AN ABSTRACT OF THE DISSERTATION OF

Leanne K. Cusack for the degree of Doctor of Philosophy in Public Health presented on December 5, 2014.

Title: Evaluating the Public Health Risks of Methylmercury Exposure and Benefits from Omega-3 Fatty Acids and Selenium from Fish Consumption.

Abstract approved: _____

Anna K. Harding

Although fish are a nutritious food source, they also are the main source of methylmercury exposure in U.S. populations. This research examined the risks from methylmercury and benefits from omega-3 fatty acids and selenium from fish consumption. The first study provided the first region-specific quantitative risk/benefit analysis for nine commonly consumed freshwater fish species in the Columbia River Basin; which is home to many Native American Tribes and subsistence fishermen who consume large quantities of locally caught fish. (Donatuto and Harper 2008; Harper and Harris 2008)(Donatuto and Harper 2008; Harper and Harris 2008) My results showed that mountain whitefish and rainbow trout provided a net benefit for cardiovascular risk and improved infant visual response memory scores across all consumption rates in all subregions in which they were sampled. The second study examined the associations between region of residence, demographic characteristics and total blood mercury concentrations in a nationally representative sample of women of childbearing age, using the 1999-2010 NHANES database. Women who live in the Atlantic and Pacific coastal regions have the highest, and women in the Midwest have the lowest, methymercury concentrations in their blood.

The third study analyzed intra- and inter-specific variability in selenium: mercury molar ratios in ten species of freshwater fish from the Columbia River Basin to determine the potential application for fish consumption guidelines. Variability in selenium:mercury molar ratios was high within the fish species regardless of fish size. This variability within fish species warrants caution in using selenium:mercury ratios in risk assessment calculations. Overall, this research

demonstrates the complexities involved in crafting guidelines that consider both the risks and benefits of fish consumption.

Evaluating Public Health Risks of Methylmercury Exposure and Benefits from Omega-3 Fatty Acids and Selenium from Fish Consumption

by
Leanne K. Cusack

A DISSERTATION

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Doctor of Philosophy

Presented December 5, 2014
Commencement June 2015

Doctor of Philosophy dissertation of Leanne K. Cusack presented on December 5, 2014

APPROVED:

Major Professor, representing Public Health

Co- Director of the School of Biological and Population Health Sciences

Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

Leanne K. Cusack, Author

ACKNOWLEDGEMENTS

I would like to express my sincerest gratitude to Dr. Anna Harding for her continuous guidance throughout this process. I was lucky to have such a kind and helpful advisor that was willing to meet with me often, revise version after version of my paper. Thank you for providing me with so many great research and teaching opportunities. I would like to thank Dr. Molly Kile who always took the time to meet with me to hash out any problems and to ensure I was heading in the right direction. Your approach helped me become a better researcher. I would like to thank Dr. Ellen Smit who helped guide me through the process of working with large national datasets. You always make sure that the student's best interest is being pursued. I appreciate your passion and approach for problem solving that is so similar to mine and I hope to achieve the work life balance that you maintain.

To Dr. Barbara Harper, thank you for bringing this topic to our attention. You brought a unique perspective to the dissertation which is so important and so interesting. To Dr. Dave Stone, I am pretty lucky to have a graduate representative that is so knowledgeable in my research area. Thank you for always meeting with me to discuss possible areas of research and ways in which to pursue these possibilities. I would like to thank Dr. John Molitor for his help and guidance with these manuscripts. I was lucky to have the input and guidance from so many knowledgeable people along the way. Thank you Helen Reuda (EPA Region 10) and Collin Eagles-Smith (USGS) for providing me with data. Thank you, Dr. Bruce Hope for helping me to brainstorm ideas.

To my family and Chris's family, thank you for helping us out in so many ways so we could get through this. We truly appreciated all the help along the way and could not have done it without you. To my husband Chris, thank you for being so patient, understanding and flexible throughout the process. Completing two PhD's and having two kids along the way was not the simplest way to go, but we have managed! And to my amazing girls, Ayla and Lilly, thank you for being so great! Your laidback and sweet nature has certainly made the whole process easier than it could have been. And to all the dear friends we have made along the way, thank you for everything.

CONTRIBUTION OF AUTHORS

Dr. Anna Harding, Dr. Molly Kile, Dr. Ellen Smit, Dr. Dave Stone and Dr. Barbara Harper provided substantial contributions to the conception, design and interpretation of the data as well as the critical review and editing of the manuscripts

TABLE OF CONTENTS

TABLE OF CONTENTS (Continued)

LIST OF FIGURES

LIST OF FIGURES (Continued)

LIST OF TABLES

LIST OF ABBREVIATIONS AND ACRONYMS

AHA	American Heart Association
AMI	Acute myocardial infarction
ATSDR	Agency for Toxic Substances and Disease Registry
BIHg	Blood inorganic mercury
BOHg	Blood organic mercury (methylmercury)
BTHg	Blood total mercury
C	chemical concentration in fish tissue
CVD	cardiovascular disease
CDC	Centers for Disease Control
CHD	coronary heart disease
CWA	Clean Water Act
CRITFC	Columbia River Intertribal Fish Commission
CTUIR	Confederated Tribes of the Umatilla Indian Reservation
CRB	Columbia River Basin
DEQ	Department of Environmental Quality
DF	detection frequency
DHA	docosahexaenoic acid
DOE	Department of Energy
EMAP	Environmental Monitoring and Assessment Program
EPA	Eicosapentaenoic acid
FCR	Fish Consumption Rate
FDA	Food and Drug Administration
FS	fillet with skin
FW	fillet without skin
H-THg	Hair Total Mercury
IHg	inorganic mercury
LLD	lower limit of detection
LOD	limit of detection
LOAEL	lowest observed adverse effect level
MAX	maximum
MDC	minimum detectable concentration
MF	modifying factor
MIN	minimum
MI	myocardial infarction
NCHS	National Center for Health Statistics
NHANES	National Health and Nutrition Examination Survey
NLFA	National Listing of Fish Advisories
ND	not detected
NOAEL	no observable adverse effect level
PUFAs	polyunsaturated fatty acids
RfD	reference dose
THg	Total mercury
U.S. EPA	United States Environmental Protection Agency
UF	uncertainty factors

WB	whole body
USDA	U.S. Department of Agriculture
USEPA	U.S. Environmental Protection Agency
USGS	United States Geological Survey
VRM	visual recognition memory
WW	Wet weight

Units

ng/kg	nanograms per kilogram (ppt)
µg/kg	micrograms per kilogram (ppb)
g/day	grams per day
mg/kg	milligram per kilogram (ppm)
kg	kilogram
kg/g	kilogram per gram
mg/kg-day	milligram per kilogram-day

Evaluating the Public Health Risks of Methylmercury Exposure and Benefits from Omega-3 Fatty Acids and Selenium from Fish Consumption

CHAPTER 1 - INTRODUCTION

Fish is an important part of a balanced diet (Sidhu 2003). Fish and fish oil contain omega-3 polyunsaturated fatty acids (omega-3s) and selenium that play an important role in human health (Sidhu 2003). Fish also have a cultural importance for many communities and constitute a significant global commodity. Furthermore, fishing is an important commercial, recreational and subsistence activity (Close et al. 2002; Harper and Harris 2008).

Despite the benefits from eating fish, fish consumption is also the main exposure pathway for methylmercury. Mercury is a naturally occurring element in the earth's crust. It is a highly reactive heavy metal which is seldom found as a free element in nature (Mozaffarian 2009). In its elemental form, mercury is released into the environment through human activities such as mining and mine tailings, coal burning, trash incineration and industrial emissions and runoff (Egeland and Middaugh 1997; Pirrone et al. 2010; Streets et al. 2011). It is also emitted from chlorine production, dental amalgams, thermometers, and batteries (Mozaffarian 2009). When released into the air, mercury then deposits from rain and is eventually deposited into surface water where it accumulates in streams, lakes and oceans. There, microbial action of bacteria in the water and sediments can transform the inorganic forms of mercury into methylmercury. This organic form of mercury can be absorbed by fish, both through contact with the water they live in, and through the food chain. Tissue concentrations in fish are a function of local mercury contamination and on the size, life span and predatory nature of each fish species (Mozaffarian 2009). Methylmercury biomagnifies up the food chain such that long-lived predatory fish (which are also valued as a human food source) accumulate higher concentrations (Williams and Stern 2005).

Methylmercury is considered to be more toxic than inorganic mercury because of its ability to cross biological membranes such as the blood brain barrier and placental barrier. Subsequently, fetuses of pregnant women are susceptible to intoxication (Aschner and Aschner 1990; Mahaffey 1999; Silbernagel et al. 2011). The majority of mercury present in fish is methylmercury and approximately 90-95% is found in the muscle or fillet (Mahaffey et al.

2003). Methylmercury can also be found to a lesser extent in organs such as the liver but these parts are rarely consumed by the general U.S. population (Mahaffey et al. 2003).

There is a significant public health concern associated with the health effects from chronic-low dose exposure due to modest fish consumption. One serious health risk is present for fetuses who are exposed *in utero*. Minimata Bay, Japan was heavily contaminated by a petrochemical and plastics company that was discharging methylmercury contaminated wastewater into the bay and contaminating the fish. Residents in this area relied heavily on fish as a food source. Awareness of increased sensitivity of the human fetus to methylmercury occurred following the birth of infants showing severe cerebral palsy-like symptoms in Minimata Bay, Japan, despite the mothers displaying little to no manifestation of methylmercury poisoning (Harada 1995). This marked the historic recognition of the brain and nervous system as the primary target organ for methylmercury poisoning which resulted in a discernible distal sensory disturbances, constriction of visual fields, ataxia, dysarthria, auditory disturbances, and tremors (Clarkson et al. 2003; Harada 1995; McAlpine and Araki 1959).

Two longitudinal cohort studies, from the Faroe Islands and the Seychelles, have been following children through their teenage years, assessing neuropsychological performance as a function of current, childhood, and *in utero* exposure to methylmercury from fish consumption. The Faroe Islands study found a consistent association between neurobehavioral deficit and *in utero* exposure (Grandjean et al. 1997). No effects were observed initially in the Seychelles cohort (Davidson et al. 2000; Myers et al. 1996; Myers et al. 2003). A recent study of the Seychelles 9-year-old cohort has revealed a decrease in fine motor function which is associated with higher fetal exposure levels ($\geq$10 $\mu g\ g^{-1}$). Investigators believe that adverse effects may become apparent in the higher-order cognitive functions that develop with maturity (Davidson et al. 2006; Van Wijngaarden et al. 2006).

Another serious health concern is that methylmercury is a risk factor for cardiovascular endpoints through a variety of different mechanisms (Mergler et al. 2007; Mozaffarian 2009). These include cardiovascular disease (coronary heart disease, acute myocardial infarction (AMI), ischemic heart disease), blood pressure and hypertension effects as well as alterations in heart rate variability (Chan and Egeland 2004; Mergler et al. 2007).

In contrast to the negative health effects from fish consumption due to the exposure of methylmercury in fish, considerable evidence indicates that there are also numerous health

benefits. Consumption of fatty fish reduces coronary heart disease (CHD) mortality, which is the leading cause of death in developed and most developing nations (Mozaffarian 2006). Ingestion of fish has been associated with improvement of blood lipid profiles, lower blood pressure (Mozaffarian 2006), improvement in rheumatoid arthritis (Kremer 2000), enhanced eye and brain development (Fleith and Clandinin 2005), and improvement in neurologic and psychological disorders such as depression, schizophrenia, and Parkinson's disease (Calon and Cole 2007). Fish provide several beneficial components including those associated with consumption of omega-3s, improvement of cardiovascular endpoints and enhanced brain development. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) are typical and abundant omega-3s found in fresh and saltwater seafood and are the main focus when evaluating the benefits of fish consumption (Stern and Korn 2011).

Fish provide nutrients and high quality protein; yet fish-consuming populations must decide whether the benefits of eating fish outweigh the risks from also consuming methylmercury and other contaminants. In the U.S., many states have responded to the potential risks of methylmercury in fish by issuing fish consumption advisories. These advisories provide advice on reducing the risk of adverse health effects associated with methylmercury from eating fish caught in local waters. Most fish consumption advisories are based solely on risks of methylmercury, and do not attempt to incorporate the benefits from omega-3 fatty acids (Stern and Korn 2011).

The mere perception of risk regarding methylmercury in fish is often enough to discourage some from eating particular fish, and can lead to women in particular avoiding fish altogether (Hightower 2008). However, given the benefits to be gained from fish consumption, a continued trend away from fish consumption is of public health concern.

Region-specific Risk-benefit Analysis

The Columbia River Basin has frequent fish advisories in effect due to mercury contamination in different rivers, reservoirs and lakes. The Columbia River Basin is home to recreational fishers, high-end fish consumption populations, and many Native American tribes who rely upon fish for cultural and subsistence activities (Close et al. 2002; Harper and Harris 2008). It is, therefore, desirable to provide fish consumption advice that not only focuses on the risks from methylmercury, but also considers the benefits from the omega-3 fatty acids found in

fish. In this way we can address both immediate and cumulative long-term dietary benefits and risks while still offering species-specific consumption advice. No study, however, has used a quantitative approach to determine both the risks of methylmercury concentrations in fish and the benefits derived from the omega-3 fatty acids from consuming fish caught in the Columbia River Basin. The objective of this study was to provide region-specific risk-benefit analysis for nine commonly consumed fish species in the Columbia River Basin using a risk/benefit model developed by Ginsberg and Toal (Ginsberg and Toal 2009). This quantitative method for addressing key aspects of the fish risk/benefit issue by analyzing the health trade-offs from eating different fish species (Ginsberg and Toal 2009). Their approach uses established dose-response relationships found in previous studies for omega-3 fatty acid and methylmercury for common endpoints: cardiovascular disease in adults (CHD mortality of first myocardial infarction [MI]) and neurodevelopment in 6-month-old infants (visual recognition memory [VRM]).

This study is the first to apply Ginsberg and Toal's quantitative approach to fish species in the Columbia River Basin. Risk/Benefit Indices (RBIs) for fish consumption, using established dose–response relationships for positive and negative impacts on cardiovascular and neurodevelopmental health endpoints, were generated for fish species in the Columbia River Basin. This approach has the potential to help guide future fish consumption advisories and to provide species-specific fish consumption advice for nine commonly consumed fish species within the Columbia River Basin.

Methylmercury and Fish Consumption using NHANES Data

The second aspect of this research analyzed the associations between geography, fish consumption patterns and methylmercury exposure for women of childbearing age in the U.S. using data from the 1999-2010 National Health and Nutrition Examination Survey (NHANES). NHANES is a continuous national survey that evaluates the health and nutritional status of the non-institutionalized U.S. population. NHANES has been evaluating exposure to environmental chemicals for more than three decades. Blood mercury analysis was included since 1999. This study combined these data with the dietary component of the survey to determine the association between methylmercury and fish consumption in women of childbearing age (ages 16-49).

Children and developing fetuses are highly susceptible to the effects of mercury exposure. Mercury concentrations in fish and shellfish species can range from < 0.1 ppm for shellfish such as oysters and mussels, to many parts per million in the high end predatory fish such as tuna, swordfish and shark (Mahaffey et al. 2003). Freshwater fish such as walleye and northern pike have also been found to contain high concentrations of methylmercury (Munn and Short 1997). Therefore, the type of fish consumed as well as the quantity of fish consumed will both contribute to exposure levels.

This study investigated the role that geography has on methylmercury concentrations for women of childbearing age in the US and the trends in fish consumption by using NHANES data from 1999-2010. This study reinforces and expands upon previous observations that dietary exposure via fish consumption is an important route for methylmercury intake by the general population, and especially for racial/ethnic groups with higher fish consumption. Increased understanding of the fish species contributing to high levels of methylmercury levels in women of childbearing age and the demographic characteristics associated with these fish species will help focus interventions and recommendations to at risk sub-populations. Results from this study will provide a better understanding of the demographics and types of fish associated with high levels of methylmercury which can help physicians provide more targeted advice to women of childbearing age.

Selenium: Mercury Molar Ratios

The third aspect of this research examined the intra- and inter-specific variability in selenium: mercury molar ratios in ten species of freshwater fish from the Columbia River Basin in order to evaluate their potential application for fish consumption guidelines. While the benefits from omega-3 fatty acids are well known, recent studies have shown that selenium may offer a protective buffer to the negative health effects of methylmercury (Burger et al. 2012; Gochfeld et al. 2012; Kaneko and Ralston 2007). Selenium is a trace mineral that is essential to health. It is found in fish and seafood, as well as eggs, meat and vegetables (Choi et al. 2008). A deficiency level has been identified at low levels, but it is toxic at high levels and is regulated in the body (Eisler 1987). Selenium is an essential part of selenoproteins. These are important in antioxidant enzymes and are catalysts for the production of the thyroid hormone (Rayman 2000). The exact physiological functions that selenium exert in the brain are still not understood;

however, studies have found that selenium and certain selenoproteins continue to be maintained despite prolonged selenium deficiencies (Chen and Berry 2003; Whanger 2000). Studies have also shown that the content and intake of selenium varies considerably both within and between countries because of differences in geography, agronomic practices, food availability and preferences (Combs 2001; Rayman 2000).

Selenium's ability to prevent mercury toxicity is not new, as its significance has been recognized for more than 40 years (Kaneko and Ralston 2007). Since that time studies have demonstrated selenium's ability to counteract the adverse impacts of mercury exposure (Beijer and Jernelöv 1978; Iwata et al. 1973; Ohi et al. 1976; Watanabe 2001). Methylmercury is an irreversible selenoenzyme inhibitor (Watanabe et al. 1999) which impair both selenoprotein form and function. Methylmercury has a high binding affinity for selenium and thus excess selenium may chelate methylmercury and protect selenoproteins. Conversely, methylmercury may be viewed as creating a relative selenium deficiency. Studies on the protective effects of selenium are not conclusive. Newland et al. (2008) examined the effects of fetal methylmercury exposure to adults using animal models, and found that that diets rich in selenium did not uniformly protect against methylmercury. In 2010, Park and Mozaffarian reported that although fish consumption has shown a substantial reduction in cardiovascular risk, clinical trials have demonstrated mixed and inconclusive results for cardiovascular risk when selenium and methylmercury are both considered.

A group of researchers (Peterson et al. 2009; Ralston 2008; Ralston et al. 2008; Sørmo et al. 2011) have strongly suggested that excess selenium protects against mercury toxicity and that a selenium: mercury molar ratio greater than 1 is largely protective against the adverse effects of mercury. They argue that the selenium: mercury molar ratios should therefore be incorporated in risk assessment and regulation regarding mercury and fish consumption in humans. Others have maintained that although these ideas are intriguing and should be examined further, that it is unlikely that a single molar ratio would operate across different endpoints or effects (e.g. development, cognition, coordination, locomotion, and visual acuity) and species (Burger and Gochfeld 2012).

Molar ratios in fish can vary substantially in different water bodies due to differing amounts of either mercury or selenium concentrations (Reash 2012). Further, a greater understanding of the intra- and inter-specific variability in the molar ratios of edible fish tissue

must be pursued before selenium: mercury molar ratios may be viably considered in future risk assessments. The intra-specific variability in the selenium: mercury molar ratio needs to be sufficiently low in order to be useful in a regulatory context or in the issuance of consumption advice. It is also important to gain a better understanding of the different molar ratios between species to determine if this will help consumers make sound decisions about what species to eat by choosing fish low in mercury and high in selenium.

While a number of papers have examined the amounts and effects of methylmercury in fish, few papers have examined selenium: mercury molar ratios in fish. Studies investigating selenium: mercury molar ratios are more common in marine species as marine fish are known to have high concentrations of selenium (Burger and Gochfeld 2012; Burger et al. 2012; Burger and Gochfeld 2013; Burger et al. 2013; Gochfeld et al. 2012; Kaneko and Ralston 2007; Ralston 2008; Raymond and Ralston 2009). However, data on the molar ratios from freshwater fish are particularly limited, in part because the focus has been solely on the mercury levels that pose a risk to humans. The few papers that have studied molar ratios in freshwater fish have found varying, but not generally conflicting, results.

In addition, researchers have called for additional data regarding molar ratios in freshwater fish in order to examine the selenium: mercury molar ratios in individual fish. Burger and Gochfeld (2012) advise that it is also useful to compare the molar ratios for the same species from different regions and that more data are needed before meaningful ratios can be inferred for many species (Burger and Gochfeld 2012).

This aspect of the dissertation examined intra- and inter-specific variability in selenium: mercury molar ratios in ten species of fish from the Columbia River Basin. This is the first study to examine the intra- and inter-specific variability in selenium: mercury molar ratios from freshwater fish species in the Columbia River Basin. In addition, this research sought to determine if the intraspecific variation in the molar ratio is sufficiently low in order to use in developing fish consumption advice and regionally specific risk management decisions within the Columbia River Basin.

Specific Aims: The overall goal of this research was to examine the risks from methylmercury and benefits from omega-3 fatty acids and selenium from fish consumption. This goal was pursued in three different studies. The first study provided a regionally specific risk/benefit

analysis for nine commonly consumed freshwater fish species in the Columbia River Basin. The second study examined the intra- and inter-specific variability in selenium: mercury molar ratios in ten species of freshwater fish from the Columbia River Basin. The third study examined the regional variations in methylmercury distribution and fish consumption for the U.S. population using data from the 1999-2010 NHANES data base.

Specific Aim 1: To provide a region -specific risk/benefit analysis for nine commonly consumed freshwater fish species in the Columbia River Basin based on 4 different fish consumption values.

Hypothesis 1a: There is no net risk in terms of adult cardiovascular risk from bridgelip sucker, channel catfish, mountain whitefish, walleye, sturgeon, smallmouth bass, largescale sucker, rainbow trout or Chinook salmon caught in the Columbia River Basin. Species that yield a positive result will from Equation 1 will show a net benefit whereas a result less than 1 signifies an increase in risk.

Hypothesis 1b: There is no net risk to infant visual response memory from maternal consumption of bridgelip sucker, channel catfish, mountain whitefish, walleye, sturgeon, smallmouth bass, largescale sucker, rainbow trout or Chinook salmon caught in the Columbia River Basin. Species that yield a positive result will from Equation 2 will show a net benefit whereas a result less than 1 signifies an increase in risk.

Specific Aim 2: To analyze the associations between geography, fish consumption patterns and methylmercury exposure for women of childbearing age in the U.S. using data from the 1999-2010 NHANES.

Hypothesis 2a: There is no regional variation in total whole blood methylmercury levels in women of childbearing age in the U.S. between 1999 and 2010.

Hypothesis 2b: There is no change in the amount of fish consumed by women of childbearing age in the U.S. between 1999 and 2010.

Specific Aim 3: To examine intra- and interspecific variability in selenium: mercury molar ratios in ten species of fish from Columbia River Basin to characterize the potential application of this approach for fish consumption guidelines.

*Hypothesis 3a***:** Selenium: mercury molar ratios vary by fish species in the Columbia River Basin.

Hypothesis 3b: Selenium: mercury molar ratios vary within fish species in the Columbia River Basin.

Significance and Justification

Consumers are justifiably confused with hearing media reports advocating the benefits of fish while at the same time seeing health advisories that advise against eating fish that are high in methylmercury (Cohen et al. 2005; Ginsberg and Toal 2009; Oken et al. 2005). Methylmercury contamination poses a particular challenge to public health because the main exposure is through fish consumption which has known benefits for human health (Mergler et al. 2007).

My study uses a quantitative approach to examine the risks and benefits related to consumption of fish in the Columbia River Basin and offers useful advice for individuals. This approach highlights the beneficial aspects of fish and still cautions against riskier local fish species. My research also contributes new knowledge regarding selenium: mercury molar ratios in freshwater fish by using data from the Columbia River Basin which have not previously been analyzed. This information is needed to determine the utility of using selenium: mercury molar ratios if fish advisories and is the first study to examine the intra- and inter-specific variability in selenium: mercury molar ratios from freshwater fish species in the Columbia River Basin.

Finally, my research examines the geographical and temporal trends in fish consumption and whole blood methylmercury levels at the national level. NHANES sampling may not be consistent across survey cycles with respect to the representation of coastal areas, and thus observed differences may be due to geographical location of the participants. For this reason it is important to take into consideration geographical residence when determining trends in both blood methylmercury and fish consumption trends for women of childbearing age in the U.S. This study expanded upon previous work by using additional cycles of NHANES data (from 2005-2010) and the restricted geographical data to examine regional trends. Increased understanding of the fish species contributing to high levels of methylmercury levels in women

of childbearing age and the demographic characteristics associated with these fish species will help focus interventions and recommendations to at risk sub-populations. Results from this study will provide a better understanding of the demographics and types of fish associated with high levels of methylmercury which can help public health and health care professionals provide more targeted advice to women of childbearing age.

The study evaluating selenium: mercury molar ratios will be part of a series of papers being developed by the Western North America Mercury Synthesis Project. This project is based on collaboration between the Biodiversity Research Institute and the U.S. Geological Survey involving an interdisciplinary team of scientists and policy experts; it focuses on a tri-national synthesis of mercury cycling and bioaccumulation throughout Western North America with the intent of quantifying the influence of land use, habitat, and climatological factors on mercury risk. While the main focus is on larger landscape questions, exposure and effects of mercury on fish, wildlife and humans will also be examined. This study is the only research in the Western North America Mercury Synthesis Project devoted to human health aspects of mercury exposure. The selenium: mercury molar ratio study is useful to public health officials at the Oregon Health Authority who are interested in the effects of the molar ratios on mercury toxicity and the potential to incorporate these ideas and results into crafting fish consumption advisories.

CHAPTER 2 – LITERATURE REVIEW

Fish is a vital source of food and an important part of a balanced diet. The nutritional benefits garnered from fish relate to the utilization of proteins of high biological value, as well as both minerals and vitamins contained in fish (Sidhu 2003). Fish and fish oil contain omega-3 polyunsaturated fatty acids (PUFAs) and selenium that are known to play an important role in human health (Sidhu 2003). Fish also have a cultural importance for many communities and constitute a significant global commodity. Fishing is an important commercial, recreational and subsistence activity (Hughes 2014).

Health Risks from Methylmercury

The perception of fish as a healthy food has been tempered by concern regarding the potential harm from exposure to methylmercury present in fish (Chan and Egeland 2004; Mahaffey 1999; Mozaffarian 2009; Rice et al. 2003; Rice 2004). Mercury is a naturally occurring element in the earth's crust. It is third, after arsenic and lead, on the 2011 Agency for Toxic Substances and Disease Registry (ATSDR) priority list of 275 hazardous substances (Agency for Toxic Substances and Disease Registry 2013). The list includes substances that present the most significant potential threats to human health in the United States. Mercury is a highly reactive heavy metal which is seldom found as a free element in nature (Mozaffarian 2009). In its elemental form, mercury is released into the environment through human activities such as coal burning, trash incineration, mining and industrial emissions (Egeland and Middaugh 1997; Pirrone et al. 2010; Streets et al. 2011). It is also emitted from chlorine production, dental amalgams, thermometers and batteries (Mozaffarian 2009). Released into the air, mercury then deposits into surface water where it accumulates in streams, lakes and oceans. There, microbial action in the water and sediment transform inorganic mercury into methylmercury. This organic form of mercury can be absorbed by fish, both through contact with the water they live in, and through the food chain. Tissue concentrations in fish are a function of local contamination and on the size, life span and predatory nature of each fish species (Mozaffarian 2009). Methylmercury biomagnifies up the food chain by a factor of greater than 1 million; larger, long-lived predatory fish (those which are also valued as a human food source) accumulate higher concentrations (Williams and Stern 2005).

Methylmercury is considered to be more toxic than inorganic mercury because of its ability to cross biological membranes, such as the blood brain barrier and placental barrier. This renders fetuses of pregnant women susceptible to intoxication (Aschner and Aschner 1990; Mahaffey 1999; Silbernagel et al. 2011). Methylmercury found in fish is the primary mercury species of interest to human health, as it is more reactive and potentially toxic than elemental or inorganic mercury.

The majority of mercury present in fish is methylmercury and approximately 90-95% is found in the muscle or fillet (Mahaffey et al. 2003). Methylmercury can also be found to a lesser extent in organs such as the liver but these parts are rarely consumed by the general U.S. population (Mahaffey et al. 2003). Mercury is able to strongly bind to sulfhydryl groups, and this can alter the activity of a variety of enzymes, ion channels and receptors (Ralston et al. 2008).

Chronic exposures to methylmercury from prolonged intakes (such as 1-2 fish meals per day for 10+ years) of fish that have high levels of methylmercury can produce sensorimotor symptoms in adults; however, these can be reversed when mercury exposure is eliminated (Mozaffarian 2006). The increased sensitivity of the human fetus to methylmercury was established following the birth of infants showing severe cerebral palsy-like symptoms in Minimata Bay, Japan, despite the mothers displaying little to no manifestation of methylmercury poisoning (Harada 1995). This marked the historic recognition of the brain and nervous system as the primary target organ for methylmercury poisoning (Clarkson et al. 2003; Harada 1995; McAlpine and Araki 1959). The increased sensitivity to the fetal developing nervous system was reinforced in the early 1970s in the Iraqi poisoning outbreak. The outbreak occurred due to consumption of seed grain that had been treated with organomercurial fungicide containing methylmercury (Bakir et al. 1973). Children of pregnant women exposed *in utero* developed severe motor and sensory impairments and delayed mental development (Amin-Zaki et al. 1974). Symptoms expressed by the offspring exposed *in utero* bore a similar resemblance to those in Japan but within a shorter time period.

However, the majority of the population is not exposed to high doses of methylmercury. The public health concern is focused on the health effects from chronic-low dose exposure due to modest fish consumption. A body of evidence has been developed demonstrating that methylmercury is a risk factor for cardiovascular endpoints through a variety of different mechanisms (Mergler et al. 2007; Mozaffarian 2009). These include cardiovascular disease

(coronary heart disease, acute myocardial infarction [AMI], ischemic heart disease), blood pressure and hypertension effects as well as alterations in heart rate variability (Chan and Egeland 2004; Mergler et al. 2007).

Based on the severe adverse effects from methylmercury exposure in Japan and Iraq, a number of prospective epidemiological studies were performed with the intent of evaluating the effects of chronic low dose methylmercury exposure from consuming seafood during pregnancy. Several of these studies from different parts of the world (Crump et al. 1998; Grandjean et al. 1997; Grandjean et al. 1998; Jedrychowski et al. 2006; Oken et al. 2005) reported poorer neurological status and slower development in newborns, infants, and/or children exposed to methylmercury *in utero* and/or during early childhood.

Two major longitudinal cohort studies, from the Faroe Islands and the Seychelles, have been following children through teenage years. These studies have assessed neuropsychological performance as a function of current, childhood and *in utero* exposure. The study conducted in the Faroe Islands found a consistent association between neurobehavioral deficit and *in utero* exposure despite omitting from this study children whose mother's hair mercury levels were above 10 μg g^{-1}(Grandjean et al. 1997). No effects were observed initially in the Seychelles cohort (Davidson et al. 2000; Myers et al. 1996; Myers et al. 2003). A recent study of the Seychelles 9-year-old cohort has revealed a decrease in fine motor function which is associated with higher fetal exposure levels (≥10 μg g^{-1}). It has been suggested by the investigators that adverse effects may become apparent in the higher-order cognitive functions that develop with maturity (Davidson et al. 2006; Van Wijngaarden et al. 2006).

Health benefits from Omega-3 fatty Acids in Fish

Although there has been a demonstrated negative health impact from fish consumption due to the exposure to methylmercury in fish, considerable evidence indicates that there is also a wide range of health benefits from eating fish. Consumption of fatty fish reduces coronary heart disease (CHD) mortality, which is the leading cause of death in developed and most developing nations (Mozaffarian 2006). Ingestion of fish has been associated with improvement of blood lipid profiles, lower blood pressure (Mozaffarian 2006), improvement in rheumatoid arthritis (Kremer 2000), enhanced eye and brain development (Fleith and Clandinin 2005), and improvement in neurologic and psychological disorders such as depression, schizophrenia, and

Parkinson's disease (Calon and Cole 2007). Fish provide several beneficial nutrients, though most of the research on the benefits of fish consumption has focused on omega-3s, cardiovascular endpoints and enhanced brain development. Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are typical and abundant polyunsaturated fatty acids (PUFAs) found in fresh and saltwater seafood (Stern and Korn 2011).

Prospective cohort studies and clinical trials have shown a stable decline in mortality from coronary heart disease (CHD) with the an increase in omega-3 intake (Mozaffarian 2006). These studies revealed that CHD benefits were the strongest for oily fish such as salmon, herring, and sardines, which contain higher levels of omega-3s relative to leaner fish such as cod, catfish and halibut (Mozaffarian 2006).

DHA has been associated with an improvement in neurocognitive and ocular function throughout many different stages of life. Effects have been seen in the increased visual acuity of newborns (Uauy et al. 2003), better scores on neurodevelopmental test batteries (Fleith and Clandinin 2005; Oken et al. 2005) as well as in preventing neurological effects in adults (Calon and Cole 2007). Crucial growth periods occur in the brain between the beginning of the third trimester of gestation until approximately 18 months after birth (Innis 1991; Oken et al. 2005).

Fish Consumption Advisories

Different species of fish have varying amounts of both methylmercury and omega-3 fatty acids. There are fish which are known to contain high levels of omega-3s and relatively low levels of methylmercury such as anchovies, sardines, herring, and salmon. These fish will pose net benefits and as such consumption should be encouraged. There are also fish which are known to contain high levels of methylmercury and low levels of omega-3s, such as swordfish and shark, will pose a net risk and should be avoided. It becomes challenging when trying to develop advisories for fish that have intermediate levels of methylmercury and omega-3s, such as tuna, snapper, bluefish, sea bass, freshwater bass, and pike (Stern 2011).

Although fish provide nutrients and high quality protein, fish-consuming populations must decide whether the benefits of eating fish outweigh the risks from methylmercury and other contaminants. Federal, state and local governments issue fish consumption advisories when fish are unsafe to eat. The advisories generally give suggestions regarding specific fish to avoid or specific amounts considered safe to be consumed. In 2004, the EPA and FDA issued a joint fish

consumption advisory for selecting and eating fish or shellfish (U.S. EPA 2012). They recommend avoiding shark, swordfish, king mackerel, or tilefish due to high levels of mercury and to consume up to 12 ounces (2 average meals) a week of a variety of fish and shellfish that are lower in mercury (U.S. EPA 2012). The website lists shrimp, canned light tuna, salmon, pollock, and catfish as examples of the most commonly eaten fish that are low in mercury. The advisory reminds consumers to check local advisories about the safety of fish in their area and if no advisory is available, to consume up to 6 ounces (one average meal) per week of fish caught in local waters, but to not consume any other fish during that week to keep intake as low as possible (U.S. EPA 2012).

