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Methylmercury Exposure via Canned Tuna Fish Consumption and Breast Cancer

Jennifer Bodenrader
Walden University

Walden University

College of Health Sciences

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Jennifer Bodenrader

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Dr. Aaron Mendelsohn, Committee Chairperson, Public Health Faculty
Dr. Vasileios Margaritis, Committee Member, Public Health Faculty
Dr. James Rohrer, University Reviewer, Public Health Faculty

Chief Academic Officer
Eric Riedel, Ph.D.

Walden University
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Abstract

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Dissertation Submitted in Partial Fulfillment

of the Requirements for the Degree of

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Abstract

Widespread consumption of canned tuna fish since the 1950s may explain some of the increase in breast cancer prevalence in the United States and Europe. Although canned tuna is the primary source of human exposure to methylmercury, its role as an estrogen activating metalloestrogen has been overlooked in the etiology and incidence of breast cancer. Carcinogenic theory asserts that increased exposure to estrogen elevates the risk of breast cancer. The purpose of this population-based, case control study was to examine the association between canned tuna consumption, total blood mercury, and breast cancer in the NHANES 2003-2006 surveys. A multivariable logistic regression model representing 138,747,398 U.S. adult females, controlling for covariates, was applied to investigate whether canned tuna consumption or blood mercury level had a relationship to breast cancer. According to study results, women who reported eating canned tuna at one level of increased frequency out of 11 had a 6.8% increased odds of being diagnosed with breast cancer (p =0. 000 OR 1.068 and 95% CI 1.067-1.069). Women with only a 0.01 Ug/L increase in total blood mercury level were found to have a 0.2% increased odds of being diagnosed with breast cancer (p =. 000 OR 1.002 and 95% CI 1.002-1.003). Additional research individuating the canned tuna fish variable in nutrition, fish, mercury, and breast cancer studies is recommended. This research contributes to positive social change by providing evidence to improve understanding and specification of canned tuna fish in future research and better identification of methylmercury levels in canned tuna fish for public knowledge.

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Dedication

This dissertation is dedicated to the courageous women who, in the prime of their lives, were diagnosed with breast cancer, especially my dear friend Shelly Carter, whose attitude, love of life, and can-do spirit will inspire me forever. I wish to thank my steadfast spouse and loving children who supported me through this endeavor during the busiest of times. And lastly, to Grunky, who gave me the idea in the first place and Grammie, for her love.

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Chapter 1: Introduction to the Study

Introduction

Though environmental exposures are believed to explain the geographic differences in breast cancer prevalence and account for up to half of all breast cancer incidence, the identification and quantification of environmental risk factors for breast cancer remains elusive (California Breast Cancer Research Program, 2013). Exposure to naturally occurring metals that accumulate in the body over time and activate estrogen (metalloestrogens) are one group of environmental risk factors that contribute to breast carcinogenesis (Byrne, Divekar, Storchan, Parodi, & Martin, 2013). One of these metalloestrogens, methylmercury, has been consumed regularly in canned tuna fish since the 1950s in high breast cancer incidence countries, and this relationship to breast cancer incidence has been unrecognized and understudied (Byrne et al., 2013; Food and Agricultural Organization of the United Nations [FAO], 2014c; Gerstenberger, Martinson, & Kramer, 2010; Globefish Research Program, 2004; Sala-Vila & Calder, 2011).

In this chapter, I describe the research problem, research questions and hypotheses, study purpose, theoretical framework, nature of the study, underlying assumptions, strengths and limitations, and significance of the study.

Background

Consumption of canned tuna fish is the primary source of human exposure to methylmercury (Iavicoli, Fontanta, & Bergamaschi, 2009). Methylmercury is a naturally occurring estrogen activating metal compound and has been designated a probable human

carcinogen (Byrne et al., 2013). Human exposure to estrogen activating metal compounds is closely related to breast cancer carcinogenesis (Mohammadi, Bakhtiari, & Khodabandeh, 2014). Though methylmercury levels in canned tuna fish differ based on tuna size, type, and location, this information is largely unavailable to consumers, researchers, and government agencies (Gerstenberger et al., 2010; Sala-Vila & Calder, 2011; United Nations Environment Programme [UNEP], 2002). According to the small body of literature on canned tuna fish, there is a variation in methylmercury levels, with albacore and even chunk light regularly exceeding levels of concern designated by the United States Department of Agriculture (USDA), Food and Drug Administration (FDA) and the United States Environmental Protection Agency (EPA; Groth, 2010a; Groth, 2012b; Karimi, Fitzgerald, & Fisher, 2012; Suppin et al., 2005; Thompson & Lee, 2009).

In nutrition studies of breast cancer and fish, canned tuna is rarely individuated and instead is typically combined with other types of fish to test hypotheses related to the protective role of fish in breast cancer incidence (Florea & Busselberg, 2011; Yaghoubi & Barlow, 2007; Zadnick & Pompe-Kirn, 2007). Findings from fish consumption and breast cancer studies are highly varied showing increased risk, decreased risk, and no difference in breast cancer risk (Engeset et al., 2006; Franceschi et al., 2006; Kim et al., 2009; Stripp et al., 2003; Terry, Rohan, & Wolk, 2003; Vatten, Solvoll, & Loken, 2006; Yuan, Wang, Ross, Henderson, & Yu, 1995). The role of methylmercury exposure as an estrogen activator in canned tuna fish may explain some of the confounding findings present in fish and breast cancer literature (Daniel et al., 2011). Canned tuna fish consumption also shares some historic, geographic, racial/ethnic, educational, and

socioeconomic parallels with breast cancer incidence (Bray, McCarron, & Parkin, 2004; Campbell & Owen, 1994; Cancer Research UK, 2013; Daniel et al., 2011; FAO, 2004b; Globefish, 2004).

Research of the association between methylmercury exposure via consumption of canned tuna fish and breast cancer contributes to environmental knowledge of breast cancer risk factors. It may also provide information to improve the future delineation of fish variables in nutrition and breast cancer research. To evaluate the relationship between canned tuna fish consumption and breast cancer incidence, I used data from the 2003-2004 and 2005-2006 National Health and Nutrition Examination Survey (NHANES) that individuated the canned tuna fish variable with the outcome of breast cancer (NHANES, 2014c).

