating (Use of Figure is authorized by USFDA 2017b)

Some agencies, such as World Health Organization (WHO), Food and Agriculture Organization, and Agency for Toxic Substance & Disease Registry (ASTDR) have similar dose estimates to the EPA's RfD. The WHO's Joint FAO/WHO Expert Committee on Food Additives (JECFA) in 2003 set a provisional tolerable weekly intake (PTWI) of 1.6 µg of MeHg per kg body weight (equivalent to 0.2µg/kg/day). This PTWI was derived from risk assessment procedures using epidemiological data and is used in risk characterization. It includes a safety factor to account for uncertainty in the exposure-response data and pharmacokinetics of MeHg (Meyers et. al 2015) The ASTDR sets a Minimal Risk Level (MRL) - an estimate of the daily

human exposure to a hazardous substance that is likely to be without appreciable risk of adverse non-cancer health effects over a specified duration of exposure, for hazardous substances. A final MeHg MRL of 0.3µg/kg/day for chronic dose from a developmental endpoint was set in 1999.

The screening values for each category can be calculated using the equation below

$$Screening\ Value\ (SV) = \frac{(RfD) * (BW)}{(CR)}$$

Where:
SV=screening value for a noncarcinogen (µg/g)
RfD = reference dose (µg mercury/kg-d)
BW = body weight (kg)
CR = mean daily consumption rate of the species of interest (g/d)

The screening value is the highest average amount of mercury in fish that would not exceed the reference dose at a given consumption rate.

The consumption rate (CR) can be calculated using the equation below

$$CR = Serving\ size\left(\frac{oz}{serving}\right) * \frac{28.3\ g}{oz} * weekly\ servings\left(\frac{servings}{week}\right) * \frac{1\ wk}{7\ day}$$

The factors used in these calculations are:

1. Dose

- **USEPA IRIS Reference dose** (for chronic oral exposure to MeHg) = 0.1 µg/kg/day. Or
- ASTDR's Minimal Risk Level (for chronic oral exposure to MeHg) = 0.3 µg/kg/day. Or
- JECFA's provisional tolerable weekly intake = 1.6 µg/kg (equivalent to 0.2µg/kg /day)
- FDA's action level of 1ppm mercury (wet weight) = 0.5µg/kg/day

2. Body Weight: 70-80kg

The estimated body of a pregnant woman is 75 kg (165 pounds), as derived from the National Health and Nutrition Examination Survey (NHANES) 1999−2006. The mean recommended value for adults (80 kg) is different from the 70 kg commonly assumed in U.S. EPA risk assessments. Therefore, a body weight of 70 kg is used for all adults, including women of reproductive age, to calculate the consumption limits, while 14.5 kg is used for children.

3. Serving size: 4 ounces before cooking or 8-12 ounces for a variety of low-MeHg fish

The serving size of 4 ounces (113 grams) before cooking is based on FDA reference amounts customarily consumed per eating occasion (RACC) for fish and shellfish without sauce. RACCs are used by manufacturers to determine their label serving sizes, which are used as the basis for nutrient declarations on Nutrition Facts labels on food packages. The U.S. Department of Health and Human Services and U.S. Department of Agriculture recommend that women who are pregnant or breastfeeding should consume at least 8 and up to 12 ounces of a variety of seafood per week, from choices that are lower in MeHg (USFDA 2017b, USDHHS and USDA 2015).

4. Weekly Serving: 1, 2, or 3

The USFDA recommends 2-3 servings of fish a week from the best choices or 1 serving from the good choice. Children from the age of 2 are advised to be served with 1-2 servings of fish a week. When sport fish are caught, it is important to check for advisories on that fish before consumption (fig 2.5) (USFDA 2017c). Table 2.3 shows the advised weekly fish servings based on the mercuric concentration.

Table 2.3: The screening value for fish categories and number of fish servings per week. (USFDA 2017c)

Weekly fish servings	Screening value (µg/g)	Chart category
0	> 0.46	Choices to Avoid
1	≤ 0.46	Good Choices
2	≤ 0.23	Good Choices
3	≤ 0.15	Best Choices

Consumption advisories function strictly as guidance. Individual tolerance for risk and personal preferences for seafood varieties will probably continue to drive most consumers' seafood choices. Hence, a public provided with better information can generally be expected to make sounder choices (Groth 2010).

CHAPTER III

MATERIALS AND METHODS

3.1 Lake Alan Henry

Lake Alan Henry (33°03′33.0″N, 101°03′09.8″W) is a reservoir sited on the Double Mountain Fork Brazos River in West Texas. The lake has a surface area of 11.65 km^2, a mean depth of 12.19 m, and a maximum depth of 30.48 m. It is owned and operated by the City of Lubbock Parks and Recreation Department and known to attract a large number of fishermen and boaters because it is one of the premier bass fishing lakes in Texas.

3.2 Sample collection

Three Staff of the Texas Parks and Wildlife Department (TPWD) Canyon, TX office led by Mr. John Clayton along with the author of this work collected 13 fish samples from Alan Henry Reservoir.

The TPWD selected 18 sampling sites that were subdivided into three subsites in order to account for a spatial representation of the study area (Fig. 3.1). Fish species collected are predators, therefore have a unique potential for mercury bioaccumulation and are usually consumed by local anglers and their families.

Fish collection was conducted during the day and mid-night hours using the TPWD Sea Ark 1872-Pro boat equipped with a 2 rigid, non-metallic fiber glass boom and a Smith–Root Model

GPP 7.5 Electrofishing unit with system settings of 7.0 amps and 500 volts to stun fish that crossed the electric field in the water.

Stunned fish were retrieved using a dip net, but only samples of interest were retained and each retained sample was placed in a pre-labeled plastic freezer bag, in order to avoid cross-contamination. Samples were placed in a large cooler containing wet ice in other to ensure tissue preservation.

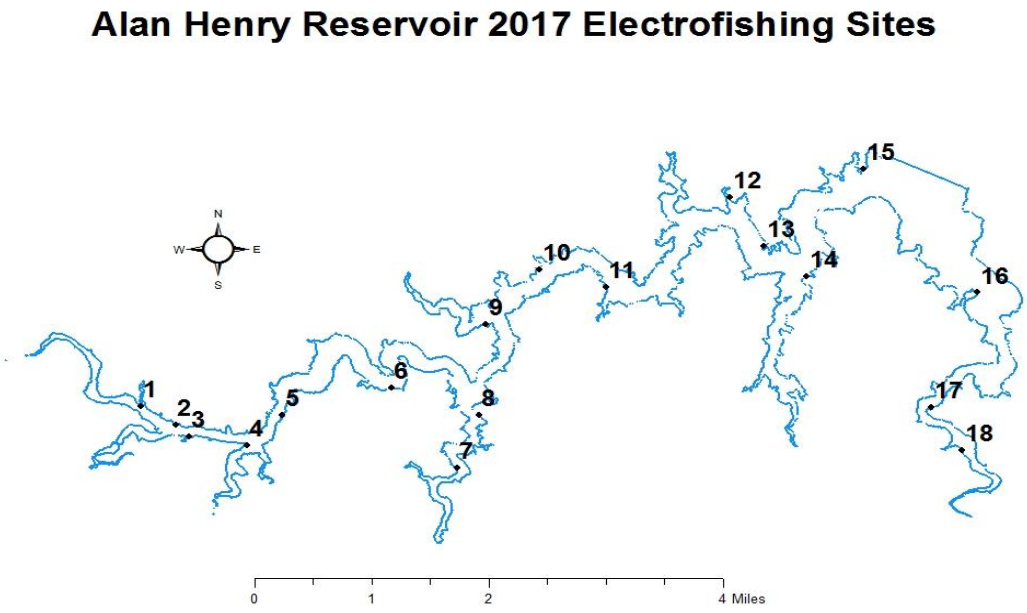

Fig. 3.1 Spatial representation of the sampling points in Lake Alan Henry Reservoir. Texas

3.3 Sample Preparation

The WTAMU researcher processed the fish samples immediately on arrival at the WTAMU Facility (Environmental Laboratory; ANS 312). The weight, using an electronic scale,

and total length (measured from the tip of the nose to the tip of the tail) of each fish sample were measured to the nearest gram (g) and millimeter (mm), respectively. After weighing and measuring each fish sample, two skin-off fillets were prepared using a clean cutting board covered with non-oil, heavy duty aluminum foil. Simultaneously, the remains of the fish after fillet were extracted (hence called "total fish body") and homogenized using a Guide Series (Model No: MG-207425) stainless steel electric meat grinder. The fillet knife and the grinder were washed with distilled water and the aluminum foil changed and disposed of appropriately immediately after each sample was processed. Each fillet and corresponding ground total fish body were separately enveloped in fresh aluminum foil and then kept in different unused pre-labeled plastic freezer bags before being temporarily stored in a secure freezer at -20° Celsius.

The prepared frozen samples, 26 in total (Fish Fillet and Total Fish Body) were shipped a week after sample collection and preparation by a commercial carrier with a preference for overnight priority delivery to the Geochemical and Environmental Research Group (GERG) Laboratory at Texas A&M University, College Station, Texas for Total Mercury Analysis. GERG laboratory's Dr. Terry Wade acknowledged the receipt of the samples.