These fish consumption advisories are designed to reduce the risk of adverse health effects or health problems from eating fish caught in local waters, and nearly all fish consumption advisories for methylmercury are based solely on risks (Stern and Korn 2011). Fish consumption advisories can be issued for a specific water body or a water body-type such as lakes, reservoirs and rivers. Advisories can include recommendations to limit or avoid certain types of fish. They can apply to locally caught fish or fish purchased at stores or restaurants. They can be issued either to the general public, which includes recreational and subsistence fishermen, or for sensitive populations such as pregnant or nursing females and children. This approach of examining only risks often results in regulatory advisories that emphasize restrictions to minimize methylmercury exposure, but leave out the more substantial positive effects of fish consumption. This is a particularly controversial issue for pregnant women, who might be dissuaded from eating any fish during pregnancy. This is unfortunate, as consuming certain fish during pregnancy may be very healthy for both the mother and the developing fetus (Oken et al. 2012). For some people the mere perception of risk is enough to keep them from eating fish (Hightower 2008). Communicating these risks and benefits to the public in an easily understood fashion is therefore very important to provide clarity to this issue. In the past, state health departments in all 50 states have issued advisories warning the public about consuming certain species of fish in certain water bodies. Currently, there are advisories in the U.S. for 33 different chemical contaminants, including mercury. In the Columbia River Basin, the majority of direct exposure of the population to mercury occurs through the consumption of contaminated fish. Oregon currently has 12 fish advisories in effect due to mercury contamination (OHA 2014).

Blood Mercury Concentrations

The U.S. EPA has set a reference dose of 0.1 µg/kg body weight/day as an exposure level that does not elicit adverse effects. A reference dose is "an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily oral exposure to the human population (including sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime" (Agency for Toxic Substances and Disease Registry 2013; U.S. EPA 2013). A reference dose is generally used in U.S. EPA's non-cancer health assessments. The reference dose is equivalent to a benchmark dose of 5.8 µg/L of whole blood. A benchmark dose is a dose that produces a predetermined change in response rate of an adverse effect when compared to the background. The reference dose was based on cord blood measurements with fetal blood mercury concentrations of 5.8 µg/L. However, more recent studies have found that the differences between maternal and cord blood concentrations may be due to the bioaccumulation of methylmercury across the placenta (Butler Walker et al. 2006; Mahaffey et al. 2003; Mergler et al. 2007; Morrissette et al. 2004; Stern and Smith 2003). Cord blood mercury concentration may be on average as much as 70% greater than maternal blood mercury concentrations (Stern and Smith 2003). Results from these studies have led researchers to question the use of 5.8 µg/L as a reference value and suggest that 3.5µg/L may be more appropriate. Using the most recently available published estimates by Mahaffey et al. (2009) (who used 1999-2004 NHANES data), 10.4% of all women surveyed had blood mercury levels ≥ 3.5 µg/L and 4.7% had levels ≥ 5.8 µg/L which includes , respectively, 6.92 and 3.1 million women in the United States.

Quantitative Approach for Risk-benefit Analysis

Ginsberg and Toal (2009) developed a quantitative method for addressing key aspects of the fish risk/benefit issue by analyzing the health trade-offs from eating different fish species (Ginsberg and Toal 2009). The approach uses established dose-response relationships found for omega-3 fatty acid and methylmercury for common endpoints: cardiovascular disease in adults (CHD mortality of first myocardial infarction (MI)) and neurodevelopment in 6-month-old infants (visual recognition memory [VRM]) from previous studies. The end points measured for adults are very similar because both are measuring the health of coronary arteries; the CHD end point includes fatal MI and sudden death (Mozaffarian 2006), and when the first MI is not

necessarily fatal (Guallar et al. 2002). The omega-3 fatty acid benefit used in the equation is taken directly from the reported slope for change in relative risk per 100 mg/day intake of EPA + DHA (Mozaffarian 2006). However, the dose-response fails to take into account the effects of methylmercury. This could lead to an underestimate of the actual relationship, or suggest a plateau in benefit which, in reality, is an indication of methylmercury toxicity (Ginsberg and Toal 2009). The dose-response estimates for methylmercury effects on MI are based on the relationship between toenail mercury and MI odds ratios (Guallar et al. 2002). This approach uses the DHA-adjusted slope from Guallar et al. (2002) in the analysis. Because MI odds ratios often overestimate relative risks and the omega-3 fatty acid cardiovascular benefits described by Mozaffarian and Rimm (2006) were in terms of relative risk, the data from Guallar et al. (2003) were converted to relative risk using an equation provided by Zhang and Yu (1998) in order to provide a reasonable estimate of relative risk. Guallar et al. (2003) found that the dose-response for methylmercury effects on MI risk has a hair mercury threshold of 0.51 ppm before there is any evidence of adverse effects. For this reason, Ginsberg and Toal (2009) used this threshold in their estimate but acknowledge that is it a source of uncertainty.

Methylmercury and omega-3 fatty acids both affect infant VRM in opposing directions. VRM is a test that evaluates an infant's ability to translate a stimulus, such as a photograph, into memory and recognize a new stimulus as new and better to the old stimulus (Ginsberg and Toal 2009). The VRM test has been demonstrated to be predictive of IQ in later developmental stages (Rose and Feldman 1995). The slope used by Ginsberg and Toal for hair mercury effect on VRM score was taken from 135 mother-infant pairs in a study by Oken et al. (2005), who adjusted the slope according to self-reported fish consumption by the mothers.

Ginsberg and Toal (2009) used the following dose-response functions to estimate the effect of one or more fish meals on the outcomes measures:

Equation 1

$$Net\ risk/benefit\ for\ adult\ CHD = [(omega-3\ FA\ mg/meal) \times (no.meals/week) \times (1\ week/7\ days) \times (14.6\%\ lower\ risk/100\ mg\ omega-3\ FA)] - \{[(hair\ Hg\ change/fish\ meal) \times (no.meals/week)] - (0.51\ ppm\ hair\ Hg) \times (23\%\ higher\ risk/1\ ppm\ hair\ Hg)$$

Equation 2

$$Net\ risk/benefit\ for\ infant\ VRM = [(omega-3\ FA\ mg/meal) \times (no.meals/week) \times (1\ week/7\ days) \times (2\ VRM\ points/100\ mg\ omega-3\ FA)] - [(hair\ Hg\ change\ per\ fish\ meal) \times (no.meals/week) \times (7.5\ VRM\ points/1\ ppm\ hair\ Hg)]$$

A positive result from these equations indicate a net health benefit, whereas a result less than 1 signifies an increased risk. The risk/benefit equations include an exposure component which is based on the number of fish meals eaten per week along with both methylmercury and omega-3 fatty acid content from fish. The estimates of omega-3 fatty acids for specific fish species is based on data from the U.S. Department of Agriculture for omega-3 fatty acids, DHA and EPA (USDA 2013).

Methylmercury concentrations in fish (µg/g) were converted to a hair methylmercury concentration (µg/g) using a one-compartment model that relates methylmercury intake to hair mercury as used in the U.S. EPA's reference dose for mercury (Ginsberg and Toal 2000; Rice et al. 2003). A 6-oz meal size was used in the equation to match the recommendation used in the joint FDA/U.S. EPA seafood consumption advisory of two meals per week equivalent to 12 oz. of fish (U.S. EPA 2004). Using Guallar et al.'s (2002) dose response required the conversion of toenail mercury biomonitoring data to hair mercury. This was accomplished with the factor developed by Ohno et al. (2007) in which hair mercury in micrograms per gram = 2.44 × toenail mercury in micrograms per gram.

An important part of the risk/benefit equation is estimating the number of meals per week consumed by the population. The FDA's recommended consumption rate is a 170g meal size. But in 2011, Oregon's Department of Environmental recognized a fish consumption rate of 175g/day, an increase up from 17.5 g/day (Department of Environmental Quality 2011). This rate, however, may not reflect the fish consumption rate for subsistence or tribal populations. Oregon is home to a number of different Native American tribes. It has been suggested that disproportionate exposures can occur for sensitive or high fish consuming populations, such as Native Americans, when national average data are used to characterize risks (Donatuto and Harper 2008). For this reason we also considered the fish consumption rate of 540 g/day

suggested by Harris and Harper (1997) as the average consumption rate for traditional and subsistence fishers of the Confederated Tribes of the Umatilla Indian Reservation.

Methylmercury and Fish Consumption at the National Level

The National Health and Nutrition Examination Survey (NHANES) is a continuous national survey that evaluates the health and nutritional status of the non-institutionalized US population (CDC 2013). NHANES has served as a source of information on human exposure to environmental chemicals for more than three decades. Blood mercury analysis has been included in NHANES reports since 1999. These data combined with the dietary component of the survey allows researchers to determine the association between methylmercury and fish consumption in U.S. women of childbearing age (16-49). Therefore, different fish species consumptions along with quantity consumed can lead to varying levels of methylmercury exposure.

Once in the gastrointestinal tract, approximately 95% of the methylmercury is absorbed (Berglund et al. 2005). Within tissues, methylmercury is slowly demethylated to Hg^{2+} (Dock et al. 1994; Vahter et al. 1995). Total mercury concentration (THg) in blood is often used to measure of methylmercury exposure. It is assumed that the inorganic mercury (IHg) exposure leading to the THg in blood is much lower than from MeHg (Grandjean et al. 1992; Grandjean et al. 1997; Schober et al. 2003; Weil et al. 2005).

The concentration of total mercury in hair (H-THg) is also used as a measure of methylmercury exposure. It is assumed that > 80% of mercury in hair is in the form of methylmercury (Cernichiari et al. 1995). Mercury is assimilated in hair during development in the hair follicle is in equilibrium with the concentration of each mercury species in blood (Kershaw et al. 1980). Although it has been proposed that H-THg reflects inorganic mercury exposure at low methylmercury exposure in populations with no or low fish consumption (Berglund et al. 2005). The total mercury concentration in urine is used as a measure of IHg exposure, as methylmercury is excreted primarily via the bile and feces (about 90%; as IHg) and only to a limited extent (about 10%) in urine as IHg (Berglund et al. 2005; Clarkson 1990). Previous studies have shown the association between fish consumption and methylmercury in women of childbearing age (Mahaffey 2004; Mahaffey et al. 2008; Kathryn R Mahaffey et al. 2009; McDowell et al. 2004; Schober et al. 2003).

Previous NHANES Studies Examining Methylmercury Exposure

Due to the developing fetus susceptibility to methylmercury, NHANES surveys a subset of woman of childbearing age about their fish consumption habits. The data collected by NHANES for women ages 16–49 years are whole blood total mercury concentrations (μg/L), 24-h dietary recall data, and 30-day finfish and shellfish frequency of consumption and species of fish/shellfish consumed.

Schober et al. (2003) were one of the first groups to use NHANES data to examine the extent of methylmercury exposure to children and women of reproductive age using a nationally representative sample. The study found that mercury concentrations increased with age, and found that blood mercury levels were three times higher in women than in children despite no difference in number of fish servings consumed in the previous 30 days. However, these researchers did not include the 'other' race/ethnicity group in the analysis. Mercury levels in non-Hispanic Blacks were higher than in non-Hispanic Whites and Mexican Americans. This may be explained in part due to differences in toxico-kinetics, dose-body size relationships, dose frequency or unknown exposure in adults (Schober et al. 2003). Rather than examining fish by species, the study analyzed fish and shellfish consumption as a dichotomous variable (yes/no response), and also included the number of fish/shellfish meals consumed in the past 30 days.

McDowell et al. (2004) used hair mercury levels of children and women of childbearing age from the 1999-2000 cycles of NHANES data to assess similar associations as well as examining the relationship between total blood and hair mercury. The authors found that non-Hispanic black and Mexican-American children had higher levels of hair mercury than non-Hispanic white children. For adult females the opposite trend was found, with non-Hispanic white females having the highest hair mercury levels. In both children and adults, an increase in fish consumption corresponded to higher levels of hair mercury. A weighted Pearson correlation between log blood and log hair mercury was estimated at 0.67 for children and 0.79 for women.

Mahaffey et al. (2004) used the 1999-2000 NHANES dataset for the purpose of describing the association between total mercury in the blood, and calculated organic mercury intake of mercury from fish and shellfish for women of childbearing age. The authors found higher concentrations of mercury among the oldest age group, 30-49, compared to younger women. Participants who identified as 'other' race/ethnicity had the highest levels of mercury.

Non-Hispanic black women had the highest fish and shellfish consumption and women in the 30-39 age category consumed more fish than any other age group. 55% of the observed variability in blood methylmercury in women aged 30-39 yrs was explained by the quantity of fish and shellfish consumed.

A study by Hightower et al. (2006) focused on the blood mercury levels among those classified as "other" racial/ethnic group. This study found that of adult female participants who self-identified as 'other', 16.59% had blood mercury levels ≥ 5.8µg/L and 27.26% had levels ≥ 3.5µg/L (Hightower et al. 2006). Reports using NHANES data from 1999-2002 prior to this had not examined blood mercury levels in this race/ethnicity category. This group is of potential concern because it includes populations known to consume higher quantities of fish including Asians, Pacific Islanders and Native Americans.

All of these studies reported significant associations between race/ethnicity and methylmercury exposure from fish consumption. Women who self-identified as 'Other' race/ethnicity had the highest blood mercury concentrations when included in the analysis and Non-Hispanic Blacks had the greatest concentrations when 'Other' was not included.

Regional Variation and Temporal Trends in Fish Consumption and Methylmercury Exposure

A further study by Mahaffey et al., (2009) investigated the regional variations of methylmercury concentrations and fish consumption using NHANES data from 1999-2004. The authors used census regions (northeast, south, Midwest and west) and proximity to coastal areas to assess the associations between methylmercury and fish consumption, and also examined temporal trends in patterns of fish consumption and mercury levels. They found that the northeast had the highest *percentage* of women of childbearing age with mercury concentrations ≥ 3.5µg/L (19%) although the south had the largest *number* of women with ≥ 3.5µg/L (1.21 million) due to the elevated population in that region. When taking in to account costal proximity (living within 50 miles of a coastal area or the Great Lakes) all coastal areas showed elevated exposure as compared to their neighboring inland regions except in the Great lakes. Their analysis of temporal trends did not show any statistically significant differences between the three cycle years included, 1999-2000, 2001-2002 and 2003-2004.

Health effects associated with methylmercury exposure should focus on long-term exposure (Rice et al. 1989). In order to assess the association of long term exposure, it is necessary to determine whether chronic mercury exposure is increasing or decreasing over time in the U.S. population. As Mahaffey et al. (2009) have described the trends from 1999-2004, there is further need to determine the current state of methylmercury exposure. Exposure information for women of childbearing age is significant because of the potential health effects for developing fetuses.

A recent study by Birch et al. (2014) expanded upon previous research investigating the trends in blood mercury concentrations, fish consumption and mercury intake in women of childbearing age. They found that blood methylmercury concentrations in the 1999-2000 NHANES survey cycle to be significantly higher than the mean for later survey cycles, but they observed no trend in the amount of fish being consumed. The differences found may be attributable to the NHANES sampling design. The sampling may not be consistent across survey cycles with respect to the representation of coastal areas, and thus observed differences may be due to geographical location of the participants. For this reason it is important to take into consideration geographical residence when determining trends in both blood methylmercury and fish consumption trends for women of childbearing age in the U.S.

This study investigated the role that geography has on the trends in fish consumption and methylmercury concentrations for women of childbearing age in the U.S. by using NHANES data from 1999-2010. This study reinforces and expands upon previous observations that dietary exposure via fish consumption is an important route for methylmercury intake by the general population, and especially for racial/ethnic groups with higher fish consumption. Increased understanding of the fish species contributing to high levels of methylmercury levels in women of childbearing age and the demographic characteristics associated with these fish species will help focus interventions and recommendations to at risk sub-populations. Results from this study will provide a better understanding of the demographics and types of fish associated with high levels of methylmercury which can help public health and health care professionals provide more targeted advice to women of childbearing age.

Selenium:mercury Molar Ratios in Freshwater Fish

The benefits derived from fish consumption are not limited to those obtained from omega-3 fatty acids. Although recent studies have centered mainly on levels of mercury and omega-3s in different fish species, attention is now focusing on the levels and protective effects of selenium (Burger et al. 2012b; Gochfeld et al. 2012; Kaneko and Ralston 2007). Selenium is a trace mineral that is essential to health. It is found in fish and seafood, as well as in eggs, meat and vegetables (Choi et al. 2008). Although a deficiency level has been identified, it is also toxic at high levels and is regulated in the body (Eisler 1987). Selenium is a major component of selenoproteins, and are known to be important in antioxidant enzymes and catalysts for the production of the thyroid hormone (Rayman 2000). The exact physiological functions that selenium exerts in the brain are still not understood; however, studies have found that selenium and certain selenoproteins continue to be maintained despite prolonged selenium deficiencies (Chen and Berry 2003; Whanger 2000). Studies have also shown that content of foods and intake of selenium varies considerably both within and between countries due to differences in geography, agronomic practices, food availability and preferences (Combs 2001; Rayman 2000).

Selenium's Role in Protecting Against Mercury Toxicity

Selenium's ability to prevent mercury toxicity has been recognized for more than 40 years (Kaneko and Ralston 2007). Data from many studies have demonstrated selenium's ability to counteract the adverse impacts of mercury exposure using selenium derived from various fish species (Beijer and Jernelöv 1978; Iwata et al. 1973; Ohi et al. 1976; Watanabe 2001). Selenium's ability to diminish the toxicity of mercury has been well established in toxicology studies of insects, fish, bird and mammal species investigated to date (Cuvin-Aralar and Furness 1991; Kaneko and Ralston 2007; Peterson et al. 2009; Ralston et al. 2006).

Mercury and methylmercury have a high binding affinity for selenium and are irreversible selenoenzyme inhibitors. Thus, excess selenium may chelate mercury and protect selenoproteins or, conversely, mercury may be viewed as creating a relative selenium deficiency (Watanabe et al. 1999). A recent study showed that high maternal exposure to methylmercury in animals inhibits selenium-dependent enzyme activity in the brain while selenium supplementation is protective (Berry and Ralston, 2008). Recent attention is now shifting focus to determine if the toxicity of methylmercury is due to impaired selenium-dependent enzyme

synthesis or activity (Ralston 2008; Ralston et al. 2008; Raymond and Ralston 2009; Watanabe et al. 1999).

Studies regarding the protective effects of selenium are mixed and are not conclusive. Newland et al. (2008) examined the effects of selenium on adults exposed to fetal methylmercury using animal models, and found that diets rich in selenium did not uniformly protect against methylmercury effects. Park and Mozaffarian (2010) reported that although fish consumption has shown a substantial reduction in cardiovascular risk, clinical trials have demonstrated mixed and inconclusive results for cardiovascular effects of selenium and mercury. The interaction between selenium and mercury are complex and thus warrant further examination.

Selenium:Mercury Molar Ratios

Ganther et al. (1972) first proposed that a selenium:mercury molar ratio of 1:1 may provide a protective effect against mercury toxicity in fish. Luten et al. (1980) drew a similar conclusion with respect to both fresh and saltwater fish. Ralston et al. (2008) found that methylmercury in rats could not be predicted using methylmercury tissue concentrations alone and that toxicity was directly related to the selenium:mercury molar ratio in tissue. They found that the molar ratio is very sensitive to the denominator since selenium is an essential trace element and is physiologically regulated. Peterson et al. (2009) suggest that benchmark values for mercury toxicity in human and wildlife species based solely on mercury levels may exaggerate the mercury toxicity potential compared to an assessment that is based on selenium:mercury molar ratios (Peterson et al. 2009).

Ralston and others (Peterson et al. 2009; Ralston 2008; Ralston et al. 2008; Sørmo et al. 2011) have argued that excess selenium protects against mercury toxicity and that a selenium:mercury molar ratio greater than 1 is largely protective against the adverse effects of mercury. They advocate for incorporating the selenium:mercury molar ratios in risk assessment and regulation regarding mercury and fish consumption in humans. While Burger and Gochfeld (2012) have maintained that these ideas are intriguing, and should be examined further, they have also suggested that it is unlikely that a single molar ratio would operate across different endpoints or effects (e.g. development, cognition, coordination, locomotion, and visual acuity) and species.

The actual selenium:mercury molar ratio and contributing mechanisms that would protect against mercury toxicity remain unclear. If there were a universal and mutual bioavailability in which all selenium in the body was able to and did in fact bind to mercury in a 1:1 ratio, this would leave an inadequate amount of selenium to then synthesize enzymes and carry out its essential role. This would indicate that a protective molar ratio would indeed need to be greater than one, but how much greater than one is not clear.

Molar ratios in fish can vary substantially in different water bodies due to differing amounts of either mercury or selenium concentrations (Reash 2012). Before selenium:mercury molar ratios can be considered in future risk assessments, more information is needed regarding the intra- and inter-specific variability in the molar ratios of edible fish tissue. The intra-specific variability in the selenium:mercury molar ratio needs to be sufficiently low, and therefor reliable and consistent, in order to be useful in a regulatory context or in the issuance of consumption advice. It is important to understand if the ratios are sufficiently consistent within a species to be useful in advising consumers. It is also important to gain a better understanding of the different molar ratios between species to determine if this will help consumers make sound decisions about what species to eat by choosing fish low in mercury and high in selenium.

While there are a significant number of papers that examine the amounts and effects of methylmercury in fish, few papers have examined the selenium:mercury molar ratios in fish. Studies examining selenium:mercury molar ratios are becoming more common in marine fish species as they are known to have high concentrations of selenium (Burger and Gochfeld 2012; Burger et al. 2012b; Burger and Gochfeld 2013; Burger et al. 2013; Gochfeld et al. 2012; Kaneko and Ralston 2007; Ralston 2008; Raymond and Ralston 2009). However, data on the molar ratios from freshwater fish are particularly limited, in part, because the focus has been on the mercury levels that pose a risk to humans.

The few studies that have examined molar ratios in freshwater fish have produced inconsistent results. Peterson et al. (2009) found selenium:mercury molar ratios between 2.22-54.33 for 10 different species of fish from western U.S. streams. Cappon and Smith (1981) found ratios between 0.51 – 3.70 for six species of fish from lakes in New York. Burger et al. (2001) found ratios between 0.68 – 12.51 for 11 different fish species in the Savannah River. Burger et al. (2012) found ratios between 3.35 – 29.36 for 6 different fish species in Tennessee. Peterson et al. (2009) compared the excess methylmercury relative to the selenium:mercury molar ratio in

whole fish to human health criteria. However, most fish consumers eat only the fillet tissue of fish.

Both Peterson et al. (2009) and Burger et al. 2013) assert the need for more data on the varying molar ratios in freshwater fish and on establishing the selenium:mercury molar ratios of whole fish vs. filets. According to Burger and Gochfeld (2012), it is also useful to compare the molar ratios for the same species from different regions. In addition, more data are needed before meaningful ratios can be inferred for many species (Burger and Gochfeld 2012).

This is the first study to examine the intra- and inter-specific variability in selenium:mercury molar ratios from freshwater fish species in the Columbia River Basin.

CHAPTER 3 – MANUSCRIPT

A Quantitative Approach to Determine Cardiovascular and Neurodevelopment Risks and Benefits from Methylmercury and Omega 3 Fatty Acids in Fish Consumed in the Columbia River Basin

Leanne K. Cusack, MPH[1]

Anna K. Harding, PhD[1]

Molly Kile, PhD[1]

Barbara Harper, PhD[1,2]

Dave Stone, PhD[3]

[1]School of Biological and Population Health Sciences, College of Public Health and Human Sciences, Oregon State University, Corvallis Oregon

[2]Department of Science and Engineering, Confederated Tribes of the Umatilla Indian Reservation, Pendleton, Oregon State University

[3]Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, Oregon

Journal:

Environmental Health Perspectives

Submission Pending

ABSTRACT

Background: Fish are an important part of a well-balanced diet as it contains high quality proteins, as well as polyunsaturated fatty acids that are known to play an important role in human health. Consumption of fish, however, is the primary route of exposure to methylmercury in humans. A quantitative risk-benefit analysis was conducted with fish consumed in the Columbia River Basin, an area that is home to numerous fishing populations, including subsistence anglers and various cultural practices around fish.

Methods: We apply Ginsberg and Toal's (2009) quantitative risk-benefit approach to analyze risks of methylmercury and benefits of omega-3 fatty acids for nine different fish species found in the Columbia River Basin.

Results: The concentrations of methylmercury found in each fish species sampled varied by region and by species. In general, mountain whitefish and rainbow trout provided a net benefit in terms of both cardiovascular risk and neurodevelopmental across all consumption rates in all subregions in which they were sampled.

Conclusions: Species that provide a net benefit for cardiovascular risk in one region may not have the same benefits in other regions and may not necessarily provide an improvement in neurodevelopment within the same region. These findings highlight the importance of careful and clear communication of information regarding fish consumption and care needs to be given to ensure that the correct information will be interpreted by the consumer.

Key Words: risk/benefit, methylmercury, Columbia River Basin, fish consumption fish advisories, cardiovascular risk, neurodevelopment

INTRODUCTION

Fish is an important part of a balanced diet (Herger 2012; Sidhu 2003). Fish and fish oil contain omega-3 polyunsaturated fatty acids (omega-3s) and selenium that play an important role in human health (Sidhu 2003). Consumption of fish, however, is the primary route of exposure to methylmercury in humans. This presents a challenge to both consumers and public health authorities because different species of fish have varying amounts of both methylmercury and omega-3 fatty acids. Also, very little research has been done to determine which wild fish species may present more health benefits than health risks and no quantitative risk-benefit analysis has been conducted with fish consumed in the Columbia River Basin. This is an area that is home to recreational fishers, high fish consumption populations, and has many Native American tribes that rely upon fish for cultural and subsistence activities (Close et al. 2002; Harper and Harris 2008).

Docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA), are common and abundant omega-3s found in fresh and saltwater fish (Stern and Korn 2011). Consumption of fatty or oily fish such as salmon, herring, and sardines, which contain higher levels of omega-3 fatty acids relative to leaner fish such as cod, catfish and halibut, is associated with reduced mortality from coronary heart disease (CHD) which is the leading cause of death in developed and most developing nations (Mozaffarian 2006). For adults, ingestion of fish has been associated with improvement of blood lipid profiles, lower blood pressure (Mozaffarian 2006), improvement in rheumatoid arthritis (Kremer 2000), reduced risk of heart disease for the general population (IOM 2006) and improvement in neurologic and psychological disorders such as depression, schizophrenia, and Parkinson's disease (Calon and Cole 2007).

Fish oils such as DHA have been associated with a number of beneficial effects on neurocognitive and ocular function in both early and later stages of life, such as increased visual acuity of newborns (Uauy et al. 2003) and better scores on neurodevelopmental test sequences (Fleith and Clandinin 2005; Oken et al. 2005). The growth of the human brain is the fastest between the beginning of the third trimester of gestation and about 18 months after birth (Innis 1991; Oken et al. 2005). It is at this stage that the demand for omega-3s is the greatest. Studies have demonstrated that insufficient supplies of omega-3s and other nutrients during this critical period may result in deficits in brain development (Innis 1991; Oken et al. 2005).

The perception of fish as a healthy food, however, has been rightfully tempered by concern regarding the potential harm from exposure to methylmercury present in fish (Chan and Egeland 2004; Domingo 2007; Mahaffey 1999; Mozaffarian 2009; Oken et al. 2005; Rice et al. 2003; Rice 2004). Larger, long-lived predatory fish, which tend to be valued as a human food source, accumulate higher concentrations of methylmercury compared to smaller, non-predatory fish. Approximately 90-95% of the methylmercury is found in the muscle or fillet of fish (Mahaffey et al. 2003).

Methylmercury is a risk factor for cardiovascular disease [coronary heart disease, acute myocardial infarction (AMI), ischemic heart disease], blood pressure and hypertension effects as well as alterations in heart rate variability (Berglund et al. 2005; Chan and Egeland 2004; IOM 2007; Mergler et al. 2007). Part of methylmercury's toxicity also stems from its ability to cross biological membranes such as the blood brain barrier and the placental barrier which can influence the developing fetus (Aschner and Aschner 1990; Mahaffey 1999; Silbernagel et al. 2011).

All 50 states have responded to the presence of methylmercury in fish by issuing fish consumption advisories warning the public about consuming certain species of fish in specific water bodies. The U.S. Environmental Protection Agency (U.S. EPA) has developed guidelines to ensure consistency among state risk assessment agencies when developing these advisories. The fish consumption advisories are based on results from a risk assessment process that integrates information on contaminant presence in fish, the potential human exposure to these contaminants, and the potential health risks of exposure. Fish advisories do not consider the health benefits from omega-3. Oregon currently has 14 fish advisories in effect due to methylmercury contamination (OHA 2014). However, Ginsberg and Toal (2009) developed a quantitative method for addressing key aspects of the fish risk/benefit issue by analyzing the health trade-offs from eating different fish species (Ginsberg and Toal 2009). This approach uses established dose-response relationships for omega-3 fatty acid and methylmercury for common endpoints which includes: (1) cardiovascular disease in adults (measuring either CHD mortality or first myocardial infarction [MI]); and (2) neurodevelopment in 6-month-old infants (visual recognition memory [VRM]). The cardiovascular end points measured for adults are very similar because both are measuring the health of coronary arteries; the CHD end point includes fatal MI and sudden death (Mozaffarian 2006), whereas the first MI is not necessarily fatal (Guallar et al.

2002). VRM is a test that evaluates an infant's ability to encode a stimulus (photograph) into memory and recognize a new stimulus as novel and preferential to the old stimulus. This test is predictive of IQ at later developmental stages (Ginsberg and Toal 2009; Rose and Feldman 1995).

We employed Ginsberg and Toal's (2009) approach to identify which locally caught fish within the Columbia River Basin are most beneficial for neurodevelopmental and cardiovascular health outcomes which might balance out the potential risks of methylmercury exposure. This approach enables us to place species that are commonly consumed by both recreational fishermen as well as many Native American tribes that reside within the Columbia River Basin into different consumption categories. While this study is not meant to imply that omega-3s eliminate the health risks of methylmercury, this research contributes data regarding which species can be consumed in order to increase omega-3s while ensuring that methylmercury exposure stays below the reference dose of 0.1 µg/kg/day. The consumption rates used in the analysis can also be compared with rates that are currently recommended by the joint fish consumption advisory issued by the Food and Drug Administration (FDA) and U.S. EPA as well as Oregon's current fish consumption rates used to set water quality standards (Department of Environmental Quality 2011; U.S. EPA 2012). Based on known methylmercury levels in common Columbia River Basin species, our goal was to determine how to obtain the recommended amount of omega-3s from fish while considering the methylmercury contamination levels and omega-3 levels of various species and locations. We examined the risks and benefits from the following species; bridgelip sucker, channel catfish, mountain whitefish, walleye, sturgeon, smallmouth bass, largescale sucker, rainbow trout or Chinook salmon caught in nine different locations throughout the Columbia River Basin.

METHODS

Geographic Location and Fish Species

Nine locations within the Columbia River Basin were included in this analysis (Table 3.3). These locations were chosen based on availability of methylmercury data in recreationally and commercially important fish species caught at these locations. Fish species were included in the following risk/benefit analysis based on a fish consumption survey conducted by the Columbia River Inter-Tribal Fish Commission (CRITFC), which is the only comprehensive

survey of fish consumption that has been conducted for the Columbia Basin (CRITFC 1994). Results of this survey indicated that the most commonly consumed fish species for members of the Nez Perce, Umatilla, Yakama, and Warm Springs Tribes were: Chinook salmon (*Oncorhynchus tshawytscha*), rainbow trout (*Oncorhynchus mykiss*), mountain whitefish (*Prosopium williamsoni)*, sturgeon (Acipenser transmontanus), walleye (Stizostedion vitreum), bridgelip sucker (*Catostomus columbianus)*, largescale sucker (*Catostomus macrocheilus*), Channel catfish (*Ictalurus punctatus)* and smallmouth bass (*Micropterus dolomieu*) (CRITFC 1994).

Methylmercury in fish tissue

Fish tissue methylmercury data used were obtained from the U.S EPA (Rueda, Helen, U.S. EPA Region 10, Compiled fish tissue methylmercury data for the Columbia Basin, excel spreadsheet, received 2/25/13). We used data from 1999 to 2006 because the analytical techniques for measuring methylmercury remained consistent during this time period. The median total methylmercury concentration was derived by averaging the methylmercury concentrations from 1999-2006 for each species by sub-region as well as a total basin-wide average. Methylmercury concentrations are presented in wet weight values as mg kg^{-1} (ppm; μg g^{-1}). All fish tissue samples are from the Columbia River Basin.

A number of samples had only whole body methylmercury concentrations. Therefore we derived methylmercury fillet concentrations using the following (Bevelhimer 1996):

$C_f = C_{wb}/ 0.7$ where:

C_{wb} = whole-body methylmercury concentration (mg/kg) wet weight

C_f = fillet methylmercury concentration (mg/kg) wet weight

Any observation that was missing the full species common name or did not specify the sample type (whole-body versus fillet) was removed from the dataset. Additionally, samples were analyzed as either single fish or as a composite of multiple fish. The number of fish in a composite sample was not always included in the dataset. Sampling theory would predict that composite samples containing large numbers of individuals should provide more accurate estimates of the mean response than samples composed of a few individuals (Wente 2004). Therefore, statistical weights were applied to each fish tissue mercury observation in the dataset so that samples of individual fish receive a statistical weight of 1 and composite samples receive

statistical weights equal to the number of fish included in the composite (Christensen et al. 2006).

USDA Omega-3 Concentrations in Fish

The risk/benefit equations contain exposure components based on the omega-3 fatty acid content of the fish which is species specific. We used the U.S. Department of Agriculture's (USDA) National Nutrient Database for Standard Reference Release 26 species specific values for fish cooked in dry heat (USDA 2013). There was no distinction between bridgelip and largescale sucker in the database, so both fish used the value derived from 'Fish, sucker, white, cooked dry heat'. The values for mountain whitefish were derived from 'Fish, whitefish, mixed species, cooked, dry heat' as mountain whitefish was not available. A category for smallmouth bass did not exist so 'Fish, bass, freshwater, mixed species, cooked, dry heat' values were used. Sturgeon data were derived from 'Fish, sturgeon, mixed species, cooked, dry heat'. Table 3.1 shows the eicosapentaenoic acid and docosahexaenoic acid content for each species.

Fish Consumption Rates

The equation was run using 4 different consumption rates. A rate of one 170g meal per week was used in order to compare to current fish consumption advisories in the Columbia River Basin (Table 3.2). Two meals per week were evaluated to be consistent with the U.S.EPA and FDA current recommendations for eating two 170g meals per week. A rate of 7.2 meals per week was used to reflect Oregon's current fish consumption estimate (175g/day). And finally a rate of 25.53 meals per week was used to reflect the traditional subsistence fish consumption rate for the Native American tribes residing in the Columbia River Basin members who consume 500lbs per capita annually (620g/day) (Harper and Harris 2008).

Methylmercury conversions using a one-compartment model

The Ginsberg and Toal's (2009) methodology necessitated that methylmercury concentrations in fish (µg/g) be converted to a hair methylmercury concentration (µg/g). This was done using a one-compartment pharmacokinetic model that relates methylmercury intake to hair mercury as used in the U.S. EPA's reference dose for methylmercury which is 0.0001mg/kg - day (Ginsberg and Toal 2000; Rice et al. 2003). The model (Figure 3.1) simulates

methylmercury uptake, distribution, and elimination according to first order processes using input parameters for adult women. A 170g meal size was used in the equation to match the recommendation used in the joint U.S. Food and Drug Administration (FDA) and U.S. EPA seafood consumption advisory. (U.S. EPA 2012).