Problem Statement

Breast cancer is the leading cause of female cancer death and is most frequently diagnosed cancer in the world (Jemal, Center, DeSantis, & Ward, 2010). Unidentified environmental and nutritional risk factors may explain the geographic differences in breast cancer prevalence and account for up to half of breast cancer incidence (California Breast Cancer Research Program, 2013). The role of the primary route of human methylmercury exposure, consumption of canned tuna fish, has been overlooked in environmental literature (Gerstenberger et al., 2009). Scholars regularly combine canned tuna fish with other fish variables in hypothesizing the role of fish in breast cancer incidence, and the varied results suggest significant confounding (Engeset et al., 2006; European Prospective Investigation into Cancer and Nutrition [EPIC], 2012a; Florea &

Busselberg, 2011; Yaghoubi & Barlow, 2007; Zadnick & Poompe-Kirn, 2007). This study builds on existing knowledge of methylmercury as an environmental and nutritional risk factor for breast cancer, and I aimed to fill gaps in the literature by assessing the frequency of canned tuna consumption and blood mercury levels and measuring their association with breast cancer with data from the 2003-2006 NHANES survey (NHANES, 2014c).

Purpose of the Study

The intent of this population-based, case control study was to measure methlymercury exposure via canned tuna fish consumption and to examine its association to breast cancer. The primary goal was to measure evidence of the association between canned tuna fish consumption frequency and breast cancer and between blood mercury levels, used as a proxy for methylmercury level (Sheehan et al., 2014), and breast cancer. A secondary goal was to evaluate the descriptive statistics of the social determinants of canned tuna consumption. The dependent variable for the study was breast cancer diagnosis. The independent variables were frequency of canned tuna fish consumption reported in the food frequency questionnaire and blood mercury levels. Covariate factors that were designated as having adequate evidence of confounding and that were available in the dataset were examined and included race/ethnicity, annual household income, education level, age of menarche, hormone therapy, obesity, age, alcohol consumption, age when breast cancer first diagnosed, age at first full-term pregnancy, parity, breast feeding, smoking cigarettes, personal history of cancer, early age at menopause, and diabetes (National Cancer Institute, Description of the Evidence, 2014; NHANES, 2014c;

University of California San Francisco Medical Center, 2015). These covariates were captured in the NHANES questionnaires related to Demographic, Reproductive Health, Medical Conditions, Smoking, Alcohol Use, and Body Measurements administered to participants (NHANES, 2014c). For the secondary study purpose, the frequency of canned tuna fish consumption was the dependent variable, and race/ethnicity, age, annual household income, and education level were used as independent variables.

NHANES was established in the 1960s to survey and collect nutrition and health data representative of the population of the United States (NHANES, 2014c). Each year since 1999 about 5,000 people throughout the country have been sampled each year to ensure that they are representative of the population. The sampling design used is described by NHANES as a "complex, multistage, probability sampling design" (Centers for Disease Control and Prevention [CDC], 2013i, p. #1). First, a complex method is used to identify sample levels based on minority groups and geographic areas to define primary sampling units. Then these units are further segregated into local neighborhoods. Within each neighborhood, homes are chosen randomly, over or under sampling to maintain representativeness. Lastly, individual participants within households are randomly selected within sociodemographic categories (CDC, 2013i). The survey for participants includes demographics, medical questions, food/nutrition questionnaires, in-person health evaluations, and laboratory testing. Though the food frequency questionnaire has been administered by the NHANES, the variable of canned tuna fish appears to only have been individuated during two survey periods, 2003-2004 and 2005-2006.

Research Question and Hypotheses

1. Is there a relationship between consumption of canned tuna fish and breast cancer?

$H_0$1: There is no relationship between consumption of canned tuna fish and breast cancer.

*H1*1: There is a relationship between consumption of canned tuna fish and breast cancer.

2. Is there a relationship between total blood mercury level and breast cancer?

$H_0$2: There is no relationship between total blood mercury level and breast cancer.

$H_1$2: There is a relationship between total blood mercury level and breast cancer.

3. What is the frequency of women's canned tuna fish consumption for different age groups, race/ethnicities, annual household income, and education level?

(This question was addressed with descriptive statistics [i.e., no specific hypothesis was tested]).

Theoretical and Conceptual Framework

The two theoretical perspectives supporting this research were carcinogenesis theory and medical geography. Carcinogenesis theory is a conceptual framework based on evidence that estrogen stimulates breast cell development and cumulative exposure to estrogen increases breast cancer risk (Henderson, Ross, & Bernstein, 1988). Exposure to the metalloestrogen methylmercury increases the risk of breast cancer (Byrne et al.,

2013). The theory of medical geography focuses on investigation of the cultural, social, and geographic environment to understand the spatial differences and etiology of disease (Paul, 1985). This exploration of methylmercury exposure via canned tuna fish consumption, through the literature review and research question results, is a continuance of efforts to understand differences in breast cancer incidence by geography and social determinants (Sheehan et al., 2014).

In this study, the primary association between methylmercury via canned tuna fish and breast cancer is understood to interact via carcinogenesis theory. Carcinogenesis theory is applied in research questions related to the consumption of canned tuna fish, blood mercury level, and breast cancer. The descriptive statistics of the social determinants of canned tuna fish consumption were explored from a medical geography perspective. Parallels between the social determinants in both breast cancer and canned tuna consumption are reflected in the literature review and a research question exploring the age, race/ethnicity, education level, and annual household income and canned tuna fish consumption frequency of participants. Both theories are described in greater detail in Chapter 2.

Nature of the Study

Every 2 years since 1999 (with additional surveys back to the 1960s), NHANES has sampled a representative cross section of the United States with nutritional and health based surveys and health evaluations (NHANES, 2014c). A population-based, case control design was applied to this existing dataset for the two survey periods, 2003-2004 and 2005-2006, in which the canned tuna fish variable was individuated. A population-

based case control is a type of case control design that is applied to the population-based NHANES (Hopper, Bishop, & Easton, 2005). The design is also useful in initial identification of whether an association exists between an exposure and an outcome (Lewallen & Courtright, 1998), like canned tuna consumption and breast cancer.

Key study dependent variables included breast cancer diagnosis and frequency of canned tuna consumption. Independent variables included frequency of canned tuna consumption; total blood mercury level; and social determinant variables of age, race/ethnicity, education level, and annual household income. Covariates included race/ethnicity, annual household income, education level, age of menarche, hormone therapy, obesity, age, alcohol consumption, age when breast cancer first diagnosed, age at first full-term pregnancy, parity, breast feeding, smoking cigarettes, personal history of cancer, early age at menopause, and diabetes (National Cancer Institute, 2014a; University of California San Francisco Medical Center, 2015).

Definition of Terms

Age of menarche: Reported age range of first menstrual period (NHANES, 2008a).

Alcohol consumption: Defined in answer to a question of having consumed at least 12 drinks of any kind of alcoholic beverage in any 1 year (NHANES, 2008a).

Blood methylmercury level: The level of methylmercury measured in a person's blood, also referred to as its proxy total blood mercury level.

Breast feeding: Defined in answer to the question of having ever breastfeed a child (NHANES, 2008a).