3.4 Sample Laboratory Analysis

The GERG laboratory, using its established Standard Operating Procedure (SOP- 0006 and SOP 0202), analyzed fish fillet and total fish body obtained from Lake Alan Henry Reservoir for organic mercury. The GERG's SOP for Mercury Analysis involves two phases. These are:

1. Digestion of tissue samples prior to mercury analysis using the Cold Vapor Atomic Absorption Spectrophotometry (CVAAS)

This SOP describes the preparation of tissue samples for mercury analysis using the CVAAS. In summary, before samples can be analyzed by CVAAS, the mercury in the tissue must be converted to the Hg2+ form. The fish tissue was digested using a modified version of EPA method 245.6. Fish Tissue samples are homogenized in the original sample containers with a Tekmar Tissumizer, or equivalent, and a subsample is freeze-dried for percent moisture determination and analysis. Very small samples may be macerated manually before or after freeze-drying with a Teflon spatula to minimize sample loss. The freeze-dried subsample is ground to a fine powder. The homogenized sample is weighed into a polypropylene centrifuge tube, acid is added, and the sample is heated. After cooling, deionized water, potassium permanganate and potassium persulfate are added to each tube and the tubes are heated. Before analysis, a solution is added to reduce excess permanganate and the digestate is brought to a final volume of 40 mL with deionized water. The digestates are then ready for mercury analysis using the CVAAS technique.

i. *Laboratory Sample Preparation:*

Frozen samples received were thawed and homogenized. To the extent possible, homogenization is performed in the original sample containers using a Tekmar Tissumizer. A subsample is removed for percent dry weight and required trace element analyses. The subsample is freeze-dried and the analytical samples are transferred to the trace metals laboratory. The subsample container is labelled with a GERG-generated label that indicates the

laboratory identification number, the field identification number, the type of sample, and the sample delivery group identification. The original sample container is resealed and refrozen. A Sample Transfer Form is prepared and receipt of the prepared samples is documented. The sample identifications and related QC sample identifications for a digestion QC batch are logged into the appropriate logbook bench sheet and the freeze-dried sample is homogenized prior to digestion. The sample weight is also documented on this sheet, along with any comments regarding digestion activities.

ii. Sample Homogenization:

Freeze-dried tissue is transferred to a pre-cleaned, acid washed, Teflon vessel. An acid-washed Teflon balls are added to each container, and the samples are homogenized by shaking each container for twenty to thirty minutes using a wrist-action shaker until the sample is a homogeneous powder. Samples may alternately be mechanically homogenized using a pre-cleaned mortar and pestle.

iii. Digestion and Extraction:

a. The approximate sample weight desired for digestion is 0.05 to 0.2 grams (on a dry-weight basis) for tissues. The sample is weighed to 0.1 mg on an analytical balance and transferred to a 50 mL polypropylene centrifuge tube. The appropriate amount of spiking solution is added to the bottles designated for the Matrix Spike (MS) and Laboratory Spike Blank (LBS) prior to acid digestion.

b. 2.0 mL of concentrated nitric acid (HNO_3) is added to each tube. The tube is mixed well, capped loosely, and then placed in the hot block for 30 minutes. The temperature of the water within the hot block is 90-95°C. The reading on the hot block is generally 105-110°C.

c. After cooling, 10 mL of deionized water is added and the tube is vortexed. 10 mL of 5 % potassium permanganate ($KMnO_4$) and 5 mL of 5 % potassium persulfate ($K_2S_2O_8$) are added to each tube. If the purple-colored solution fades, additional $KMnO_4$ is added until a stable color is attained. The tube is mixed well, capped tightly, and then placed in the hot block at a water temperature of 90-95°C for 30 minutes.

d. After cooling, 5 mL of the sodium chloride/hydroxylamine sulfate is added to reduce excess permanganate and the volume is brought to 40 mL with deionized water. The sodium chloride/hydroxylamine sulfate can be replaced with 5 mL of 10% hydroxylamine hydrochloride ($NH_2OH•HCl$). The tube is mixed until manganese oxide particles are dissolved. The digestate should be colorless at this point. Additional sodium chloride/hydroxylamine sulfate (or hydroxylamine hydrochloride) solution can be added if there is still a purple color to the digestate. Samples are ready to be analyzed using the SOP for the analysis of mercury using CVAAS. Samples must be analyzed on the same day that the digestion is completed.

2. Determination of mercury by Cold Vapor Atomic Absorption Spectrometry (CVAAS).

This method is suitable for the determination of the concentration of total mercury (organic and inorganic) in the digestates resulting from the preparation of sediment, soil, and aqueous, biological and other sample matrices. The detection limit for mercury in samples is defined by the digestate concentration when it is less than or equal to the lowest calibration (0.2 ng/mL) standard.

In summary, this procedure requires that Mercury ions in a digestate be reduced by $SnCl_2$ in an acidic solution to Hg^0, and then carried through a flow cell on a stream of inert gas. The auto sampler system adds the Sn^{++} reductant in an HCl solution to the reaction chamber through a

flow injection system. A small volume of digestate or standard solution (0.5 mL) is introduced to the reaction chamber. Reaction of Hg^{++} with the tin results in the reduction of Hg^{++} to Hg^{o}. The elemental Hg is stripped out of the solution by an argon gas stream and passes into the quartz cell positioned in the path of light source from a mercury lamp. Elemental mercury absorbs radiant energy at 253.7 nm from the mercury lamp. The absorbance (peak height) is measured as a function of mercury concentration by an atomic absorption spectrophotometer. A photometric detector measures the luminous intensity of monochromatic light that has passed through the sample. The attenuation of the light is directly proportional to the concentration of the mercury vapor, which is quantified using a standard curve. Using the FIMS system, readings are obtained for all calibration standards, sample digestates and QC sample digestates.

i. *Instrumentation*

The cold vapor mercury analyzer used in this laboratory is a Perkin Elmer Flow Injection Mercury System (Model FIMS-400). The FIMS consists of a single beam high-sensitivity spectrophotometer with a high-energy mercury source, a precision long-path absorption cell (26 cm) and an integrated flow injection sample-delivery system. The FIMS combines cold vapor mercury AA with the flow injection technique, which enables cold vapor mercury AA procedures to be fully automated for mercury determinations in the low ng/mL levels. This instrument is equipped with a Perkin Elmer AS-90 auto-sampler and controlled by a PC computer with Win-AA Lab software.

ii. *Calibration and Standardization*

Calibration standards are purchased as single element mercury solutions. As required, calibration standards are diluted using the appropriate Class A volumetric glassware and/or

calibrated pipettes with a 5% or 10% nitric acid solution, depending on the analytical procedure being used. A six (6) point calibration curve is used for mercury analyses by CVAA. Standard 5 (S5) is the high concentration solution, Standard 0 (S0) is the blank, and Standards 1, 2, 3 and 4 (S1, S2, S3 and S4) are intermediate standard concentrations. The five mercury calibration standards are prepared at concentrations of 0.2, 1.0, 2.0, 5.0 and 10.0µg/L. Three or four drops of 5% KMnO4 solution are added to all standards in order to match the matrix and to keep the Hg++ ion stable. A linear line fit is used to evaluate the calibration curve with either zero or a calculated intercept.

iii. Quality Control

EPA Method 1620 key elements for CVAA quality control are summarized in Table 3.1

Table 3.1: Key elements of EPA Method 1620 CVAA Quality Control

Elements	Control Limit Criteria	Frequency
1. Initial and Periodic Analytical Requirements		
- Instrument Detection Limit (IDL)	Must meet minimum levels (MLs) specified in contract or Method 1620	Within 30 days of start of contract analysis and annually during contract period or after major instrument adjustment.
- Initial Precision and Accuracy (IPAR)	Recovery range of 85-115% for Hg; %RSD meets Method 1620.	Initial for FIMS system, annually thereafter.
2. QC Requirements for Hg Analysis by FIMS System		
- Instrument Calibration	Minimum of a blank and five standards; correlation coefficient of 0.995 or better. Re-sloping acceptable if preceded and followed by CCB/CCV.	Each time the instrument is set-up (initialized) and then each 24 hr during a continuous run.
- Initial Calibration Blank (ICB)	Absolute value ≤ ML. If fails, recalibrate and reprocess.	Immediately after system calibration and at beginning of each analytical run.
- Initial Calibration Verification Standard (ICV)	Within ± 10% of the true value. If fails, stop analysis, recalibrate.	After every ICB.
- Continuing Calibration Blank (CCB)	Absolute value ≤ ML. When fails, recalibrate, reanalyze back to last passing CCB.	A minimum of every 10 analyses, or every 2 hr during analysis, and at end.
- Continuing Calibration Verification Standard (CCV)	Within ± 10% of the true value. If fails, stop, recalibrate, reanalyze back to last acceptable ICV or CCV. Same CCV must be used for entire sample batch.	After every CCB.
- Laboratory Control Sample (LCS)	Recovery within ± 15%; if fails, terminate analysis, recalibrate or re-digest and reanalyze.	Once LCS per sample set or episode, whichever is more frequent.[1]
- Procedural Blank (PB), (Preparation or Method Blank)	Absolute value ≤ ML; if fails, the associated samples > ML and ≤ 10 ML must be reprocessed. Samples > 10 ML can be reported.	One PB with each sample set prepared
	Recovery within ± 15%; repeat analysis if fails; then recalibrate or re-digest if fails.	
	Recovery within ± 15%; repeat analysis if fails; then recalibrate if fails; if this continues to fail dilute by 10, re-analyze, report, and qualify.	
- DORMs (SRMs) [2]		At least one SRM per sample set prepared
- Digested Matrix Spike (MS) [3]	Same recovery criteria as for MS. Acceptable RPD ≤ 20%; report and qualify failure.	At least 10% of samples analyzed per matrix per sample set.
- Digested Matrix Spike Duplicate (MS) [4]		At least 10% of samples analyzed per matrix per sample set.

Note 1:A sample set or batch is a group of up to 20 field samples prepared at the same time and associated with the same QC samples.
Note 2:Analysis of standard reference materials (SRM) as a LCS requires a recovery ± 20% of certified values for complete digestions only.
Note 3:Samples to be spiked can be specified by the clients; the concentration of Hg in the matrix spike solutions is 1- 5 times the background sample level or 5 – 50 times the ML for non-detects.
Note 4:RPD = Relative percent difference between spike recovery results for matrix spike and matrix spike duplicate

a. Instrument Criteria

The standard working conditions for the FIMS system are provided in Table 3.2

Table 3.2: Recommended Working Conditions for FIMS 400 System

Wavelength	253.7 nm
Lamp	HCL
Slit	0.7 nm
Cell temperature	50° C
Integration time	15 sec.
Data processing	peak height
Argon gas supply pressure	52 psig (360 kPa)
Sample loop volume	500 µl
Argon carrier gas flow	70 - 100 mL/min
3% HCl carrier flow rate	9 - 11 mL/min
10% $SnCl_2$ reductant flow rate	5 - 6 mL/min
Pump 1 speed for prefill and fill	100 rpm
Pump 1 speed for injection	0 rpm
Pump 2 speed	120 rpm
Prefill and fill time	25 sec.
Injection time	15 sec

b. Calibration Criteria

- The correlation coefficient (r) should be greater than or equal to 0.9950 for the calibration curve used for mercury concentration calculations.
- The calculated value for the concentration of mercury in each standard used for the calibration curve should be within plus or minus 5% of the true value for the concentration of each standard.
- The absolute value of the concentration for mercury in the calibration blank should be less than the ML or CRDL for each project.
- The characteristic mass (M_0) is used to determine if the instrument is in good condition or if there is any contamination of Hg during analyses. The standard M_0 for Hg should be 136 pg. with 0.0044 Absorbance-seconds with a required QC acceptance of ± 20%

variation. Corrective actions should be taken for too low or too high M_0 values, which may include instrumental maintenance and/or re-preparation of calibration solutions.