The model derives hair mercury concentrations from a specified methylmercury dose in a single meal by assuming that 95% of methylmercury that is ingested from any fish meal will be absorbed from the gastrointestinal tract and that 5% of the absorbed dose is distributed within a blood volume of 5L (Ginsberg and Toal 2000, 2009). The model also assumes that methylmercury clearance from blood (primarily by means of metabolism to inorganic mercury followed by fecal excretion) occurs at a rate of 1.4% per day for a blood half-life of 49.5 days, and that the ratio of the hair concentration (in ppb) to blood concentration (mg/L) is 250 (Ginsberg and Toal 2000). This is the same model the U.S.EPA used to calculate the current methylmercury reference dose (U.S. EPA 2014).

Ginsberg and Toal's (2009) approach uses the following equations to evaluate the cardiovascular (CHD) and neurodevelopment (VRM) risks and benefits. Benefits for adult CHD are defined in the following equations as a percent reduction in relative risk per 100 mg of omega-3s per day and risk is defined as increased relative risk per 1 ppm hair mercury for coronary heart disease. Benefits of infant visual recognition memory are defined as an increase of 2 VRM points per 100 mg of omega-3s per day and the risk is defined as decrease in 7.5 VRM points per 1 ppm hair mercury. Species that yield a positive result from these equations have a net benefit, whereas a result < 1 signifies an increased risk. The same input parameters were used in equations 1 and 2.

Equation 1

$$
\begin{aligned}
&Net\ risk/benefit\ for\ adult\ CHD \\
&\quad = [(omega-3\ FA\ mg/meal) \times (no.meals/week) \times (1\ week/7\ days) \\
&\quad \times (14.6\%\ lower\ risk/100\ mg\ omega \\
&\quad -3\ FA)] - \{\left[\left(hair\ Hg\frac{change}{fish}meal\right)\right. \\
&\quad \times \left.\left(no.\frac{meals}{week}\right)\right] - (0.51\ ppm\ hair\ Hg)\} \\
&\quad \times (23\%\ higher\ risk/1\ ppm\ hair\ Hg)
\end{aligned}
$$

Equation 2

$$Net\ risk/benefit\ for\ infant\ VRM = [(omega-3\ FA\ mg/meal) \times (no.meals/week) \times (1\ week/7\ days) \times (2\ VRM\ points/100\ mg\ omega\ -3\ FA)] - [(hair\ Hg\ change\ per\ fish\ meal) \times (no.meals/week) \times (7.5\ VRM\ points/1\ ppm\ hair\ Hg)]$$

The risk/benefit equation was run for each species in 9 different locations (Table 3) throughout the Columbia River Basin.

Statistical Analysis

The omega-3 cardiovascular benefit used in the equation was in terms of improved relative risk. Relative risk in this equation is the ratio of the probability of coronary heart disease occurring in an exposed group (those who consume fish) to the probability of coronary heart disease occurring in a non-exposed group (those who do not consume fish).

RESULTS

Table 3.4 shows the mean, median, minimum and maximum methylmercury concentrations by species and different locations in the Columbia River Basin. Overall walleye had the highest median methylmercury concentrations in the Columbia River Basin, although methylmercury concentrations varied considerably between different locations. The lowest median concentration of methylmercury for all species in the Columbia River Basin was found in Chinook salmon. Subregion1704, the Upper Snake region, had the highest average methylmercury concentration in the Basin and subregion 1702, the Upper Columbia, had the lowest average methylmercury concentrations in fish.

Cardiovascular endpoints

Figure 3 shows the net benefit or risk of fish consumption on coronary heart disease (CHD) mortality and myocardial infarctions (MI) for various fish consumption rates of individual fish species found in the Columbia River Basin. The percent improvement in relative

risk varies by fish species and by location. These results suggest that the cardiovascular benefits of omega-3s are greater than the risk from methylmercury for many fish consumption rates and for many fish species analyzed in the Columbia River Basin.

Across all regions we see that there are only two subregions, 1708 and 1709, in which all species of fish sampled provided a net benefit for CHD. However, in subregions 1701 and 1702 all fish provided a net benefit when consumed once per week. In subregions 1703 and 1707 all fish sampled could be consumed up to a rate of 25 meals per week and obtain a net benefit with the exception of largescale sucker in subregion 1703 and catfish in subregion 1707 which do not provide a benefit at any consumption rate.

Neurodevelopmental endpoints

The estimated risk/benefits for predicted VRM scores based on different fish consumption patterns are described in Figures 3.4a and b. The percent improvement in the predicted change in VRM scores varies by fish species and by location, and the pattern is not similar to those for cardiovascular risk. In each region, fish species that had provided a net benefit in terms of CHD do not necessarily provide a benefit in terms of neurodevelopment. Approximately half of the fish species sampled in all of the subregions provide a negative predicted change in VRM scores. For example, smallmouth bass provided a net benefit for adult CHD at all consumption rates in subregion1701 but would result in a negative predicted change in VRM scores across all consumption rates. Where largescale sucker and walleye were safe to consume in terms of adult CHD in some regions, they result in a negative predicted change in VRM score in every region sampled.

Neurodevelopment and cardiovascular risks by species

When considering the net risks and benefits on CHD and VRM scores of fish consumption by species we find that mountain whitefish, rainbow trout, Chinook salmon, bridgelip sucker and consuming a fish basket confer a net benefit in terms of CHD across all consumption rates in each region that they were sampled. The net benefit refers to optimal omega-3intake while limiting the methylmercury exposure to less than 0.1 µg/kg/day for daily ingestion. The first three species also had the lowest overall basin-wide methylmercury concentrations with mountain whitefish having a median concentration of 0.078 µg/g; Chinook

salmon having a median of 0.013 µg/g; and, rainbow trout having a median of 0.066 µg/g (see Table 3.4). Bridgelip sucker which has moderately high levels of omega-3s (1046 mg/g) had net improvements in relative risk of CHD but did not have a consistent direction in predicted change in VRM scores. This species had a very minor positive effect on VRM scores for two of the regions and a negative effect on VRM scores for the other two regions in which it was found. Benefits from the other species varied enough by region that no consistent advice could be given.

An improvement in VRM scores was seen across all fish consumption rates for mountain white fish and rainbow trout in all regions. Both walleye and sturgeon had net risks at all fish consumption rates. Smallmouth bass and largescale sucker were the most variable in terms of net benefit and risk for both end points. No consistent pattern was evident for these species throughout the different regions. While both have relatively high amounts of omega-3s (1046 mg/g in largescale sucker and 1297 mg/g in smallmouth bass), their methylmercury concentrations varied considerably. Sturgeon showed a positive effect for percent improvement in relative risk and a negative effect of VRM scores. Catfish had a net benefit for both CHD and neurodevelopment in one location, 1702, where it had substantially lower median methylmercury concentrations and was found to have a net risk on both endpoints in all other locations. Walleye was found to have a net benefit for cardiovascular disease when less than 7.2 meals were consumed per week in subregion 1702, however it displayed a net risk for VRM score in this region. In the other subregions it had a net risk for both endpoints across all consumption rates.

DISCUSSION

This study is the first to conduct a region-specific and species-specific risk and benefit analysis for locally caught fish within the Columbia River Basin. The effects were modeled based on different fish consumption rates that are reported in the region. These estimated effects on cardiovascular health and VRM assume long-term consumption of fish at these intake rates. The present research highlights the findings that methylmercury and omega-3 concentrations found in some species varies enough such that it would be unwise to provide fish consumption advice for the entire Columbia River Basin, nor for an entire state. The data show that some species have generally lower methylmercury concentrations and relatively high omega-3s and can be consumed at much greater frequency than the recommended amount of twice per week.

With respect to methylmercury, very few species can be safely consumed at the rates which were traditionally consumed by local Native American tribes (25 meals/week or 620g/day).

We observed that at all intake rates and all geographical regions in the Columbia River Basin, mountain whitefish, rainbow trout and Chinook salmon all have positive predicted net cardiovascular benefits and VRM scores. This can be attributed to the high amount of omega-3s found in these species (mountain whitefish - 2740 mg/g, rainbow trout - 1680 mg/g and Chinook salmon - 2953 mg/g respectively) and the lowest median methylmercury concentrations (whitefish - 0.078 µg/g, Chinook salmon - 0.013 µg/g rainbow trout - 0.066 µg/g. When omega-3 concentrations were lower, such as in bridgelip sucker (1046 mg/g) we observed a positive predicted net benefit to CHD but did not observe any consistent pattern for VRM scores. This species had a very minor positive effect on VRM scores for two of the regions and a negative effect on VRM scores for the other two regions in which it was found. This is likely due to the range of methylmercury values found in this species (see Table 3.4). Sturgeon showed a positive effect for percent improvement in relative risk and a negative effect of VRM scores. This is likely caused by the methylmercury threshold of 0.51 ppm for cardiovascular endpoints and not in the infant neurodevelopment equation because white sturgeon had a median methylmercury concentration of 0.101 µg/g in the only location it was sampled.

In regions such as 1701, 1703 and 1705 we saw worsening of relative risk of CHD when largescale sucker was consumed more than once per week, yet in other regions it could be consumed up to 25 meals per week. When looking at the median mercury levels in these regions we see a clear divide in which regions 1701, 1703 and 1705 have median values close to the U.S.EPA's recommended level of 0.3ppm of mercury in fish for safe consumption. We see similar patterns for smallmouth bass leading to deleterious effects in terms of both CHD and VRM scores in the three regions (1704, 1705 and 1706) in which the median values of methylmercury in each of those subregions are equal to or greater than 0.3ppm.

In terms of neurodevelopment, it appears that mountain whitefish and bridgelip sucker can be consumed at a rate of 25 meals per week to achieve a net benefit of omega-3s while limiting the predicted negative effects attributed to methylmercury, but only in limited regions. Data for Chinook salmon were only from one region but demonstrated a net benefit of omega-3s when consumed as much as 25 meals per week. Rainbow trout, mountain whitefish, largescale sucker and bridgelip sucker can be consumed at these rates to attain positive cardiovascular benefits but

again, only in a limited amount of regions and only if there are no other contaminants contributing to risk.

In each region that mountain whitefish and rainbow trout were sampled (apart from subregion 1703), our analysis showed that they can be consumed at least 7 times per week without posing a risk to cardiovascular or neurodevelopmental endpoints. While there is no consistent rate throughout the Basin, this smallmouth bass appears to provide a net benefit for both endpoints when consumed once or twice per week in different subregions. This is considerably more that the statewide advisory limiting the consumption to 1-2 times per month and the difference entirely reflects whether or not benefits from omega-3s are included in the equation while still maintaining methylmercury exposures less than 0.1 µg/kg/day.

Studies have indicated that the mere perception of risk is causing people to avoid fish consumption (Hightower 2008). This analysis has demonstrated that even if pregnant women are advised to eat no more than 2 meals per week of certain fish, the benefits outweigh the risks in terms of cardiovascular and neurodevelopment.

LIMITATIONS

Several limitations were identified in this research. Similar to other toxicological studies, uncertainties exist in the underlying dose-response relationships that serve as the basis for the conclusions regarding fish consumption and predictive health outcomes. For example, when estimating the potential risks of myocardial infarctions, the equation used a threshold of 0.51 ppm since no adverse effects were evident below this level. Ginsberg and Toal (2009) acknowledge that the appearance of this threshold may be related to measurement error and variability in the baseline population that could obscure any measured effect below this level. This would mean that if there was an effect on MI below 0.51 ppm in hair, the slope may be different than seen at higher body burdens. The dose-response relationships used in the risk/benefit equation are supported by available data but do contain uncertainties.

The data provided by the U.S.EPA were collected from state and federal databases as well as private data sources. There were not an equal number of samples throughout each region, nor were each species equally represented. Both Chinook salmon and sturgeon were only sampled in two different locations. The number of samples for each species varied by study and therefore by location. The average mercury concentrations found and reported for each subregion

are dependent on the number of samples from that region and therefore may not be an accurate representation of the average methylmercury found in fish in that region.

Some researchers collected whole-body samples; others collected fillets with skin on or with skin off. In this analysis, we treated fillets with skin on as fillets with skin off. A previous study found, on average, that skin-off samples were 5% higher than skin-on samples, although concentrations in some paired samples were very similar or identical (Serdar 2001). While this applied to a very small proportion of total fish tissue samples, our method might underestimate methylmercury concentrations.

The data did not always indicate the number of fish included in a sample for all observations, so statistical weights were assigned on the basis of the number of fish in the sample where indicated and assumed to be 1 (an individual fish sample) where not indicated. Because composite samples containing large numbers of individuals would provide more accurate estimates of the mean response than samples composed of a few individuals, this could also underestimate methylmercury concentrations. However, this method followed previous studies (Christensen et al. 2006; Wente 2004).

When determining the omega-3 content in the fish species used, we used national estimates from the USDA National Nutrient Database for Standard Reference Release 26 similar to previous studies (Ginsberg and Toal 2009; Loring et al. 2010; USDA 2013). Because we are using local methylmercury values, using concentrations of omega-3s found in locally caught fish would be more appropriate when possible.

It is also important to consider that mercury is not the only contaminant found in this, nor are omega-3s the only benefits we derive from fish consumption. An equation that included other risks and benefits from fish would be an important direction for future research.

CONCLUSIONS AND RECOMMENDATIONS

The concentrations of methylmercury found in each fish species sampled varied by region and by species. However, we found that both mountain whitefish and rainbow trout provided a net benefit in terms of both CHD risk and improved VRM scores across all consumption rates in all subregions on which they were sampled. Both walleye and sturgeon had net risks at all fish consumption rates. Smallmouth bass and largescale sucker were the most variable in terms of net benefit and risk for both end points. Species that provide a net benefit for

CHD in one region may not have the same benefits in other regions and may not necessarily provide an improved VRM score within the same region. This makes generating general fish consumption advice based on either species or location difficult.

Future work should focus on gathering omega-3 fatty acid levels for locally caught and consumed fish species. This would remove some of the uncertainty and augment the approach considerably. Dose-response relationships for other beneficial components of fish, such as selenium, and from other contaminants such as PCBs, DDT or PBDE, should also be included in this equation in the future. While an important goal of public health officials is to assess and communicate risks, generalized fish consumption advice may encourage people to avoid fish species that are beneficial to their health. These findings highlight the importance of careful and clear communication of information regarding fish consumption and care needs to be given to ensure that the correct information will be interpreted by the consumer.

Table 3.1. EPA and DHA values for select fish species from the USDA National Nutrient Database for Standard Reference Release 26.

Species	EPA (mg)	DHA (mg)	Total (mg)
Bridgelip Sucker	415	631	1046
Largescale Sucker	415	631	1046
Channel Catfish	170	233	403
Mountain Whitefish	690	2050	2740
Walleye	187	490	677
Sturgeon	423	202	625
Smallmouth Bass	518	779	1297
Rainbow Trout	796	884	1680
Chinook Salmon	1717	1236	2953

Table 3.2. Range of ingestion rates used in the analysis.

g/day	meals/week	Rationale
24.29	1	To compare to current fish consumption advisories in the Columbia River Basin
48.57	2	U.S.EPA and FDA current recommendations of 12 ounces per week
175	7.2	Oregon's current fish consumption rate
620	25.53	Traditional subsistence fish consumption rate (Harper and Harris 2008)

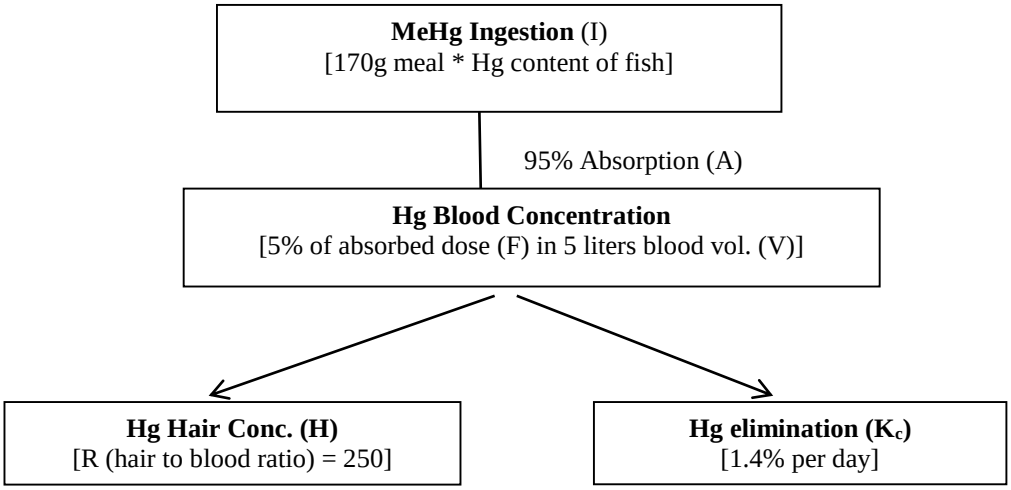
Table 3.3. Description and location of Subregion used in this analysis

Sub-region	Area Description/Location	State Covered	Area (sq. mi.)
1701	Kootenai-Pend Oreille-Spokane: The Kootenai, Pend Oreille, and Spokane River Basins	Idaho, Montana, and Washington.	203
1702	Upper Columbia: The Columbia River Basin above the confluence with the Snake River Basin, excluding the Yakima River Basin	Washington.	22600
1703	Yakima. The Yakima River Basin	Washington.	6210
1704	Upper Snake: The Snake River Basin to and including the Clover Creek Basin	Idaho, Nevada, Utah, Wyoming	3560
1705	Middle Snake: The Snake River Basin below the Clover Creek Basin to Hells Canyon Dam	Idaho, Nevada, Oregon.	36700
1706	Lower Snake: The Snake River Basin below Hells Canyon Dam to its confluence with the Columbia River	Idaho, Oregon, Washington.	35200
1707	Middle Columbia: The Columbia River Basin below the confluence with the Snake River Basin to Bonneville Dam	Oregon, Washington.	29800
1708	Lower Columbia: The Columbia River Basin below Bonneville Dam, excluding the Willamette River Basin	Oregon, Washington.	6250
1709	Willamette: The Willamette River Basin	Oregon.	11400

Table 3.4. Median (mean), Max and Min methylmercury ug/g by species and location.

	White Sturgeon	Walleye	Smallmouth bass	Rainbow Trout	Mountain Whitefish	Largescale Sucker	Chinook Salmon	Channel catfish	Bridgelip Sucker
Columbia River Basin	0.13 (0.15)	0.56 (0.51)	0.29 (0.33)	0.07 (0.11)	0.08 (0.11)	0.18 (0.22)	0.02 (0.02)	0.20 (0.22)	0.23 (0.21)
1701			0.14 (0.18)	0.08 (0.08)	0.05 (0.05)	0.28 (0.24)			
1702		0.21 (0.26)	0.11 (0.11)	0.04 (0.05)	0.04 (0.06)	0.07 (0.06)		0.02 (0.03)	0.04 (0.04)
1703			0.16 (0.16)	0.13 (0.13)	0.15 (0.15)	0.39 (0.38)			
1704		0.97 (0.97)	0.52 (0.83)	0.06 (0.08)	0.08 (0.18)	0.19 (0.19)			0.09 (0.09)
1705			0.29 (0.32)	0.06 (0.15)	0.05 (0.05)	0.26 (0.27)		0.25 (0.28)	0.23 (0.28)
1706		0.05 (0.05)	0.42 (0.32)	0.04 (0.04)	0.09 (0.13)	0.16 (0.18)		0.02 (0.05)	
1707			0.14 (0.23)	0.05 (0.15)	0.01 (0.02)	0.11 (0.14)		0.30 (0.30)	0.12 (0.12)
1708	0.13 (0.15)				0.21 (0.19)	0.12 (0.12)			
1709			0.17 (0.22)	0.08 (0.18)	0.06 (0.06)	0.10 (0.24)	0.02 (0.02)		
Max Conc.	0.19	1.38	1.23	0.670	0.51	1.62	0.05	0.56	0.86
Min Conc.	0.06	0.06	0.02	ND*	0.01	0.01	0.01	0.02	0.04

*ND- Not Detected



Model equation: $H = \frac{(I*A*(1-K_c))*F*R}{V}$

Figure 3.1. One-compartment pharmacokinetic model for methylmercury adapted from Ginsberg and Toal (2000)

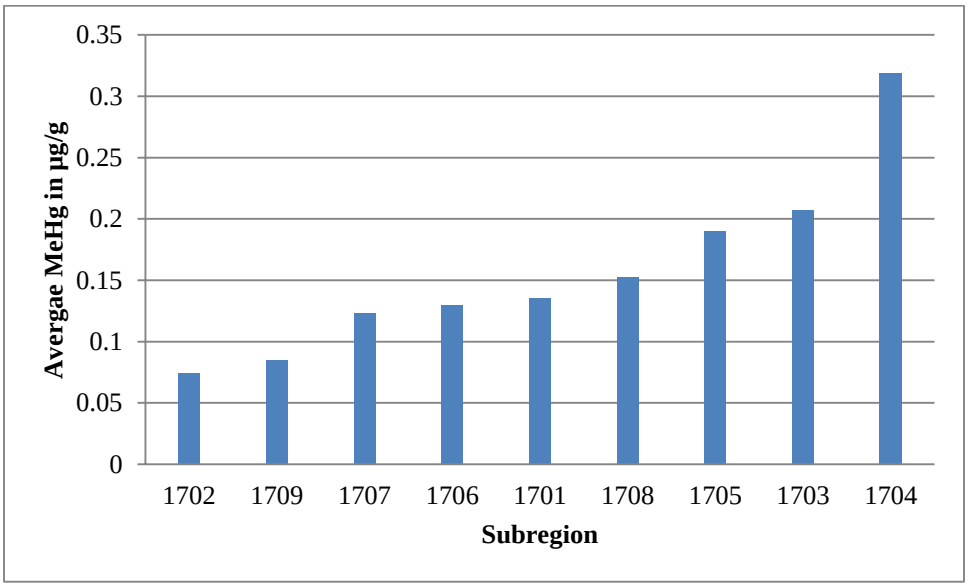


Figure 3.2. Average MeHg in µg/g for various subregions in the Columbia River Basin

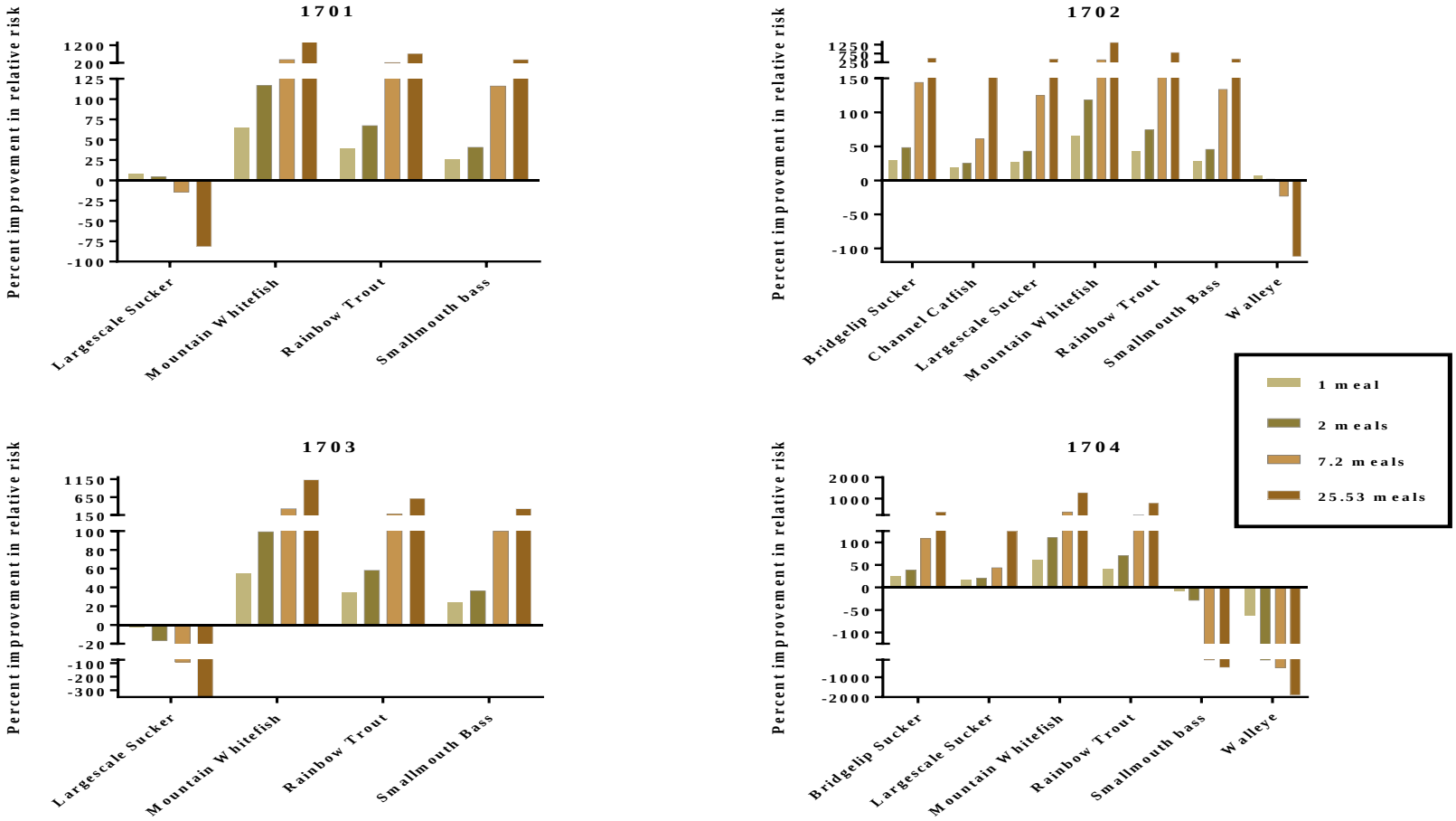


Figure 3.3a. Estimated net effect of MeHg and Omega 3s on cardiovascular risk for various fish consumption rates in the Columbia River Basin

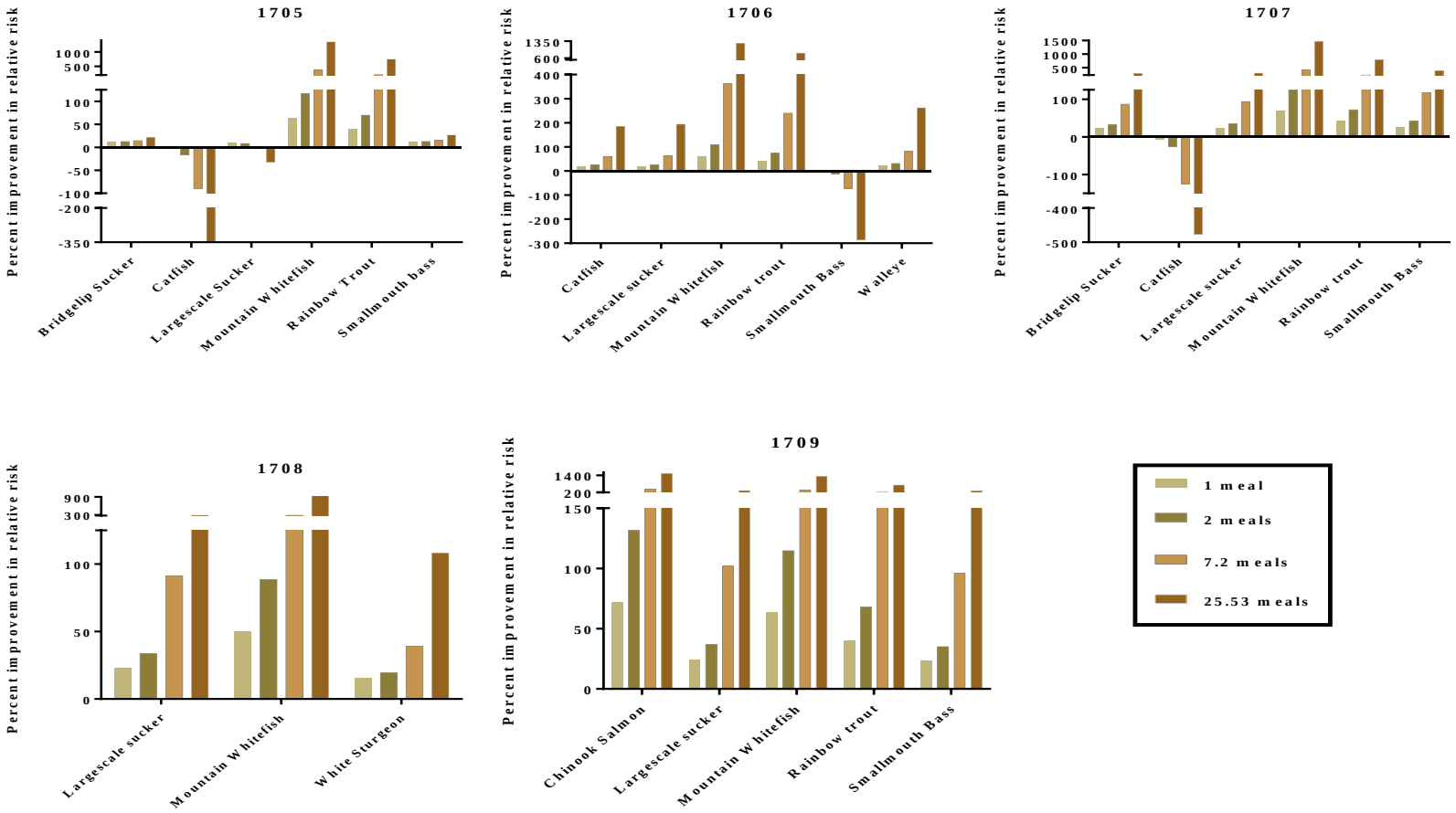


Figure 3.3b. Estimated net effect of MeHg and Omega 3s on cardiovascular risk for various fish consumption rates in the Columbia River Basin

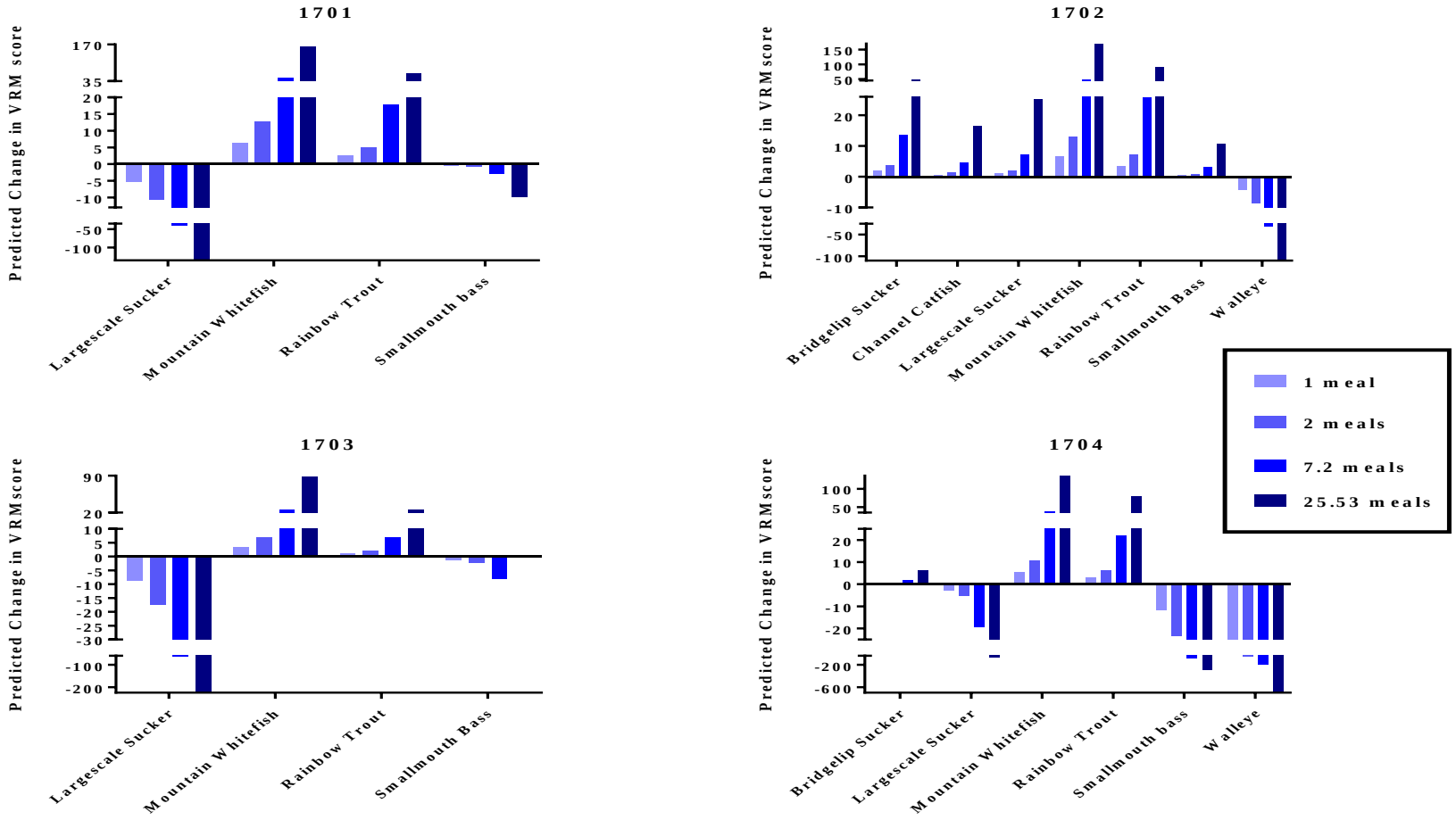


Figure 3.4a. Estimated net effect of MeHg and Omega 3s on neurodevelopment at 6 months of age for various consumption rates in the Columbia River Basin

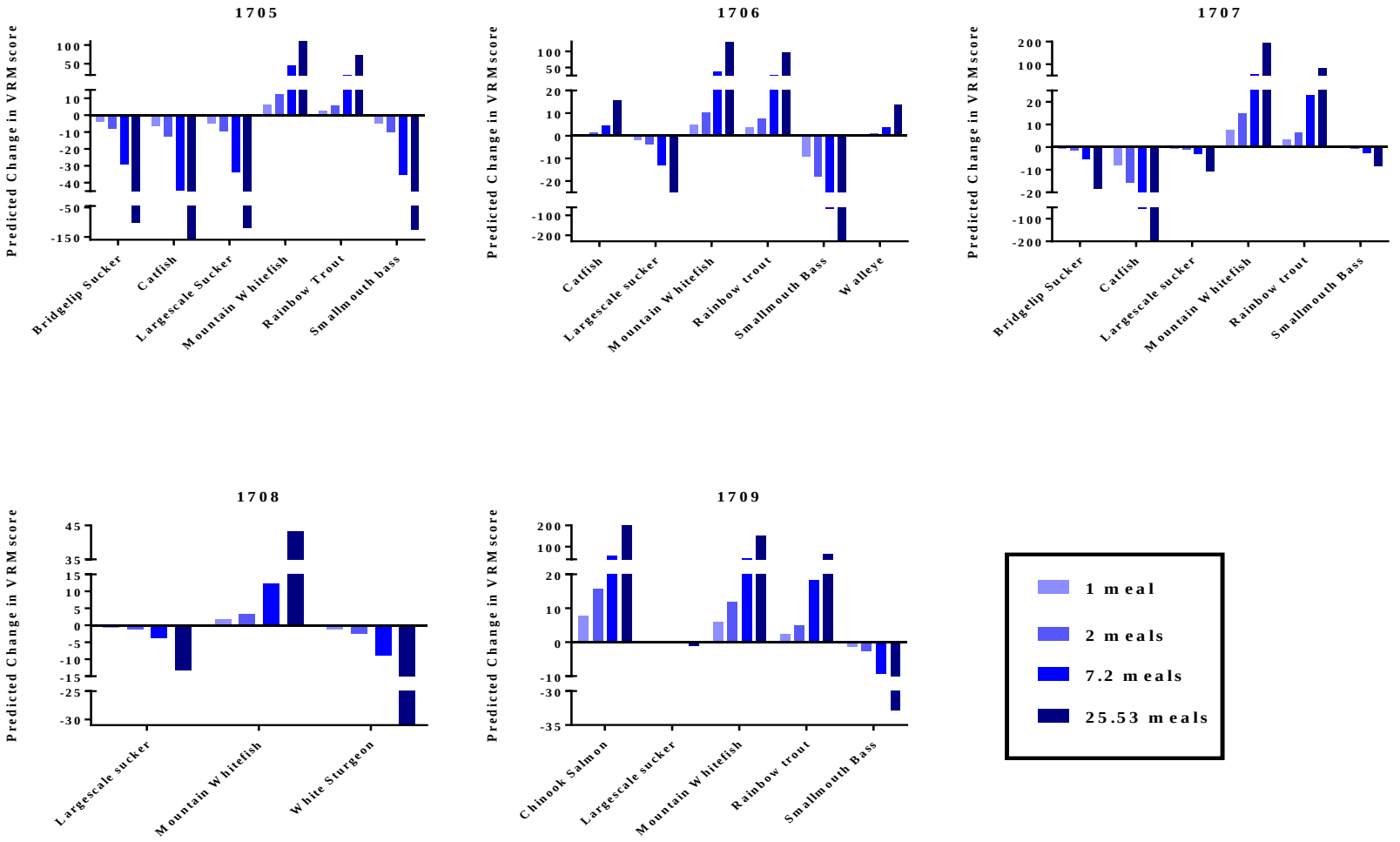


Figure 3.4b. Estimated net effect of MeHg and Omega 3s on neurodevelopment at 6 months of age for various consumption rates in the Columbia River Basin

CHAPTER 4 – MANUSCRIPT

Regional Variations in Blood Mercury Concentrations and Temporal Trends in Fish Consumption in Women of Child Bearing Age in the United States using NHANES data from 1999-2010

Leanne K. Cusack, MPH[1]

Ellen Smit, PhD[1]

Molly Kile, PhD[1]

Anna K. Harding, PhD[1]

[1]School of Biological and Population Health Sciences, College of Public Health and Human Sciences, Oregon State University, Corvallis Oregon

Journal:

Environmental Health Perspectives

Submission Pending

ABSTRACT

Background: Fish and shellfish are the primary routes of exposure to methylmercury in the US. Part of methylmercury's toxicity stems from its ability to cross biological membranes, such as the blood brain barrier and placental barrier. This renders the fetus particularly susceptible to methylmercury intoxication.