Canned tuna fish: Any kind of tuna fish packaged in cans or pouches.

Cases: Women reporting having been told by a health professional they had breast cancer in the NHANES 2003-2006 survey.

Controls: Women reporting not ever being told they had breast cancer by a health professional in the NHANES 2003-2006 survey.

Early birth: Defined as having had a full-term pregnancy prior to age 20, which has been found to reduce risk of breast cancer (National Cancer Institute, 2011b).

Exposure: The delivery of methlymercury to the human body from consuming tuna fish.

Hormone therapy: Defined by answers to questions of ever taking birth control pills and ever using female hormones (NHANES, 2006).

Late birth: Defined as having had a full-term pregnancy after age 30. Breast cancer risk has been shown to increase in women who give birth over age 30 (National Cancer Institute, 2011b).

Social determinants: The social, cultural, and economic conditions to which people are born that impact future health. For this study, ethnicity/race, age, education level, and annual household income were used to represent social determinants.

Total blood mercury level: Used as a proxy for Methylmercury level (Sheehan et al., 2014).

Assumptions

The primary assumptions underlying this study were that reported differences in the frequency of canned tuna fish consumption reflect corresponding differences in levels

of methylmercury exposure, and the measurement of total blood mercury level (Hg) is an accurate proxy for blood methylmercury level (MeHg; Sheehan et al., 2014). Levels of methylmercury in canned tuna fish are highly varied, unknown, unidentified (Gerstenberger et al., 2010; Sala-Vila & Calder, 2011; UNEP, 2002), and are not captured in the NHANES food frequency questionnaire (NHANES, 2014c). Therefore, for the analysis of Research Question 1, it was assumed that people reporting eating canned tuna fish more often have significantly higher lifetime methylmercury exposure from canned tuna fish than people reporting eating canned tuna fish less often. For Research Question 2, total blood mercury level (Hg) was considered an accurate biomarker proxy for MeHg based on previous validation (Sheehan et al., 2014).

Limitations and Scope of the Study

The results from this case control design were unable to provide any evidence of causation and were limited to inference of the association between canned tuna fish consumption and breast cancer and total blood mercury level and breast cancer (Lewallen & Courtright, 1998). The case control design tends to be subject to selection bias (Lewallen & Courtright, 1998). NHANES' cross sectional sampling design includes all members of the population, regardless of health status, and complex procedures were used to ensure population representativeness to mitigate the inherent selection bias in the case control design (Public Health Action Support Team, 2011). Important breast cancer risk factors that were not included in the NHANES survey include the BRCA gene and family history of breast cancer (NHANES, 2014c; UCSF, 2015). Though controlling for covariates was primary to this analysis, residual confounding, especially related to the

BRCA gene and family history of breast cancer, was anticipated. As described in Chapter 4 some covariates were inappropriate for inclusion in the final model due to question design or collinearity. Confounding from other unidentified genetic and/or environmental risk factors for breast cancer was possible. Confounding from significant nonmethylmercury type mercury exposures (organic and elemental) was not anticipated but possible during Research Question 2 analysis (FDA, 2014b).

Though consumption of canned tuna fish has been established as the primary source of methylmercury exposure in humans (Ashraf, 2006; Boadi, Twumasi, Badu, & Osei, 2011; Burger & Gochfeld, 2004; Burger, Stern, & Gochfeld, 2005; Carrington & Bolger, 2002; Dabeka, McKenzie, Forsyth, & Conacher, 2004; FDA, 2002a; Globefish Research Program, 2004; Groth, 2010a; Hightower & Moore, 2003; Khansari et al., 2006; Ikem & Egiebor, 2005; Jaffry & Brown, 2008; Laxe & Gamallo, 2008; Moon et al., 2011; Rahimi et al., 2010; EPA, 1997c; Voegborlo, El-Methnani, & Abedin, 1999; Xue, Zartarian, Liu, & Geller, 2012), the inability to precisely and cumulatively measure or estimate methylmercury exposure from canned tuna fish for Research Question 1 was a primary limitation of this study. As will be described in Chapter 2, it is estimated that fish consumption accounts for 90% of human methylmercury exposure, and canned tuna is the single most consumed fish in the world, but information needed to estimate MeHg levels in cans of tuna is unavailable (Burger & Gochfeld, 2006; Gerstenberger et al., 2010; Sala-Vila & Calder, 2011). Trends in the literature reflect higher mean MeHg levels in white albacore tuna and lower MeHg mean levels in chunk light, but with significant variability (Groth, 2012b; Karimi et al., 2012). According to the small body of

canned tuna fish literature, there are higher MeHg levels in canned tuna tested in the United States compared to other areas of the world, but U.S. studies are grossly overrepresented (Suppin et al., 2005; Thompson & Lee, 2009).

However, the results from the measurement of total blood mercury level in Research Question 2 provide insight. I found evidence of an association of the general category of canned tuna and the frequency of total blood mercury level; this implies that greater sensitivity (specific MeHg levels in canned tuna) is not warranted to address Research Question 1. If evidence of an association was found between total blood mercury level and breast cancer but not canned tuna fish frequency and breast cancer, this would have implied that greater sensitivity in MeHg levels in canned tuna was warranted to accurately measure MeHg exposure.

The Food Frequency questionnaire used in the NHANES survey did not allow for a comparison between types of tuna because these categories of tuna are not differentiated (NHANES, 2014c). However, because even slightly elevated levels of methylmercury exposure can be toxic to humans and MeHg accumulates in the body over time (Mahalakshmi, Balakrishnan, Indira, & Srinivasanm 2011; Rahim et al., 2010), the frequencies captured in the food frequency questionnaire (NHANES, 2008a) allowed for a comparison between those that report consuming canned tuna frequently and those that do not. The food frequency questionnaire asked for canned tuna consumption in 11 levels, from *never* to *2 or more times a day* (NHANES, 2008a). The scope of measuring methylmercury exposure via canned tuna consumption was limited to presuming that

those that report eating more frequently have higher lifetime methylmercury exposures from canned tuna fish than those that report eating less frequently.

Canned tuna fish consumption has some intriguing parallels with breast cancer incidence historically (both increased since the 1940s), socioeconomically, educationally, by race/ethnicity (both higher in Caucasian women of higher education and income), and geography (both highest in North America and Western/Northern Europe; Bray et al., 2004; Campbell & Owen, 1994; Cancer Research UK, 2013; FAO, 2004b; Globefish, 2004). The NHANES dataset included questions related to age, education level, annual household income, and race/ethnicity (NHANES, 2014c). Fejerman et al. (2010) compared ancestral groupings with breast cancer incidence and found that the risk of breast cancer increased by 20% with every 25% increase in European ancestry. This increase is believed to be caused primarily by unidentified environmental factors in the United States (Fejerman et al., 2010). One underlying reason to conduct this research was to examine methylmercury exposure via canned tuna consumption as one unidentified environmental factor in breast cancer incidence (Byrne et al., 2013). In this study, I only examined results, through descriptive statistics, of the canned tuna consumption levels of groups by age, education level, household income, and race/ethnicity. The purpose was to identify canned tuna consumption by these groups to see if they broadly parallel the known social determinants of breast cancer. Inference from the findings of this question to breast cancer was limited to exploring broad trends that may or may not overlap and did not address association or causation.