- An initial calibration blank (ICB) is run after the calibration standard solutions at the beginning of the analytical sequence, prior to the initial calibration verification standard (ICV). A continuing calibration blank (CCB) is analyzed every 10 samples or every 2 hours, whichever is sooner, and at the completion of the analytical sequence, prior to the continuing calibration verification standard (CCV). Both of these blanks can be the same diluted acid solution, usually 5% HNO_3 solution.
- An initial calibration verification standard solution (ICV) is analyzed at the beginning of the analytical sequence for the verification of the calibration curve. A continuing calibration verification standard solution (CCV) is analyzed every 10 samples or 2 hours (whichever is sooner), and at the completion of the analytical sequence. This is a midlevel standard made from second source mercury standard, and the same solution is used for the ICV and the CCV.

  For further calibration verification, analysis of an EPA or NIST-traceable analytical standard using the new calibration standards should meet all QC acceptance criteria (see Standard Reference Material).

c. Criteria for QC Samples for Analytical Batch

All data from QC samples are evaluated for a specific digestion batch and analytical sequence before making decisions regarding corrective actions. However, contamination of the method blank normally requires the full investigation of the contamination source, and complete re-preparation and analysis of the batch, unless the concentration in all samples is 10 times that determined in the blank.

i. Method Blank

- A Method Blank is used to demonstrate that the sample preparation, digestion, and analytical procedure are free of contamination. The blank is carried through all steps in the preparation procedure but contains no sample matrix. A method blank is required for each set of 20 or fewer samples in a preparation/digestion batch.
- The QC acceptance criteria for the method blank specifies that if mercury is present in concentrations > 2 times the CRDL or ML, the preparation/digestion batch requires re-digestion, unless the concentration in all samples is 10 times that found in the method blank.
- Analyst discretion is used when contamination is present which does not adversely affect the overall analytical effort.

ii. Duplicates

- A sample duplicate (DUP) is used to evaluate matrix homogeneity and analytical precision in the presence of a sample matrix. The duplicate is prepared from the same homogenized aliquot as the original sample and is required with each set of 20 or fewer samples.
- The QC acceptance criterion for duplicate analyses is that the Relative Percent Difference (RPD) between the original and its duplicate should not exceed 20%. The DUP QC acceptance criteria are advisory only non-compliance may not require re-digestion of the entire analytical batch. However, corrective action may still be indicated, which may include reanalysis of the DUP and the original sample, instrument maintenance and/or recalibration, or re-digestion of the original sample and its duplicate.

- The RPD is considered invalid and is not evaluated when the mercury concentrations determined are less than the 10 times the ML or CRDL.

iii. Matrix Spike and Matrix Spike Duplicate

- The Matrix Spike (MS) sample is a fortified sample used to evaluate analytical accuracy in the presence of a sample matrix. The MS is prepared from the same homogenized aliquot as the original sample and is required with each set of 20 or fewer samples.
- A Matrix Spike Duplicate (MSD) is used to evaluate both analytical accuracy and precision in the presence of a sample matrix. When required, the MSD is prepared from the same homogenized aliquot as the original sample and is prepared with each set of 20 or fewer samples.
- The QC acceptance criteria for mercury in the MS and MSD are a percent recovery of 85% to 115% of the spiked amount.
- The QC acceptance criteria are invalid when the spiked amount is not sufficient to increase the mercury concentrations in the sample by at least 50% (or more, as the contract requires).
- If a Matrix Spike Duplicate (MSD) has been included with the analytical batch, the percent recovery results obtained from the MS and MSD samples should agree within a Relative Percent Difference (RPD) of 20%.
- The MS and MSD QC acceptance criteria are advisory only and results outside the acceptance criteria do not require re-digestion of the entire analytical batch. However, corrective action may still be indicated, which may include reanalysis of the original

sample and its MS/MSD, instrument maintenance and/or recalibration or re-digestion of the original sample, and its MS/MSD.

iv. Laboratory Blank Spike

- When there is inadequate sample, a difficult matrix, the absence of a valid SRM, or required by contract, a Laboratory Blank Spike (LBS) may be used to evaluate analytical accuracy of the method, as well as instrument performance. The LBS is also referred to as a Laboratory Control Sample (LCS), and may be required with each set of 20 or fewer samples.
- The QC acceptance criteria for mercury recovery in the LBS are 85 to 115% of spiked Hg amount.

d. Analytical Criteria for Sample

- Analyze samples and all related QC samples under the same conditions as standards.
- Review the data to determine that the % RSD is within plus or minus 5% for the replicate analyses performed by the instrument. Samples that do not meet this criterion should be re-analyzed and may require re-digestion if the criterion is not met on the second analysis. Samples with low concentrations (< 2 times the MDL) are acceptable with higher RSD values.
- Review the data to verify that all analytical determinations are within the range of the calibration curve. Samples at concentrations that are higher than the highest calibration standard used to calibrate the instrument must be diluted and reanalyzed.

iv. Analytical Procedure

a. Turn on FIMS and allow it to warm up for 30-45 minutes, until the energy level is stable.

b. Make the necessary chemical solutions for the analyses of mercury, including 3% HCl solution as carrier, and 10% $SnCl_2$ solution used as the reductant of Hg^{2+} in the standards and digestates.

c. Prepare the mercury calibration standards at concentrations of 0.2, 1.0, 2.0, 5.0 and 10.0 µg/L from a commercially available concentrated mercury standard.

d. Open the FIMS software and choose the appropriate analytical method according to the sample matrix type and the requirements of clients. Click on the Sample Information icon and enter QC samples and client sample identification, weight, dilution factor, and target concentration units. Verify entered information with the location of QC samples and client samples in the auto-sampler.

e. Set appropriate rinse and uptake time (not less than 10 seconds and 25 seconds, respectively).

f. Adjust the FIMS system to make sure it is operating under optimum conditions. This includes but is not limited to changing of the peristaltic pump tubings, changing of the liquid-gas separator membrane, adjusting the pump speed and pressure, etc.

g. Load the calibration standard solutions and sample digestate solutions into the auto sampler. Wipe out spills immediately, before they can cause contamination or damage.

h. Analyze the standard calibration curve. Review the ICV reading and depending on the percent recovery of the standard (90% - 110%), accept the curve or rerun the curve if it is not within accepted QC criteria.

i. Analyze samples and all related QC samples under the same conditions as standards.

j. Review the data to determine that the %RSD is within plus or minus 5% for the replicate analyses performed by the instrument. Samples that do not meet this criterion should be re-analyzed and may require re-digestion if the criterion is not met on the second analysis. Samples with low concentrations (< 2 times the MDL) are acceptable with higher RSD values.

k. Review the data to verify that all analytical determinations are within the range of the calibration curve. Samples at concentrations that are higher than the highest calibration standard used to calibrate the instrument must be diluted and reanalyzed.

v. Data Analysis and Calculations

Sample analysis results are calculated by comparing sample peak height, area, or absorbance against the standard curve. Calculations may be made using a best fit linear regression analysis of the standards and blanks. Using the least squares best fit method, the slope and y-intercept of the line for the standards and the blank were calculated.

i. Calculation of mercury in sample.

$$[\boldsymbol{Hg}]ng/g = \frac{(\mathrm{P}h - y\ intercept) * V * d}{S * Sw}$$

Where:

Ph is the sample peak height; **V** is the digestate volume in ml, **d** is the dilution factor, **S** is the slope of the standard regression line (peak height/ng), and **Sw** is the sample weight digested in grams.

ii. Calculation of Relative Percent Difference (%RPD).

$$\%RPD = \frac{200*(abs.(A-B))}{(A+B)}$$

Where:

A is the measured concentration in Sample A (or MS),

B is the measured concentration in Duplicate B (or MSD).

iii. Calculation of Percent Recovery (%R).

$$\%R = \frac{100*(A-B)}{T}$$

Where:

A is the measured concentration after spiking,

B is the measured concentration before spiking,

T is the true concentration of the spike

3.5 Risk-Based Consumption Concentration.

Allowable consumption concentrations (Health Assessment Comparison Value) of contaminated fish, based on a contaminant's noncarcinogenic health effects was calculated using the below equation. The MeHg concentration in fish tissue samples was compared to the comparison value derived from the maximum acceptable oral dose for Methylmercury.

$$\boldsymbol{Health\ Assessment\ Comparison\ Value}\ (\boldsymbol{CV}) = \frac{(AODmax) * (BW)}{(CR)}$$

Where:

AOD_{max} is the maximum acceptable oral dose ($mgkg^{-1day-1}$)

BW is the Body Weight (Kg),

CR is the consumption rate (g/day)

The body weight, consumption rate, and measure of central tendency used to derive a Maximum

Acceptable Oral Dose differs among the USEPA and the three coastal states, as shown in Table 3.3 below. The USEPA and California use the USEPA-established Reference Dose (RfD) as the Maximum Acceptable Oral Dose. On the other hand, Texas and Alaska use the ASTDR-established Minimum Risk Level (MRL) and the Alaska-Specific Acceptable Daily Intake, respectively.

Table 3.3. Comparison of Body Weight, Consumption Rate, and Measures of Central Tendency used by the USEPA, Texas, California, and Alaska.

	Mercury			
	USEPA	Texas	California	Alaska
Body Weight (Kg)	70	70	70	60
Consumption Rate (g/day)	17.5	30	32	46.5
Maximum Acceptable Oral Dose (mg/kgday)	0.0001	0.0003	0.0001	0.00056
Measures of Central Tendency	Arithmetic Mean, Median	Arithmetic Mean	Arithmetic mean	Median

Hazard Quotients (HQ)

The HQ, according to the USEPA (2017b), is the ratio of the potential exposure to the substance and the level at which no adverse effects are expected. This is calculated using the following equation:

$$HQ = \frac{MeHg\ Conc.}{HACV}$$

Where:

HQ = Hazard Quotient
MeHg Conc. = Methylmercury concentration in Fish Tissue
HACV= The Health Assessment Comparison Value, which serves as an indicator of no adverse effects.