Methods: Data from the National Health and Nutrition Examination Survey (NHANES), 1999-2010 (n=9597) were used to determine trends in total whole blood and methylmercury concentrations within coastal and non-coastal regions of the US for women of childbearing age, and the associations with age, race/ethnicity, income and fish consumption.

Results: Statistically significant differences were found in both mean blood methylmercury and blood total mercury concentrations across survey cycles, but there was no consistent trend over time. All women who lived in coastal regions had greater blood mercury concentrations relative to women living inland. Residents of the Atlantic coast had both the highest total fish consumption in the past 30 days as well as the highest blood mercury concentrations.

Conclusions: U.S. women of childbearing age who live in coastal regions consumed more fish meals per month and had higher whole blood MeHg concentrations compared to women living in the Midwest. In particular, women who lived in the Atlantic or Pacific coastal regions had the highest fish intake and the highest blood MeHg concentrations. A better understanding of the demographics associated with high levels of methylmercury and the types of fish contributing to this will help physicians give more targeted advice to women.

Keywords: NHANES, methylmercury, fish consumption, regional variations, coastal

INTRODUCTION

The general population is exposed to methylmercury, a known neurotoxicant, from fish consumption(McDowell et al. 2004). However, methylmercury concentrations vary by species. For instance, the concentrations of mercury in fish and shellfish species ranges from < 0.1 ppm for shellfish such as oysters and mussels, to many parts per million for high end predatory fish such as tuna, swordfish and shark (Mahaffey et al. 2003). Methylmercury concentrations are also known to vary within and among fish species across the U.S. by more than 10-fold (Kathryn R Mahaffey et al. 2009). Freshwater fish such as walleye and northern pike have also been found to have high concentrations of methylmercury (Harper and Harris 2008; Rose and Feldman 1995). Additionally, methylmercury exposure also depends on the amount of each species consumed.

Women of childbearing age are the target audience for fish advisories due to the greater sensitivity of the developing fetus to methylmercury's toxicity. In the U.S., the Food and Drug Administration and the Environmental Protection Agency (U.S.EPA) have issued a joint advisory for pregnant women, women who may become pregnant, nursing mothers and young children. The advisory recommends avoiding eating specific types of fish that contain high levels of mercury, including tilefish, shark, swordfish, and king mackerel, as well as limiting the intake of albacore tuna to 6 ounces per week (U.S. EPA 2012). The advisory also states that fish is a healthy food due to its unique nutrient profile and that women of child bearing age should consume fish low in mercury up to twice a week. Communicating the risks and benefits of seafood consumption is challenging due to the complex nature of the message in order to have the intended consequences of improved health. Yet data shows this is not necessarily the case. Following the advisory issued jointly by the FDA and U.S. EPA, a longitudinal study observed that pregnant women reduced fish consumption to levels below beneficial amounts rather than substituting fish known to have lower levels of methylmercury (Oken et al. 2003). This is an important public health concern because the risks posed from reducing or eliminating fish consumption (of fish containing low levels for methylmercury) during pregnancy has been found to be greater than the risks of harm from exposure to contaminants such as mercury (Karouna-Renier et al. 2008).

This issue is further underscored by regional differences in fish consumption. In the United States, fish consumption varies by region. Data collected from 1999-2004 in the National

Health and Nutrition Examination Survey (NHANES) showed that women of child bearing age in the Northeast had the highest percentage of women with mercury concentrations ≥ 3.5μg/L in their blood, the U.S. EPA's reference value that is likely to be without an appreciable risk of deleterious effects (Agency for Toxic Substances and Disease Registry 2013; U.S. EPA 2013); (Mahaffey et al. 2009). Yet another study that used NHANES data from 1999-2010 observed no change in fish consumption in women of reproductive age even though blood methylmercury concentrations appear to be decreasing since 2000(Birch et al. 2014). However, it is unclear from these studies if regional fish consumption practices were a factor in the different mercury exposure or whether women of childbearing age are adopting the intended practices of choosing fish species with lower mercury levels.

This study fills this gap in knowledge about the regional distributions of fish consumption patterns and blood methylmercury levels in women of childbearing age in the U.S. This research will be useful to clinical providers and public health agencies as they develop more targeted fish consumption advice to these women.

METHODS

Study Population

NHANES is a continuous national survey that evaluates the health and nutritional status of the non-institutionalized US population conducted by the National Center for Health Statistics. This study was limited to data from women of childbearing age (16-49 yrs of age) in 6 consecutive cycles of NHANES spanning from 1999 to 2010 (n= 9597). In addition to the publically available data, this analysis used the respondent's county as a geographic unit. This is a restricted variable and special permission to access this data was granted from the National Center for Health Statistics (NCHS).

Fish Consumption Data

Participants completed an interview that asked them to recall the number of times they ate each of 31 types of fish or shellfish in the previous 30 days. No data were collected on the amount of each species consumed. Frequency of fish and shellfish consumption across the 30-day recall period was calculated as total consumption and by type of fish consumed *a*) tuna, *b*) large predator fish (shark and swordfish), *c*) marine fish (fish sticks, haddock, mackerel, porgy,

salmon, sardines, sea bass, unknown, other unknown, pollock and flatfish) *d*) freshwater fish (catfish, perch, pike, trout, bass and walleye) and *e*) marine shellfish (crab, crayfish, lobster, mussels, oyster, scallops, shrimp, other shellfish, unknown other shellfish).

Blood Mercury Data

The analytical method for measuring total mercury (tHg) and inorganic mercury (iHg) in blood have been described in detail by the NCHS (CDC 2013; Jones N.D.) Briefly, tHg and iHg were measured using cold-vapor atomic absorption spectrophotometry with detection limits of 0.14 μg/L for tHg and 0.4 μg/L for iHg. Methylmercury in blood (MeHg) is calculated by subtracting iHg from tHg. Since the limit of detection (LOD) for iHg is larger than the LOD for tHg this approach may result in negative values. To address this problem, we followed the protocols described by Mahaffey et al. (2009) where MeHg = tHg - iHg if the difference is ≥ 0. If the difference is < 0, MeHg = 0.2 μg/L which is one half of LOD. If it is assumed that MeHg has the same LOD as iHg, then MeHg can be set equal to the LOD of iHG (Kathryn R Mahaffey et al. 2009). Population prevalence estimates were obtained for blood mercury levels ≥ 5.8 μg/L and ≥ 3.5 μg/L which are two reference values used by the U.S. EPA (U.S. EPA 2012; USEPA).

Geographical Data

The participant's county or county equivalent was used to categorize participants as being either coastal or non-coastal. Coastal or non-coastal groups were and then further categorized into eight different regional groups; Atlantic Coast, Northeast, Great Lakes, Midwest, South, Gulf of Mexico, West and Pacific Coast. Any county that bordered the Pacific or Atlantic Ocean, the Gulf of Mexico or the Great Lakes was considered coastal. Any county whose center point was within 25 miles of any coast was also considered coastal (See Supplemental Table 4.1 for a list of coastal counties). The coastal regions were identified based on their proximity to the nearest largest water body.

Covariates

Demographic data were included in the analysis as potential confounders. These covariates included: race/ethnicity (Non-Hispanic White, Non-Hispanic Black, Other Hispanic,

Mexican American and Other), age (16-19, 20-29, 30-39 and 40-49 years of age) and income (<$20,000, $20,000-$44,999, $45,000-$74,999 and $75,000+), and survey cycle year.

Statistical Analysis

Trends over time were assessed using simple linear regression with only 'time' (year of NHANES survey release) as a predictor. Blood mercury levels were natural log transformed to approximate a normal distribution. Linear regression models were used to evaluate the association between blood mercury concentrations and estimated 30-day fish and shellfish consumption (total and by type of fish); race/ethnicity; income; time, geographical location and age.

Dependent variables included: total blood mercury, the natural logarithm of the total blood mercury, methylmercury, and the natural logarithm of methylmercury, in order to compare the differences between using total mercury and MeHg.

Independent variables included were: estimated 30-day fish and shellfish consumption (total and by type of fish); race/ethnicity; income; time, geographical location and age.

Following NCHS guidance, the 2-year mobile examination center weights were used for all analyses reported in this study (CDC 2013). We performed all analysis using SAS, version 9.2 (SAS Institute Inc., Cary, NC).

Population prevalence estimates for each census and coastal region were obtained for blood mercury levels ≥ 5.8 μg/L and ≥ 3.5 μg/L using appropriate sample weights, in order to determine the percentage of the population that has blood mercury levels greater than the current and suggested reference doses set by the U.S.EPA.

RESULTS

Regional Trends in Whole Blood Methylmercury Concentrations

The percentage of women by geographic census region and coastal status that had MeHg concentrations ≥ 3.5μg/L and ≥ 5.8μg/L are presented in Table 4.1. Using the average MeHg across all survey cycles (1999-2010), average MeHg concentrations were greater in coastal regions compared to non-coastal regions. Also, women in the Northeast had the highest percentage of MeHg concentrations ≥5.8μg/L (5.66%) and ≥ 3.5 μg/L (12.51%). When coastal regions were added into the analysis, further spatial heterogeneity was observed and all coastal regions had higher MeHg concentrations relative to their neighboring inland regions as seen in

Figure 4.1. The arithmetic and geometric mean of MeHg concentration by region and coastal status for women of childbearing age are presented in Table 4.2. Women living in coastal regions had higher mean MeHg levels (1.12 µg/L; 95% CI 1.05 µg/L -1.20 µg/L) compared to those living in non-coastal areas (0.74 µg/L; 95% CI 0.70 µg/L -0.78 µg/L). Additionally, women living in the Atlantic and Pacific coastal region had the highest average MeHg concentration of 1.35 µg/L, 95% CI 1.22 µg/L -1.50 µg/L and 1.19 µg/L, 95% CI 1.09 µg/L -1.31 µg/L, respectfully. Women in the inland Midwest had the lowest average MeHg concentrations of 0.65 µg/L (95% CI 0.61 µg/L -0.68 µg/L).

Trends in Fish Consumption

The unweighted sample size and weighted frequency of fish meals in the past 30 days for each survey cycle is displayed in Figure 4.2 The total number of fish meals consumed by US women of childbearing age differed across the 6 survey cycles (p=0.05). Compared to 2009-2010, total fish meal consumption was significantly less in 1999-2000 (β=-1.04 meals, SE =0.34, p=0.003), 2003-2004 (β=-0.61, se =0.31, p=0.05) and in 2007-2008 (β=-0.73, se =0.29, p=0.01) (Data not shown). Also, the percentage of people who reported not eating fish in the past month was 22% in 2009-2010 and 26% in 1999-2000 indicating that in recent years, a greater percentage of women aged 16-49 were consuming fish. While there was no consistent trend over time, this data indicates that these women, on average, are consuming more fish meals per month in 2009-2010 compared to earlier years. The data also showed that the frequency of marine fish meals (p=0.01) and shellfish meals (p=0.02) differed by survey cycles. Both marine fish and shellfish consumption has been increasing slightly each year since 1999 with the exception of 2007-2008. No difference in the mean number of fish meals were observed for freshwater fish (p=0.24), tuna (p=0.09) or large predatory fish (p=0.55) (Data not shown).

Fish consumption by age, income, and race/ethnicity

Age, income, and race/ethnicity were associated with higher fish consumption in bivariate analysis. Specifically, as age increased so did total fish consumption (β=0.09, se =0.005, p=<0.0001), marine fish (β=0.04, se =0.002, p=<0.0001), freshwater fish (β=0.008, se =0.001, p=<0.0001), tuna (β=0.02, se =0.002, p=<0.0001), shellfish (β=0.03, se =0.003, p=<0.0001) and large predatory fish (β=0.0006, se =0.0002, p=<0.002). Increased income was

associated with increased total fish consumption (p=<0.001), marine fish (p=<0.001), tuna (p=<0.001), shellfish (p=<0.001) and large predatory fish (p=0.003). Income was not associated with freshwater fish consumption (p=0.10). Finally, participants who self-identified as 'Other' consumed the greatest amount of total fish with a mean of 6.37 (SE=0.48, p=<0.001) fish meals per month and Mexican Americans were consuming the least with a mean of 3.01(SE=0.11, p=<0.001) meals per month. The 'Other' category consumed the greatest amount of marine fish in the last 30-days, 2.5 (SE= 0.33, p=<0.001) and Mexican Americans consumed the least amount, 0.74 (SE=0.04, p=<0.001). Freshwater fish was consumed the most by Non-Hispanic Blacks with 0.61 meals in the last 30 days (SE=0.05, p=<0.001) and consumed the least by Mexican Americans, 0.18 (SE=0.01, p=<0.001). Tuna was consumed the most frequently by Non-Hispanic whites, 1.17 (SE=0.04, p=<0.001) and by Non-Hispanic Blacks the least, 0.60 (SE=0.04, p=<0.001). Swordfish/shark was consumed the most by frequently by Non-Hispanic whites, 0.02 (SE=0.004, p=<0.001) and by Non-Hispanic Blacks the least, 0.006 (SE=0.003, p=0.02). Shellfish was consumed the most frequently by the 'Other' category, 2.59 (SE=0.21, p=<0.001) and the least by Mexican Americans, 1.37 (SE=0.05, p=<0.001) (Figure 4.3).

Fish consumption by region

Fish species consumed by survey participants varied by region (Figure 4.4). In all regions except the Inland West and Inland Midwest, shellfish was the most commonly consumed item. Women living in the Gulf of Mexico coastal region consumed the most freshwater while the Inland Northeast consumed the least. The Pacific Coast consumed the most marine fish and the Gulf of Mexico region consumed the least amount. Tuna was consumed fairly similarly in the Great Lakes, Inland Midwest and Inland Northeast and the lowest consumption was found in the Gulf of Mexico. Shellfish was consumed in the greatest quantity in the Gulf of Mexico and consumed the least in the Great Lakes. Swordfish and shark were consumed less than 1% in all regions (Table 4.3).

Associations between blood mercury and fish consumption by region, time and demographic variables

Blood MeHg concentrations were associated with income (p<0.0001), age (p<0.0001), race (p<0.0001), total fish meals (p<0.0001), and region (p<0.0001). Blood MeHg

concentrations increased with increasing age and income, with those with a household income of ≥$75,000 having higher MeHg concentrations (β=0.47, se =0.08, p=<0.0001) than those reporting a household income less than $20,000. Non-Hispanic Whites and Mexican American had lower MeHg concentrations compared to Non-Hispanic Blacks and other Hispanic races although those self-identifying as 'Other' had the highest MeHg concentrations. On average, people who self-identified as 'Other' race had more MeHg in their blood β=0.52, se =0.09, p=<0.0001) compared to those in the Non-Hispanic White category. We also see a difference between region of residence with the Atlantic Coast having the highest (β=0.31, se =0.13, p=0.02) and the inland Midwest having the lowest (β=-0.40, se =0.11, p=0.0006) methylmercury concentrations compared to the Inland South while controlling for all other variables. Swordfish and shark are associated with the highest methylmercury exposure (β=0.56, se =0.20, p=0.006), followed by tuna (β=0.12, se =0.02, p=<0.0001), shellfish (β=0.08, se =0.01, p=<0.0001), freshwater fish (β=0.071, se =0.023, p=0.003) and marine fish (β=0.66, se =0.023, p=0.004) (Table 4.4).

DISCUSSION

This study observed that U.S. women of childbearing age that live in coastal regions consumed more fish meals per month and had higher whole blood MeHg concentrations compared to women living in the Midwest. In particular, women who lived in the Atlantic or Pacific coastal regions had the highest fish intake and the highest blood MeHg concentrations. Compared to a previous study by Mahaffey et al. (2009) we see a decrease in the geometric mean blood mercury concentrations for women residing in the Atlantic coast, from 1.55 µg/L to 1.35 µg/L, and the Gulf of Mexico, from 0.96 µg/L to 0.88 µg/L, an increase residing in the Inland Northeast from 0.77 µg/L to 0.85 µg/L and no change in other regions when adding in more recent NHANES survey cycles (K. R. Mahaffey et al. 2009).

These findings are consistent with previous data that found that regional differences in exposure may be present even within a single state due to location of residence (coastal/non-coastal), type of fish, and consumption rates (Karouna-Renier et al. 2008; Patch et al. 2005; Warner 2007). Residents of the Atlantic coast had both the highest total fish consumption in the past 30 days as well as the highest blood mercury concentrations. Local consumption choices can greatly affect levels of mercury in a specific population (Legrand et al. 2005). Women residing

in the Atlantic coast consume shellfish in the greatest quantity followed by marine fish compared to women in the Gulf of Mexico who consumed the second highest total amount of fish, yet had lower levels of mercury.

The analysis found a statistically significant difference in the reported frequency of total fish consumption and blood methylmercury levels across survey cycles. Total fish consumption is higher in recent years, with an increase in marine and shellfish consumption and a decrease in freshwater fish consumption. There is consistent evidence demonstrating that awareness of fish advisories leads to lower blood methylmercury levels (Anderson et al. 2004; Karouna-Renier et al. 2008). We found that there was no reduction in mean mercury levels however there was a reduction at the 90th percentile. This would suggest that women who are consuming fish at the greatest rate may be making more informed choices regarding methylmercury in fish when choosing which fish to consume. However, only a small percentage of the sample is consuming fish at a rate of twice a week as recommended by the most recent U.S. EPA /FDA advisory. Fish consumption varies by race/ethnicity (Kathryn R Mahaffey et al. 2009) with Asian populations consuming the most fish.

While few studies have assessed awareness of federal fish consumption advisories, awareness of state fish consumption advisories among women of childbearing ages ranges between 20-30% (Anderson et al. 2004; Imm et al. 2005; Knobeloch et al. 2005) This could be due in part to the fact that state fish advisories are often targeted to residents who frequently consume recreationally caught fish through means such as inclusion and dissemination of information with sport-fishing licenses (Anderson et al. 2004). Similar to other studies, we see that a greater percentage of the population consumes marine fish as opposed to freshwater fish (Anderson et al. 2004). Studies have also shown that minorities, in particular Non-Hispanic Blacks, are less likely to be aware of advisories (Anderson et al. 2004; Karouna-Renier et al. 2008). We found Non-Hispanic Blacks consume the most freshwater, marine and shellfish second only to the 'Other' category.

Therefore, considering total methylmercury exposure, advisories need to be comprehensive and include both sport and commercial species. New methods of disseminating this information need to be considered in order to increase awareness among women of childbearing age.

Advisory outreach efforts are often targeted toward low-income subsistence anglers (Anderson et al. 2004). Our data would suggest greater efforts are needed to reach older women

at the upper end of the socioeconomic scale. Similar to other studies we found that overall fish consumption increases with reported increases in family household income and age (Mahaffey et al. 2003; Kathryn R Mahaffey et al. 2009). However we saw a decrease in consumption of freshwater fish as income categories increased.

While fish consumption advisories are important for making informed decisions, education is also needed to enable women to recognize and select an appropriate combination of shellfish and fish to obtain important nutritional benefits of fish while maintaining mercury levels below the threshold. In order to reach diverse subgroups within a population it is often necessary to design messages for specific audiences.

The present study helps to identify those groups that are at risk for excess methylmercury exposure. In order to reach these populations, outreach and informational material should be provided to both public health agencies and medical care professionals (Burger, 2005) and outreach should be targeted to include those that have high exposures or are unaware of advisories- this includes women living in coastal regions, Non-Hispanic Black women, women who identify themselves as 'Other', consumers of marine fish and women of higher socioeconomic status (Katner et al. 2010). The goal is to promote consumption of fish that are beneficial for health and provide the public with specific examples of fish that are most beneficial (Smith and Sahyoun 2005) .

The American Medical Association encourages physicians and other medical care providers to "assist in educating patients about the relative mercury content of fish and shellfish products and make patients aware of the advice contained in both national and regional consumer fish consumption advisories" (AMA, 2004). A better understanding of the demographics associated with high levels of methylmercury and the types of fish contributing to this will help physicians give more targeted advice to women. Exposure information for women of childbearing age is particularly important because of the potential exposures and risks to developing fetuses.

LIMITATIONS

Although the use of the 30-day food frequency data could potentially have dietary recall error associated with it, it should not affect the results or conclusions of this study though as the methodology in the data collection across study years has not changed. It is unlikely that the

recall bias that is inherent in food frequency questionnaires would differ from one survey cycle to the next. In addition, fish and shellfish are generally easily identifiable foods and therefore more readily recalled than other food groups (Mahaffey et al. 2003). The validity of the dietary recall for fish consumption has been found to be greater than all other food groups (Karvetti and Knuts 1985; MacIntosh et al. 1996).

CONCLUSIONS AND RECOMMENDATIONS

This analysis allowed us to determine which demographic variables are associated with both fish consumption and blood mercury concentrations. Place of residence, age, race/ethnicity, income and type of fish being consumed all play an important role in exposure to methylmercury in fish. All coastal regions had greater blood mercury concentrations relative to their inland neighbors. U.S. women of childbearing age (16-49) that live in the Atlantic and Pacific coastal regions having the highest methylmercury concentrations and women living in the Midwest have the lowest methylmercury concentrations. We also found that the number of fish meals consumed by women of childbearing age differs by region with the highest intake associated with coastal regions. Women in the Atlantic coast are consuming the most total fish and women in the Midwest are consuming the least. Total fish consumption has been slowly increasing from 1999-2010. The fact that blood methylmercury concentrations are decreasing and fish consumption is increasing may be due to the fact that women are making more informed choices when it comes to fish consumption.

Substituting fish with high methylmercury concentrations for fish containing lower levels of methylmercury among women of childbearing age may provide important developmental benefits and few negative impacts. However, decreasing fish consumption altogether is substantial public health concern because the risks posed from reducing or eliminating fish consumption during pregnancy, due to loss of nutrients, has been found to be greater than the risks of harm from exposure to contaminants such as mercury (Hibbeln et al. 2007). Risk managers and physicians need to consider the target demographics for fish consumption advisories, how populations will respond to fish these advisories, how those responses will influence nutrient intake and methylmercury exposure, and the affect this will in turn have on public health (Cohen et al. 2005).

Table 4.1. Percentage of participants with methylmercury concentrations ≥ 3.5µg/L and ≥ 5.8µg/L by U.S. Census region and coastal status for all years combined.

	U.S. Census Region				Coastal Status		Pr>F
Methylmercury	**Northeast**	**South**	**Midwest**	**West**	**Coastal**	**Noncoastal**	
Percentage ≥ 3.5 µg/L	12.51	7.17	1.91	9.21	12.01	4.37	<0.001
Percentage ≥ 5.8 µg/L	5.66	2.83	0.78	3.76	5.32	1.61	<0.001

Table 4. 2. Blood total mercury (µg/L), women 16-49 years of age, by region and coastal status, NHANES 1999-2010

	N	**Arith. Mean** (95% CI)		**Geometric Mean** (95% CI)		**Selected percentiles** (95% CI)							
Coastal Region						25th		50th		75th		90th	
Atlantic coast	1662	2.41	(2.13,2.69)	1.35	(1.22,1.50)	0.64	(0.57,0.72)	1.37	(1.22,1.53)	2.89	(2.47,3.38)	5.36	(4.65,6.18)
Gulf of Mexico	541	1.41	(0.42,2.41)	0.88	(0.49,1.59)	0.49	(0.26,0.95)	0.83	(0.48,1.43)	1.60	(1.02,2.49)	2.99	
Pacific Coast	1566	1.97	(1.75,2.19)	1.19	(1.09,1.31)	0.59	(0.54,0.65)	1.20	(1.05,1.36)	2.38	(2.07,2.73)	4.47	(3.77,5.31)
Great Lakes	708	1.09	(1.04,1.13)	0.78	(0.75,0.82)	0.46	(0.41,0.51)	0.82	(0.78,0.85)	1.39	(1.31,1.48)	2.13	(1.83,2.48)
Inland West	1237	1.33	(1.14,1.52)	0.85	(0.74,0.97)	0.45	(0.4,0.51)	0.89	(0.78,1.02)	1.59	(1.39,1.82)	3.09	(2.69,3.55)
Inland Midwest	1289	0.94	(0.88,1.01)	0.65	(0.61,0.68)	0.36	(0.3,0.42)	0.68	(0.63,0.73)	1.19	(1.12,1.27)	1.92	(1.71,2.15)
Inland South	2449	1.11	(0.98,1.23)	0.71	(0.64,0.78)	0.39	(0.34,0.45)	0.70	(0.63,0.77)	1.29	(1.16,1.43)	2.30	(1.98,2.67)
Inland North East	729	1.56	(1.36,1.75)	0.88	(0.74,1.03)	0.40	(0.25,0.66)	0.90	(0.73,1.10)	1.78	(1.49,2.13)	3.42	(3.05,3.83)
Coastal Status													
Coastal	4477	1.92	(1.76,2.08)	1.12	(1.05,1.20)	0.56	(0.51,0.61)	1.09	(1.00,1.19)	2.17	(1.98,2.37)	4.30	(3.86,4.78)
Non-coastal	5704	1.18	(1.09,1.26)	0.74	(0.70,0.78)	0.39	(0.37,0.42)	0.73	(0.68,0.78)	1.39	(1.30,1.48)	2.50	(2.27,2.75)

Table 4.3. Fish consumption among women 16-49 years of age participating in NHANES from 1999-2010 [mean ± SE (95% CI)]

Region	Type of Fish	Mean ± SE	95% CI
Inland Midwest	Freshwater fish	0.29 ± 0.024	(0.28,0.29)
	Marine Fish	1.25 ± 0.068	(1.25,1.25)
	Tuna	0.97 ± 0.053	(0.97,0.97)
	Shellfish	1.27 ± 0.095	(1.26,1.27)
	Swordfish/Shark	0 ± 0.002	(0,0)
	total	3.78 ± 0.14	(3.77,3.79)
Great Lakes	Freshwater fish	0.48 ± 0.041	(0.48,0.49)
	Marine Fish	1.12 ± 0.066	(1.11,1.12)
	Tuna	1.13 ± 0.069	(1.12,1.13)
	Shellfish	1.33 ± 0.088	(1.32,1.33)
	Swordfish/Shark	0.01 ± 0.001	(0.01,0.01)
	total	4.07 ± 0.186	(4.06,4.09)
Inland South	Freshwater fish	0.39 ± 0.039	(0.39,0.39)
	Marine Fish	1.22 ± 0.09	(1.22,1.22)
	Tuna	0.98 ± 0.067	(0.97,0.98)
	Shellfish	1.59 ± 0.104	(1.59,1.59)
	Swordfish/Shark	0.01 ± 0.002	(0.01,0.01)
	total	4.19 ± 0.218	(4.18,4.19)
Inland West	Freshwater fish	0.19 ± 0.042	(0.19,0.19)
	Marine Fish	1.66 ± 0.084	(1.65,1.66)
	Tuna	1.28 ± 0.054	(1.28,1.28)
	Shellfish	1.57 ± 0.089	(1.57,1.58)
	Swordfish/Shark	0.01 ± 0.006	(0.01,0.01)
	total	4.71 ± 0.179	(4.7,4.72)
Gulf of Mexico	Freshwater fish	0.53 ± 0.023	(0.52,0.53)
	Marine Fish	1.36 ± 0.042	(1.36,1.37)
	Tuna	0.87 ± 0.022	(0.87,0.88)
	Shellfish	2.76 ± 0.047	(2.76,2.77)
	Swordfish/Shark	0.01 ± 0.001	(0.01,0.01)
	total	5.54 ± 0.052	(5.53,5.54)
Inland Northeast	Freshwater fish	0.09 ± 0.003	(0.09,0.09)
	Marine Fish	1.29 ± 0.038	(1.28,1.29)
	Tuna	1.17 ± 0.055	(1.16,1.17)
	Shellfish	1.67 ± 0.073	(1.67,1.68)
	Swordfish/Shark	0.02 ± 0.004	(0.02,0.02)
	total	4.24 ± 0.156	(4.23,4.25)
Pacific Coast	Freshwater fish	0.21 ± 0.024	(0.21,0.21)
	Marine Fish	2.02 ± 0.105	(2.01,2.02)

	Tuna	1.07 ± 0.074	(1.07,1.07)
	Shellfish	2.19 ± 0.114	(2.18,2.19)
	Swordfish/Shark	0.03 ± 0.012	(0.03,0.03)
	total	5.52 ± 0.21	(5.51,5.53)
Atlantic coast	Freshwater fish	0.19 ± 0.022	(0.19,0.19)
	Marine Fish	1.82 ± 0.057	(1.81,1.82)
	Tuna	1.28 ± 0.046	(1.28,1.29)
	Shellfish	2.46 ± 0.077	(2.46,2.46)
	Swordfish/Shark	0.04 ± 0.008	(0.04,0.05)
	total	5.8 ± 0.14	(5.79,5.81)

Table 4.4. Associations between blood mercury and fish consumption by region, time and demographic variables

	Parameter	Standard Error	p value
Intercept	-1.95	0.18	<.0001
Survey Cycle			
1999-2000	0.09	0.11	0.4255
2001-2002	0.11	0.12	0.3877
2003-2004	0.05	0.13	0.7018
2005-2006	-0.50	0.15	0.0011
2007-2008	0.08	0.12	0.4913
2009-2010	0.00	0.00	.
Income			
$75,000+	0.47	0.08	<.0001
$45,000-$74,999	0.22	0.08	0.0090
$20,000-$44,999	0.08	0.08	0.2730
<$20,000	0.00	0.00	.
Race/Ethnicity			
Mexican American	-0.05	0.06	0.4107
Other Hispanic	0.17	0.11	0.1174
Other	0.52	0.09	<.0001
Non-Hispanic Black	0.30	0.05	<.0001
Non-Hispanic White	0.00	0.00	.
Age			
40-49	0.30	0.06	<.0001

30-39	0.30	0.06	<.0001
20-29	0.15	0.06	0.0116
16-19	0.00	0.00	.
Fish Consumption/month			
9+	1.82	0.08	<.0001
5-8	1.33	0.07	<.0001
1-4	0.69	0.08	<.0001
0	0.00	0.00	.
Region			
Atlantic Coast	0.31	0.13	0.0165
Gulf Coast	-0.28	0.14	0.0379
Pacific Coast	0.25	0.12	0.0386
Great Lakes Coast	-0.15	0.12	0.2253
Inland West	-0.08	0.12	0.5029
Inland Midwest	-0.40	0.11	0.0006
Inland Northeast	-0.38	0.12	0.0016
Inland South	0.00	0.00	.

Figure 4.1. A map of methylmercury concentration (geometric mean and 95% Confidence Interval (µg/L)) by coastal/inland regions for all years combined.