Significance of the Study

The results of this study are useful to better understand and substantiate canned tuna's role as the primary route of methylmercury exposure in the human population. Examining the association between canned tuna consumption, total blood mercury levels, and breast cancer enhances and expands upon current knowledge of metalloestrogens and environmental risk factors in breast cancer incidence. On a practical level, this research contributes to social change by providing support for better understanding and specification of canned tuna fish in future breast cancer research and better identification of methylmercury levels in canned tuna fish for public knowledge.

Summary

Consumption of canned tuna fish is the primary route of methylmercury exposure in humans and is believed to contribute to breast cancer carcinogenesis as a metalloestrogen through carcinogenic theory (Byrne et al., 2013; Henderson et al., 1988; Iavicoli et al., 2009). In this case control study, I examined the association between canned tuna consumption, total blood mercury level, and breast cancer in the population-based NHANES survey from 2003-2006 (NHANES, 2014c). The primary research questions focused on exploring the frequency of canned tuna fish consumption, total blood mercury levels, and breast cancer through the application of a logistic regression model. Consumption of canned tuna fish has historic, geographic, educational, socioeconomic, and ethnic/racial parallels to breast cancer incidence as understood through a medical geography conceptual framework (Bray et al., 2004; Campbell & Owen, 1994; Cancer Research UK, 2013; Daniel et al., 2011; FAO, 2004b; Globefish,

2004; Paul, 1985). The secondary research question examined the social determinants of canned tuna consumption through results of descriptive statistics.

This study enhances current knowledge of environmental and nutritional breast cancer risk factors by measuring canned tuna fish consumption, understood as the primary route of methylmercury exposure, and breast cancer from both a carcinogenic theory and medical geography perspective. In the next chapter a more thorough description of the underlying theoretical frameworks and literature review supporting the study are provided.

Chapter 2: Literature Review

Introduction

Breast cancer is the leading cause of female cancer death and the most frequently diagnosed cancer in the world (Jemal et al., 2010). In 2008, approximately 1.4 million women were newly diagnosed with breast cancer and incidence continues to increase (Jemal et al., 2010; World Health Organization [WHO], 2013a). The geographic differences in breast cancer incidence are striking with a variance between countries of greater than 500% (Jemal et al., 2010). Environmental and nutritional factors are believed to explain the marked regional geographical differences in incidence of breast cancer and are estimated to account for between 50% and 67% of worldwide breast cancer cases (Bray et al., 2004; California Breast Cancer Research Program, 2013; The National Cancer Institute, 2013c; Strumylaite, Mechonsina, & Tamasauskas, 2010).

In one body of environmental breast cancer literature, researchers hypothesize that increased exposure to environmental metals since the 1950s have increased breast cancer risk through the activation of estrogen in the body (Byrne et al., 2013). Scholars are increasingly supporting the likelihood that exposure to estrogen activating metals (metalloestrogens) also effects estrogen levels in vitro. The metalloestrogens nickel, chromium, and cadmium have been established human carcinogens, and mercury, lead, and copper have been determined to be probable carcinogens. Over time metalloestrogens accumulate throughout the human body and in human breast tissue (Byrne et al., 2013). Tumor scholars have found significantly higher levels of metalloestrogens in malignant and benign breast tissues than in healthy tissue (Mohammadi et al., 2014).

Mercury has been identified by the WHO (2014c) as one of the "ten chemicals of major public health concern" (p. #2). Researchers of population-based studies of the metalloestrogen mercury and its relationship to breast cancer have focused primarily on occupational and environmental exposure to inorganic mercury compounds (WHO, 1997d). The most toxic, widespread, and common form of mercury is the organic compound methylmercury, and 90% of human methylmercury exposure is through consumption of fish (State of New Jersey Department of Environmental Protection [NJDEP] 2010; Mahalakshmi et al., 2011).

In studies of fish as vehicles of methylmercury exposure, scholars have primarily focused on sporting fish caught near sites of mercury release and large occasionally eaten high methylmercury containing commercial fish (Gerstenberger et al., 2010). Surprisingly underrepresented is the single most consumed fish in the world, and the primary route of human exposure to methylmercury, canned tuna fish (Ashraf, 2006; Boadi et al., 2011; Burger & Gochfeld, 2004; Burger et al., 2005; Carrington & Bolger, 2002; Dabeka et al., 2004; FDA, 2002a; Gerstenberger et al., 2010; Globefish Research Program, 2004; Groth, 2010a; Hightower & Moore, 2003; Khansari et al., 2006; Ikem & Egiebor, 2005; Jaffry & Brown, 2008; Laxe & Gamallo, 2008; Moon et al., 2011; NJDEP, 2010; Rahimi et al., 2010; EPA, 1997c; Voegborlo et al., 1999; Xue et al., 2012).

Not only is canned tuna underrepresented as the most significant methylmercury exposure in the population, it is regularly combined with other fish variables to test hypotheses related to the protective role of fish in nutrition and breast cancer research (Daniel et al., 2011; Hjartaker, 2003; Karimi et al., 2012; Sala-Vila & Calder, 2011). It is

plausible that a lack of differentiation of the canned tuna variable for its role as a breast carcinogen may explain some of the unidentified confounding present in fish and breast cancer research (Daniel et al., 2011; Hjartaker, 2003; Karimi et al., 2012; Sala-Vila & Calder, 2011).

The widespread introduction of the canned tuna fish industry in the 1950s and the high consumption levels of canned tuna fish in Europe and the United States provide an unexamined parallel to historic and geographic differences in breast cancer prevalence (Boffetta, Merler, & Vainio, 1993; Cancer Research UK, 2013; FAO, 1996a; FAO, 2004b; Karimi et al., 2012; Miyake, Guillotreau, Sun, & Ishimura, 2010). Though current evidence points to unidentified nutritional factors as integral to breast cancer etiology and geographic differences in incidence (Bray et al., 2004; Gray, 2008; Jevtic et al., 2010; Parkin, Boyd, & Walker, 2011), it appears the relationship between methylmercury exposure via canned tuna fish and breast cancer remains largely unexamined.