An **HQ > 1** implies that human health adverse noncancer effects are likely to occur, while a **HQ ≤ 1** implies that adverse noncancer effects are not likely.

Total Mercury (Hg) and Methylmercury (MeHg)

In this study, total mercury (THg) in fish samples was analyzed, and was used interchangeably with MeHg. This substitution was based on the results of scientific studies that showed that about 71.91- 98.1% of THg in fish and shellfish tissue is present largely as MeHg (Jung et al 2013, Marrugo-Negrete et al. 2008, Kehrig et al 2001, NAS 1991, and Tollefson 1989). In addition, the USEPA recommends that THg should be analyzed and a conservative assumption be made that all THg is present as MeHg (USEPA 2000).

CHAPTER IV

RESULTS AND DISCUSSION

4.1 Quality Assurance

The accuracy of the test method was determined by method blank, sample duplicate, matrix spike, standard reference material: DORM-4 (Dogfish muscle) and laboratory Blank Spike. These quality assurance tests agree well with the Quality Control (QC) Criteria and therefore attest to the accuracy of the method. The quality assurance results are presented in Table 4.1.

Table 4.1: Quality Assurance result of laboratory analysis

Sample Matrix - Fish Tissue (Fillet)			
	Mercury (µg/g)	Quality Control Recovery	Relative Percentage Difference (%RPD)
Sample	0.14	-	-
Blank	0.001	-	-
Duplicate	0.144	-	2.817
DORM-4	0.413	99.28	-
Matrix Spike	0.321	91.88	-
Laboratory Blank Spike	0.197	98.5	-

4.2 Fish Sample Total Mercury (Methylmercury) Concentrations

MeHg concentrations in the fish samples (Fillet and Whole body) are summarized in Table 4.2. The medians, lower and upper quartile MeHg concentration of both fish species are shown in Figure 4.1. Complete analytical results for the fish samples (Largemouth Bass and Alabama Spotted Bass) are presented in Appendix A.

Table 4.2: Mercury Concentration in Fish from Lake Alan Henry Reservoir, 2017

		Mercury Concentration (µg/g)		
Species	**Sample**	**Mean ± SD**	**95% UCL**	**Range**
Large Mouth *(n= 8)*	Fillet	0.855 ±0.658	1.623	0.034-2.263
Large Mouth *(n= 8)*	Whole Body	0.540 ±0.570	1.29	0.161-1.807
Alabama Bass *(n=5)*	Fillet	0.429 ±0.210	0.626	0.088-0.373
Alabama Bass *(n=5)*	Whole Body	0.240 ±0.111	0.346	0.14-0.64

Fillets from all fish samples were analyzed for MeHg. The mean mercury concentration of the Alabama Spotted Bass fillet (0.429 ±0.19 µg/g) was lower than that of Largemouth Bass (0.854±0.66 µg/g). There was no significant difference between the mean MeHg concentrations analyzed in both fish samples ($p= 0.14$, $p>0.05$; Mann-Whitney U-test *see Appendix B). This agrees with a previous study conducted in 2008 by the Texas Department of Health Services (DSHS) in the same area (DSHS 2010). The results obtained from this research demonstrated a positive relationship between fish size and fish tissue MeHg, and this agrees with several well-established studies (Burger and Gochfeld 2011, Cizdziel et. al 2002, Simonin et. al 2008).

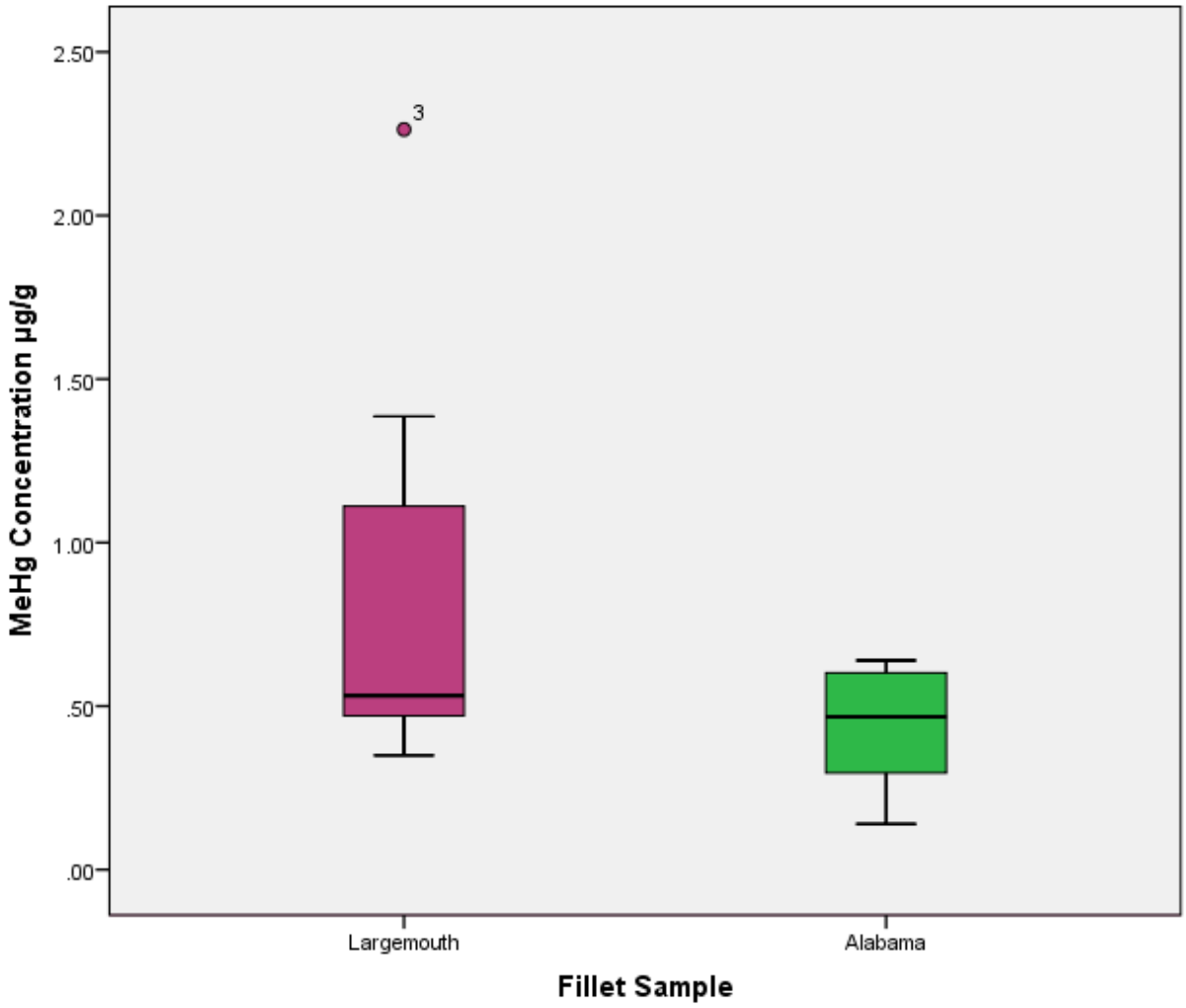

Fig 4.1: Box plot of MeHg concentration in fillet samples of Alabama Spotted Bass and Largemouth Bass

This study showed that fish length or size is important to the accumulation of MeHg in fish tissue regardless of the age and tropic level of the fish. MeHg concentrations in different size classes of Alabama Spotted Bass and Largemouth Bass were determined (see Appendix A) in order to evaluate the differences in consumers' exposure to MeHg when fish of different sizes are consumed. The mean MeHg concentrations in different size classes (small, medium and

large) of Alabama Spotted and Largemouth Bass was highest in the large size Largemouth Bass (1.495µg/g) and in the medium size Alabama Spotted Bass (0.621 µg/g). Also, small sizes of Largemouth Bass (0.435 µg/g) and Alabama Spotted Bass (0.218 µg/g) had the lowest MeHg concentrations. Concentration of fish MeHg against fish sizes (fig. 4.2) showed a positive and significant correlation with sizes of fish. (Alabama Spotted Bass: R= 0.61, Largemouth Bass; R= 0.89). This relationship is a result of efficient MeHg bioaccumulation and biomagnification by the increasing sizes of the fish species via the food chain pathway (Southworth, Peterson and Ryon 2000). It can also be a function of high consumption rate and higher energy allocation to activity cost as the size of fish increases (Trudel and Rasmussen 2006). However, other factors such as the specific sites (locales and region) where the fish were collected also affected the concentration of MeHg in fish in spite of their length.

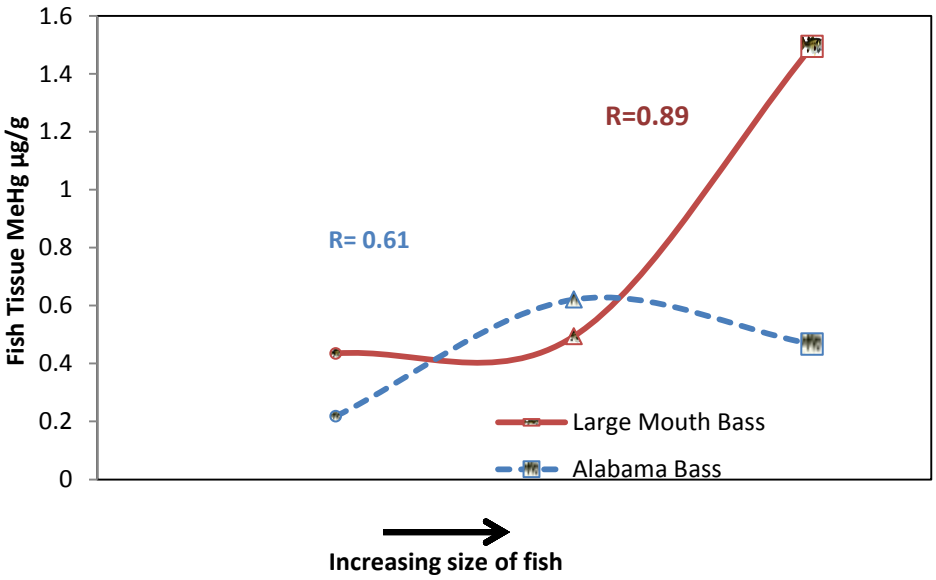

Fig 4.2: Correlation of fish MeHg concentration against fish sizes of Largemouth Bass and Alabama Spotted Bass.