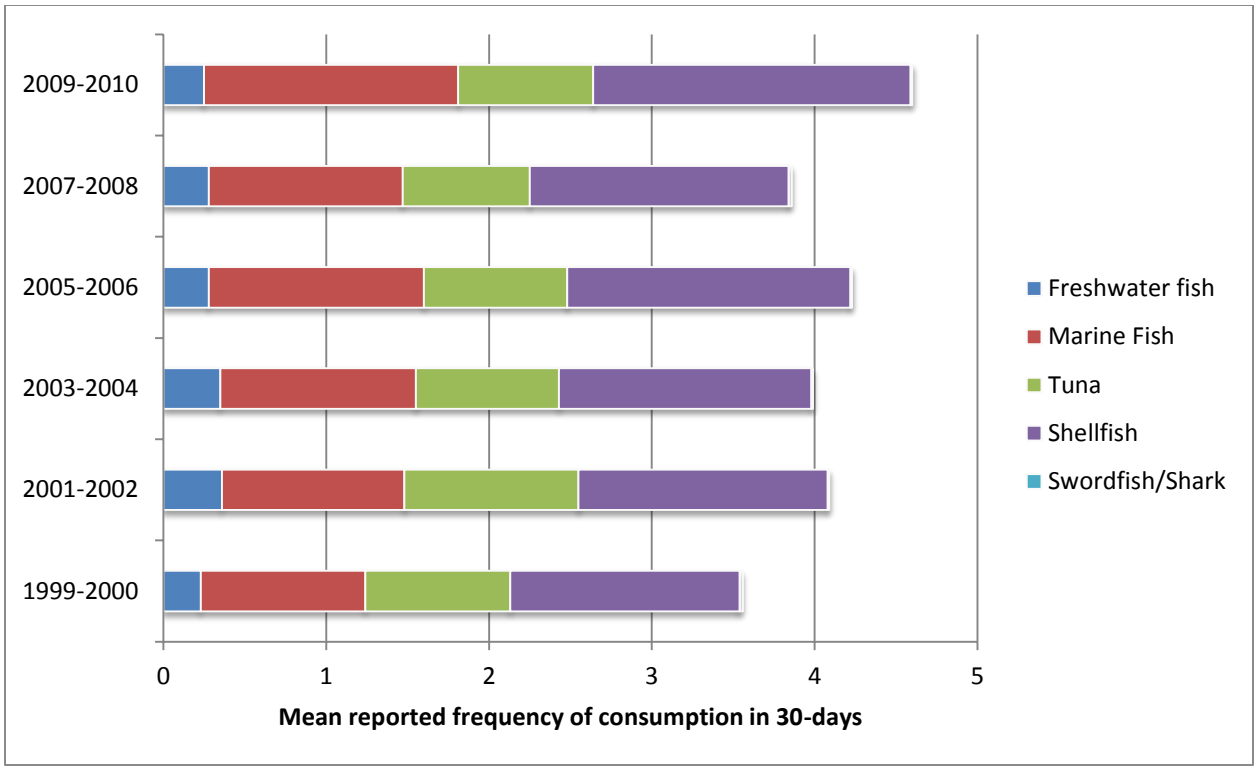


Figure 4.2. Mean reported fish consumption by species in NHANES participant women aged 16-49 years, by survey cycle.

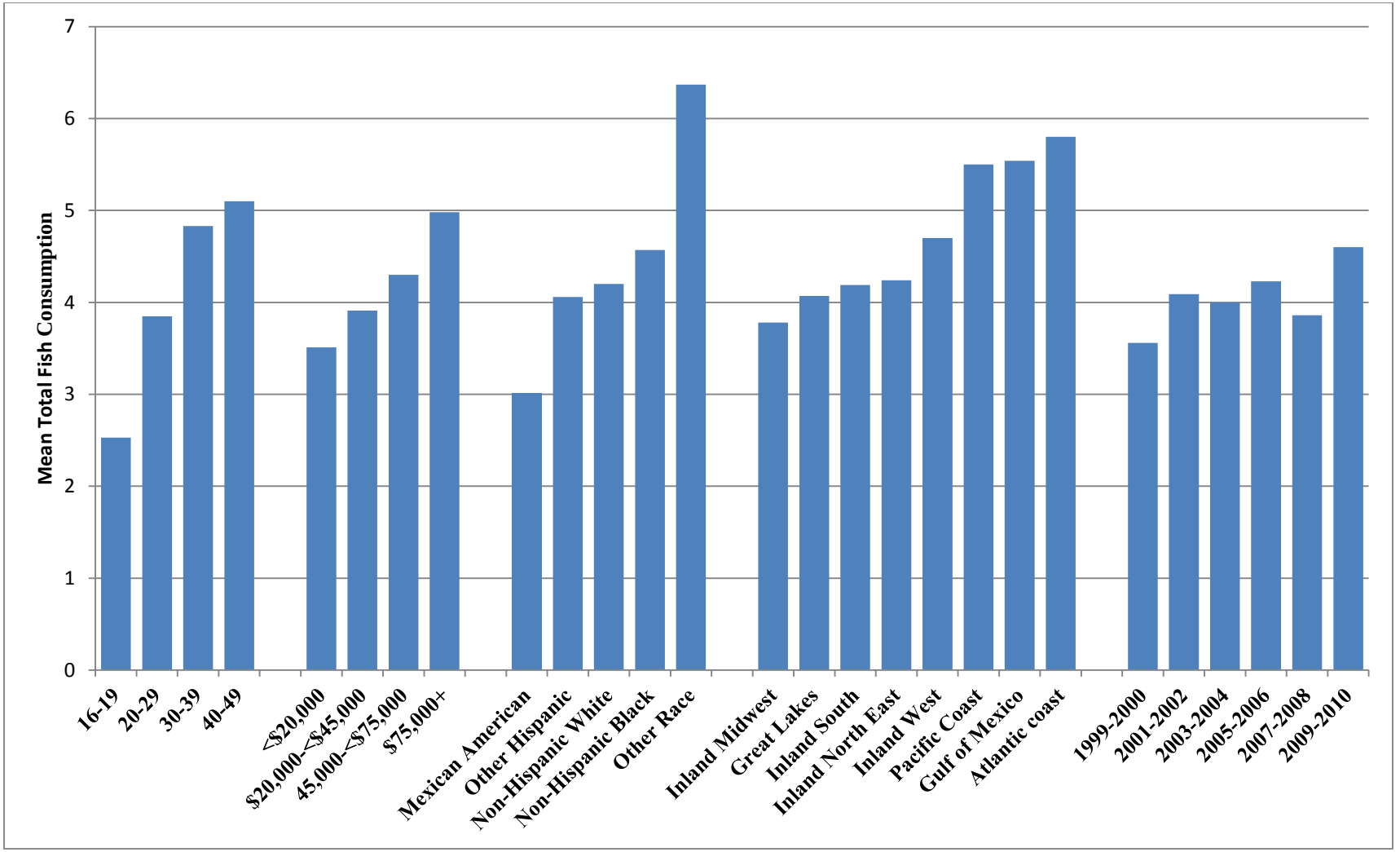


Figure 4.3. Mean total fish consumption for women of childbearing age in the U.S. by demographic variable, region and survey cycle

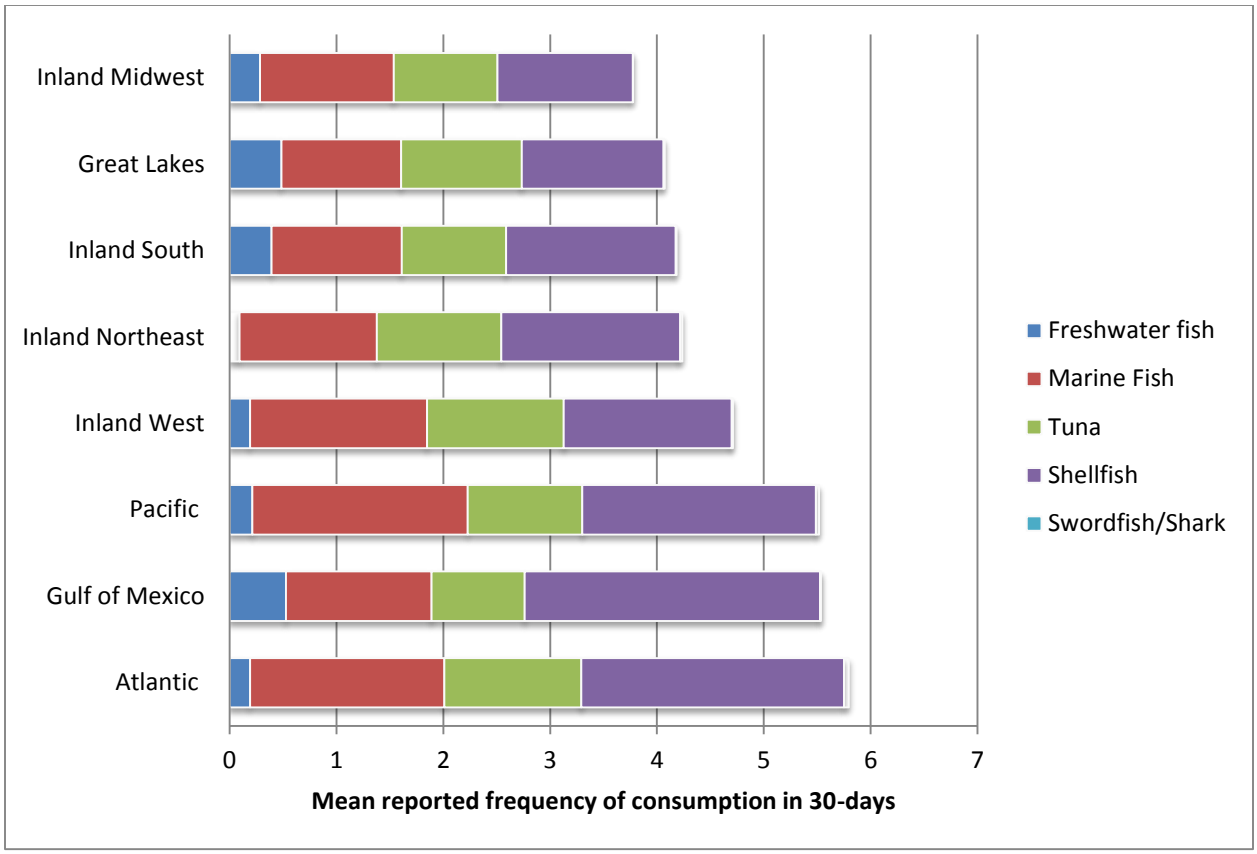


Figure 4.4. Mean reported fish consumption by species in NHANES participant women aged 16-49 years, by region for all years combined.

CHAPTER 5 - MANUSCRIPT

Selenium: Mercury Molar Ratios in Freshwater Fish in the Columbia River Basin: Potential Applications for Regionally Specific Fish Consumption Advisories

Leanne K. Cusack, MPH[1]
Collin Eagles-Smith, PhD[2]
Anna K. Harding, PhD[1]
Dave Stone, PhD[3]

[1]School of Biological and Population Health Sciences, College of Public Health and Human Sciences, Oregon State University, Corvallis Oregon
[2]United States Geological Survey, Corvallis Oregon
[3]Department of Environmental and Molecular Toxicology, Oregon State University, Corvallis, Oregon

Journal:
Environmental Research
Submission Pending

ABSTRACT

Background: Fish are full of beneficial nutrients such as selenium and omega-3s and are an excellent source of low fat protein. However, fish are also the primary source of methylmercury for humans. States issue fish consumption advisories based solely on the risks that methylmercury pose to human health. Selenium has well established protective effects against mercury toxicity. It has recently been suggested the selenium: mercury molar ratio be considered in risk management. The exact molar ratio that would confer protection is still being determined. In order for the ratio to be useful in a risk assessment context, it would need to have low variability within species.

Methods: We examined 10 different freshwater fish species found within the Columbia River Basin in order to determine the inter- and intra-specific variability in the selenium: mercury molar ratios.

Results: We found significant variation in selenium: mercury molar ratios, within and between species. The mean selenium: mercury ratios were negatively correlated with mean mercury levels for all individual fish combined but not with mean length.

Conclusions: The considerable variability in Selenium: mercury molar ratios varied substantially within fish species renders this approach impractical for consideration in risk assessment. However, providing the selenium and mercury to consumers may be helpful when consumers are deciding which fish species to consume.

Key Words: selenium, mercury, molar ratios, Columbia River Basin, fish consumption fish advisories

INTRODUCTION

Fish is an important part of a healthy diet. Fish and seafood are significant sources of low fat protein and contain omega-3 polyunsaturated fatty acids (omega 3s) and selenium that play an essential role in human health (Sidhu 2003). The perception of fish as a healthy food has been tempered by concern regarding the potential harm from exposure to methylmercury present in fish (Chan and Egeland 2004; Mahaffey 1999; Mozaffarian 2009; Rice et al. 2003; Rice 2004). Studies have reported poorer neurological status and slower development in newborns, infants, and/or children exposed to methylmercury *in utero* and/or during early childhood (Crump et al. 1998; Grandjean et al. 1997; Grandjean et al. 1998; Jedrychowski et al. 2006; Oken et al. 2005).

All 50 states have responded to the presence of methylmercury in fish by issuing fish consumption advisories warning the public about consuming certain species of fish in specific water bodies (U.S. Environmental Protection Agency [U.S. EPA] 2012). The U.S. EPA has developed guidelines to ensure consistency among state risk assessment agencies when developing these advisories. The fish consumption advisories are based on results from a risk assessment process that integrates information on contaminant presence in fish, the potential human exposure to these contaminants, and the potential health risks of exposure (U.S. EPA 2012).

Although there has been a demonstrated negative health impact from fish consumption due to exposure to methylmercury, considerable evidence indicates that there is also a wide range of health benefits. While recent studies have primarily focused on levels of mercury and omega-3s in different fish species, attention is now being given to the levels and protective effects of selenium with respect to fish consumption (Burger et al. 2012b; Gochfeld et al. 2012; Kaneko and Ralston 2007). Selenium is found in fish and seafood, as well as in eggs, meat and vegetables (Choi et al. 2008). Studies have demonstrated that content of foods and intake of selenium varies considerably both within and between countries due to differences in geography, agronomic practices, food availability and preferences (Combs 2001; Rayman 2000).

Selenium is a trace mineral that is essential to health and is regulated in the body (Rayman 2000). However, both selenium deficient and selenium toxicity have been identified (Eisler 1987). Selenium is a constituent of selenoproteins, which are known to be important in antioxidant enzymes and serves as catalysts for the production of the thyroid hormone (Rayman

2000). The exact physiological functions that selenium exerts in the brain are still not understood; however, studies have found that selenium and certain selenoproteins continue to be maintained despite prolonged selenium deficiencies (Chen and Berry 2003; Whanger 2000). Higher levels of selenium have also been associated with a decrease in non-fatal heart attacks (Mozaffarian 2009).

The interaction between selenium and mercury are complex and not fully understood. Mercury and methylmercury have a high binding affinity for selenium and are irreversible selenoenzyme inhibitors. Excess selenium may chelate mercury and protect selenoproteins or, conversely, mercury may be viewed as creating a relative selenium deficiency (Watanabe et al. 1999). A recent study showed that high maternal exposure to methylmercury in animals inhibits selenium-dependent enzyme activity in the brain while selenium supplementation is protective (Berry and Ralston, 2008).Recent attention is now shifting focus to determine if the toxicity of methylmercury is due to impaired selenium-dependent enzyme synthesis or activity (Ralston 2008; Ralston et al. 2008; Raymond and Ralston 2009; Watanabe et al. 1999).

Selenium's ability to prevent mercury toxicity has been recognized for more than 40 years (Beijer and Jernelöv 1978; Iwata et al. 1973; Kaneko and Ralston 2007; Ohi et al. 1976; Watanabe 2001). Selenium's ability to diminish the toxicity of mercury has been well established in all insect, fish, bird and mammal species investigated to date (Cuvin-Aralar and Furness 1991; Kaneko and Ralston 2007; Peterson et al. 2009; Ralston et al. 2006; Ralston and Raymond 2013).

Ganther (1972) and others have posed that selenium to mercury molar ratio of 1:1 may provide a protective effect against mercury toxicity from fish and therefore, be incorporated in risk assessment and regulation regarding mercury and fish consumption in humans. (Ganther et al. 1972; Peterson et al. 2009; Ralston and Raymond 2013; Ralston 2008; Ralston et al. 2008; Raymond and Ralston 2009). The idea that selenium: mercury molar ratios may confer protection against mercury toxicity has been a topic of increasing research, interest and controversy. Ralston et al. (2008) found that methylmercury in rats could not be predicted using methylmercury tissue concentrations alone and that toxicity was directly related to the selenium: mercury molar ratio in tissue (Ralston et al. 2008). The study found that the molar ratio is very sensitive to the denominator since selenium is an essential trace element and is physiologically regulated. Peterson et al. (2009) suggest that benchmark values for mercury toxicity in human

and wildlife species based solely on mercury levels may exaggerate the mercury toxicity potential compared to an assessment that is based on selenium: mercury molar ratios (Peterson et al. 2009).

While others researchers agree that these ideas are intriguing, and should be examined further, they also suggest that it is unlikely that a single molar ratio would operate across different endpoints or effects (e.g. development, cognition, coordination, locomotion, and visual acuity) and species (Burger and Gochfeld 2012).

The actual selenium: mercury molar ratio and contributing mechanisms that would protect against mercury toxicity remains unclear. If there were a universal and mutual bioavailability in which all selenium in the body was able to, and did bind to mercury in a 1:1 ratio, this would leave an inadequate amount of selenium to synthesize enzymes and carry out its essential role (Burger et al. 2013). This suggests that a protective molar ratio would need to be greater than one, but how much greater than one is not clear.

Molar ratios in fish can vary substantially in different water bodies due to differing amounts of either mercury or selenium concentrations (Reash 2012). In addition, the intra-specific variability in the selenium: mercury molar ratio needs to be sufficiently low and consistent in order to be considered in a regulatory context or in the issuance of consumption advice. It is important to determine if the ratios are adequately consistent within a species to be useful in advising consumers. It is also important to gain a better understanding of the different molar ratios between species to determine if this will help consumers make sound decisions about what species to eat by choosing fish low in mercury and high in selenium.

Data on the selenium: mercury molar ratios from freshwater fish are particularly limited, in part because the focus has been solely on the mercury levels that pose a risk to humans. Studies examining selenium: mercury molar ratios are more common in marine fish species as they are known to have high concentrations of selenium (Burger and Gochfeld 2012; Burger et al. 2012b; Burger and Gochfeld 2013; Burger et al. 2013; Gochfeld et al. 2012; Kaneko and Ralston 2007; Ralston 2008; Raymond and Ralston 2009). At present there are a very limited number of studies that present data on mean selenium: mercury molar ratios for individual fish, and there is very little knowledge of individual fish that have molar ratios that are either above or below a suggested protective level. Both Peterson et al. (2009) and Burger et al. (2012) have asserted the need for more data on the varying molar ratios in freshwater fish from different

regions before meaningful ratios can be inferred for many species (Burger et al. 2012a; Gochfeld et al. 2012; Peterson et al. 2009).

Past studies have generally focused on mercury levels in fish species due to risks posed by mercury; but the protective characteristics of selenium in fish are now being examined as well (Burger and Gochfeld 2011, 2012; Burger et al. 2012b; Kaneko and Ralston 2007). While many studies still do not report the levels of selenium, it is important to understand how the selenium: mercury molar ratios vary in freshwater fish before using molar ratios can be used for fish consumption advisories.

Gaining a better understanding of intra- and inter-specific variability in selenium: mercury molar ratios in ten species of fish from the Columbia River Basin will be directly useful for developing fish consumption advice and regionally specific risk management decisions. The Columbia River Basin is home to many Native American Tribes and subsistence fishermen that consume large quantities of locally caught fish (Donatuto and Harper 2008; Harper and Harris 2008). Subsequently, we conducted a study to determine the selenium, mercury and selenium: mercury molar ratio, as well as the intra- and interspecific variability in these measurements in 10 fish species caught in the Columbia River Basin.

METHODS

Data were obtained from the United States Geological Survey (USGS) Western North America Mercury Synthesis Project, and included fish total mercury and selenium concentrations from a suite of State and Federal databases and monitoring programs. Fish species analyzed were from the Columbia River Basin and include: yellow perch (*Perca flavescens*) (n= 43), smallmouth bass (*Micropterus dolomieu*) (n= 95), cutthroat trout (*Oncorhynchus clarkii*) (n= 6), rainbow trout (*Oncorhynchus mykiss*) (n= 12), brown trout (*Salmo trutta*) (n= 420), mountain whitefish (*Prosopium williamsoni*) (n= 20), white sturgeon (*Acipenser transmontanus*) (n= 32), walleye (*Sander vitreus*) (n= 10), black crappie (*Pomoxis nigromaculatus*) (n= 12) and Chinook salmon (*Oncorhynchus tshawytscha*) (n= 9). These fish species are commonly consumed both within the Basin and in other parts of the country.

Total mercury and total selenium levels were measured without speciation and reported in µg/g on a wet weight basis for mercury and dry weigh for selenium. Total mercury is an accepted approximation of methylmercury, as 90-95% of total mercury present in fish is methylmercury

(Karouna-Renier et al. 2008; Knobeloch et al. 2005; McKelvey et al. 2007). Mercury concentrations reported in whole fish were converted to fillet concentrations using the following (Bevelhimer 1996):

$C_f = C_{wb}/ 0.7$ where:

C_{wb} = whole-body methylmercury concentration (mg/kg)

C_f = fillet methylmercury concentration (mg/kg)

Molar ratios were calculated by dividing the mean concentration (in µg/g) by the molecular weight for mercury and selenium. For each species the mean selenium: mercury molar ratio was calculated from the average selenium and average mercury levels, following the method used by Burger et al. (2012a). Calculating the mean molar ratio in this way gives a different result than calculating a molar ratio for each individual fish and then taking the mean molar ratio from those (Burger et al. 2012a). We also examined and calculated the individual ratio for each fish species.

Both mercury and selenium were highly skewed so they were log-transformed prior to correlation analysis. A one-way analysis of variance was used to examine the differences in both mercury and selenium levels and the selenium: mercury molar ratios between species. Pearson's correlation was used to examine the relationships between molar ratios and body length of fish, mean selenium and mean mercury levels. The level of significance was set at $\alpha < 0.05$.

RESULTS

Overall (n=259) there was a weak but statistically significant positive correlation between mercury and selenium concentrations (Pearson $r = 0.21$; $p < 0.001$). However, this correlation was present only for walleye and mountain whitefish. There was a significant positive correlation between mercury and length (Pearson $r = 0.36$; $p<0.005$) but not for selenium and length across all species.

Differences among species in selenium: mercury molar ratios

There were significant interspecific variations in mean selenium and mean mercury levels as well as the selenium: mercury molar ratio (Table 5.1). The mean molar ratios for each species were all above 1, with the exception of three individual fish from two species, smallmouth bass and walleye, in which molar ratios were below 1. There was a wide range in selenium: mercury

molar ratios, from 2.9:1to 29.4:1 with the lowest ratios found in walleye, smallmouth bass and cutthroat trout and the highest ratios were found in Chinook salmon. The range in mean mercury levels was greater than the range in mean selenium levels. Mean mercury levels were highest in walleye, 0.32 ± 0.06 µg/g and lowest in Chinook salmon, 0.02 ± 0.001µg/g. Mean selenium concentrations were highest in brown trout, 0.99 ± 0.17 µg/g, and lowest in cutthroat trout, 0.21 ± 0.05 µg/g. Species with higher levels of mercury generally had lower molar ratios. Among species, the range in mercury levels (0.02 ppm -0.32 ppm) was less than the range among selenium levels (0.21ppm - 0.99 ppm) (Table 5.2).

There was a significant negative correlation between mean selenium: mercury ratios and mercury levels (Figure 5.1). Total length was significantly correlated with selenium: mercury ratios (Figure 5.2) for all of the fish species together. Selenium and mercury both contribute to the molar ratio; mercury had a strong negative correlation (Pearson $r = -0.66$, $p = <0.0001$) and selenium had a positive significant relationship (Pearson $r = 0.43$, $p = <0.0001$).

Differences within species in selenium: mercury molar ratios

The coefficient of variation (CV) for concentrations of selenium, mercury and molar ratios within a fish species gives an indication of the reliability of the mean selenium: mercury molar ratio for each species. Concentrations of selenium vary more than the concentration of mercury in each fish species (Table 5.2). While there is no gold standard threshold, a low CV indicates higher reliability and a high CV is an indication of low reliability. We found that walleye had the lowest CV and cutthroat trout had the highest CV.

We also examined individual variation in the molar selenium: mercury ratios by plotting them against length, which is an indication of size. Figures 5.3a and 5.3b display the molar ratios by length for each individual in all fish species evaluated. The molar ratio of one is displayed on the figures to correspond to this suggested protective ratio; the line at 5 is shown for convenience, as it is still uncertain which ratio is required to offer protection.

For the seven species for which we had lengths, the length for five species were negatively correlated with the selenium: mercury ratio, but the correlations were not statistically significant. A negative correlation means that as size increases, the selenium: mercury ratio decreases. It was negatively correlated in yellow perch and positively correlated in all remaining fish species but the correlation was not statistically significantly. A positive correlation means

that as mercury increases selenium concentration also increases. The selenium and mercury levels were positively and significantly correlated in two fish species, mountain whitefish and smallmouth bass. Yellow perch and cutthroat trout were positively correlated but they were not statistically significant.

The molar ratios for all individual fish indicated that there was variation present for all species (n=252). Four fish showed selenium: mercury molar ratios less than one; three smallmouth bass and a rainbow trout. Five species - walleye, cutthroat trout, sturgeon, black crappie and brown trout had molar ratios greater than 2. All individual whitefish and yellow perch had molar ratios greater than 5. However, 50% of the individual whitefish and 83% perch had molar ratios greater than 10. Smallmouth bass has three fish below 1 and the remaining fish had molar ratios ranging from 1-17.8. Chinook salmon had the highest selenium: mercury molar ratios with all individuals having ratios greater than 10. Rainbow trout had 1 fish with a molar ratio less than one and the remainder of the fish were greater than 6 with a range from 6.4-20.

DISCUSSION

Interspecific and intraspecific variations in Se:Hg molar ratios

This study measured mercury and selenium concentrations ten freshwater fish species commonly consumed in the Columbia River Basin. All fish showed significant variation in mean selenium and mean mercury concentrations as well as selenium: mercury molar ratios.

Selenium is an essential trace element that is regulated in the body at low concentrations and occurs naturally in the aquatic environment (Caldwell et al. 2009; Kathryn R Mahaffey et al. 2009) . Mercury, however, has no known biological function. Fish absorb mercury directly through their gills or through consumption of other fish species that contain mercury. Mercury is bound to the protein in the fish tissue which results in the larger, longer-lived predatory fish having greater concentrations of mercury (McDowell et al. 2004; Munn and Short 1997; Willett 2012). However, bottom feeding fish such as sturgeon may also accumulate greater concentrations of mercury through either direct contact with contaminated sediment or by eating benthic invertebrates and epibenthic organisms (MacIntosh et al. 1996). We found that concentrations of selenium found in fish varied more than the concentrations of mercury in fish. These results are contrary to what Burger et al (2014) have found however concentrations of

selenium in the environment can vary considerably and further data is needed to gain a better understanding of how these concentrations can vary geographically.

We observed that the selenium: mercury molar ratio is largely dependent on the selenium concentration in the fish. The interspecific variation in mean molar ratios, is expected though, given that larger fish and those that are higher on the trophic scale tend to have higher levels of mercury (Anderson et al. 2004; Fleming et al. 1995; Karvetti and Knuts 1985). Our results showed that the top level predators such as smallmouth bass, walleye and brown trout had higher amounts of mercury and lower selenium: mercury molar ratios. We observed a significant relationship between mean mercury levels and the molar ratios for all fish species except Chinook salmon, cutthroat trout and mountain whitefish. These findings are consistent with previous studies in both freshwater and marine fish species (Burger and Gochfeld 2012; Burger et al. 2012a; Burger et al. 2012b).

Apart from salmon, much of the freshwater fish that people consume is self-caught and any association between length and molar ratio has the potential to help recreational fishermen predict molar ratios found within a fish and be helpful in determining which self-caught fish to consume. Length, however, is not a reliable gauge for selenium: mercury molar ratios. For example, yellow perch were the smallest fish species sampled and had a relatively high molar ratio of 18:1. Conversely walleye were the largest fish and had a molar ratio of 2.94:1. However, the mean length of black crappie was similar to yellow perch yet it had a molar ratio of 10:1. Similarly, the mean length of brown trout was similar to walleye; yet, it had a molar ratio of 9.7:1. Yellow perch was the only species in which the selenium: mercury ratio increased with size. At low mercury concentrations, the size relationships may not hold, which could explain the previous finding (Smith and Sahyoun 2005). While we found no significant association between length and mercury concentrations for any individual species, we did find that across all species there was a significant positive correlation between length and mercury (Pearson $r = 0.36$; $p = 0.005$). These findings may be partly due to small sample sizes. We did not find a correlation between length and molar ratios but these findings are consistent with previous findings in both freshwater and marine fish, in which there was not a significant correlation between mean molar ratios and mean length (Burger and Gochfeld 2012; Burger et al. 2012a; Burger et al. 2012b).

Freshwater and Saltwater Selenium: Mercury Molar ratios

Previous studies investigating the selenium: mercury molar ratios in freshwater fish have found the ratios to vary from 3.5-29.4 in six species in Tennessee (Burger et al. 2012a); 0.68 – 12.51 in eleven species in the Savannah River (Burger et al. 2001); 2.22-54.33 in ten species in Western US streams (Peterson et al. 2006); 0.51 - 3.70 in six species from lakes in New York (McDowell et al. 2004). Our study found molar ratios varying from 2.94-29.38 in ten different freshwater fish species. While our range is similar to other studies, the species and geographical areas samples were not always similar. Burger and colleagues also sampled yellow perch and black crappie in Savannah. They found ratios of 3.27 and 3.6, respectively while we found ratios of 18.07 and 10.08. Both species had similar levels of selenium but their study found much higher (3-6 times greater) levels of mercury in yellow perch and black crappie. The crappie in Tennessee were more similar having mean mercury levels of 0.05 µg/g (compared to 0.09 µg/g in ours) and mean selenium of 0.42 µg/g (0.35 µg/g in ours). The molar ratio for black crappie in Tennessee was 21.09 compared to the molar ratio of 10.08 for crappie in the Columbia River Basin. The differences in the ranges of the molar ratios may be due to the geographical areas being studied, levels of selenium and mercury in the environment or the number of fish species being investigated.

Comparing mercury and selenium levels with fish studies elsewhere can be complicated because some studies measure mercury in whole bodies and others measure mercury in the muscle tissue. Studies have shown that mercury levels are generally higher in muscle tissue than in the whole body of fish (Bevelhimer 1997). The use of both wet and dry weight in the literature provides additional challenges. Burger et al. (2001) found that concentrations expressed on a wet weight basis are about 18% of the level expressed on a dry weight (Burger et al. 2001). Our data measured wet weight and all data was converted to levels in fillets.

Many studies have investigated the ranges of selenium: mercury molar ratios in saltwater fish and have consistently shown an excess of selenium over mercury in terms of concentration (Burger and Gochfeld 2011; Burger et al. 2013; Gochfeld et al. 2012; Ralston 2008). The molar ratios in marine fish vary considerably between regions due to the wide range of sizes, tropic level and foraging methods. Molar ratios found in these studies varied from 0.46 to 17.65 for 15 different species in Hawaii (Kaneko and Ralston 2007); 0.36 to 60 for 19 recreationally caught species in New Jersey (Burger and Gochfeld 2012); 2.69 to 46.42 for 15 fish species in the

Aleutian Islands, Alaska (Burger et al. 2012b); and 1.23 to 67.21 from 21 different fish species purchased in New Jersey and Chicago markets (Burger and Gochfeld 2013). All of these studies have found greater variations in the selenium: mercury molar ratios compared to the freshwater studies, which is likely due to the wide range of species, and because many of them are long-lived predatory fish.

Implications for use of molar ratios in risk assessment

Current fish consumption advisories issued by State agencies are based solely on the risks associated with fish consumption (Department of Health and Welfare 2014; OHA 2014; Washington State Department of Health 2014). When examining the risks associated with mercury, there are two different sets of guidance values, tissue concentration and daily or weekly ingestion, used to protect human health from methylmercury exposure. The U.S.EPA recommends a level of less than 0.3 ppm of mercury in fish tissue for safe consumption (U.S. EPA 2013). In fish from the Columbia River Basin, there was a wide range of mean mercury values found. Using the U.S.EPA's criterion only one species, walleye, had mean mercury levels greater than 0.3 ppm. However, based on an assessment using the U.S.EPA's level of less than 0.3 ppm for safe consumption for all species combined (n= 252 for all species combined), 10.6% of the individual fish had mercury levels greater than 0.3 ppm and would not be recommended for consumption by humans.

Understanding the variability in molar ratios is important if they are to be used in risk assessment. Despite a species having a mean molar ratio greater than one, our data demonstrated that it is possible for individual fish within that species to have molar ratios less than one. While we observed this for only 4 individual fish, Burger and colleagues have found this to occur much more frequently (Burger et al. 2001; Burger and Gochfeld 2011, 2012; Burger et al. 2012b; Burger and Gochfeld 2013; Burger et al. 2013; Gochfeld et al. 2012). The differences observed between studies may be attributed to the different species being examined and the vary levels of mercury found in the environment.

It is also important to gain a better understanding of the toxicological significance of selenium in its protective responses to mercury exposure and whether the speciation of selenium plays a role. There is currently no agreement of which ratio confers protection. In the event that there were extremely high levels of both mercury and selenium, the ratio would suggest

protection when in reality both mercury and selenium may actually exceed toxicological benchmarks.

Ralston and others (2008) argue that selenium concentration should also be considered in the assessment of human health risks to mercury exposure and that a selenium: mercury molar ratio greater than 1:1 will offer protection against mercury exposure (Peterson et al. 2009; Ralston et al. 2006; Ralston 2008; Raymond and Ralston 2009). However, it is still unclear the exact ratio required to be protective for all populations. Protective levels of molar ratios may be different for women of childbearing ages and children and high fish consumers compared to the general population. Regardless of the value which may offer protection, if the molar ratio is to be used in crafting fish consumption advisories, the molar ratios should be consistent within a species.

Previous studies have shown that concentrations of mercury and selenium as well as the selenium: mercury molar ratios vary both seasonally and yearly (Gochfeld et al. 2012). Studies have also demonstrated that fish species in supermarkets may be mislabeled (Serdar and County 2001). Despite a person having an understanding of molar ratios and which fish to purchase, the mislabeling and seasonal variability make it difficult for a person to actually know what the molar ratio of the fish may be.

Fish are an important source of high quality protein, selenium and omega-3s, and consumption should be encouraged. Fish are also an important food source for Native Americans and subsistence fishermen (Donatuto and Harper 2008; Harper and Harris 2008). Recent studies have shown that the U.S. EPA/FDA fish consumption advisories may be discouraging people from eating fish (McCann P. 2005; Oken et al. 2003). Basing fish consumption advisories on information that includes benefits as well as risks could encourage people to eat more fish. However, given the variability found in the molar ratios of freshwater fish in the Columbia River Basin, incorporating the selenium: mercury molar ratios into fish consumption advisories in this area may be premature and is not yet supported by any studies to date.

CONCLUSIONS AND RECOMMENDATIONS

This paper examined the inter- and intra-specific variability of the selenium: mercury molar ratios in edible fresh water fish species found in the Columbia River Basin. Variation in selenium: mercury was high within the fish species regardless of sample size, which may due to

either the trophic level of the fish or levels of selenium or mercury in the environment. The levels of selenium found in fish are regulated which should make it more consistent within fish species (Burger and Gochfeld 2013; Caldwell et al. 2009). However, we found that the variability in selenium was greater than the variability of mercury concentrations in fish species sampled. The variation in ratios is not surprising given the known variability of methylmercury both within and between fish.

The considerable variability of molar ratios found in individual fish species creates a challenge to use selenium: mercury molar ratios in fish consumption advisory notifications. However, providing the selenium, mercury and omega-3 information to consumers may be helpful when consumers are deciding which fish species to consume. Because mercury plays such an important role in the selenium: mercury molar ratio, at the present time it is advisable to base fish consumption on known mercury concentrations until more is understood about the complex interactions between selenium and mercury.