The purpose of this chapter is to examine what is known about canned tuna fish as a route of human methylmercury exposure, its role in breast cancer research, and geographic and historical parallels between breast cancer incidence and canned tuna consumption. First, the two theoretical frameworks of carcinogenesis theory and medical geography used for this study will be described and literature search strategy explained. Major sections of the literature review include breast cancer significance; geographic patterns; parallels to canned tuna consumption; methylmercury and canned tuna fish; mercury and breast cancer; and studies of diet, fish, canned tuna, and breast cancer.

Literature Search Strategy

The PubMed, Google, Google scholar, Walden University Library, Globefish (largest organization for fisheries in the world, includes data from Info fish), Info fish (organization for fisheries in Asia, Oceana, Middle East, Africa, and Latin America), Food Standards Australia New Zealand, Food and Agricultural Organization of the United Nations (FOA), Eurostat and European Prospective Investigation Into Cancer (via the WHO International Agency for Research on Cancer) search engines were used for publications with an open date range for different combinations of the terms *metal(s), mercury, methylmercury, tuna, fisheries, tuna fish, canned tuna, canned fish, albacore, fish, seafood, food, nutrition, nutrition survey, diet, dietary patterns, commercial fish, consumption, markets, imports, canned food, canned seafood, cancer, carcinogenic theory, medical geography, Geographic Information Systems (GIS), Spatial, breast cancer, endocrine disruptors, United States, U.S., North America, Europe, Northern Europe, Southern Europe,* and *Australia, Asia.* New leads via literature references, authors with numerous publications on a topic, government websites (e.g., Food Standards Australia/New Zealand, United States Department of Agriculture [USDA], WHO, International Agency for Research on Cancer [IARC]), news articles, cancer study registries, and publications) were identified and followed to locate pertinent and current literature and information. More literature search strategies will be described for each section.

For the section on canned tuna and geographic methylmercury biomarkers, the terms *canned tuna, tuna, fish, seafood, consumption, mercury, methylmercury, blood,*

hair, biomarkers, United States, region, Europe, North America, global, and *geography* were used in the Google scholar, PubMed, and Walden University library search engines. No date range restrictions were included in the search. The purpose of this search was to find articles specific to methylmercury biomarker mapping in high breast cancer areas by country and the world. Mahaffey, Clickner, and Jeffries (2009) synthesized the body of biomarker studies in the United States and used mapping. Sheehan et al. (2014) mapped the body of available biomarker studies in the world. Sheehan et al. claimed to be the first to map all currently available biomarker data globally. Both authors described the body of biomarker literature as composed of small local studies. No other regionally mapped biomarker studies were located.

I attempted to identify and review every study ever conducted on the methylmercury concentrations of canned tuna fish for the section on studies of methylmercury concentrations in Canned Tuna Fish. Using no date range restrictions, the terms *tuna, canned tuna, mercury,* and *methylmercury* were used in the Walden University, Google Scholar, Pub Med, WHO, CDC, Info Fish, Globefish, USDA search engines, and web sites. The search engines Google and Yahoo were also used to identify articles or websites that made references to canned tuna and methylmercury studies. These leads were read and when a peer-reviewed study referenced, the study was searched for by name and/or author to locate it. No limitation by date was included in the search. Inclusion of the country terms *United States, Europe, North America, Middle East, South America, Australia*, and *Canada* was also used in an attempt to capture studies conducted in other countries. The references used in individual canned tuna and

methylmercury publications were reviewed to locate additonal studies. Descriptions of studies used by the USDA to establish methylmercury levels were searched for by name, author, and year to locate and include. After it appeared all studies were included and the search was exhausted, sections were organized by studies conducted in the United States and other countries.

The goal of the section on canned tuna markets was to find country-specific information on which kinds of canned tuna, and how much canned tuna, was consumed since the birth of the canned tuna industry in the 1950s. The terms *tuna fish, tuna, canned tuna*, *fish, commercial fish, consumption, markets, imports, canned food, canned seafood*, and names of countries and regions (*Europe, United States, North America, worldwide, Australia*) were used in the Walden University, Pub Med, Google Scholar, Globefish, Info fish, Google, and Yahoo search engines with no date range restrictions. Pertinent information was time-consuming to locate and difficult to find. Small sections of country-specific and regional government reports of fish markets and imports contained the information sought. Unfortunately, market-level data of canned tuna purchased is privately held by grocery corporations and is not publically accessible.

The goal of the literature search for the section on mercury, hormones, and breast cancer research was to find review and summary articles on the relationship between methylmercury and hormones and hormones and breast cancer. The terms *mercury, methylmercury, endocrine, hormones,* and *breast cancer* were used in the Walden Library, Google Scholar, and Pub Med search engines. No date range restrictions were used in the initial search. Then, articles most pertinent to the contents of the research

question and underlying theories (medical geography and carcinogenic theory) and published in the last 5 years were included.

The purpose of the literature search strategy for the section on mercury and cancer population-based research was to identify and include the major and historical studies used to assess the carcinogencity of mercury by the U. S. Environmental Protection Agency and FDA. Both websites were read for references to population-based studies used in carcinogenic determinations. These references were searched for by name and/or author and were included in the study. In addition, the terms *mercury* and *cancer* were used in the Walden University, Google Scholar, and Pub Med search engines to identify pertinent literature with no date range restrictions. All identified population-based studies were included. After all studies were identified, they were prioritized by the year 2000 and after.

For the section on diet, fish, canned tuna, and breast cancer, two strategies were used. First, a search for all nutrition-related literature that identifed, included, and/or discussed canned tuna fish and breast cancer with no date range restrictions was undertaken. Second, articles were included on how diet and/or fish consumption variables were used in breast cancer research and summarized or reviewed for the relationship between fish, diet, nutrition, and breast cancer. The search terms *diet, fish, tuna, canned tuna, nutrition,* and *breast cancer* were used in the Walden University, Pub Med, European Investigation Into Nutrition and Google Scholar search engines. Reviews published in the previous 12 years were included.

Theoretical and Conceptual Framework

The purpose of this study was to examine the relationship between canned tuna fish and breast cancer from two perspectives: the theory of carcinogenesis and medical geography (Henderson et al., 1988; Mead, 2000). According to the carcinogenesis perspective, cumulative exposure to estrogen increases the risk of breast cancer (Henderson et al., 1988); this perspective was applied to this study to understand the role of canned tuna fish as a vessel of methylmercury exposure. Because methylmercury is an estrogen activating environmental compound, exposure to it increases breast cancer risk. The concept of medical geography (Mead, 2000) was applied to examine the history of the canned tuna fish industry, geographic consumption, and cultural preferences for canned tuna fish to determine parallels with breast cancer history and geographical incidence.