4.3. Fish fillet and whole-body Methylmercury: Differences

The relationship between the MeHg concentrations in the fillet and whole-body (excluding the fillet) of Alabama Spotted and Largemouth Bass is illustrated in Fig. 4.3. The solid line is included as an expected reference point if MeHg concentration of whole body and fillet were equal. Paired t-tests (Appendix C) showed that fish fillet MeHg concentrations were significantly different from the fish whole-body MeHg concentrations for both Alabama Spotted Bass ($p<0.05$) and Largemouth Bass ($p<0.05$). Mann Whitney U-Test was also used to check the statistical differences between the MeHg concentrations in fish fillet and wholebody for both fish species. The result of the U-test, as shown in Appendix C, shows a statistical difference ($p<0.05$) between MeHg in fish fillet and that in fish wholebody for both Alabama Bass and Largemouth Bass. The MeHg concentrations were higher in fillet than in the whole body of both Alabama Spotted Bass and Largemouth Bass. This result therefore reveals that a significant amount of MeHg is absorbed by the tissue of fish, rather than the whole body. Recreational fishers may therefore be exposed to a heightened fish MeHg concentration when consuming fish tissues or fillets over the whole body.

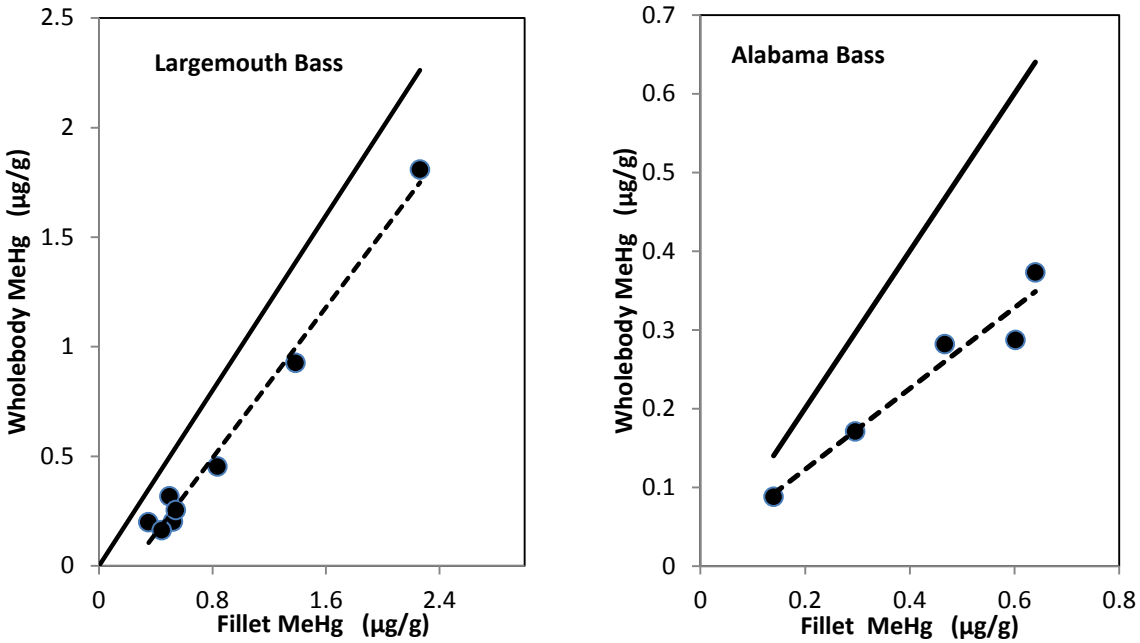

Fig 4.3: Relationship between MeHg concentrations in the fillet and whole- of Alabama Spotted and Largemouth Bass. The solid line shows the expected reference point if MeHg concentration in whole body and fillet of Largemouth Bass and Alabama Spotted Bass were equal.

4.4 Lake Alan Henry Fish Concentrations: 2008 and 2017

The objectives of this study were to acquire data that will be used to estimate the potential health risk of MeHg to humans from fish consumption from the Lake Alan Henry reservoir. This study also compares the results obtained to that of a similar study conducted by the DSHS in 2008. Used were the same assumptions for fish consumption rate, average body weight, daily human exposure and the arithmetic mean of Fish Fillet MeHg concentration as the DSHS research in 2008. The screening value against the arithmetic mean fish MeHg was derived using the HACV equation. The ASTDR Minimal Risk Level (MRL) for MeHg (0.003 mg/kg/day) was used in the previous studies in order to ensure uniformity; the same was used in this study. The MRL of 0.0003 mg mercury/kg/day was based on neurodevelopmental

endpoints of the Seychelles study on fetal chronic exposed *in utero* to MeHg from maternal fish ingestion (Davidson et. al 1998). The Largemouth Bass MeHg concentration for both years (Table 4.3) exceeded the safe MeHg consumption level (0.7 mg/kg) in fish. This study contrasts the previous study as it revealed that consumption of Alabama bass is not likely to cause any potential risk to humans. This contrast might be due to differences in the number of samples collected, the size or length of the samples, as well as the water chemistry and lake activity during both years. However, the present study agreed with the previous one and therefore confirms that consumption of Largemouth Bass (HQ = 1.6) may cause potential harm to humans.

Table 4.3: Mean Minimum and Maximum MeHg concentration and Hazard Quotient of Alabama Spotted Bass and Largemouth Bass for 2008 and 2017 Study.

	Alabama Spotted Bass		Largemouth Bass	
	Mean MeHg (min- max)	Hazard Quotient(HQ)	Mean MeHg (min- max)	Hazard Quotient(HQ)
2008	**1.367**±0.870 (0.331-2.321)	2	**1.136**±0.378 (0.143-1.789)	1.6
2017	0.429 ±0.210 (0.088-0.373)	0.6	**0.855** ±0.658 (0.034-2.263)	1.2

*MRL= 0.0003mg/kg/day BW= 70kg CR= 30g SV= **0.7**

4.5 Fish Advisory Protocol comparison of California, Alaska and Texas.

The Allowable consumption concentrations (Health Assessment Comparison Value) of contaminated fish calculated using the equation listed in section 3.5 of this text, yielded the following results for Methylmercury, as shown in Table 4.4.

Table 4.4. Health Assessment Comparison Values used by the USEPA, Texas, California, and Alaska for Methylmercury in fish.

Mercury (Methylmercury) mg/kg			
USEPA	**Texas**	**California**	**Alaska**
0.4	0.7	0.22	0.72

MeHg concentrations in fish tissue samples were derived employing the measure of central tendency used by the USEPA, Texas, California, and Alaska. These were compared to their respective Health Assessment Comparison Values (HACVs). Hazard Quotients (HQs) were calculated to determine the potential presence or absence of risk.

The results obtained are shown in Table 4.5 below.

Table 4.5. Largemouth and Alabama Spotted Bass MeHg concentrations, Hazard quotients and the Health Assessment Comparison Values used by the USEPA, Texas, California, and Alaska

		Methylmercury			
		Largemouth Bass		Alabama Bass	
	HACV (mg/kg)	MeHg Conc. (mg/kg)	HQ	MeHg Conc. (mg/kg)	HQ
USEPA	0.4	0.85475	**2.1**	0.429	**1.1**
Texas	0.7	0.85475	**1.2**	0.429	0.6
California	0.22	0.85475	**3.9**	0.429	**2**
Alaska	0.72	0.532	0.7	0.467	0.6

The MeHg concentration for Alaska is lower compared to that of the USEPA, Texas, and California, which may be due to the small sample size. A small sample size can affect the MeHg concentration when the median is used, which is the case in Alaska. As previously stated, the arithmetic mean is used by the USEPA and other states.

The HQ for the USEPA and California exceeds 1 for both Largemouth and Alabama Spotted Bass, which means potential adverse health risks cannot be ruled out. The HQ for Texas is > 1 for Largemouth Bass and < 1 for Alabama Spotted Bass, indicating the likelihood of risk and the absence thereof, respectively. In contrast, HQs for Alaska are < 1 for both Largemouth and Alabama Spotted Bass, indicating that potential risk is not likely.

Based on the results obtained from this study, advisories should be issued for both Largemouth and Alabama Spotted Bass in California. In Texas, an advisory should be issued only for Largemouth Bass. In the state of Alaska, there is no need for an advisory for either species. However, if the USEPA protocol were to be used in Texas, California, and Alaska, our results suggest fish consumption advisories would need to be issued for both fish species in these other states.

CONCLUSION

According to the results of this study, Methylmercury concentrations in fish tissue ranged from 0.14 mg/kg to 0.64 mg/kg for Alabama Spotted Bass and from 0.034 mg/kg to 2.263 mg/kg for Largemouth Bass. Further research may be conducted with a larger sample size for Largemouth Bass, as there is a discrepancy between the results of this study and those obtained in 2008 from the same site. Considering the small sample size of Alabama Bass from both studies, we can hypothesize either that this species exists in low numbers in the reservoir, or that sampling efforts should be improved in order to obtain a more representative sample size.

As far as toxicological values are concerned, there is a hierarchy of databases to consult for these values. First in order comes the USEPA Integrated Risk Information System (IRIS) database, followed by the USEPA's Health Effects Assessment Summary Tables (HEAST), and then any other databases, including HSDB, the National Cancer Institute's Chemical Carcinogenesis Research Information System (CCRIS), EPA's GENE-TOX, and the National Institute of Occupational Safety and Health's (NIOSH's) Registry of Toxic Effects of Chemical Substances (RTECS) (USEPA 2000). The state of Texas does not follow this hierarchy, however; instead, Texas uses the Agency for Toxic Substances and Disease Registry's (ATSDR) Minimal Risk Level (MRL) as the reference dose, at a consumption rate of 30 g/day. Alaska uses the Alaska-Specific Acceptable Daily Intake, at a consumption rate of 46.5 g/day, specifically tailored to women of childbearing age and a body weight of 60 kg. This is due to the different geographic location, lifestyle, and dietary habits of the Alaskan population (Hamade 2014), which should not be directly compared to parameters applied to the contiguous United States. California uses a consumption rate of 32 g/day, with a recommended intake of half the amount for children and women between the ages of 18 and 45. The USEPA RfD, at a recreational consumption rate of 17.5 g/day, applies to the general population of the contiguous United States.