In addition, further research is needed to identify species fished in environments with known mercury and selenium contamination. More information is also needed on the selenium levels found in individual fish before generalizations can be made.

Table 5.1. Total Mercury and Selenium concentrations (ug/g, wet weight) and Selenium: Mercury Molar ratios in fish species collected in the Columbia River Basin.

Common Name	Scientific Name	N	Mercury mean ± SE	Selenium mean ± SE	Hg nmol/g wet wt.	Se nmol/g wet wt.	Se:Hg	Se:Hg ratio correlation with length tau (p)
Walleye	*Sander vitreus*	10	0.32 ± 0.06	0.37 ± 0.04	1.60	4.69	2.94	-0.60 (NS)
Smallmouth bass	*Micropterus dolomieu*	95	0.23 ± 0.02	0.39 ± 0.05	1.15	4.33	3.78	-0.11 (NS)
Cutthroat trout	*Oncorhynchus clarkii*	6	0.12 ± 0.03	0.21 ± 0.05	0.60	2.63	4.39	-0.40 (NS)
White sturgeon	*Acipenser transmontanus*	32	0.11 ± 0.01	0.30 ± 0.02	0.55	3.79	6.91	
Rainbow trout	*Oncorhynchus mykiss*	12	0.10 ± 0.01	0.36 ± 0.04	0.50	4.52	9.07	-0.06 (NS)
Brown trout	*Salmo trutta*	20	0.26 ± 0.07	0.99 ± 0.17	1.30	12.60	9.72	-0.40 (NS)
Black crappie	*Pomoxis nigromaculatus*	12	0.09 ± 0.02	0.35 ± 0.03	0.45	4.52	10.08	-0.33 (NS)
Mountain whitefish	*Prosopium williamsoni*	20	0.14 ± 0.03	0.84 ± 0.15	0.70	10.60	15.19	-0.41 (NS)
Yellow perch	*Perca flavescens*	43	0.06 ± 0.01	0.43 ± 0.03	0.30	5.40	18.07	0.32 (NS)
Chinook salmon	*Oncorhynchus tshawytscha*	9	0.02 ± 0.001	0.23 ± 0.04	0.10	2.93	29.38	
		259						
ANOVA (p)			22.78 (<0.0001)	9.36 (<0.0001)			26.77 (<0.0001)	

Table 5.2. Coefficient of Variation (CV) for Mercury, Selenium and Se:Hg Molar Ratios in 10 Freshwater Fish Species Caught in the Columbia River Basin

Common Name	**N**	**Mercury CV**	**Selenium CV**	**Selenium: Mercury Molar Ratio CV**
Walleye	10	28.7 %	28.1%	29.4%
Smallmouth bass	95	36.1%	48.6%	60.2%
Cutthroat trout	6	31.8%	38.7%	93.1%
White sturgeon	32	21.9%	23.6%	53.6%
Rainbow trout	12	19.7%	58.9%	58.3%
Brown trout	20	58.6%	284.5%	67.5%
Black crappie	12	22.1%	25.5%	33.1%
Mountain whitefish	20	33.3%	99.6%	35.5%
Yellow perch	43	14.4%	40.3%	58.6%
Chinook salmon	9	5.9%	37.0%	44.8%

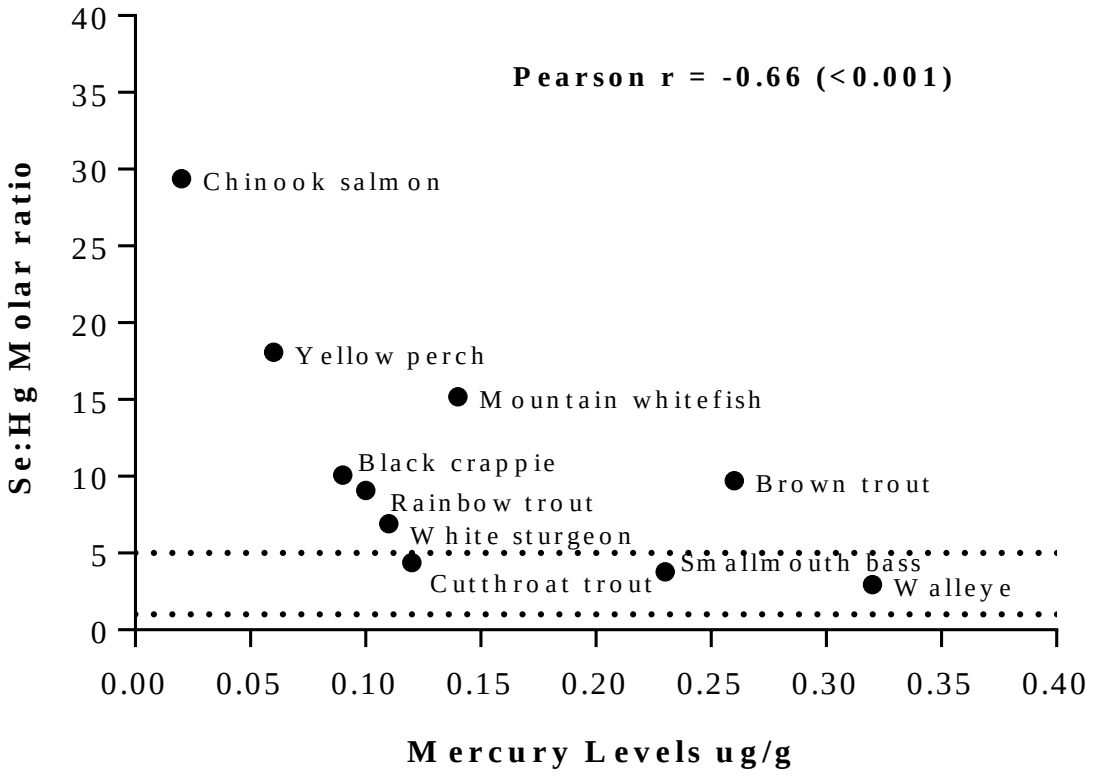


Figure 5.1. Relationship between selenium: mercury molar ratios to concentration of total mercury in freshwater fish found in the Columbia River Basin.

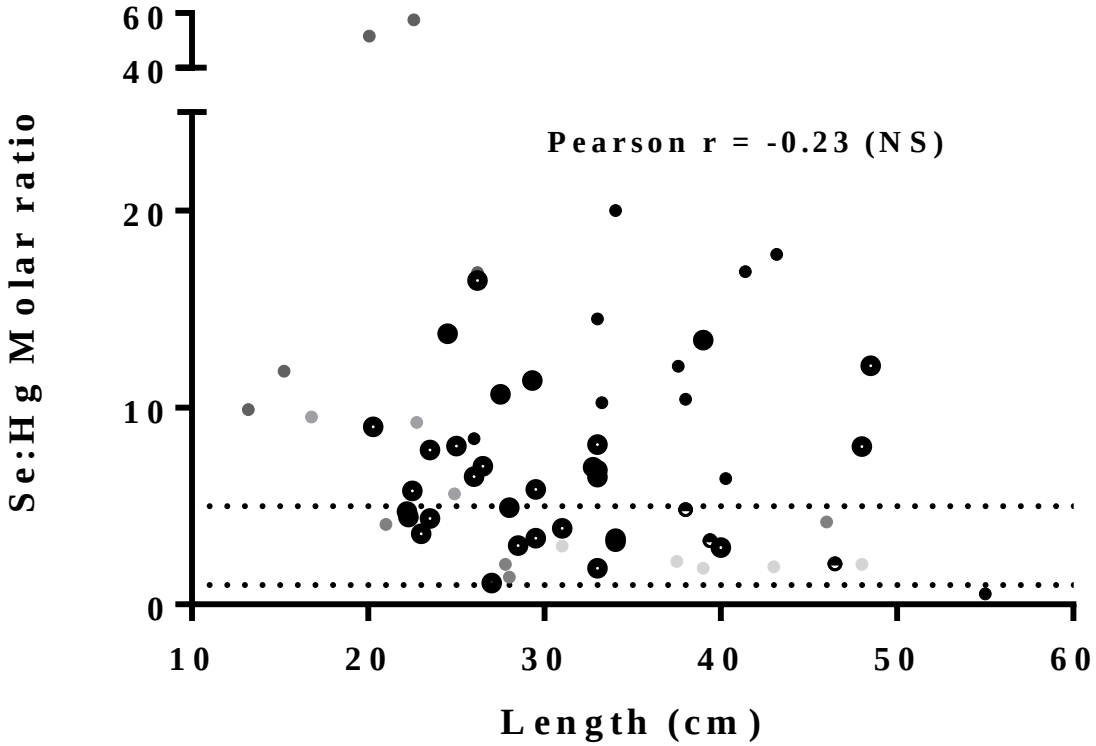


Figure 5.2. Relationship between selenium: mercury molar ratios to length for freshwater fish species found in the Columbia River Basin.

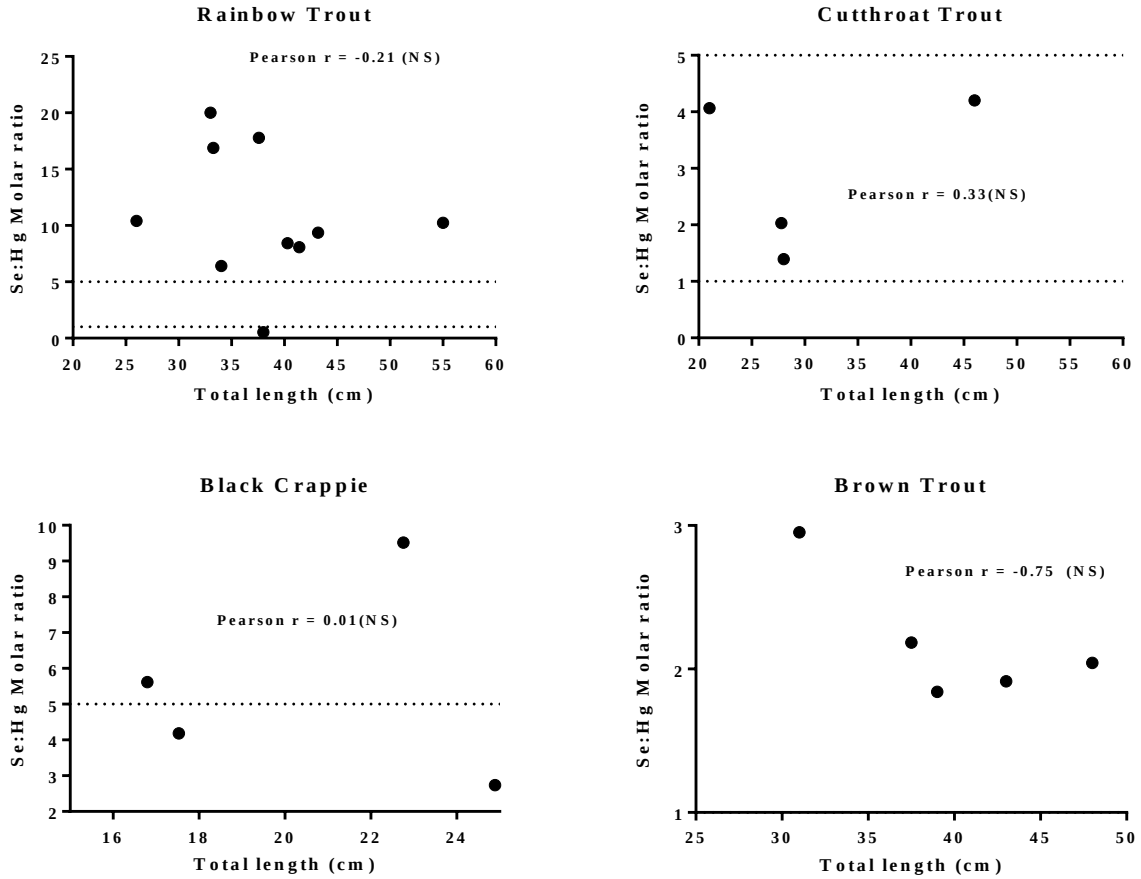


Figure 5.3a. Se: Hg Molar ratios for individual fish, by species, as a function of total length.

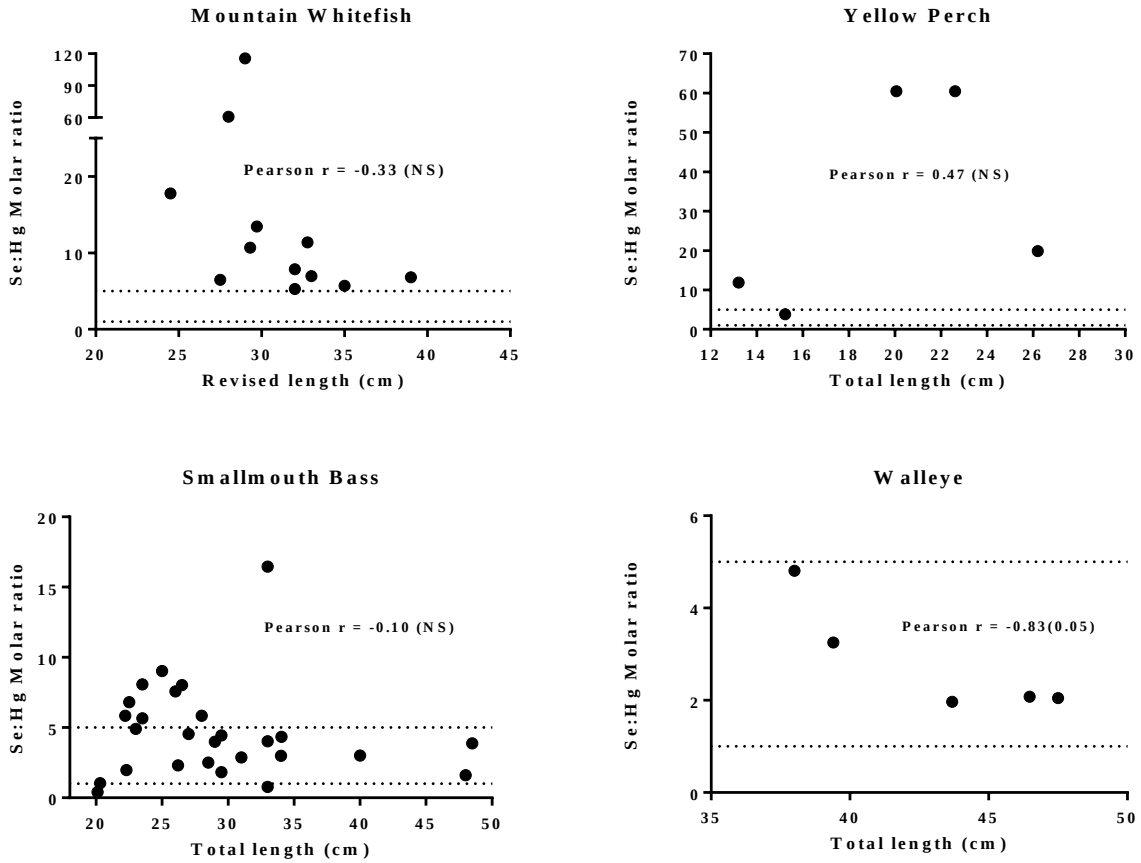


Figure 5.3b Se:Hg molar ratios for individual fish, by species, as a function of total length.

CHAPTER 6 – SUMMARY AND CONCLUSIONS

Fish are an important part of a balanced diet by providing nutrients and high quality protein. However, fish-consuming populations must decide whether the benefits of eating fish outweigh the risks from also consuming methylmercury and other contaminants. All 50 states in the U.S. have responded to the potential risks of methylmercury in fish by issuing fish consumption advisories, which provide advice on reducing the risk of adverse health effects from eating fish caught in local waters. Most fish consumption advisories for methylmercury are based solely on risks, and do not attempt to balance the risks and benefits. Given the benefits to be gained from fish consumption, a continued trend away from fish consumption is of public health concern. This research examines both the positive and negative health aspects of fish consumption using three different approaches.

The first manuscript used an integrated risk/benefit model developed by Ginsberg and Toal's (2009) to quantify net adult cardiovascular benefits or risks and net infant neurodevelopment benefits and risks based on mercury and omega 3 concentrations. This study focused on fish species in the Columbia River Basin, which has frequent fish advisories in effect due to mercury contamination in different rivers, reservoirs and lakes.

Using a quantitative risk/benefit analysis coupled with regionally specific fish contamination data, study found that the concentrations of methylmercury found in each fish species sampled varied by region within the Columbia River Basin and varied by species. The exception to this was that mountain whitefish, rainbow trout and chinook salmon provided a net benefit in terms of both CHD risk and improved VRM scores across all consumption rates in all sub-regions on which they were sampled. Species that provide a net benefit for coronary heart disease in one region may not have the same benefits in other regions and may not necessarily provide an improved visual recognition memory score within the same region. This makes generating general fish consumption advice based on either species or location difficult. Current fish consumption advisories in Oregon are region specific but in many regions the advice is to not consume any resident species. Following this advice would mean a loss of potentially valuable benefits from the omega 3s found in mountain whitefish, rainbow trout and chinook salmon in many of these regions. Statewide mercury advisories are in effect for smallmouth and largemouth bass in both Washington and Idaho. The advisory states that women of childbearing age and children should consume no more than 2 meals of bass per month. Our results indicated

that smallmouth bass provided a net risk in terms of infant neurodevelopment in all regions that it was sampled when taking into account the omega 3s found in this species.

Future work should focus on gathering omega-3 fatty acid levels for locally caught and consumed fish species. This would remove some of the uncertainty and augment the approach considerably. Dose-response relationships for other beneficial components of fish, such as selenium, and from other contaminants such as PCBs, DDT or PBDE, should also be included in integrated risk/benefit models in the future. While an important goal of public health officials is to assess and communicate risks, generalized fish consumption advice may encourage people to avoid fish species that are beneficial to their health. These findings highlight the importance of careful and clear communication of information regarding fish consumption and care needs to be given to ensure that the correct information will be interpreted by the consumer.

The second manuscript investigated geographical differences in methylmercury concentrations and fish consumption for women of childbearing age in the U.S. and the trends in fish consumption by using National Health and Nutrition Examination Survey (NHANES) data from 1999-2010. This study reinforced and expanded upon previous observations that dietary exposure via fish consumption is an important route for methylmercury intake by women of childbearing age, and especially for racial/ethnic groups with higher fish consumption.

One of the major findings of this research was that all coastal regions had greater blood mercury concentrations relative to their inland neighbors after controlling for other confounders. U.S. women of childbearing age (16-49) who live in the Atlantic and Pacific coastal regions have the highest blood methylmercury concentrations and women living in the Midwest have the lowest blood methylmercury concentrations. The number of fish meals consumed by women of childbearing age differs by region with the highest intake associated with coastal regions. Women in the Atlantic coast are consuming the most total fish and women in the Midwest are consuming the least amount of total fish. Total fish consumption has been slowly increasing from 1999-2010. The fact that blood methylmercury concentrations are decreasing and fish consumption is increasing may be due to the fact that women are making more informed choices when it comes to fish consumption.

However, only 17% of women of childbearing age are consuming fish at a rate of twice a week as recommended by both the American Heart Association and the U.S. EPA/FDA advisory. In 2009-2010, approximately 25% of women of childbearing age in the U.S. were not

consuming any fish at all and the mean consumption was 4.6 meals in the previous 30 days. The results of this study indicate that fish consumption advice needs to be tailored to specific regions in order to help women of childbearing age make more informed choices to increase consumption of fish which are low in methylmercury and high in omega 3s.

Increased understanding of the fish species contributing to high levels of methylmercury levels in women of childbearing age and the demographic characteristics associated with these fish species will help focus interventions and recommendations to at risk sub-populations. In order to reach these populations, outreach and informational material should be provided to both public health agencies and medical care providers. Outreach should be targeted to include those who have high exposures or are unaware of fish consumption advisories - this includes women living in coastal areas, Non-Hispanic Black women, women who self-identify as 'Other', consumers of marine fish and women of higher socioeconomic status.

The third manuscript focused on the inter- and intraspecific variability of selenium: mercury molar ratios for ten freshwater fish species commonly consumed in the Columbia River Basin. While the benefits from omega-3 fatty acids are well known, recent studies have shown that selenium may offer a protective buffer to the negative health effects of methylmercury. This study sought to determine if the intraspecific variation in the molar ratio is sufficiently low in order to use molar ratios in developing fish consumption advice and regionally specific risk management decisions within the Columbia River Basin.

Variation in concentrations of selenium, mercury and selenium: mercury molar ratios were high within the fish species regardless of sample size, which may due to either the trophic level of the fish or levels of mercury and selenium in the environment. The considerable variability of molar ratios within individual fish species renders this impractical for consideration in risk assessment and creates a challenge to use selenium: mercury molar ratios in fish consumption advisory notifications. Because mercury plays such an important role in the selenium: mercury molar ratio, at the present time it is advisable to base fish consumption on known mercury concentrations until more is understood about the complex interactions between selenium and mercury. However, providing the selenium, mercury and omega-3 information to consumers may be helpful when consumers are deciding which fish species to consume.

In addition, further research is needed to identify species fished in environments with known mercury and selenium contamination. More information is also needed on the selenium levels found in individual fish before generalizations can be made.

The research presented in this dissertation demonstrates the complexities involved in crafting fish consumption advisories that take into account both the risks and benefits of fish consumption. While we know that fish are a valuable and nutritious food source, they are also the main exposure for methylmercury in the U.S. Regional variations, demographic characteristics and types of fish consumed all play an important role in methylmercury exposure. Taking into account the positive aspects of fish consumption, such as omega-3s and selenium, could be the way forward when devising fish consumption advice. However, more research is needed to understand selenium's ability to ameliorate the effects of mercury toxicity before we can be confident about its protective effects. Additional research would also be beneficial to better understand the omega-3 fatty acid levels for locally caught and consumed fish species so that consumers are not discouraged from eating fish as a healthy component of their diet.

REFERENCES

Agency for Toxic Substances and Disease Registry. 2013. Toxfaqs™ for mercury. Available: http://www.atsdr.cdc.gov/toxfaqs/tf.asp?id=113&tid=24 [accessed 08/05 2013].

Amin-Zaki L, Elhassani S, Majeed MA, Clarkson TW, Doherty RA, Greenwood M. 1974. Intra-uterine methylmercury poisoning in iraq. Pediatrics 54:587-595.

Anderson H, Hanrahan L, Smith A, Draheim L, Kanarek M, Olsen J. 2004. The role of sport-fish consumption advisories in mercury risk communication: A 1998–1999 12-state survey of women age 18–45. Environ Res 95:315-324.

Aschner M, Aschner JL. 1990. Mercury neurotoxicity: Mechanisms of blood-brain barrier transport. Neurosci Biobehav Rev 14:169-176.

Bakir F, Damluji S, Amin-Zaki L, Murtadha M, Khalidi A, Al-Rawi N, et al. 1973. Methylmercury poisoning in iraq. Science 181:230-241.

Beijer K, Jernelöv A. 1978. Ecological aspects of mercury--selenium interactions in the marine environment. Environ Health Perspect 25:43.

Berglund M, Lind B, Björnberg KA, Palm B, Einarsson Ö, Vahter M. 2005. Inter-individual variations of human mercury exposure biomarkers: A cross-sectional assessment. Environ Health 4:20.

Bevelhimer MS. 1996. Relative importance of temperature, food, and physical structure to habitat choice by smallmouth bass in laboratory experiments. Trans Am Fish Soc 125:274-283.

Bevelhimer MS, Beauchamp, J.J., Sample, B.E., Southworth, G.R. 1997. Estimation of whole-fish contaminant concentrations from fish fillet data.

Birch RJ, Bigler J, Rogers JW, Zhuang Y, Clickner RP. 2014. Trends in blood mercury concentrations and fish consumption among us women of reproductive age, nhanes, 1999–2010. Environ Res.

Burger J, Gaines KF, Boring CS, Stephens WL, Snodgrass J, Gochfeld M. 2001. Mercury and selenium in fish from the savannah river: Species, trophic level, and locational differences. Environ Res 87:108-118.

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 409:1418-1429.

Burger J, Gochfeld M. 2012. Selenium and mercury molar ratios in saltwater fish from new jersey: Individual and species variability complicate use in human health fish consumption advisories. Environ Res 114:12-23.

Burger J, Gochfeld M, Jeitner C, Donio M, Pittfield T. 2012a. Selenium: Mercury molar ratios in freshwater fish from tennessee: Individual, species, and geographical variations have implications for management. EcoHealth 9:171-182.

Burger J, Gochfeld M, Jeitner C, Donio M, Pittfield T. 2012b. Interspecific and intraspecific variation in selenium:Mercury molar ratios in saltwater fish from the aleutians: Potential protection on mercury toxicity by selenium. Sci Total Environ 431:46-56.

Burger J, Gochfeld M. 2013. Selenium and mercury molar ratios in commercial fish from new jersey and illinois: Variation within species and relevance to risk communication. Food Chem Toxicol 57:235-245.

Burger J, Jeitner C, Donio M, Pittfield T, Gochfeld M. 2013. Mercury and selenium levels, and selenium:Mercury molar ratios of brain, muscle and other tissues in bluefish (pomatomus saltatrix) from new jersey, USA. Sci Total Environ 443:278-286.

Butler Walker J, Houseman J, Seddon L, McMullen E, Tofflemire K, Mills C, et al. 2006. Maternal and umbilical cord blood levels of mercury, lead, cadmium, and essential trace elements in arctic canada. Environ Res 100:295.

Caldwell KL, Mortensen ME, Jones RL, Caudill SP, Osterloh JD. 2009. Total blood mercury concentrations in the us population: 1999-2006. Int J Hyg Environ Health 212:588-598.

Calon F, Cole G. 2007. Neuroprotective action of omega-3 polyunsaturated fatty acids against neurodegenerative diseases: Evidence from animal studies. Prostaglandins, Leukotrienes and Essential Fatty Acids 77:287-293.

CDC CfDCaP. 2013. National health and nutrition examination survey questionnaire. Available: http://www.cdc.gov/nchs/nhanes/nhanes_questionnaires.htm [accessed Nov 21 2013].

Cernichiari E, Toribara TY, Liang L, Marsh DO, Berlin M, Myers GJ, et al. 1995. The biological monitoring of mercury in the seychelles study: Methylmercury and human health. Neurotoxicology 16:613-627.

Chan HM, Egeland GM. 2004. Fish consumption, mercury exposure, and heart diseases. Nutr Rev 62:68-72.

Chen J, Berry MJ. 2003. Selenium and selenoproteins in the brain and brain diseases. J Neurochem 86:1-12.

Choi AL, Cordier S, Weihe P, Grandjean P. 2008. Negative confounding in the evaluation of toxicity: The case of methylmercury in fish and seafood. Crit Rev Toxicol 38:877-893.

Christensen VG, Wente SP, Sandheinrich MB, Brigham ME. 2006. Spatial variation in fish-tissue mercury concentrations in the st. Croix river basin, minnesota and wisconsin, 2004.U. S. Geological Survey.

Clarkson TW. 1990. Human health risks from methylmercury in fish. Environ Toxicol Chem 9:957-961.

Clarkson TW, Magos L, Myers GJ. 2003. The toxicology of mercury—current exposures and clinical manifestations. New Engl J Med 349:1731-1737.

Close DA, Fitzpatrick MS, Li HW. 2002. The ecological and cultural importance of a species at risk of extinction, pacific lamprey. Fisheries 27:19-25.

Cohen JT, Bellinger DC, Connor WE, Kris-Etherton PM, Lawrence RS, Savitz DA, et al. 2005. A quantitative risk–benefit analysis of changes in population fish consumption. Am J Prev Med 29:325-334. e326.

Combs GF. 2001. Selenium in global food systems. Br J Nutr 85:517-547.

Council OE. ND. Chapter 2: Mercury. Available: http://www.oeconline.org/our-work/healthier-lives/pollutioninpeople/report/chapter2 [accessed 5/13 2013].

CRITFC CRI-TFC. 1994. A fish consumption survey of the umatilla, nez perce, yakama, and warm springs tribes of the columbia river basin. Columbia River Intertribal Fish Commission (CRITFC) Technical Report no 94-3.

Crump KS, Kjellström T, Shipp AM, Silvers A, Stewart A. 1998. Influence of prenatal mercury exposure upon scholastic and psychological test performance: Benchmark analysis of a new zealand cohort. Risk Anal 18:701-713.

Cuvin-Aralar MLA, Furness RW. 1991. Mercury and selenium interaction: A review. Ecotoxicol Environ Saf 21:348-364.

Davidson PW, Palumbo D, Myers GJ, Cox C, Shamlaye CF, Sloane-Reeves J, et al. 2000. Neurodevelopmental outcomes of seychellois children from the pilot cohort at 108 months following prenatal exposure to methylmercury from a maternal fish diet. Environ Res 84:1.

Davidson PW, Myers GJ, Weiss B, Shamlaye CF, Cox C. 2006. Prenatal methyl mercury exposure from fish consumption and child development: A review of evidence and perspectives from the seychelles child development study. Neurotoxicology 27:1106-1109.

Department of Environmental Quality. 2011. Human health toxics water quality standards. Available: http://www.deq.state.or.us/wq/pubs/factsheets/standards/HumanHealthToxicsRM.pdf [accessed 05/25 2014].

Department of Health and Welfare. 2014. The idaho fish consumption advisory program. Available: http://healthandwelfare.idaho.gov/Health/EnvironmentalHealth/FishAdvisories/tabid/180/default.aspx [accessed 05/25 2014].

Dock L, Rissanen R-L, Vahter M. 1994. Demethylation and placental transfer of methyl mercury in the pregnant hamster. Toxicology 94:131-142.

Domingo JL. 2007. Omega-3 fatty acids and the benefits of fish consumption: Is all that glitters gold? Environ Int 33:993-998.

Donatuto J, Harper BL. 2008. Issues in evaluating fish consumption rates for native american tribes. Risk Anal 28:1497-1506.

Egeland GM, Middaugh JP. 1997. Balancing fish consumption benefits with mercury exposure. Science 278:1904-1905.

Eisler R. 1987. Mercury hazards to fish, wildlife, and invertebrates: A synoptic review:Fish and Wildlife Service, US Department of the Interior.

Fleith M, Clandinin M. 2005. Dietary pufa for preterm and term infants: Review of clinical studies. Crit Rev Food Sci Nutr 45:205-229.

Fleming L, Walking S, Kaderman R, Levin B, Ayyar D, Bizzio M, et al. 1995. Mercury exposure in humans through food consumption from the everglades of florida. Water, Air, Soil Pollut 80:41-48.

Ganther H, Goudie C, Sunde M, Kopecky M, Wanger P, Hoh S, et al. 1972. Selenium: Relation to decreased toxicity of methylmercury added to diets containing tuna. Science,(Wash) 175:1122-1124.

Ginsberg GL, Toal BF. 2000. Development of a single-meal fish consumption advisory for methyl mercury. Risk Anal 20:41-48.

Ginsberg GL, Toal BF. 2009. Quantitative approach for incorporating methylmercury risks and omega-3 fatty acid benefits in developing species-specific fish consumption advice. Environ Health Perspect 117:267.

Gochfeld M, Burger J, Jeitner C, Donio M, Pittfield T. 2012. Seasonal, locational and size variations in mercury and selenium levels in striped bass (morone saxatilis) from new jersey. Environ Res 112:8-19.

Grandjean P, Weihe P, Jørgensen P, Clarkson T, Cernichiari E, Viderø T. 1992. Impact of maternal seafood diet on fetal exposure to mercury, selenium, and lead. Archives of Environmental Health: An International Journal 47:185-195.

Grandjean P, Weihe P, White RF, Debes F, Araki S, Yokoyama K, et al. 1997. Cognitive deficit in 7-year-old children with prenatal exposure to methylmercury. Neurotoxicol Teratol 19:417-428.

Grandjean P, Weihe P, White RF, Debes F. 1998. Cognitive performance of children prenatally exposed to “safe” levels of methylmercury. Environ Res 77:165-172.

Guallar E, Sanz-Gallardo MI, Veer Pvt, Bode P, Aro A, Gómez-Aracena J, et al. 2002. Mercury, fish oils, and the risk of myocardial infarction. New Engl J Med 347:1747-1754.

Harada M. 1995. Minamata disease: Methylmercury poisoning in japan caused by environmental pollution. CRC Crit Rev Toxicol 25:1-24.

Harper BL, Harris SG. 2008. A possible approach for setting a mercury risk-based action level based on tribal fish ingestion rates. Environ Res 107:60-68.

Herger LaLE. 2012. Assessment of mercury in fish tissue from select lakes of northeastern oregon Available: http://www.epa.gov/region10/pdf/publications/reports/hg_in_fish_tissue_ne_or_feb2012.pdf [accessed 05/24 2014].

Hibbeln JR, Davis JM, Steer C, Emmett P, Rogers I, Williams C, et al. 2007. Maternal seafood consumption in pregnancy and neurodevelopmental outcomes in childhood (alspac study): An observational cohort study. The Lancet 369:578-585.

Hightower JM, O'Hare A, Hernandez GT. 2006. Blood mercury reporting in nhanes: Identifying asian, pacific islander, native american, and multiracial groups. Environ Health Perspect 114:173-175.

Hightower JM. 2008. Diagnosis: Mercury: Money, politics, and poison:Island Press.
Hughes RM. 2014. Recreational fisheries in the USA: Economics, management strategies, and ecological threats. Fish Sci:1-9.

Imm P, Knobeloch L, Anderson HA. 2005. Fish consumption and advisory awareness in the great lakes basin. Environ Health Perspect 113:1325.

Innis SM. 1991. Essential fatty acids in growth and development. Prog Lipid Res 30:39-103.
IOM IoM. 2006. Seabood choices: Balancing benefits and risks. National Academy Press, Washington, DC (2006).

IOM IoM. 2007. Seafood choices: Balancing benefits and risks:The National Academies Press.
Iwata H, Okamoto H, Ohsawa Y. 1973. Effect of selenium on methylmercury poisoning. Res Commun Chem Pathol Pharmacol 5:673.

Jedrychowski W, Jankowski J, Flak E, Skarupa A, Mroz E, Sochacka–Tatara E, et al. 2006. Effects of prenatal exposure to mercury on cognitive and psychomotor function in one-year-old infants: Epidemiologic cohort study in poland. Ann Epidemiol 16:439-447.

Jones RAaRL. N.D. Laboratory procedure manual Available: http://www.cdc.gov/nchs/data/nhanes/nhanes_99_00/lab06_met_blood_mercury.pdf [accessed 02/22 2013].

Kaneko JJ, Ralston NV. 2007. Selenium and mercury in pelagic fish in the central north pacific near hawaii. Biol Trace Elem Res 119:242-254.

Karouna-Renier NK, Ranga Rao K, Lanza JJ, Rivers SD, Wilson PA, Hodges DK, et al. 2008. Mercury levels and fish consumption practices in women of child-bearing age in the florida panhandle. Environ Res 108:320-326.

Karvetti R, Knuts LR. 1985. Validity of the 24-hour dietary recall. J Am Diet Assoc 85:1437-1442.

Katner A, Sun M-H, Suffet M. 2010. An evaluation of mercury levels in louisiana fish: Trends and public health issues. Sci Total Environ 408:5707-5714.