Theory I: Exposure to Estrogens Increases Breast Cancer Risk

The first conceptual framework applied to this study was the carcinogenesis theory that total cumulative exposure to estrogens increases breast cancer risk (Henderson et al., 1988). Beaston (1896) first hypothesized that ovarian function was primary to the etiology of breast cancer after removing ovaries in two women resulted in the slowdown and reversal of breast tumor development. Lacassagne (1932) reported that female hormones had a role in carcinogenesis, evidenced by 100% of the mice developing breast tumors after weekly injections of hormones (Regato, 1993). However, Bittner (1942) is often cited as the origin of the theory that estrogens cause cancer (Henderson et al., 1988).

After reviewing the body of animal, laboratory, and population research on the carcinogenic role of estrogen, Henderson et al. (1988) refined the estrogen cancer theory into three hypotheses of carcinogenic pathways. For breast cancer, Henderson et al. described estrogen as the "primary stimulant for breast cell proliferation" (p. #248). Hendersen et al. explained that increased risk among most known risk factors (including age of menarche, not breast feeding, hormone therapy, and advanced pregnancy age) increases the number of years that a woman is exposed to higher levels of estrogen. Henderson et al. hypothesized that "cumulative exposure of breast tissue to bioavailable estrogens" is what determines breast cancer risk (p. #248).

Davis, Axelrod, Bailey, Gaynor, and Sasco (1998) described an "emerging paradigm" in understanding estrogen-related risk factors for breast cancer etiology (p. # 523). There are three times of estrogen exposure in breast cell development: during fetal, prepubescent, and perimenopausal periods Exposures to high levels of estrogen in utero may make breast cancer cells more susceptible to future exposure. Later, during times of prepubescent and perimenopausal periods, increased estrogen exposure can change the overall hormone levels in breast tissue, increasing breast cancer risk (Davis et al., 1998). Davis et al. (1993) published a medical hypothesis that exposure to natural or synthetic compounds that effect estrogen may be a cause of geographic differences and increased incidence in breast cancer. In their review of current evidence of the mechanisms by which environmental metals activate estrogen (metalloestrogens) in breast cancer, Byrne et al. (2013) stated that the high incidence of breast cancer incidence is caused to some degree by exposure to environmental estrogens. Exposure to metalloestrogens, including

methylmercury, activates estrogen in vitro and plays a role in breast cancer carcinogenesis, but overall the relationship of estrogen activating environmental metals and breast cancer remains understudied (Byrne et al., 2013).

Assumptions underlying the carcinogenic theory that estrogen causes breast cancer specific to this dissertation include the following:

1. Estrogen stimulates breast cell development (Henderson et al., 1988).
2. The majority of known risk factors including never being pregnant, never breast feeding, having a child later in life, early menarche, late menopause, and hormone replacement therapy increase breast cancer risk by increasing lifetime exposure to estrogen (Davis et al., 1998).
3. The metalloestrogen methylmercury activates estrogen and exposure to it increases breast cancer risk (Byrne et al., 2013).

Theory II: Medical Geography

The second conceptual framework used in this study was medical geography. The origins of medical geography can be traced back to Hippocrates who identified the importance of environment to human health (Harvard University Library, 2014). Snow applied the concept of medical geography to map and understand the spatial environment surrounding the cholera outbreak in London in 1854 (McLeod, 2000). Paul (1985) claimed that by the 1980s, the medical geography field had grown and developed seven loosely defined interrelated conceptual frameworks.

The seven frameworks described by Paul (1985) include disease ecology, the oldest and most widely applied framework that is used to understand disease as a

"maladaptation between organism, culture and environment, requiring the coincidence in time and space of agent, pathogen and host" (p. #400). The second framework, disease mapping, dates back to the late 1700s. Physicians mapped cases of yellow fever in New York and discovered it came from contagious immigrants who arrived on ships in New York harbor. During the late 1800s to mid 1900s, many local, regional, and worldwide disease maps were created using complex techniques. Since the mid 1950s disease mapping has focused less on illustration and more on using maps as investigatory tools. The third concept, associative analysis, was established in the 1960s and is used with statistics to test hypothesis of risk factors with geographic scales. Studies of disease trends by scales of region and culture use this approach. Fourth, disease diffusion was established in the late 1880s, but became fully used in the 1960s. Similar to disease ecology, researchers use disease diffusion to identify the influence of environmental mechanisms contributing to disease pathology by viewing time and total environment at the same time. Disease diffusion has been vital to understanding the spread of disease in studies of river blindness and infectious disease. Fifth, the geography of nutrition was established in the 1970s after two medical geographers completed a report on aspects of nutrition for different cultures throughout the entire world. Nutrition geography is aligned with disease ecology. Aspects of nutritional geography that have been studied include climate, soil, and cultural food practices. Lastly, Paul (1985) described the geography of health care that is concerned with the spatial distribution of health services and ethnomedicine and medical pluralism that is concerned with the spatial distribution of indigenous and modern medical practices.

In addition to researchers using a medical geography perspective to understand regional differences in breast cancer incidence, some have applied it to understand geographic differences in methylmercury exposure. For example, Sheehan et al. (2014) completed a systematic review of 164 studies of children and women's methlymercury (MeHg) biomarkers. Local high fish-consuming communities in the Arctic, coastal Southeastern Asia, coastal Mediterranean, coastal Western Pacific and those living near rivers close to gold mines were hypothesized to be at highest risk of methylmercury toxicity because of their high consumption of locally caught fish. Sheehan et al.'s (2014) study will be covered in greater detail in the methylmercury section of this chapter. It is included here to illustrate medical geography's application in understanding methylmercury exposure.

Summary

Carcinogenic theory asserts that exposure to estrogen activating compounds increases breast cancer risk (Henderson et al., 1988). In this study carcinogenic theory is the plausible pathway to explain how methylmercury exposure increases breast cancer risk. Both breast cancer incidence and canned tuna consumption have occurred with marked historic and regional trends. In this study application of medical geography has framed the parallels between consumption of canned tuna and increased breast cancer prevalence.

Breast Cancer Significance

Breast cancer is the leading cause of female cancer death and the most frequently diagnosed cancer in the world (Jemal et al., 2010). Incidence continues to increase

worldwide (WHO, 2013a). GLOBOCAN (2012) estimates that for the year 2012, worldwide incidence for female breast cancer was 1,676,633 cases with 521,817 breast cancer deaths, accounting for approximately 23% of all new cancer diagnosis and 14% of all cancer deaths throughout the world (Jemal et al., 2011). Prevalence of breast cancer by country and region varies by more than 500 percent and closely reflects geographic differences in mortality (Jemal et al., 2010). North America, Australia, New Zealand, Northern Europe, and Western Europe have the highest breast cancer incidence (Jemal et al., 2011).