Fish consumption advisories are an important tool, informing the inhabitants of the United States about consumption of recreational fish that may be contaminated with levels of MeHg in order to prevent adverse public health hazards. There are probably a number of water bodies in the states of Texas, Alaska and California that may contain high levels of fish MeHg concentrations but without consumption advisories. These water bodies are likely to be issued

consumption advisories if the USEPA protocol is used. Apparently, there is a discrepancy between the USEPA methods and those of the examined coastal states of the United States.

As a result of the findings in this study and for the sake of public health protection, there is a need for discussion among states and federal scientists (risk assessors, toxicologists, etc.), as well as legislators of the United States to determine which appropriate method should be employed before the issuance of a fish consumption advisory.

REFERENCES

AMAP/UNEP. (2015). "Global Mercury Modelling: Update of Modelling Results in the Global Mercury Assessment 2013." *Arctic Monitoring and Assessment Programme, Oslo, Norway/UNEP Chemicals Branch, Geneva, Switzerland*. **36**.

Asano, S., Eto, K., Kurisaki, E., Gunji, H., Hiraiwa, K., Sato, M., Sato, H., Hasuike, M., Hagiwara, N. and Wakasa, H. (2000). "Acute inorganic mercury vapor inhalation poisoning." *Pathology International*, 50,**169–174.**

ATSDR. Agency for Toxic Substances and Disease Registry . (2015). "Toxicological Profile for Mercury."*Toxic Substances Portal, ASTDR.* https://www.atsdr.cdc.gov/toxprofiles/TP.asp?id=115&tid=24#bookmark07. Accessed on 24April, 2018.

Axelrad D. A., Bellinger D. C., Ryan L. M., and Woodruff T. J. (2007). "Dose–Response Relationship of Prenatal Mercury Exposure and IQ: An Integrative Analysis of Epidemiologic Data." *Environmental Health Perspectives*; *115*(4), **609–615.**

Bernhoft R. A. (2012). "Mercury toxicity and treatment: a review of the literature." *Journal of Environmental and Public Health*, vol. 2012, Article ID 460508, **10 pp**.

Biester H., Bindler R., Martinez-Cortizas A., and Engstrom D.R. (2007). "Modeling the Past Atmospheric Deposition of Mercury Using Natural Archives." *Environmental. Sci. Technol.; 41* (14):**4851–4860.**

Bradley M.A.,Barst B. D., and Basu N .(2017). "A Review of Mercury Bioavailability in Humans and Fish." *Int. J. Environ. Res. Public Health;*14(2): **169.**

Burger J., and Gochfeld M. (2009). "Perceptions of the risks and benefits of fish consumption: Individual choices to reduce risk and increase health benefits." *Environmental Research*; 109(3): **343–349.**

Burger J., Gochfeld M. (2011). "Mercury and selenium levels in 19 species of saltwater fish from New Jersey as a function of species, size, and season." *Sci Total Environ;* 409(8):**1418-29.**

Carl H. Lamborg, Chad R. Hammerschmidt, Katlin L. Bowman, Gretchen J. Swarr, Kathleen M. , et al.(2014). "A global ocean inventory of anthropogenic mercury based on water column measurements." *Nature* 512, **65–68.**

Celo V., Lean D.R.S, and Scott S. L. (2006). "Abiotic methylation of mercury in the aquatic environment." *Science of The Total Environment,* 368(1)**:126-137.**

Chang T. C., You S. J., Yu B. S., Chen C. M., and Chiu Y. C. (2009) "Treating high-mercury-containing lamps using full-scale thermal desorption technology," *Journal of Hazardous Materials*, 162(2-3), **967–972.**

Chehimi L., Roy V., Jeljeli M., Sakly M. (2012). "Chronic exposure to mercuric chloride during gestation affects sensorimotor development and later behavior in rats" *Behav. Brain Res*., 234, **43-50**

Cizdziel J. V., Hinners T.A., Pollard J.E., Heithmar E.M., Cross C.L. (2002). "Mercury concentrations in fish from Lake Mead, USA, related to fish size, condition, trophic level, location, and consumption risk." *Arch Environ Contam Toxicol*. 43(3)**:309-17.**

Compeau G.C., Bartha R. (1987). "Effect of salinity on mercury-methylating activity of sulfate-reducing bacteria in estuarine sediments." *Applied and Environmental Microbiology* 53(2): **261-265.**

Das K., Lepoint G., Leroy Y., Bouquegneau J.M. (2003). "Marine mammals from the southern North Sea: feeding ecology data from $\delta^{13}C$ and $\delta^{15}N$ measurements" *Mar. Ecol. Prog. Ser.*; 263, **287–298**

Davidson P.W., Myers G. J., Cox C., Axtell C., ….., Clarkson T.W. (1998). "Effects of prenatal and postnatal methylmercury exposure from fish consumption on neurodevelopment: outcomes at 66 months of age in the Seychelles Child Development Study." *JAMA.* 280(8)**:701-707.**

(DSHS). Department of State Health Services, Austin, Texas, Division for Regulatory Services Policy, Standards, and Quality Assurance Unit, Seafood and Aquatic Life Group.. (2010). "Characterization of Potential Adverse Health Effects Associated with Consuming Fish from Alan Henry Reservoir Garza and Kent Counties, Texas" *Alan Henry Reservoir RC 2008:* **pp 45.**

Dorea J.G., Barbosa A.C., Silva G.S. (2006) Fish mercury bioaccumulation as a function of feeding behavior and hydrological cycles of the Rio Negro, Amazon *Comparative Biochemistry and Physiology Part C:* 142(3–4) **275-283.**

Ekstrom E.B., Morel F.M., and Benoit J. M. (2003). "Mercury Methylation Independent of the Acetyl-Coenzyme A Pathway in Sulfate-Reducing Bacteria." *Applied and Environmental Microbiology,* 69 (9): **5414-5422.**

Fernandes A.B., Barros F.L., Peçanha, F. M., Wiggers, G. A., Frizera V. P., Ronacher S. M., … Valentim V.D.(2012)."Toxic Effects of Mercury on the Cardiovascular and Central Nervous Systems." *J. Biomed. Biotechnol. Int.;* 949048.

Fitzgerald W.F. and Mason R. P. (1997). "Biogeochemical cycle of mercury in the marine environment. In: Sigel A, Sigel H, editors." *Metal Ions in Biological Systems. New York: Marcel Dekker* ,**53-111.**

FAO. Food and Agriculture Organization of the United Nations. (2016). "The State of World Fisheries and Aquaculture 2016: Contributing to food security and nutrition for all." *Rome.***200pp**

Food and Agriculture Organization of the United Nations. (2016). "The State of World Fisheries and Aquaculture; Contributing to food security and nutrition for all." *Rome;***200 .**

Forsyth D., Casey V., Dabeka R. W., McKenzie A. (2004). "Methylmercury levels in predatory fish species marketed in Canada." *Food Addit. Contam.*; 21(9):**849-856.**

Genchi G., Sinicropi M. S., Carocci A., Lauria G., and Catalano A. (2017). "Mercury Exposure and Heart Diseases." *International Journal of Environmental Research and Public Health*; *14*(1), **74**.

Gochfeld Michael. (2003). "Cases of mercury exposure, bioavailability, and absorption." *Int J Environ Res Public Health, Ecotoxicology and Environmental Safety;* 56(1):**174-179.**

Government of Canada (GOC). (2013). "Methylmercury in Fish*". Mercury in the food chain.* https://www.canada.ca/en/environment-climate-change/services/pollutants/mercury-environment/health-concerns/food-chain.html. Accessed on 22 April 2018.

Groth E. 3rd. (2010). "Ranking the contributions of commercial fish and shellfish varieties to mercury exposure in the United States: Implications for risk communication" *Environmental Research* 110:**226–36.**

Guallar E., Sanz-Gallardo M. I., Van'T Veer, P., Bode P., Aro A., Gómez-Aracena J., ... Kok F. J. (2002). "Mercury, fish oils, and the risk of myocardial infarction." *New England Journal of Medicine, 347*(22), **1747-1754.**

Gworek B., Dmuchowski W., Baczewska A.H., Brągoszewska P., Bemowska-Kałabun O., Wrzosek-Jakubowska J. (2017). "Air Contamination by Mercury, Emissions and Transformations—a Review." *Water, Air, and Soil Pollution*. 228(4), **123**.

Hamade A. K. (2014). "Fish Consumption Advice for Alaskans: A Risk Management Strategy To Optimize the Public's Health" *Department of Health and Social Services, State of Alaska;***38.** http://dhss.alaska.gov/dph/Epi/eph/Documents/fish/FishConsumptionAdvice2014.pdf Accessed on 26 March 2018.

Hamade A. K. (2014). "Fish Consumption Advice for Alaskans: A Risk Management Strategy to Optimize the Public's Health." *Alaska Scientific Advisory Committee for Fish Consumption, Section of Epidemiology, Division of Public Health, Department of Health and Social Services State of Alaska.* Updated July 21, 2014: **78**

Health Canada. (2007). "Human Health Risk Assessment of Mercury in Fish and Health Benefits of Fish Consumption."https://www.canada.ca/en/health-canada/services/food-nutrition/reports publications/human-health-risk-assessment-mercury-fish-health-benefits-fish-consumption.html Accessed on 23 March 2018.

Hines M.E, Poitras E.N., Covelli S., Faganeli J., Emili A., Zizek S., Horvat M. (2012). "Mercury methylation and demethylation in Hg-contaminated lagoon sediments (Marano and Grado Lagoon, Italy)." *Estuarine, Coastal and Shelf Science,* 113: **85-95.**

Hong Y.-S., Kim Y.-M., and Lee K.-E. (2012). "Methylmercury Exposure and Health Effects." *Journal of Preventive Medicine and Public Health*; 45(6): **353–363.**

Hosseini M., Nabavi S. M. B., and Parsa Y. (2013). "Bioaccumulation of Trace Mercury in Trophic Levels of Benthic, Benthopelagic, Pelagic Fish Species, and Sea Birds from Arvand River, Iran." *Biological Trace Element Research*, *156*(1-3), **175–180.**

Hsi H.C., Hsu Y.W., Chang T.C., et al. (2016). "Methylmercury concentration in fish and risk-benefit assessment of fish intake among pregnant versus infertile women in Taiwan." *PLoS ONE* 11(5):**e0155704.**

Jan, A. T., Azam, M., Siddiqui, K., Ali, A., Choi, I., & Haq, Q. M. R. (2015). "Heavy Metals and Human Health: Mechanistic Insight into Toxicity and Counter Defense System of Antioxidants." *International Journal of Molecular Sciences*, *16*(12), **29592–29630**.