Kershaw TG, Dhahir PH, Clarkson TW. 1980. The relationship between blood levels and dose of methylmercury in man. Archives of Environmental Health: An International Journal 35.

Knobeloch L, Anderson HA, Imm P, Peters D, Smith A. 2005. Fish consumption, advisory awareness, and hair mercury levels among women of childbearing age. Environ Res 97:220-227.

Kremer JM. 2000. N− 3 fatty acid supplements in rheumatoid arthritis. The American journal of clinical nutrition 71:349s-351s.

Legrand M, Arp P, Ritchie C, Chan HM. 2005. Mercury exposure in two coastal communities of the bay of fundy, canada. Environ Res 98:14-21.

Loring PA, Duffy LK, Murray MS. 2010. A risk–benefit analysis of wild fish consumption for various species in alaska reveals shortcomings in data and monitoring needs. Sci Total Environ 408:4532-4541.

MacIntosh DL, Spengler JD, Ozkaynak H, Tsai L-h, Ryan PB. 1996. Dietary exposures to selected metals and pesticides. Environ Health Perspect 104:202.

Mahaffey KR. 1999. Methylmercury: A new look at the risks. Public Health Rep 114:396.

Mahaffey KR, Clickner RP, Bodurow CC. 2003. Blood organic mercury and dietary mercury intake: National health and nutrition examination survey, 1999 and 2000. Environ Health Perspect 112:562-570.

Mahaffey KR. 2004. Fish and shellfish as dietary sources of methylmercury and the ω-3 fatty acids, eicosahexaenoic acid and docosahexaenoic acid: Risks and benefits. Environ Res 95:414-428.

Mahaffey KR, Clickner RP, Jeffries RA. 2008. Methylmercury and omega-3 fatty acids: Co-occurrence of dietary sources with emphasis on fish and shellfish. Environ Res 107:20-29.

Mahaffey KR, Clickner RP, Jeffries RA. 2009. Adult women's blood mercury concentrations vary regionally in the united states: Association with patterns of fish consumption (nhanes 1999–2004). Environ Health Perspect 117:47.

Mahaffey KR, Clickner RP, Jeffries RA. 2009. Adult women's blood mercury concentrations vary regionally in the united states: Association with patterns of fish consumption (nhanes 1999-2004). Environ Health Perspect 117:47-53.

McAlpine D, Araki S. 1959. Minamata disease: Late effects of an unusual neurological disorder caused by contaminated fish. Arch Neurol 1:522.

McCann P. 2005. Great lakes mercury protocol. In: Proceedings of the 2005 national forum on contaminants in fish. U.S. Epa 823-r-05-006. Available: http://epa.gov/waterscience/fish/forum/2005/proceedings2005.pdf [accessed March 24th 2014].

McDowell MA, Dillon CF, Osterloh J, Bolger PM, Pellizzari E, Fernando R, et al. 2004. Hair mercury levels in us children and women of childbearing age: Reference range data from nhanes 1999–2000. Environ Health Perspect 112:1165.

McKelvey W, Gwynn RC, Jeffery N, Kass D, Thorpe LE, Garg RK, et al. 2007. A biomonitoring study of lead, cadmium, and mercury in the blood of new york city adults. Environ Health Perspect 115:1435.

Mergler D, Anderson HA, Chan LHM, Mahaffey KR, Murray M, Sakamoto M, et al. 2007. Methylmercury exposure and health effects in humans: A worldwide concern. AMBIO: A Journal of the Human Environment 36:3-11.

Morrissette J, Takser L, St-Amour G, Smargiassi A, Lafond J, Mergler D. 2004. Temporal variation of blood and hair mercury levels in pregnancy in relation to fish consumption history in a population living along the st. Lawrence river. Environ Res 95:363-374.

Mozaffarian D. 2009. Fish, mercury, selenium and cardiovascular risk: Current evidence and unanswered questions. Int J Env Res Public Health 6:1894-1916.

Mozaffarian D REB. 2006. Fish intake, contaminants, and human health: Evaluating the risks and the benefits. JAMA 296:1885-1899.

Munn MD, Short TM. 1997. Spatial heterogeneity of mercury bioaccumulation by walleye in franklin d. Roosevelt lake and the upper columbia river, washington. Trans Am Fish Soc 126:477-487.

Myers G, Davidson P, Shamlaye C, Axtell C, Cernichiari E, Choisy O, et al. 1996. Effects of prenatal methylmercury exposure from a high fish diet on developmental milestones in the seychelles child development study. Neurotoxicology 18:819-829.

Myers GJ, Davidson PW, Cox C, Shamlaye CF, Palumbo D, Cernichiari E, et al. 2003. Prenatal methylmercury exposure from ocean fish consumption in the seychelles child development study. The Lancet 361:1686-1692.

OHA OHA. 2014. Fish and shellfish consumption. Available: https://public.health.oregon.gov/HealthyEnvironments/Recreation/FishConsumption/Pages/index.aspx [accessed 05/25 2014].

Ohi G, Nishigaki S, Seki H, Tamura Y, Maki T, Konno H, et al. 1976. Efficacy of selenium in tuna and selenite in modifying methylmercury intoxication. Environ Res 12:49-58.

Oken E, Kleinman KP, Berland WE, Simon SR, Rich-Edwards JW, Gillman MW. 2003. Decline in fish consumption among pregnant women after a national mercury advisory. Obstet Gynecol 102:346.

Oken E, Wright RO, Kleinman KP, Bellinger D, Amarasiriwardena CJ, Hu H, et al. 2005. Maternal fish consumption, hair mercury, and infant cognition in a us cohort. Environ Health Perspect 113:1376.

Oken E, Choi AL, Karagas MR, Mariën K, Rheinberger CM, Schoeny R, et al. 2012. Which fish should i eat? Perspectives influencing fish consumption choices. Environ Health Perspect 120:790.

Patch SC, Maas RP, Sergent KR. 2005. An investigation of factors related to levels of mercury in human hair:University of North Carolina-Asheville Environmental Quality Institute.

Peterson SA, Van Sickle J, Herlihy AT, Hughes RM. 2006. Mercury concentration in fish from streams and rivers throughout the western united states. Environ Sci Technol 41:58-65.

Peterson SA, Ralston NVC, Peck DV, Sickle JV, Robertson JD, Spate VL, et al. 2009. How might selenium moderate the toxic effects of mercury in stream fish of the western u.S.? Environ Sci Technol 43:3919-3925.

Pirrone N, Cinnirella S, Feng X, Finkelman R, Friedli H, Leaner J, et al. 2010. Global mercury emissions to the atmosphere from anthropogenic and natural sources. Atmospheric Chemistry and Physics 10:5951-5964.

Ralston C, Blackwell J, Ralston N. 2006. Effects of dietary selenium and mercury on house crickets(acheta domesticus l.): Implications of environmental co-exposures. Environmental Bioindicators 1:98-109.

Ralston N, Raymond L. 2013. Selenium status and intake influences mercury exposure risk assessments. Selenium in the environment and human health:203.

Ralston NV. 2008. Selenium health benefit values as seafood safety criteria. EcoHealth 5:442-455.

Ralston NVC, Ralston CR, Blackwell Iii JL, Raymond LJ. 2008. Dietary and tissue selenium in relation to methylmercury toxicity. Neurotoxicology 29:802-811.

Rayman MP. 2000. The importance of selenium to human health. The lancet 356:233-241.

Raymond LJ, Ralston NVC. 2009. Selenium's importance in regulatory issues regarding mercury. Fuel Processing Technology 90:1333-1338.

Reash RJ. 2012. Selenium, arsenic, and mercury in fish inhabiting a fly ash exposure gradient: Interspecific bioaccumulation patterns and elemental associations. Environ Toxicol Chem 31:739-747.

Rice DC, Krewski D, Collins B, Willes RF. 1989. Pharmacokinetics of methylmercury in the blood of monkeys (< i> macaca fascicularis</i>). Fundam Appl Toxicol 12:23-33.

Rice DC, Schoeny R, Mahaffey K. 2003. Methods and rationale for derivation of a reference dose for methylmercury by the us epa. Risk Anal 23:107-115.

Rice DC. 2004. The us epa reference dose for methylmercury: Sources of uncertainty. Environ Res 95:406-413.

Rose SA, Feldman JF. 1995. Prediction of iq and specific cognitive abilities at 11 years from infancy measures. Dev Psychol 31:685.

Schober et al. 2003. Blood mercury levels in us children and women of childbearing age, 1999-2000. JAMA 289:1667-1674.

Serdar D, County W. 2001. Mercury concentrations in edible muscle of lake whatcom fish:Washington State Department of Ecology.

Serdar D, Johnson,J., Mueller, K. and Glen Patrick. 2001. Mercury concentrations in edible muscle of lake whatcom fish. Available: http://www.eco-3.com/LkWhcomFishHg.pdf [accessed March 23rd 2014].

Sidhu KS. 2003. Health benefits and potential risks related to consumption of fish or fish oil. Regul Toxicol Pharmacol 38:336-344.

Silbernagel SM, Carpenter DO, Gilbert SG, Gochfeld M, Groth E, Hightower JM, et al. 2011. Recognizing and preventing overexposure to methylmercury from fish and seafood consumption: Information for physicians. J Toxicol 2011.

Smith KM, Sahyoun NR. 2005. Fish consumption: Recommendations versus advisories, can they be reconciled? Nutr Rev 63:39-46.

Sørmo EG, Ciesielski TM, Øverjordet IB, Lierhagen S, Eggen GS, Berg T, et al. 2011. Selenium moderates mercury toxicity in free-ranging freshwater fish. Environ Sci Technol 45:6561-6566.

Stern AH, Smith AE. 2003. An assessment of the cord blood: Maternal blood methylmercury ratio: Implications for risk assessment. Environ Health Perspect 111:1465.

Stern AH, Korn LR. 2011. An approach for quantitatively balancing methylmercury risk and omega-3 benefit in fish consumption advisories. Environ Health Perspect 119:1043.

Streets DG, Devane MK, Lu Z, Bond TC, Sunderland EM, Jacob DJ. 2011. All-time releases of mercury to the atmosphere from human activities. Environ Sci Technol 45:10485-10491.

U.S. EPA USEPA. 2009. Columbia river basin: State of the river report for toxics Available: http://www2.epa.gov/sites/production/files/documents/columbia_state_of_the_river_report_jan2009.pdf 2014].

U.S. EPA USEPA. 2012. What you need to know about mercury in fish and shellfish. Available: http://water.epa.gov/scitech/swguidance/fishshellfish/outreach/advice_index.cfm [accessed 04/21 2013].

U.S. EPA USEPA. 2013. Mercury. Available: http://www.epa.gov/hg/exposure.htm [accessed 05/20 2013].

U.S. EPA USEPA. 2014. Methylmercury (mehg) (casrn 22967-92-6). Available: http://www.epa.gov/iris/subst/0073.htm [accessed 05/25 2014].

Uauy R, Hoffman DR, Mena P, Llanos A, Birch EE. 2003. Term infant studies of dha and ara supplementation on neurodevelopment: Results of randomized controlled trials. The Journal of pediatrics 143:17-25.

USDA USDoA, Agricultural Research Service). 2013. Usda national nutrient database for standard reference, release 26. Available: http://www.ars.usda.gov/ba/bhnrc/ndl [accessed 05/24 2014].

USEPA. Toxic release inventory. Available: http://www.epa.gov/tri/tridata/index.htm#pdr [accessed 11/03 2013].

Vahter ME, Mottet NK, Friberg LT, Lind SB, Charleston JS, Burbacher TM. 1995. Demethylation of methyl mercury in different brain sites of macaca-fascicularis monkeys during long-term subclinical methyl mercury exposure. Toxicol Appl Pharmacol 134:273-284.

Van Wijngaarden E, Beck C, Shamlaye CF, Cernichiari E, Davidson PW, Myers GJ, et al. 2006. Benchmark concentrations for methyl mercury obtained from the 9-year follow-up of the seychelles child development study. Neurotoxicology 27:702-709.

Warner K. 2007. Mercury levels in hair of coastal alabama anglers and residents. Oceana, Washington DC/ht tp://na oceana org/sites/default/files/o/fileadmin/oceana/uploads/mercury/Rodeo_Hair_Report_Final pdfS (accessed 2109 11).

Washington State Department of Health. 2014. Fish consumption advisories. Available: http://www.doh.wa.gov/CommunityandEnvironment/Food/Fish/Advisories.aspx [accessed 05/25 2014].

Watanabe C, Yoshida K, Kasanuma Y, Kun Y, Satoh H. 1999. < i> in utero</i> methylmercury exposure differentially affects the activities of selenoenzymes in the fetal mouse brain. Environ Res 80:208-214.

Watanabe C. 2001. [selenium deficiency and brain functions: The significance for methylmercury toxicity]. Nihon eiseigaku zasshi Japanese journal of hygiene 55:581.

Weil M, Bressler J, Parsons P, Bolla K, Glass T, Schwartz B. 2005. Blood mercury levels and neurobehavioral function. JAMA: the journal of the American Medical Association 293:1875-1882.

Wente SP. 2004. A statistical model and national data set for partitioning fish-tissue mercury concentration variation between spatiotemporal and sample characteristic effects. US Geological Survey Science Investigation Report 5199.

Whanger P. 2000. Selenium and the brain: A review. Nutr Neurosci 4:81-97.

Willett W. 2012. Nutritional epidemiology:Oxford University Press.

Williams LK, Stern AH. 2005. Balancing the risks and benefits of fish consumption. Vol. 142:American College of Physicians, 946-949.

APPENDICES

APPENDIX A

Fish Baskets

The equation was run for each individual species however we included a 'Fish Basket' calculation for one of the sub-regions because there was not adequate data in the other regions. The CRITFC fish consumption survey showed that approximately 92.4% of adults CRITFC members consumed salmon, 70.2% consumed rainbow trout, 22.8 % consumed mountain whitefish and 7.7% consumed suckers. We defined the fish basket as consuming half salmon and half of all other species sampled in that region. We calculated mercury concentrations by weighting the median mercury values as follows: Fish basket MeHg= 0.5*Salmon Hg+ 0.125*Largescale sucker Hg + .125*Mountain whitefish Hg + 0.125*Rainbow Trout Hg + 0.125*Smallmouth bass Hg. The values for the average omega-3s were also derived in this way.

A species-by-species approach to fish consumption advisories is meaningful because many people have a particular type of fish that they consume most often. We included a "fish basket' in subregion 1709 because this is the only region in which salmon were sampled. We defined a fish basket as consumption of half salmon and half of all other species sampled in that region. Consuming a fish basket appears to have a net benefit for neurodevelopment when consumed at a rate of 7 meals per week. The EPA's RfD of 0.1µg/kg/day for daily fish ingestion is not exceeded when consumed at this rate. While there is no methylmercury reference dose for the general population, a number of states use a value of 0.3 µg/kg/day for the general public in order to prevent neurological effects (Ginsberg and Toal 2009). A fish basket also provides a net benefit in terms of adult cardiovascular risk when consumed up to 25 meals per week.

Sources of Mercury in the Columbia River Basin

Wide ranges of methylmercury concentrations were found in fish tissue within the Columbia River Basin. However, not all species were sampled in each region, so the averages displayed represent data from varying fish samples. The majority of fish sampled were resident fish (all except chinook salmon and sturgeon depending on where they were sampled). Resident fish spend their life in the Columbia River and its tributaries so that any exposure and uptake of mercury will occur in water in the vicinity of the locations where the samples were collected. These exposures can come from either point or non-point sources. Point sources include current and past industrial discharges to the air, land and water. Non-point sources are more widespread sources such as runoff from farms, and roads and atmospheric deposition. Discharges from

industrial sources and storm water runoff from streets and other developed areas are more direct sources of mercury to streams than either air deposition or erosion. Although the concentrations being emitted to these waterbodies are low, the volume of discharge is high (U.S. EPA 2009). According to the State of the River Report for Toxics, nine of the 23 largest municipal and industrial wastewater point sources found in the U.S. portion of the Columbia River have reported discharging a total of 33 pounds of mercury per year (U.S. EPA 2009) This number may in fact be even greater than this as mercury reporting is not always required. These sources are significant at the local watershed level due to the fact that they are being directly deposited into the water.

Concentrations of methylmercury in fish can vary across the basin and it is often difficult to determine with certainty what the source may be. Historic mercury and gold mining can be important sources that load mercury directly to streams and have significant impacts at a watershed scale (U.S. EPA 2009). Current sources of mercury emissions are from the coal-fired plants located in the Columbia River Basin. One possible explanation is that it could be due to the local variations of naturally occurring mercury in the soils in each subregion. A natural source of mercury in Oregon includes deposits of cinnabar related to geothermal and volcanic activity (U.S. EPA 2009). Further exploration is needed to determine what is causing the mercury levels in each sub region.

Fish Consumption Advisories in the Columbia River Basin

The Oregon Health Authority currently lists 14 different waterbodies with a fish consumption advisory based on mercury contamination (OHA 2014). The advisories are categorized by ‘vulnerable population’ and ‘everyone else’. Vulnerable population includes children under the age of 6, women of childbearing age and people with thyroid or immune system problems. The majority of affected fish species are listed as ‘all resident fish’, with the exception of one advisory excluding rainbow trout from the warning (OHA 2014). Resident fish are species that spend their entire lives within a certain territory and do not migrate. In Portland Harbor it is advised against eating carp, bass and catfish. While Oregon does not have a statewide ban on small and largemouth bass, it does recommend limiting consumption of all resident fish to 1-2 meals *per month* in many locations. The Washington State Department of Health has issued a statewide mercury advisory stating that women of childbearing age and children should limit largemouth and smallmouth bass to 2 meals per month (Washington State

Department of Health 2014). They also provide advice based on waterbody and species. Idaho's Department of Health and Welfare currently has a statewide advisory for mercury in bass, both largemouth and smallmouth (Department of Health and Welfare 2014). This advisory issued states that women who are pregnant, planning on becoming pregnant, nursing and children under the age of 15 should not eat more than 2 meals per month of bass. The general population is advised to consume no more than 8 meals per month and to not eat any other fish during the month at these amounts of bass caught in Idaho.

APPENDIX B

Table 1. Distribution of Blood Methylmercury concentrations (µg/L) by Year, Age, Income and Race/Ethnicity for women aged 16-49 using NHANES 1999-2010.

	N	Arith. Mean		Geometric Mean		Selected percentiles (95% CI)							
		(95% CI)		(95% CI)									
All Women						25th		50th		75th		90th	
1999-2000	1640	1.67	(1.19, 2.16)	0.48	(0.31, 0.75)	0.17	(0.01, 0.40)	0.64	(0.46, 0.88)	1.72	(1.17, 2.54)	4.40	(3.28, 5.91)
2001-2002	1815	1.17	(0.97, 1.36)	0.52	(0.46, 0.59)	0.20	(0.19, 0.21)	0.52	(0.46, 0.58)	1.32	(1.13, 1.54)	2.80	(2.44, 3.21)
2003-2004	1617	1.00	(0.81, 1.19)	0.29	(0.22, 0.39)	0.20	(0.14, 0.28)	0.40	(0.28, 0.57)	1.10	(0.89, 1.36)	2.49	(1.96, 3.17)
2005-2006	1804	1.11	(0.94, 1.28)	0.57	(0.48, 0.66)	0.20	(0.17, 0.24)	0.58	(0.49, 0.69)	1.29	(1.05, 1.58)	2.72	(2.27, 3.26)
2007-2008	1510	0.95	(0.76, 1.13)	0.50	(0.43, 0.58)	0.20	(0.18, 0.22)	0.44	(0.36, 0.54)	1.01	(0.8, 1.28)	2.34	(1.80, 3.04)
2009-2010	1798	1.13	(1.00, 1.25)	0.59	(0.53, 0.66)	0.20	(0.17, 0.24)	0.54	(0.45, 0.65)	1.33	(1.13, 1.57)	2.70	(2.45, 2.98)
Age													
16-19	2478	0.70	(0.63, 0.77)	0.30	(0.26, 0.33)	0.20	(0.18, 0.22)	0.24	(0.19, 0.29)	0.76	(0.66, 0.88)	1.60	(1.45, 1.77)
20-29	2752	0.98	(0.89, 1.07)	0.40	(0.35, 0.46)	0.20	(0.19, 0.22)	0.42	(0.37, 0.47)	1.12	(1.01, 1.23)	2.38	(2.14, 2.64)
30-39	2515	1.33	(1.14, 1.51)	0.55	(0.48, 0.63)	0.20	(0.17, 0.23)	0.60	(0.53, 0.68)	1.49	(1.33, 1.67)	3.25	(2.80, 3.77)
40-49	2439	1.33	(1.20, 1.46)	0.59	(0.53, 0.65)	0.25	(0.20, 0.30)	0.66	(0.60, 0.71)	1.49	(1.35, 1.65)	3.23	(2.84, 3.67)
Income													
>20,000	2997	0.89	(0.78, 1.00)	0.35	(0.30, 0.41)	0.20	(0.18, 0.23)	0.40	(0.35, 0.46)	0.99	(0.87, 1.13)	2.20	(1.92, 2.52)
20-44,999	2896	1.07	(0.94, 1.20)	0.41	(0.36, 0.47)	0.20	(0.18, 0.22)	0.46	(0.42, 0.51)	1.15	(1.01, 1.30)	2.41	(2.13, 2.74)
45-74,999	1806	1.10	(0.98, 1.22)	0.49	(0.43, 0.56)	0.20	(0.18, 0.22)	0.53	(0.47, 0.59)	1.22	(1.09, 1.36)	2.52	(2.14, 2.97)
<75,000	1901	1.49	(1.33, 1.66)	0.71	(0.63, 0.80)	0.27	(0.23, 0.33)	0.78	(0.69, 0.88)	1.84	(1.60, 2.10)	3.74	(3.30, 4.25)
Race/Ethnicity													
Mexican American	2605	0.72	(0.66, 0.78)	0.34	(0.31, 0.38)	0.20	(0.18, 0.22)	0.40	(0.37, 0.43)	0.86	(0.80, 0.93)	1.55	(1.40, 1.71)
Non-Hispanic Black	2253	1.23	(1.09, 1.37)	0.59	(0.53, 0.66)	0.28	(0.24, 0.33)	0.69	(0.62, 0.77)	1.39	(1.24, 1.54)	2.68	(2.33, 3.08)

Non-Hispanic White	4087	1.10	(0.99, 1.22)	0.44	(0.40, 0.50)	0.20	(0.18, 0.22)	0.48	(0.44, 0.54)	1.22	(1.10, 1.35)	2.79	(2.49, 3.12)
Other Hispanic	762	1.25	(0.94, 1.56)	0.57	(0.46, 0.70)	0.21	(0.17, 0.26)	0.65	(0.58, 0.74)	1.52	(1.33, 1.73)	2.65	(2.18, 3.22)
Other	477	2.34	(1.98, 2.69)	0.99	(0.78, 1.27)	0.40	(0.25, 0.63)	1.20	(0.92, 1.55)	3.00	(2.46, 3.65)	5.73	(4.92, 6.68)

Table 2. Distribution of Blood mercury concentrations (μg/L) by Year, Age, Income and Race/Ethnicity for women aged 16-49 using NHANES 1999-2010.

	N	Arith. Mean		Geometric Mean		Selected percentiles							
			(95% CI)		(95% CI)				(95% CI)				
All Women						25th		50th		75th		90th	
1999-2000	1640	1.96	(1.46, 2.46)	1.01	(0.83, 1.25)	0.41	(0.32, 0.53)	0.94	(0.76, 1.17)	2.03	(1.47, 2.8)	4.73	(3.56, 6.28)
2001-2002	1815	1.43	(1.22, 1.64)	0.83	(0.74, 0.93)	0.38	(0.32, 0.45)	0.79	(0.71, 0.87)	1.60	(1.40, 1.84)	3.02	(2.66, 3.43)
2003-2004	1617	1.35	(1.15, 1.55)	0.82	(0.71, 0.94)	0.39	(0.31, 0.48)	0.76	(0.66, 0.88)	1.51	(1.30, 1.76)	3.04	(2.48, 3.72)
2005-2006	1804	1.45	(1.25, 1.64)	0.92	(0.82, 1.02)	0.47	(0.40, 0.55)	0.89	(0.8, 1.00)	1.64	(1.41, 1.92)	3.11	(2.77, 3.50)
2007-2008	1510	1.25	(1.06, 1.43)	0.79	(0.70, 0.88)	0.41	(0.37, 0.46)	0.76	(0.68, 0.85)	1.42	(1.23, 1.64)	2.72	(2.2, 3.36)
2009-2010	1798	1.39	(1.25, 1.54)	0.88	(0.80, 0.97)	0.44	(0.38, 0.52)	0.83	(0.73, 0.94)	1.63	(1.43, 1.86)	3.12	(2.85, 3.40)
Age													
16-19	2478	0.92	(0.85, 1.00)	0.56	(0.53, 0.600)	0.23	(0.21, 0.25)	0.53	(0.49, 0.58)	1.09	(1.00, 1.20)	1.98	(1.84, 2.14)
20-29	2752	1.24	(1.15, 1.34)	0.76	(0.71, 0.8)	0.39	(0.36, 0.43)	0.72	(0.67, 0.77)	1.44	(1.35, 1.54)	2.70	(2.46, 2.96)
30-39	2515	1.65	(1.45, 1.85)	0.95	(0.88, 1.03)	0.49	(0.45, 0.54)	0.90	(0.83, 0.96)	1.81	(1.65, 1.99)	3.60	(3.15, 4.10)
40-49	2439	1.67	(1.54, 1.80)	1.05	(0.99, 1.11)	0.57	(0.52, 0.62)	1.00	(0.94, 1.06)	1.89	(1.74, 2.05)	3.69	(3.28, 4.14)
Income													
>20,000	2997	1.17	(1.04, 1.30)	0.71	(0.66, 0.77)	0.39	(0.34, 0.45)	0.70	(0.64, 0.75)	1.30	(1.17, 1.45)	2.50	(2.22, 2.81)
20-44,999	2896	1.38	(1.24, 1.51)	0.80	(0.75, 0.85)	0.40	(0.37, 0.42)	0.79	(0.74, 0.85)	1.50	(1.39, 1.61)	2.76	(2.46, 3.11)
45-74,999	1806	1.41	(1.28, 1.53)	0.87	(0.82, 0.93)	0.49	(0.44, 0.54)	0.89	(0.84, 0.95)	1.59	(1.47, 1.73)	2.99	(2.60, 3.44)
<75,000	1901	1.80	(1.63, 1.97)	1.10	(1.01, 1.19)	0.58	(0.53, 0.63)	1.10	(1.01, 1.19)	2.19	(1.95, 2.46)	4.09	(3.71, 4.51)
Race/Ethnicity													
Mexican American	2605	1.05	(0.95, 1.14)	0.70	(0.65, 0.74)	0.39	(0.36, 0.43)	0.7	(0.66, 0.74)	1.20	(1.12, 1.28)	1.99	(1.81, 2.20)
Non-Hispanic Black	2253	1.56	(1.41, 1.70)	1.02	(0.95, 1.09)	0.59	(0.55, 0.63)	0.99	(0.92, 1.07)	1.74	(1.60, 1.90)	3.10	(2.73, 3.51)
Non-Hispanic White	4087	1.38	(1.27, 1.50)	0.82	(0.77, 0.87)	0.40	(0.37, 0.43)	0.8	(0.74, 0.85)	1.59	(1.47, 1.72)	3.18	(2.89, 3.50)
Other Hispanic	762	1.58	(1.21, 1.95)	0.97	(0.88, 1.08)	0.50	(0.43, 0.58)	0.99	(0.90, 1.10)	1.89	(1.73, 2.07)	3.03	(2.55, 3.59)

Other	477	2.72	(2.32, 3.12)	1.51	(1.33, 1.73)	0.69	(0.55, 0.88)	1.58	(1.29, 1.92)	3.45	(2.86, 4.15)	6.09	(5.41, 6.86)

Table 3. Percentages and their standard errors for frequency of fish consumption by type of fish, by NHANES survey release, income, race/ethnicity and age for women aged 16-49 years, NHANES 1999-2010.

Variable					
NHANES Survey Release					
Total Fish	N	0 times	1-4 times	5-8 times	9+ times
1999-2000	1640	26.10%	48.35%	15.12%	10.43%
2001-2002	1815	22.64%	47.82%	17.30%	12.23%
2003-2004	1617	22.88%	46.44%	17.81%	12.86%
2005-2006	1804	24.11%	42.79%	18.85%	14.25%
2007-2008	1510	25.23%	46.16%	15.63%	12.98%
2009-2010	1798	23.14%	40.71%	19.41%	16.74%
Tuna	N	0 times	1-2 times	3-4 times	5+ times
1999-2000	1640	67.87%	21.10%	6.95%	4.09%
2001-2002	1815	62.15%	24.41%	8.82%	4.63%
2003-2004	1617	66.91%	21.52%	7.30%	4.27%
2005-2006	1804	67.24%	20.51%	8.54%	3.71%
2007-2008	1510	71.92%	18.01%	6.03%	4.04%
2009-2010	1798	69.30%	18.85%	7.62%	4.23%
Marine Fish	N	0 times	1-2 times	3-4 times	5+ times
1999-2000	1640	60.24%	27.80%	7.13%	4.82%
2001-2002	1815	60.00%	27.05%	7.99%	4.96%
2003-2004	1617	55.91%	28.51%	9.21%	6.37%
2005-2006	1804	56.43%	26.27%	9.81%	7.48%
2007-2008	1510	55.83%	28.41%	9.47%	6.29%
2009-2010	1798	52.73%	26.47%	10.68%	10.12%
Marine Shellfish	N	0 times	1-2 times	3-4 times	5+ times
1999-2000	1640	52.80%	28.41%	11.34%	7.44%
2001-2002	1815	50.58%	30.14%	10.36%	8.93%
2003-2004	1617	50.65%	28.39%	12.43%	8.53%
2005-2006	1804	45.57%	31.87%	11.53%	11.03%
2007-2008	1510	50.60%	29.40%	10.66%	9.34%
2009-2010	1798	43.88%	30.42%	14.13%	11.57%
Fresh water fish	N	0 times	1-2 times	3+ times	
1999-2000	1640	88.29%	9.76%	1.95%	

	2001-2002	1815	82.53%	13.44%	4.02%	
	2003-2004	1617	82.56%	13.91%	3.53%	
	2005-2006	1804	86.70%	10.14%	3.16%	
	2007-2008	1510	85.89%	11.59%	2.52%	
	2009-2010	1798	87.82%	9.29%	2.89%	
Swordfish/Shark		N	0 times	1 time		
	1999-2000	1640	98.72%	1.28%		
	2001-2002	1815	98.79%	1.21%		
	2003-2004	1617	99.38%	0.62%		
	2005-2006	1804	99.33%	0.67%		
	2007-2008	1510	98.81%	1.19%		
	2009-2010	1798	99.39%	0.61%		
Income						
Total Fish		N	0 times	1-4 times	5-8 times	9+ times
	<$20,000	2997	27.60%	47.80%	14.00%	10.50%
	$20,000-<$45,000	2896	25.30%	45.10%	17.10%	12.50%
	45,000-<$75,000	1806	21.70%	45.10%	19.30%	13.90%
	$75,000+	1901	18.40%	41.20%	21.90%	18.50%
Tuna		N	0 times	1-2 times	3-4 times	5+ times
	<$20,000	2997	71.71%	19.02%	6.37%	2.90%
	$20,000-<$45,000	2896	68.02%	20.58%	6.63%	4.77%
	45,000-<$75,000	1806	65.23%	21.54%	8.86%	4.37%
	$75,000+	1901	60.23%	24.46%	10.10%	5.21%
Marine Fish		N	0 times	1-2 times	3-4 times	5+ times
	<$20,000	2997	62.30%	25.16%	7.71%	4.84%
	$20,000-<$45,000	2896	59.70%	26.48%	7.77%	6.04%
	45,000-<$75,000	1806	53.71%	28.90%	9.80%	7.59%
	$75,000+	1901	46.76%	31.14%	12.52%	9.57%
Marine Shellfish		N	0 times	1-2 times	3-4 times	5+ times
	<$20,000	2997	54.62%	27.89%	9.51%	7.97%
	$20,000-<$45,000	2896	50.14%	28.97%	11.88%	9.01%
	45,000-<$75,000	1806	45.46%	32.67%	12.18%	9.69%
	$75,000+	1901	41.71%	31.72%	14.05%	12.52%
Fresh water fish		N	0 times	1-2 times	3+ times	

	<$20,000	2997	85.42%	11.38%	3.20%	
	$20,000-<$45,000	2896	85.43%	11.40%	3.18%	
	45,000-<$75,000	1806	85.11%	11.96%	2.93%	
	$75,000+	1901	86.59%	10.52%	2.89%	
Swordfish/Shark		N	0 times	1 time		
	<$20,000	2997	99.60%	0.40%		
	$20,000-<$45,000	2896	99.41%	0.59%		
	45,000-<$75,000	1806	98.84%	1.16%		
	$75,000+	1901	98.05%	1.79%		
Race/Ethnicity						
Total Fish		N	0 times	1-4 times	5-8 times	9+ times
	Mexican American	2605	26.60%	51.90%	14.10%	7.40%
	Other Hispanic	762	23.80%	45.30%	17.80%	13.10%
	Non-Hispanic White	3095	24.30%	42.80%	18.50%	14.40%
	Non-Hispanic Black	2253	20.50%	45.40%	19.10%	15.00%
	Other Race	477	23.50%	30.00%	17.80%	28.70%
Tuna		N				
	Mexican American	2605	70.56%	20.27%	6.60%	2.57%
	Other Hispanic	762	68.64%	18.24%	7.22%	5.91%
	Non-Hispanic White	3095	59.70%	25.00%	9.70%	5.70%
	Non-Hispanic Black	2253	76.92%	15.76%	4.66%	2.66%
	Other Race	477	70.44%	15.93%	9.64%	3.98%
Marine Fish		N	0 times	1-2 times	3-4 times	5+ times
	Mexican American	2605	66.26%	25.22%	5.72%	2.80%
	Other Hispanic	762	54.99%	29.66%	8.40%	6.96%
	Non-Hispanic White	3095	55.50%	27.80%	9.70%	7.00%
	Non-Hispanic Black	2253	51.66%	29.07%	10.56%	8.70%
	Other Race	477	44.65%	23.48%	15.72%	16.14%
Marine Shellfish		N	0 times	1-2 times	3-4 times	5+ times
	Mexican American	2605	48.02%	34.55%	11.25%	6.18%
	Other Hispanic	762	46.98%	30.05%	13.65%	9.32%
	Non-Hispanic White	3095	50.90%	28.40%	11.10%	9.60%
	Non-Hispanic Black	2253	47.98%	28.85%	11.81%	11.36%
	Other Race	477	43.19%	20.96%	16.77%	19.08%
Fresh water fish		N	0 times	1-2 times	3+ times	