Currently known risk factors such as age, reproductive aspects, and genetics, are estimated to explain up to approximately half of breast cancer incidence (Strumylaite et al., 2010). A substantial body of migratory data and research provides evidence that environmental (including nutritional) factors play a significant role in the unidentified etiology and geographic variance of breast cancer prevalence (California Breast Cancer Research Program, 2013; Gray, 2008; Jevtic et al., 2010; Parkin et al., 2011). Environmental factors are believed to explain the dramatic geographic differences in breast cancer prevalence and account for up to half of breast cancer incidence throughout the world (California Breast Cancer Research Program, 2013). The National Cancer Institute (2013c) estimates that up to 67% of all cancer cases are affected by environmental factors.

Breast Cancer Geographic Patterns

In 2004, Bray et al. published a review on the global patterns of breast cancer incidence. They describe a marked variance in worldwide breast cancer incidence that is

attributable to differences in reproductive, nutritional, and environmental factors. Studies of lower incidence populations (e.g. Asian and Southern European) migrating to higher incidence populations (e.g. Australia and the United States) showed that within a generation, breast cancer risk increased significantly to parallel incidence rates of the migrants new higher risk country. Female breast cancer incidence increases were especially marked for those who relocated from low-risk to high-risk areas in childhood. Though breast cancer incidence has steadily increased throughout the world, lower incidence developing countries have experienced the largest recent increases. Bray et al., (2004, p. #229) describe this increase as "the result of the westernization of lifestyles" through nutritional and reproductive changes and increased exposure to oestrogen.

For the year 2008, age-standardized incidence rates for female breast cancer by region varied from a high of 89.7 per 100,000 population for Western Europe to a low of 19.3 per 100,000 population in Eastern Africa (Cancer Research UK, 2013). Below is a chart from Cancer Research UK (2013), using estimates from 2008, of breast cancer by region. Figure 1 shows estimates from 2008 of breast cancer by region.

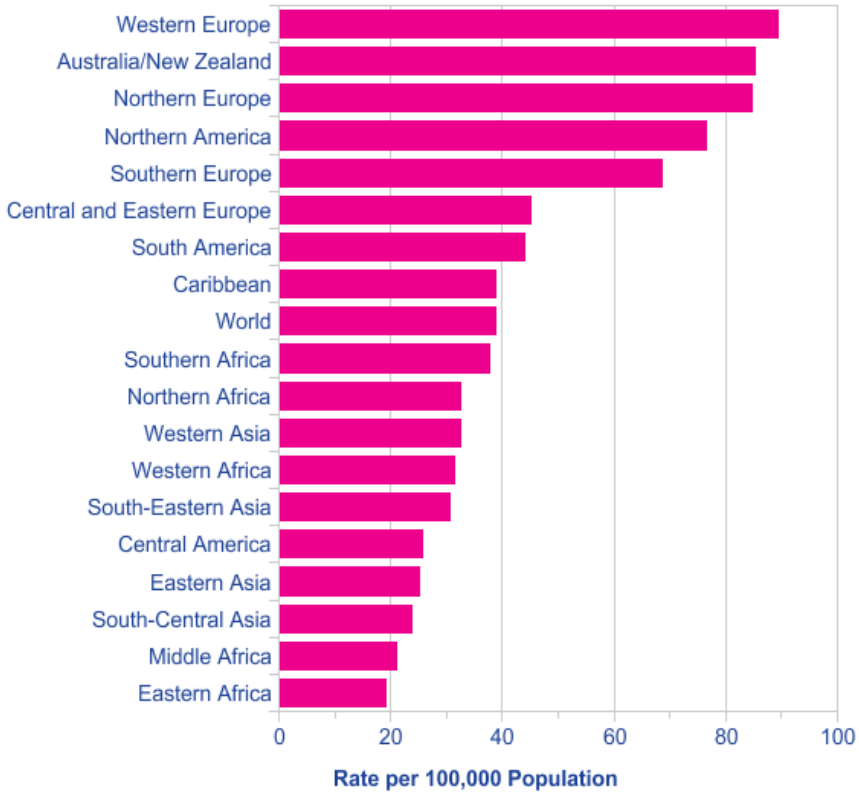

Figure 1. Female breast cancer, world age-standardized incidence rates, females, and world regions. Reprinted from Cancer Research UK, retrieved from http://www.cancerresearchuk.org/cancerinfo/cancerstats/types/breast/incidence/uk-breast-cancer-incidence-statistics. Copyright 2013 by Cancer Research UK Reprinted with permission.

Within Europe by country, female breast cancer incidence varied by almost 300%, from 145.2 per 100,000 population in Belgium to 56.5 per 100,000 population in Greece (2013). Below is a chart and map using breast cancer incidence from 2012 by European Country, from the World Health Organization, International Agency for Research on Cancer, EUCAN, (2012b).

European Age-Standardized Incidence Rates per 100,000 population, Females, EU 27 Countries (WHO, 2012b).