Karagas M. R., Choi A. L., Oken E., Horvat M., Schoeny R., Kamai E., … Korrick S. (2012). "Evidence on the Human Health Effects of Low-Level Methylmercury Exposure." *Environmental Health Perspectives*;120(6): **799–806.**

Kehrig H. A., Costa M., Moreira I., Malm O. (2001). "Methylmercury and total mercury in estuarine organisms from Rio de Janeiro, Brazil." *Environ Sci Pollut Res Int.* 8(4): **275–279**.

Kim M-K, and Zoh K-D. (2012). "Fate and Transport of Mercury in Environmental Media and Human Exposure." J *Prev Med Public Health*,45: **335-343.**

King J. K., Kostka J. E., Frischer M. E. and Saunders M.F. (2000). "Sulfate-Reducing Bacteria Methylate Mercury at Variable Rates in Pure Culture and in Marine Sediments." *Applied and Environmental Microbiology,* 66 (6): **2430-2437.**

Kris-Etherton P. M., Harris W. S. and Appel L. J. (2002). "Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease." *American Heart Association Nutrition Committee Circulation;* 106:2747–**2757.**

Kris-Etherton, P. M., Harris, W. S. and Appel, L. J. (2002). "Fish consumption, fish oil, omega-3 fatty acids, and cardiovascular disease." American Heart Association Nutrition Committee. *Circulation*. 106, **2747–2757.**

Lavoie R.A, Jardine T. D., Chumchal M. M., Kidd K A., and Campbell L. M. (2013). "Biomagnification of Mercury in Aquatic Food Webs: A Worldwide Meta-Analysis." *Environmental Science & Technology*; *47* (23), **13385-13394.**

Lewis R.J. (2005). "Sax's Dangerous Properties of Industrial Materials." *J. Am. Chem. Soc.*, 127 (8), **2794–2794.**

Mandana M. (2016). "Methylmercury and Human Embryonic Development." *Embryo Project Encyclopedia.* https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2659504/. Accessed on 23 March 2018.

Marrugo-Nerette J., Verbel J.O, Ceballos E.L, Benitez L.N. (2008). "Total mercury and methylmercury concentrations in fish from the Mojana region of Colombia." *Environ. Geochem. Health* 30(1)**:21-30.**

Mason, R. P and Sheu G. R. (2002). "Role of the ocean in the global mercury cycle." *Global Biogeochem. Cycles*, 16(4), **1093.**

Mason, R. P., Choi, A. L., Fitzgerald, W. F., Hammerschmidt, C. R., Lamborg, C. H., Soerensen, A. L., and Sunderland, E. M. (2012). "Mercury Biogeochemical Cycling in the Ocean and Policy Implications." *Environmental Research,* 119,**101–117.**

Miskimmin, B. M., Rudd J. W. M, and Kelly C. A. (1992). "Influence of dissolved organic carbon, pH, and microbial respiration rates on mercury methylation and demethylation in lake water." *Can. J. Fish. Aquat. Sci.*, 49: **17-22.**

Muhaya B.B.M., Leermakers M., Baeyens W. (1997). "Total mercury and methylmercury in sediments and in the polychaete *Nereis diversicolor* at Groot Buitenschoor (Scheldt estuary, Belgium)." *Water Air Soil Pollution;* 94:**109–23.**

Myers G. J., Davidson P. W., Watson G. E., Van Wijngaarden E., Thurston S. W., Strain J.J., Shamlaye C. F., and Bovet P. (2015). "Methylmercury exposure and developmental neurotoxicity" *Bulletin of the World Health Organization;* 93(2):**65-132.**

Nadorfy-Lopez E., Torres S. H., Finol H., Mendez M., Bello B. (2000). "Skeletal muscle abnormalities associated with occupational exposure to mercury vapors." *Histology and Histopathology*, 15(3), **673–682.**

NAS (National Academy of Sciences). (1991). "Seafood Safety. Committee on Evaluation of the Safety of Fishing Products." *National Academy Press*, Washington, DC.

National Research Council. (2000). "Toxicological Effects of Methylmercury." *Washington, DC: The National Academies Pres*s. Chapter 3, **37-71**

NEWMOA. Northeast Waste Management Officials' Association. (2018). "Volcanic-NEWMOA." http://www.newmoa.org/prevention/topichub/22/mercury_cycle.pdf. Assessed on Accessed on 2 May 2018.

Noelle E. S. (2009). "Global Biogeochemical Cycling of Mercury: A Review" *Annual Review of Environment and Resources,* 34(1), **43-63.**

O'Hara M. F., Charlap J.H., Craig R. C., Knudsen T. B. (2002). "Mitochondrial transduction of ocular teratogenesis during methylmercury exposure." *Teratology*, 65(3), **131-44.**

Oken E., Choi A.L., Karagas M.R., Marien K., Rheinberger C.M., Schoeny R., Sunderland E., Korrick S. (2012). "Which fish should I eat? Perspectives influencing fish consumption choices." *Environ. Health Perspect.*; 120, **790–798.**

Oken E., Radesky J.S., Wright R.O., Bellinger D.C., Amarasiriwardena C.J., Kleinman K.P. (2008). "Maternal fish intake during pregnancy, blood mercury levels, and child cognition at age 3 years in a US cohort." *American Journal of Epidemiology* 167: **1171–81.**

Paranjape A. R., and Hall B. D. (2017). "Recent advances in the study of mercury methylation in aquatic systems." *FACETS* , 2: **85–119.**

Park J-D and Zheng W. (2012). "Human Exposure and Health Effects of Inorganic and Elemental Mercury." *J Prev. Med. Public Health; 45:344*-**352.**

Park Jung D., Zheng W. (2012). "Human Exposure and Health Effects of Inorganic and Elemental Mercury." *Journal of Prev. Med. & Public Health*. 45(6), **344–352.**

Park K., Mozaffarian D. (2010). "Omega-3 fatty acids, mercury, and selenium in fish and the risk of cardiovascular diseases." *Curr. Atheroscler Rep.* 12, **414–422.**

Pilkington S. M., Watson R. E., Nicolaou A. and Rhodes L. E. (2011). "Omega-3 polyunsaturated fatty acids: Photoprotective macronutrients." *Exp. Dermatol***. 20**:**537–543.**

Pirrone N., Cinnirella S., Feng X., Finkelman R. B., Friedli H. R., et al. (2010). "Global mercury emissions to the atmosphere from anthropogenic and natural sources." *Atmos. Chem. Phys.,* 10, **5951–5964.**

Roman H. A., Walsh T. L., Coull B. A., Dewailly É., Guallar E., Hattis D., … Rice G. (2011). "Evaluation of the Cardiovascular Effects of Methylmercury Exposures: Current Evidence Supports Development of a Dose–Response Function for Regulatory Benefits Analysis." *Environmental Health Perspectives, 119*(5), **607–614.**

Scheuhammer, A., Meyer, M., Mark B. Sandheinrich, and Michael W. Murray. (2007). "Effects of Environmental Methylmercury on the Health of Wild Birds, Mammals, and Fish." *Ambio, 36*(1), **12-18.**

Shao D., Kang Y., Wu S., and Wong M.H. (2012). "Effects of sulfate reducing bacteria and sulfate concentrations on mercury methylation in freshwater sediments." *Science of The Total Environment,* 424:**331-336.**

Simonin H. A., Loukmas J. J., Skinner L. C., Roy K. M. (2008). "Lake variability: key factors controlling mercury concentrations in New York State fish." *Environ Pollut.* 154(1):**107-15.**
Southworth G.R., Ryon M. G., Peterson M.J. (2000). "Long-term increased bioaccumulation of mercury in largemouth bass follows reduction of waterborne selenium." *Chemosphere,* 41: **1101-1105**

Stern A. H. and Korn L. R. (2011). "An Approach for Quantitatively Balancing Methylmercury Risk and Omega-3 Benefit in Fish Consumption Advisories." *Environmental Health Perspectives*; 119(8):**1043–1046.**

Streets D.G, Devane M.K, Lu Z., Bond T.C., Sunderland E.M., Jacob D.J. (2011). "All-time releases of mercury to the atmosphere from human activities." *Environmental Science and Technology;* 45(24):**10485–10491.**

Tollefson, Linda. (1989). "Methylmercury in fish: Assessment of risk for U.S. consumers. In: The Risk Assessment of Environmental and Human Health Hazards". *A Textbook of Case Studies*, Dennis J. Paustenback (ed.), John Wiley & Sons, New York.

Torpy J.M., Lynm C., Glass R. M. (2006). "Eating Fish: Health Benefits and Risks." *Journal of the American Medical Association; 296*(15):**1926.**

Trudel M. R., Rasmussen J. B. (2006). "Bioenergetics and mercury dynamics in fish: A modeling perspective." *Can. J. Fish. Aquat. Sci.*; 63:1890–**1902.**

USDHHS and USDA. U.S. Department of Health and Human Services and U.S. Department of Agriculture.). (2015). "2015–2020 Dietary Guidelines for Americans." 8:**24.**

UNEP Chemical Branch. (2008). "The Global Atmospheric Mercury Assessment: Sources, Emissions and Transport." *UNEP-Chemical, Geneva*

USEPA. United State Environmental Protection Agency. (2018). "Advisories: What Not to Eat?" https://www.epa.gov/choose-fish-and-shellfish-wisely/fish-and-shellfish-advisories-and-safe-eating-guidelines#tabs-2 Accessed on 25 March 2018.

USEPA. United States Environmental Protection Agency. (2017a). "Methylmercury (MeHg)" https://cfpub.epa.gov/ncea/iris_drafts/recordisplay.cfm?deid=56751. Accessed on 25 March 2018.

USEPA. United States Environmental Protection Agency .(2017b). NATA: Glossary of Terms. https://www.epa.gov/national-air-toxics-assessment/nata-glossary-terms#hq. Accessed on 21 April 2018.

USEPA. United States Environmental Protection Agency. (2013). "Reference Dose (RfD): Description and Use in Health Risk Assessments" https://www.epa.gov/iris/reference-dose-rfd-description-and-use-health-risk-assessments Accessed on 25 March 2018.