	Mexican American	2605	90.02%	8.21%	1.77%	
	Other Hispanic	762	91.86%	6.82%	1.31%	
	Non-Hispanic White	3095	89.20%	8.90%	1.90%	
	Non-Hispanic Black	2253	72.79%	20.59%	6.61%	
	Other Race	477	82.18%	12.58%	5.24%	
Swordfish/Shark		N	0 times	1 time		
	Mexican American	2605	99.31%	0.69%		
	Other Hispanic	762	99.21%	0.79%		
	Non-Hispanic White	3095	98.60%	1.40%		
	Non-Hispanic Black	2253	99.60%	0.40%		
	Other Race	477	99.16%	1.05%		
Age						
Total Fish		N	0 times	1-4 times	5-8 times	9+ times
	16-19	2478	36.80%	44.90%	11.70%	6.70%
	20-29	2752	24.10%	46.70%	17.20%	12.00%
	30-39	2515	18.70%	45.80%	18.80%	16.60%
	40-49	2439	16.30%	43.60%	22.00%	18.10%
Tuna		N	0 times	1-2 times	3-4 times	5+ times
	16-19	2478	78.13%	14.33%	5.04%	2.50%
	20-29	2752	67.19%	21.00%	7.56%	4.25%
	30-39	2515	64.17%	22.70%	8.15%	4.97%
	40-49	2439	60.23%	25.17%	9.68%	4.92%
Marine Fish		N	0 times	1-2 times	3-4 times	5+ times
	16-19	2478	71.55%	20.66%	5.13%	2.66%
	20-29	2752	59.77%	26.42%	8.07%	5.74%
	30-39	2515	50.30%	30.46%	10.46%	8.79%
	40-49	2439	45.39%	32.06%	12.75%	9.80%
Marine Shellfish		N	0 times	1-2 times	3-4 times	5+ times
	16-19	2478	57.83%	27.97%	8.47%	5.73%
	20-29	2752	48.11%	31.00%	11.59%	9.30%
	30-39	2515	44.06%	30.50%	13.76%	11.69%
	40-49	2439	45.63%	29.73%	13.24%	11.40%
Fresh water fish		N	0 times	1-2 times	3+ times	
	16-19	2478	89.63%	8.43%	1.94%	

20-29	2752	86.59%	11.34%	2.07%
30-39	2515	83.98%	12.64%	3.38%
40-49	2439	82.21%	12.67%	4.93%
Swordfish/Shark	N	0 times	1 time	
16-19	2478	99.60%	0.40%	
20-29	2752	99.31%	0.69%	
30-39	2515	98.61%	1.39%	
40-49	2439	98.77%	1.23%	

Table 4. Blood MeHg concentrations g/L, by frequency of consuming fish, by NHANES survey cycle, for women 16-49 years, NHANES 1999-2010

Survey Cycle	Times eaten		Arithmetic Mean	Selected percentiles (95% CI)			
1999-2000		**N**	**(95% CI)**	**25th**	**50th**	**75th**	**90th**
	0	428	0.47 (0.38, 0.57)	0.1 (0.05, 0.16)	0.17(0.12, 0.22)	0.46 (0.35, 0.56)	1.13 (0.88, 1.39)
	1-4	793	1.27 (0.84, 1.69)	0.18 (0.14, 0.22)	0.59 (0.44, 0.73)	1.31 (0.92, 1.69)	2.88(1.66, 4.11)
	5-8	248	2.43 (1.57, 3.29)	0.50 (0.21, 0.80)	1.19 (0.78, 1.61)	2.94 (1.22, 4.65)	5.96 (2.89, 9.03)
	9+	171	3.93 (3.29, 4.56)	1.2 (0.85, 1.56)	2.72 (1.83, 3.61)	4.58 (3.98, 5.19)	10.08 (8.43, 11.74)
2001-2002							
	0	411	0.34 (0.28, 0.4)	0.13 (0.08, 0.19)	0.19 (0.13, 0.24)	0.34 (0.19, 0.49)	0.71 (0.55, 0.87)
	1-4	868	0.83 (0.70, 0.96)	0.20 (0.19, 0.21)	0.42 (0.36, 0.47)	1.01 (0.85, 1.17)	1.91 (1.48, 2.34)
	5-8	314	1.47 (1.22, 1.72)	0.38 (0.27, 0.49)	0.94 (0.73, 1.14)	1.77 (1.35, 2.20)	3.00 (2.51, 3.50)
	9+	222	2.74 (1.92, 3.56)	0.61 (0.38, 0.85)	1.62 (1.25, 1.99)	3.21 (2.40, 4.03)	6.64 (3.45, 9.82)
2003-2004							
	0	370	0.31 (0.24, 0.38)	0.10 (0.05, 0.15)	0.20 (0.10, 0.30)	0.30 (0.20, 0.40)	0.70 (0.34, 1.06)
	1-4	751	0.69 (0.56, 0.82)	0.20 (0.13, 0.27)	0.36 (0.21, 0.51)	0.80 (0.64, 0.96)	1.60 (1.27, 1.93)
	5-8	288	1.31 (1.08, 1.54)	0.32 (0.19, 0.44)	0.80 (0.61, 0.99)	1.50 (1.01, 1.99)	2.92 (2.07, 3.77)
	9+	208	2.51 (1.95, 3.08)	0.68 (0.43, 0.93)	1.46 (1.15, 1.77)	3.22 (2.21, 4.23)	5.56 (3.27, 7.86)
2005-2006							
	0	435	0.37 (0.30, 0.43)	0.20 (0.14, 0.26)	0.20 (0.14, 0.26)	0.39 (0.22, 0.56)	0.71 (0.51, 0.90)
	1-4	722	0.86 (0.66, 1.07)	0.20 (0.16, 0.24)	0.44 (0.37, 0.52)	0.93 (0.74, 1.13)	1.84 (1.21, 2.48)
	5-8	340	1.31 (1.03, 1.60)	0.44 (0.32, 0.56)	0.95 (0.79, 1.11)	1.67 (1.07, 2.27)	2.71 (1.41, 4.02)
	9+	257	2.11 (1.77, 2.45)	0.65 (0.58, 0.72)	1.35 (0.91, 1.78)	2.90 (2.32, 3.48)	4.34 (3.17, 5.51)
2007-2008							
	0	381	0.33 (0.26, 0.39)	0.19 (0.18, 0.21)	0.20 (0.18, 0.21)	0.33 (0.26, 0.4)	0.60 (0.48, 0.71)

	1-4	697	0.72 (0.57, 0.86)	0.20 (0.17, 0.24)	0.42 (0.34, 0.5)	0.82 (0.67, 0.97)	1.46 (1.11, 1.81)
	5-8	236	1.36 (1.10, 1.62)	0.40 (0.27, 0.53)	0.81 (0.6, 1.03)	1.69 (1.2, 2.18)	3.37 (2.75, 3.99)
	9+	196	2.13 (1.57, 2.69)	0.61 (0.39, 0.83)	1.27 (0.75, 1.78)	2.69 (2.03, 3.34)	5.09 (3.25, 6.94)
2009-2010							
	0	416	0.43 (0.35, 0.52)	0.20 (0.17, 0.23)	0.20 (0.17, 0.23)	0.44 (0.36, 0.51)	0.89 (0.56, 1.23)
	1-4	732	0.69 (0.61, 0.78)	0.20 (0.17, 0.23)	0.40 (0.36, 0.43)	0.92 (0.78, 1.06)	1.63 (1.35, 1.91)
	5-8	349	1.65 (1.46, 1.85)	0.41 (0.31, 0.51)	0.96 (0.78, 1.13)	2.23 (1.86, 2.6)	3.87 (3.03, 4.71)
	9+	301	2.25 (1.85, 2.66)	0.80 (0.63, 0.97)	1.43 (1.13, 1.73)	3.01 (2.46, 3.56)	4.30 (3.13, 5.47)

Table 5. Blood total mercury (µg/L), women 16-49 years of age by US Census region.

	N	Arith. Mean (95% CI)		Geometric Mean (95% CI)		Selected percentiles						(95% CI)	
Census Region						25th		50th		75th		90th	
Northeast	1525	2.02	(1.63,2.41)	1.13	(0.95,1.35)	0.59	(0.50,0.70)	1.15	(0.97,1.36)	2.30	(1.83,2.88)	4.36	(3.37,5.62)
Midwest	1946	0.97	(0.90,1.05)	0.67	(0.63,0.72)	0.39	(0.34,0.45)	0.69	(0.64,0.75)	1.20	(1.12,1.28)	1.97	(1.82,2.13)
South	3909	1.41	(1.29,1.54)	0.83	(0.77,0.89)	0.40	(0.37,0.44)	0.79	(0.74,0.85)	1.50	(1.36,1.65)	3.09	(2.81,3.41)
West	2803	1.64	(1.48,1.80)	1.00	(0.92,1.09)	0.50	(0.44,0.55)	1.00	(0.91,1.09)	1.99	(1.73,2.29)	3.67	(3.22,4.18)

Table 6. Percent of women aged 16-49 years with blood Hg and MeHg ≥ 3.5 µg/L and ≥5.8 µg/L, by NHANES survey cycle.

	Whole Blood Mercury		Methylmercury	
Year	Percentage ≥ 5.8 µg/L	Percentage ≥ 3.5 µg/L	Percentage ≥ 5.8 µg/L	Percentage ≥ 3.5 µg/L
1999-2000	7.24	15.2	6.74	14.05
2001-2002	3.77	7.87	3.27	6.44
2003-2004	2.50	7.77	1.68	6.04
2005-2006	2.67	8.44	2.36	6.66
2007-2008	2.45	6.29	2.28	4.5
2009-2010	2.38	7.44	2.22	6.79

Table 7. Percentage of examinees with BHg concentrations ≥ 3.5µg/L and ≥ 5.8µg/L by race/ethnicity and income.

BHg	Percent ≥ 3.5 µg/L	Percent ≥ 5.8 µg/L	Pr>F
Race/ethnicity			
Mexican American	3.38	0.86	<0.001
Other Hispanic	7.58	2.06	<0.001
Non-Hispanic White	8.35	3.28	<0.001
Non-Hispanic Black	7.82	3.27	<0.001
Other [a]	25.09	11.61	<0.001
Income			
< $20,000	5.54	1.59	<0.001
$20,000-$44,999	6.34	3.03	<0.001
$45,000-$74,999	7.91	3.17	<0.001
≥$75,000	13.88	5.39	<0.001

[a] Other race includes Asian, Pacific Islanders, Alaska Native/American Indian

Table 1. List of Coastal Counties

Atlantic Ocean		Gulf of Mexico		Pacific Ocean		Great Lakes
CT	Fairfield County	AL	Baldwin County	AK	Aleutians East Borough	MI	Bay County
	Hartford County		Mobile County		Aleutians West Census Area		Arenac County
	Middlesex County	FL	Alachua County		Anchorage Municipality		Alcona County
	New Haven County		Bay County		Bethel Census Area		Cheboygan County
	New London County		Calhoun County		Bristol Bay Borough		Emmet County
	Tolland County		Charlotte County		Dillingham Census Area		Alpena County
	Windham County		Citrus County		Kenai Peninsula Borough		Grand Traverse County
DE	Kent County		Collier County		Ketchikan Gateway Borough		Charlevoix County
	New Castle County		Columbia County		Kodiak Island Borough		Antrim County
	Sussex County		DeSoto County		Lake and Peninsula Borough		Leelanau County
DC	District of Columbia		Dixie County		Haines Borough		Benzie County
FL	Baker County		Escambia County		Nome Census Area		Allegan County
	Bradford County		Franklin County		North Slope Borough		Berrien County
	Brevard County		Gadsden County		Northwest Arctic Borough		Delta County
	Broward County		Gilchrist County		Prince of Wales-Hyder Census Area		Alger County
	Clay County		Glades County		Skagway Municipality		Baraga County
	Duval County		Gulf County		Valdez-Cordova Census Area		Houghton County
	Flagler County		Hamilton County		Wade Hampton Census Area		Keweenaw County
	Indian River County		Hardee County		Wrangell City and Borough		Gogebic County
	Lake County		Hendry County		Juneau City and Borough		Marquette County
	Martin County		Hernando County		Sitka City and Borough		Luce County
	Miami-Dade County		Highlands County		Yakutat City and Borough		Chippewa County
	Nassau County		Hillsborough County	CA	Alameda County		Mackinac County
	Okeechobee County		Holmes County		Contra Costa County		Schoolcraft County
	Orange County		Jackson County		Del Norte County		Menominee County
	Osceola County		Jefferson County		Humboldt County		Van Buren County
	Palm Beach County		Lafayette County		Los Angeles County		Ottawa County
	Putnam County		Lake County		Marin County		Muskegon County
	Seminole County		Lee County		Mendocino County		Oceana County
	St. Johns County		Leon County		Monterey County		Mason County
	St. Lucie County		Levy County		Napa County		Manistee County
	Union County		Liberty County		Orange County		Macomb County
	Volusia County		Madison County		San Diego County		Monroe County
GA	Bryan County		Manatee County		San Francisco County		Sanilac County
	Camden County		Marion County		San Luis Obispo County		St. Clair County
	Chatham County		Monroe County		San Mateo County		Lapeer County
	Glynn County		Okaloosa County		Santa Barbara County		Oakland County
	Liberty County		Pasco County		Santa Clara County		Wayne County

	McIntosh County		Pinellas County		Santa Cruz County		Genesee County
ME	Androscoggin County		Polk County		Solano County		Tuscola County
	Cumberland County		Santa Rosa County		Sonoma County		Huron County
	Hancock County		Sarasota County		Ventura County		Washtenaw County
	Kennebec County		Sumter County	HI	Hawaii County		Saginaw County
	Knox County		Suwannee County		Honolulu County		Midland County
	Lincoln County		Taylor County		Kalawao County		Gladwin County
	Sagadahoc County		Wakulla County		Kauai County		Kalkaska County
	Waldo County		Walton County		Maui County		Presque Isle County
	Washington County		Washington County	OR	Clatsop County		Ontonagon County
	York County	LA	Assumption Parish		Columbia County	WI	Ashland County
MD	Anne Arundel County		Cameron Parish		Coos County		Bayfield County
	Baltimore County		Iberia Parish		Curry County		Douglas County
	Calvert County		Jefferson Parish		Douglas County		Iron County
	Caroline County		Lafayette Parish		Lane County		Door County
	Cecil County		Lafourche Parish		Lincoln County		Brown County
	Charles County		Livingston Parish		Multnomah County		Marinette County
	Dorchester County		Orleans Parish		Tillamook County		Oconto County
	Harford County		Plaquemines Parish		Washington County		Kewaunee County
	Howard County		St. Bernard Parish	WA	Clallam County		Manitowoc County
	Kent County		St. Charles Parish		Clark County		Milwaukee County
	Montgomery County		St. James Parish		Cowlitz County		Ozaukee County
	Prince George's County		St. John the Baptist Parish		Grays Harbor County		Sheboygan County
	Queen Anne's County		St. Mary Parish		Island County		Racine County
	St. Mary's County		St. Tammany Parish		Jefferson County		Kenosha County
	Somerset County		Tangipahoa Parish		King County		Washington County
	Talbot County		Terrebonne Parish		Kitsap County		Waukesha County
	Wicomico County		Vermilion Parish		Mason County		Calumet County
	Worcester County	MS	Hancock County		Pacific County	OH	Ashtabula County
MA	Barnstable County		Harrison County		Pierce County		Erie County
	Bristol County		Jackson County		San Juan County		Lucas County
	Dukes County	TX	Aransas County		Skagit County		Ottawa County
	Essex County		Brazoria County		Snohomish County		Cuyahoga County
	Middlesex County		Calhoun County		Thurston County		Lorain County
	Nantucket County		Cameron County		Wahkiakum County		Lake County
	Norfolk County		Chambers County		Whatcom County		Geauga County
	Plymouth County		Galveston County				Summit County
	Suffolk County		Harris County				Medina County
NH	Rockingham County		Jackson County				Sandusky County
	Strafford County		Jefferson County				Seneca County
NJ	Atlantic County		Kenedy County				Huron County
	Bergen County		Kleberg County				Wood County
	Burlington County		Matagorda County			NY	Chautauqua County

	Camden County	Nueces County		Erie County
	Cape May County	Orange County		Cayuga County
	Cumberland County	Refugio County		Cattaraugus County
	Essex County	San Patricio County		Niagara County
	Gloucester County	Victoria County		Orleans County
	Hudson County	Willacy County		Oswego County
	Middlesex County			Monroe County
	Monmouth County			Wayne County
	Ocean County			Jefferson County
	Passaic County			Livingston County
	Salem County			Genesee County
	Union County			Ontario County
NY	Bronx County			Seneca County
	Kings County			Onondaga County
	Nassau County			Wyoming County
	New York County		MN	Carlton County
	Queens County			Cook County
	Richmond County			Lake County
	Rockland County			St. Louis County
	Suffolk County		IN	Lake County
	Westchester County			LaPorte County
NC	Beaufort County			Porter County
	Bertie County		PA	Crawford County
	Brunswick County			Erie County
	Camden County		IL	Cook County
	Carteret County			DuPage County
	Chowan County			Kane County
	Craven County			Lake County
	Currituck County			McHenry County
	Dare County			Will County
	Hyde County		KS	Allen County
	Jones County			Anderson County
	New Hanover County			Atchison County
	Onslow County			Barber County
	Pamlico County			Barton County
	Pasquotank County			Bourbon County
	Pender County			Brown County
	Perquimans County			Butler County
	Tyrrell County			Chase County
	Washington County			Chautauqua County
PA	Delaware County			Cherokee County
	Montgomery County			Cheyenne County
	Philadelphia County			Clark County

RI	Bristol County	Clay County
	Kent County	Cloud County
	Newport County	Coffey County
	Providence County	Comanche County
	Washington County	Cowley County
SC	Beaufort County	Crawford County
	Berkeley County	Decatur County
	Charleston County	Dickinson County
	Colleton County	Doniphan County
	Georgetown County	Douglas County
	Horry County	Edwards County
	Jasper County	Elk County
VA	Accomack County	Ellis County
	Arlington County	Ellsworth County
	Charles City County	Finney County
	Essex County	Ford County
	Fairfax County	Franklin County
	Gloucester County	Geary County
	Henrico County	Gove County
	Isle of Wight County	Graham County
	James City County	Grant County
	King and Queen County	Gray County
	King George County	Greeley County
	Lancaster County	Greenwood County
	Mathews County	Hamilton County
	Middlesex County	Harper County
	New Kent County	Harvey County
	Northampton County	Haskell County
	Northumberland County	Hodgeman County
	Prince William County	Jackson County
	Richmond County	Jefferson County
	Stafford County	Jewell County
	Surry County	Johnson County
	Westmoreland County	Kearny County
	York County	Kingman County
	Manassas city	Kiowa County
	Manassas Park city	Labette County
	Newport News city	Lane County
	Norfolk city	Leavenworth County
	Poquoson city	Lincoln County
	Portsmouth city	Linn County
	Suffolk city	Logan County
	Virginia Beach city	Lyon County

Williamsburg city	McPherson County
Alexandria city	Marion County
Chesapeake city	Marshall County
Fairfax city	Meade County
Falls Church city	Miami County
Hampton city	Mitchell County
	Montgomery County
	Morris County
	Morton County
	Nemaha County
	Neosho County
	Ness County
	Norton County
	Osage County
	Osborne County
	Ottawa County
	Pawnee County
	Phillips County
	Pottawatomie County
	Pratt County
	Rawlins County
	Reno County
	Republic County
	Rice County
	Riley County
	Rooks County
	Rush County
	Russell County
	Saline County
	Scott County
	Sedgwick County
	Seward County
	Shawnee County
	Sheridan County
	Sherman County
	Smith County
	Stafford County
	Stanton County
	Stevens County
	Sumner County
	Thomas County
	Trego County
	Wabaunsee County

	Wallace County
	Washington County
	Wichita County
	Wilson County
	Woodson County
	Wyandotte County

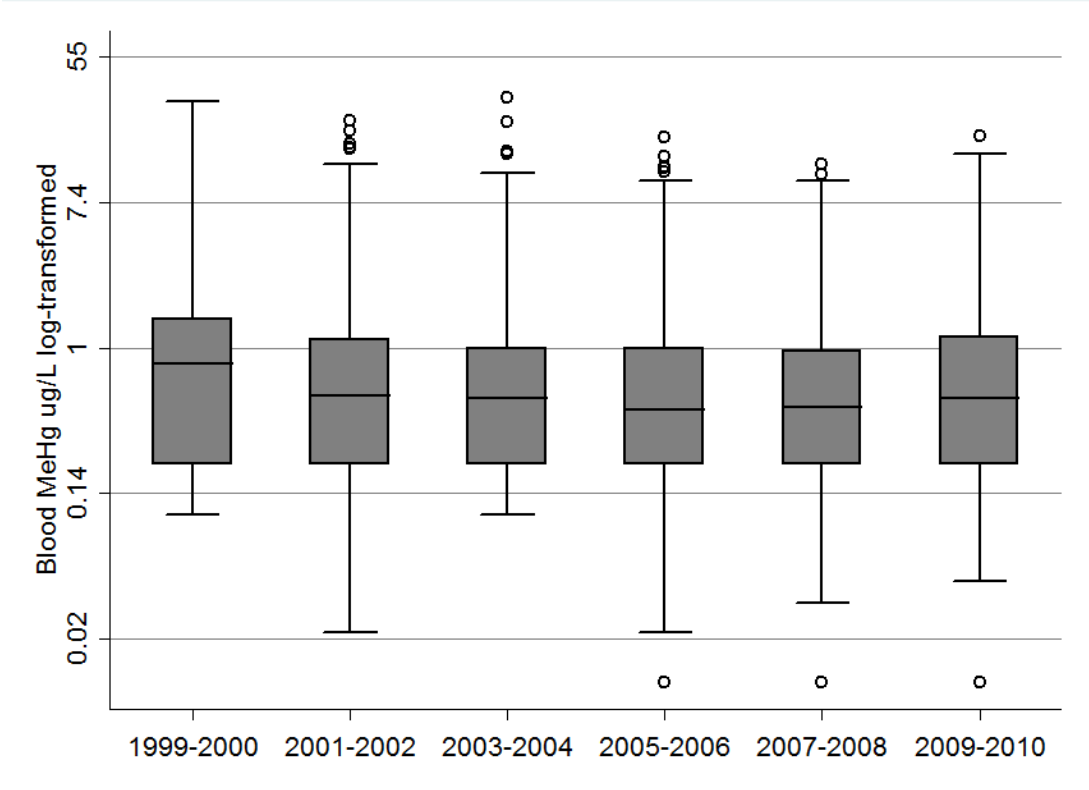


Figure 1. Distribution of blood MeHg (µg/L), by NHANES survey cycle for women age 16-49 years

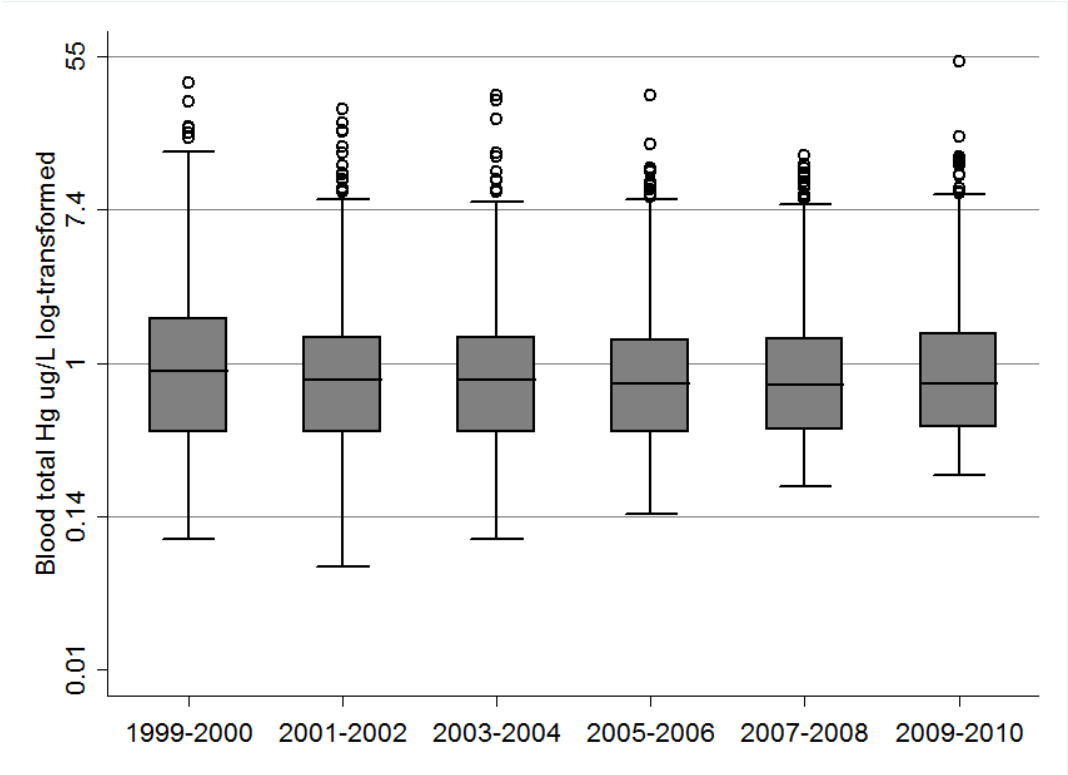


Figure 2. Distribution of blood total mercury (µg/L), by NHANES survey cycle for women age 16-49 years

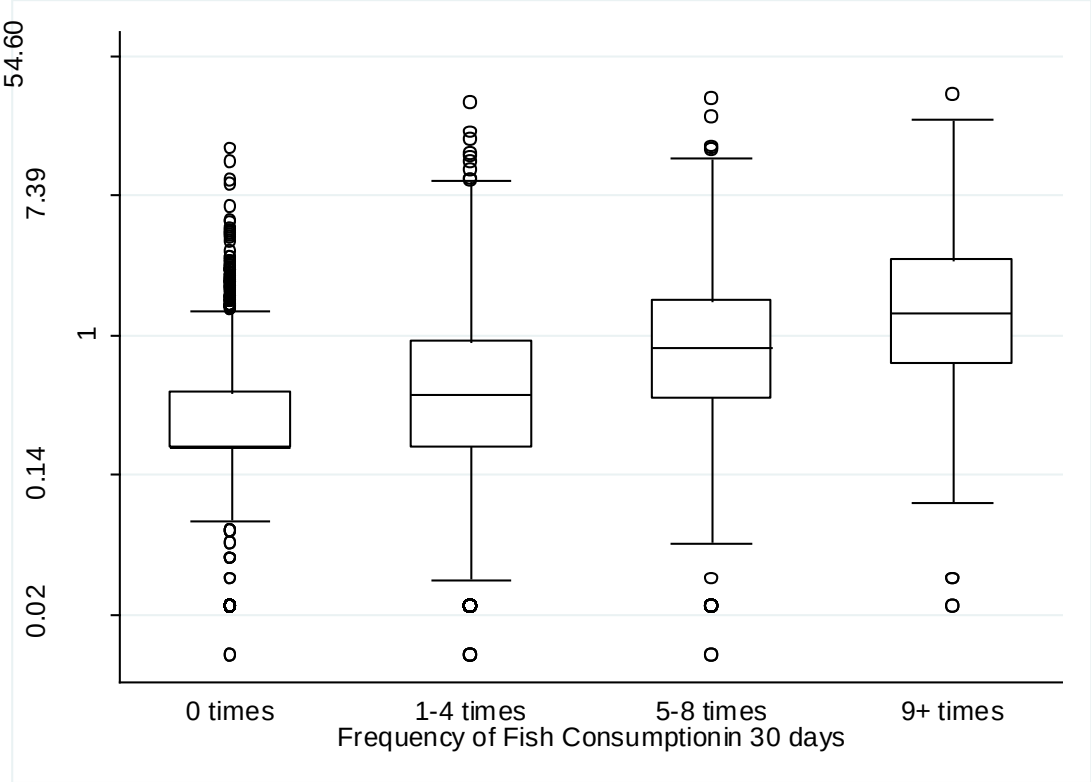


Figure 3. Distribution of blood total methylmercury (µg/L), by fish consumption for women age 16-49 years

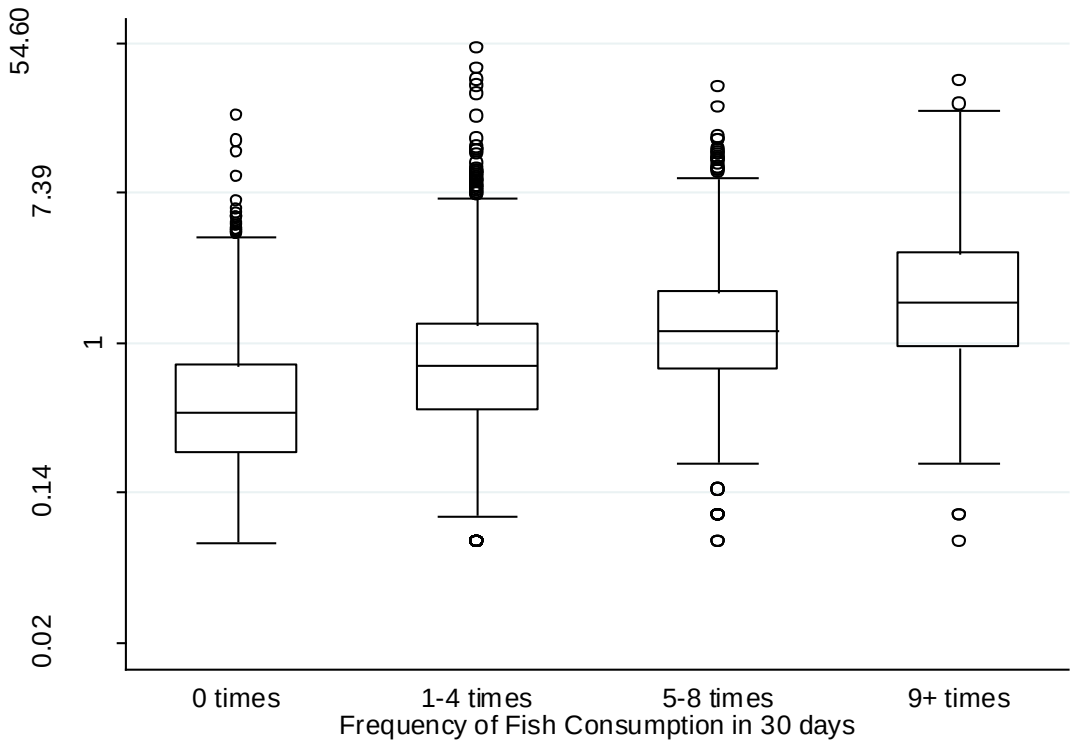


Figure 4. Distribution of blood total methylmercury (µg/L), by fish consumption for women age 16-49 years

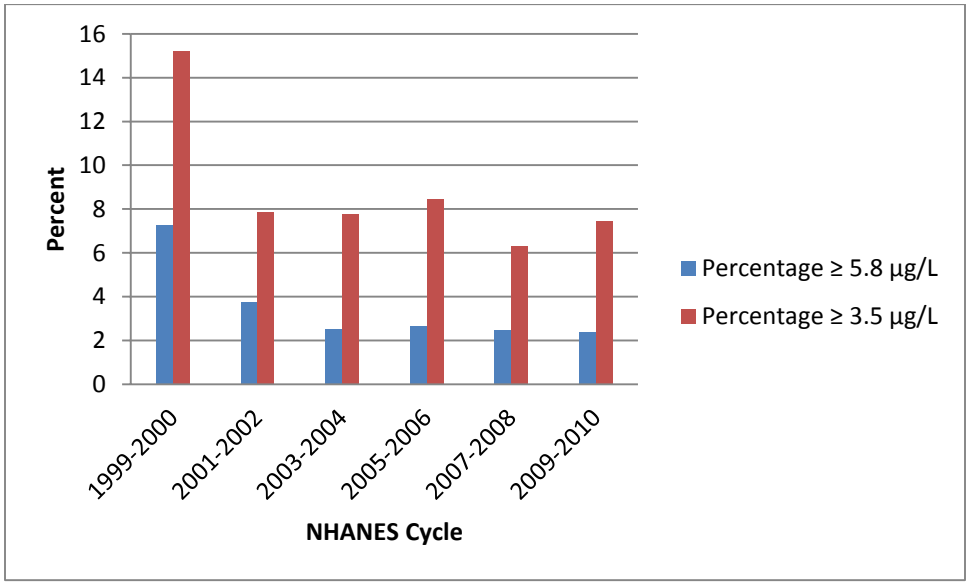


Figure 5. Percentage of women 16-49 years of age having BHg concentrations greater than those associated with exposures considered higher than the U.S. EPA's RfD for MeHg

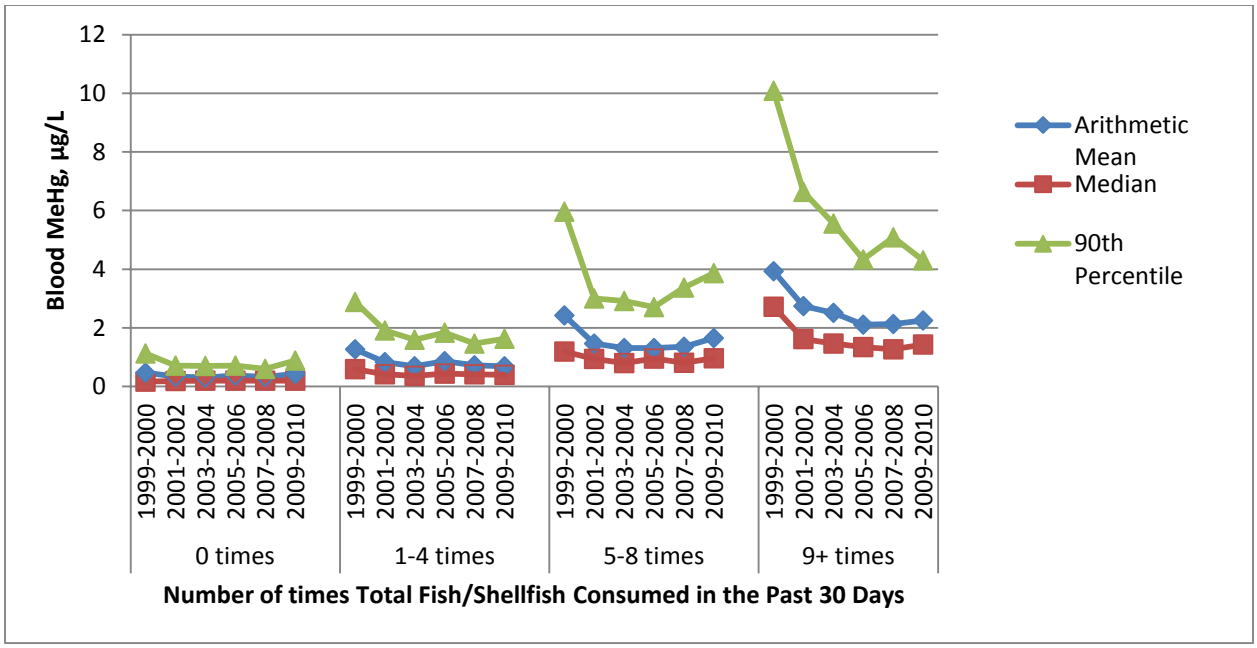


Figure 6. Mean blood MeHg concentrations by reported frequency of fish consumption in 30 days for women aged 16-49 years of age, NHANES 1999-2010