USEPA. United States Environmental Protection Agency .(2000). "Guidance for Assessing Chemical Contaminant Data for Use in Fish Advisories, Volume 2: Risk Assessment and Fish Consumption Limits, Third Edition. Pp 38-39

USFDA. United States Food & Drug Administration .(2017a). "Mercury levels in commercial fish and shell fish (1910-2010)." https://www.fda.gov/food/foodborneillnesscontaminants/metals/ucm115644.htm. Accessed on 24 April 2018.

USFDA. United States Food & Drug Administration .(2017b). "Fish: What pregnant women and parents should know." https://www.fda.gov/Food/ResourcesForYou/Consumers/ucm393070.htm Accessed on 25 March 2018.

USFDA. United States Food & Drug Administration .(2017c). "Technical Information on Development of Fish Consumption Advice - FDA/EPA Advice on What Pregnant Women and Parents Should Know about Eating Fish" https://www.fda.gov/Food/FoodborneIllnessContaminants/Metals/ucm531136.htm Accessed on 24 April 2018.

USFDA/USEPA. United States Food & Drug Administration, United States Environmental Protection Agency .(2017). "FDA and EPA issue final fish consumption advice." https://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm537362.htm Accessed on 25 March 2018.

USFDA/USEPA. United States Food & Drug Administration, United States Environmental Protection Agency. (2014). "New Advice: Pregnant Women and Young Children Should Eat More Fish." https://www.fda.gov/forconsumers/consumerupdates/ucm397443.htm. Accessed on 23 March 2018.

Ward D. M., Nislow K. H., and Folt C. L. (2010). "Bioaccumulation syndrome: identifying factors that make some stream food webs prone to elevated mercury bioaccumulation." *Annals of the New York Academy of Sciences*; *1195*, **62–83.**

Weber J.H. (1993). "Review of possible paths for abiotic methylation of mercury(II) in the aquatic environment." *Science of The Total Environment,* 26(11):**2063-2077.**

Weiner J.G., Krabbenhoft D.P., Heinz G.H., Scheuhammer A.M. (2003). "Ecotoxicology of mercury. In: Hoffman, D.J., Rattner, B.A., Burton, G.A.J., Cairns, J.J. (Eds.)." *Lewis Publishers, New York*, **409–443.**

Weiss B., Clarkson T.W., and Simon W. (2002). "Silent Latency Periods in Methylmercury Poisoning and in Neurodegenerative Disease." *Environ Health Perspect* ; (5):**851–854.**

Wilson, S., Kindbom, K., Yaramenka, K., Steenhuisen, F., Telmer, K. (2012). *Part A: global emissions of mercury to the atmosphere*. UNEP/AMAP 2012, Technical Report.

Xu L., Chen J., Niu Z., Yin L., Chen Y. (2013). "Characterization of mercury in atmospheric particulate matter in the southeast coastal cities of China." *Atmos. Pollut. Res.,*4 (4) , **454-461**

Yamashita Y., Omura Y., Okazaki E. (2005). "Total mercury and methyl mercury levels in commercially important fishes in Japan." *Fisheries Science;* 71:**1029-1035.**

Ynalvez, R., Gutierrez, J., Gonzalez-Cantu, H. (2016). "Mini-review: toxicity of mercury as a consequence of enzyme alteration." *Biometals,* 29(5), **781.**

Zioła-Frankowska A., Dąbrowski M., Kubaszewski Ł., Rogala P., Kowalski A., and Frankowski M. (2017). "An analysis of factors affecting the mercury content in the human femoral bone." *Environmental Science and Pollution Research International*; 24(1), **547–557.**

APPENDIX A

Analytical results for the fish samples (Largemouth Bass and Alabama Spotted Bass)

Gerg ID	ClientID	Fish name	Site Number	Size	Fish body Part	Matrix	Receive Date	Digestion Date	Wet weight (g)	Mercury (ug/g) Wet	Reporting Limit(Wet)
C90073	AB2S	Alabama Spotted Bass	2	Small	Fillet	tissue	10/5/2017	1/9/2018	0.685	0.14	0.006
C90074	AB2M		2	Medium	Fillet	tissue	10/5/2017	1/9/2018	0.523	0.64	0.008
C90075	AB3S		3	Small	Fillet	tissue	10/5/2017	1/9/2018	0.512	0.296	0.008
C90076	AB3M		3	Medium	Fillet	tissue	10/5/2017	1/9/2018	0.607	0.602	0.007
C90077	AB3L		3	Large	Fillet	tissue	10/5/2017	1/9/2018	0.662	0.467	0.006
C90078	ABB2S		2	Small	Wholebody	tissue	10/5/2017	1/9/2018	0.765	0.088	0.005
C90079	ABB2M		2	Medium	Wholebody	tissue	10/5/2017	1/9/2018	0.539	0.373	0.007
C90080	ABB3S		3	Small	Wholebody	tissue	10/5/2017	1/9/2018	0.556	0.171	0.007
C90081	ABB3M		3	Medium	Wholebody	tissue	10/5/2017	1/9/2018	0.523	0.287	0.008
C90082	ABB3L		3	Large	Wholebody	tissue	10/5/2017	1/9/2018	0.645	0.282	0.006
C90083	LM1S	Largemouth Bass	1	Small	Fillet	tissue	10/5/2017	1/9/2018	0.596	0.521	0.007
C90084	LM1M		1	Medium	Fillet	tissue	10/5/2017	1/9/2018	0.551	0.497	0.007
C90085	LM1L		1	Large	Fillet	tissue	10/5/2017	1/9/2018	0.516	2.263	0.008
C90086	LM2S		2	Small	Fillet	tissue	10/5/2017	1/9/2018	0.57	0.349	0.007
C90087	LM2M		2	Medium	Fillet	tissue	10/5/2017	1/9/2018	0.65	0.443	0.006
C90088	LM2L		2	Large	Fillet	tissue	10/5/2017	1/9/2018	0.499	0.836	0.008
C90089	LM3M		3	Medium	Fillet	tissue	10/5/2017	1/9/2018	0.657	0.543	0.006
C90090	LM3L		3	Large	Fillet	tissue	10/5/2017	1/9/2018	0.603	1.386	0.007
C90091	LMB1S		1	Small	Wholebody	tissue	10/5/2017	1/9/2018	0.54	0.201	0.007
C90092	LMB1M		1	Medium	Wholebody	tissue	10/5/2017	1/9/2018	0.529	0.317	0.008
C90093	LMB1L		1	Large	Wholebody	tissue	10/5/2017	1/9/2018	0.516	1.807	0.008
C90094	LMB2S		2	Small	Wholebody	tissue	10/5/2017	1/9/2018	0.521	0.198	0.008
C90095	LMB2M		2	Medium	Wholebody	tissue	10/5/2017	1/9/2018	0.499	0.161	0.008
C90096	LMB2L		2	Large	Wholebody	tissue	10/5/2017	1/9/2018	0.547	0.453	0.007
C90097	LMB3M		3	Medium	Wholebody	tissue	10/5/2017	1/9/2018	0.527	0.253	0.008
C90098	LMB3L		3	Large	Wholebody	tissue	10/5/2017	1/9/2018	0.724	0.926	0.006

APPENDIX B

Mann-Whitney U-test comparing the mean MeHg concentrations analyzed in Largemouth and Alabama Spotted Bass.

Ranks

	Group	N	Mean Rank	Sum of Ranks
Fish	Largemouth Fillet	8	8.00	64.00
	Alabama Fillet	5	5.40	27.00
	Total	13		

Test Statistics[a]

	Fish
Mann-Whitney U	12.000
Wilcoxon W	27.000
Z	-1.171
Asymp. Sig. (2-tailed)	.242
Exact Sig. [2*(1-tailed Sig.)]	.284[b]

a. Grouping Variable: Group

b. Not corrected for ties.

Descriptives

	Group			Statistic	Std. Error
MeHg Concetration µg/g	Largemouth Fillet	Mean		.8548	.23252
		95% Confidence Interval for Mean	Lower Bound	.3049	
			Upper Bound	1.4046	
		5% Trimmed Mean		.8046	
		Median		.5320	
		Variance		.433	
		Std. Deviation		.65766	
		Minimum		.35	
		Maximum		2.26	
		Range		1.91	
		Interquartile Range		.79	
		Skewness		1.761	.752
		Kurtosis		2.697	1.481
	Alabama Fillet	Mean		.4290	.09410
		95% Confidence Interval for Mean	Lower Bound	.1677	
			Upper Bound	.6903	
		5% Trimmed Mean		.4333	
		Median		.4670	
		Variance		.044	
		Std. Deviation		.21042	
		Minimum		.14	
		Maximum		.64	
		Range		.50	
		Interquartile Range		.40	
		Skewness		-.531	.913
		Kurtosis		-1.516	2.000

APPENDIX C

Paired t-text used to compare MeHg concentrations in the Fish fillet and the fish whole-body of Alabama Spotted Bass and Largemouth Bass.

Paired Samples Test

		Paired Differences					t	df	Sig. (2-tailed)
		Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference Lower	Upper			
Alabama Bass	Fillet and Whole Body	.18880	.10588	.04735	.05733	.32027	3.987	4	.016
Lrge Mouth Bass	Fillet and Whole Body	-.31525	.11488	.04062	-.41130	-.21920	-7.761	7	.000

Mann Whitney U- text used to compare MeHg concentrations in the fish fillet and the fish whole-body of Alabama Spotted Bass

Test Statistics[a]

	Alabama
Mann-Whitney U	5.000
Wilcoxon W	20.000
Z	-1.567
Asymp. Sig. (2-tailed)	.117
Exact Sig. [2*(1-tailed Sig.)]	.092[b]

a. Grouping Variable: VAR00002

b. Not corrected for ties.

Ranks

	VAR00002	N	Mean Rank	Sum of Ranks
Alabama Spotted Bass	Fillet	5	7.00	35.00
	Wholebody	5	4.00	20.00
	Total	10		

Mann Whitney U- text used to compare MeHg concentrations in the fish fillet and the fish whole-body of Largemouth Bass.

Ranks

	Matrix	N	Mean Rank	Sum of Ranks
Largemouth Bass	Fillet	8	10.63	85.00
	Wholebody	8	6.38	51.00
	Total	16		

Test Statistics[a]

	Largemouth Bass
Mann-Whitney U	15.000
Wilcoxon W	51.000
Z	-1.785
Asymp. Sig. (2-tailed)	.074
Exact Sig. [2*(1-tailed Sig.)]	.083[b]

a. Grouping Variable: VAR00002

b. Not corrected for ties.