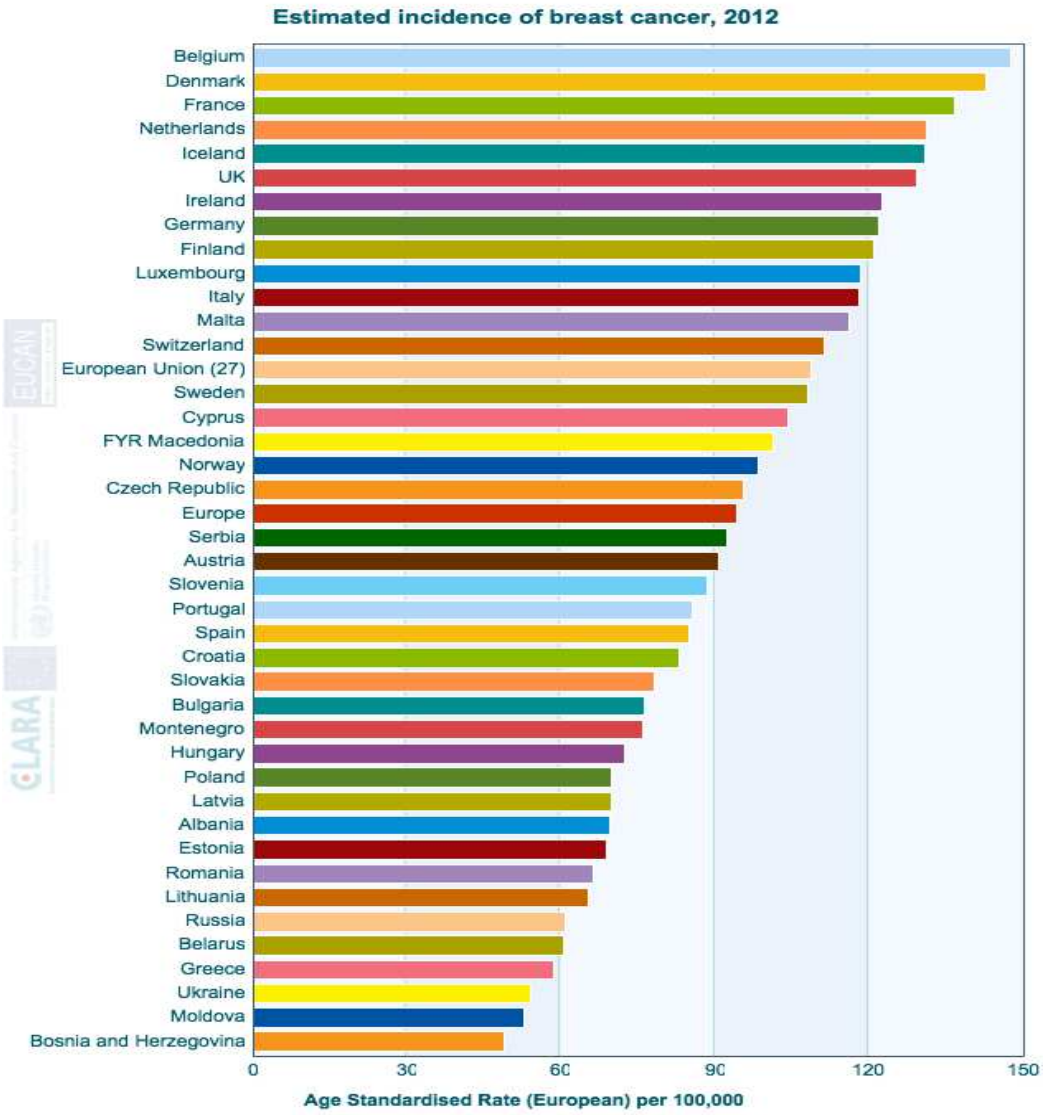

Figure 2. Estimated incidence of breast cancer, 2012. Reprinted from World Health Organization, International Agency for Research on Cancer, (IARC) EUCAN, by J. Ferlay et al., 2013 and F. Bray, J.S. Ren, E. Masuyer & J. Ferlay, 2008. Retrieved from: http://eco.iarc.fr/eucan/CancerOne.aspx?Cancer=46&Gender=2#block-map-f/. Copyright 2012 by IARC. Reprinted with permission.

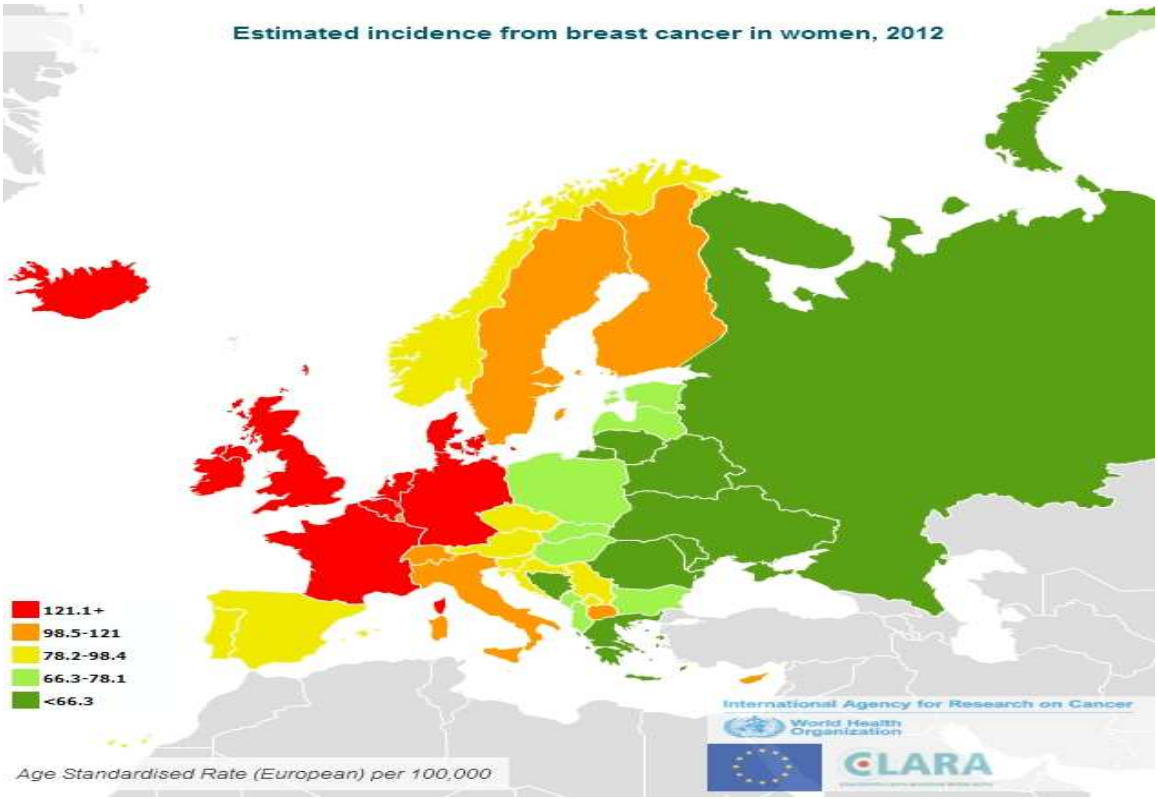

Figure 3. Estimated incidence from breast cancer in women, 2012. Reprinted from World Health Organization, International Agency for Research on Cancer, (IARC) EUCAN, by J. Ferlay et al., 2013 and F. Bray, J.S. Ren, E. Masuyer & J. Ferlay, 2008. Retrieved from: http://eco.iarc.fr/eucan/CancerOne.aspx?Cancer=46&Gender=2#block-map-f/ Copyright 2012 by IARC. Reprinted with permission.

Within Europe, the overall estimated breast cancer incidence rate in 2012 for the 27 states of the EU were 108.8 per 100,000 (Ferlay et al, 2013). Western Europe had the highest incidence of breast cancer in the world at 126.8 per 100,000 population, next Northern Europe at 120.8 per 100,000 population, then Southern Europe at 96.8 per 100,000 and lastly Central and Eastern Europe at 63.4 per 100,000 (Ferlay et al, 2013).

In the United States in 2010, female breast cancer incidence varied from 142.9 per 100,000 population in the District of Columbia to 106.3 in New Mexico (CDC, 2014l). The chart below shows female breast cancer incidence by state for the years 2007 to 2011, rates per 100,000 population. (CDC, 2014l).

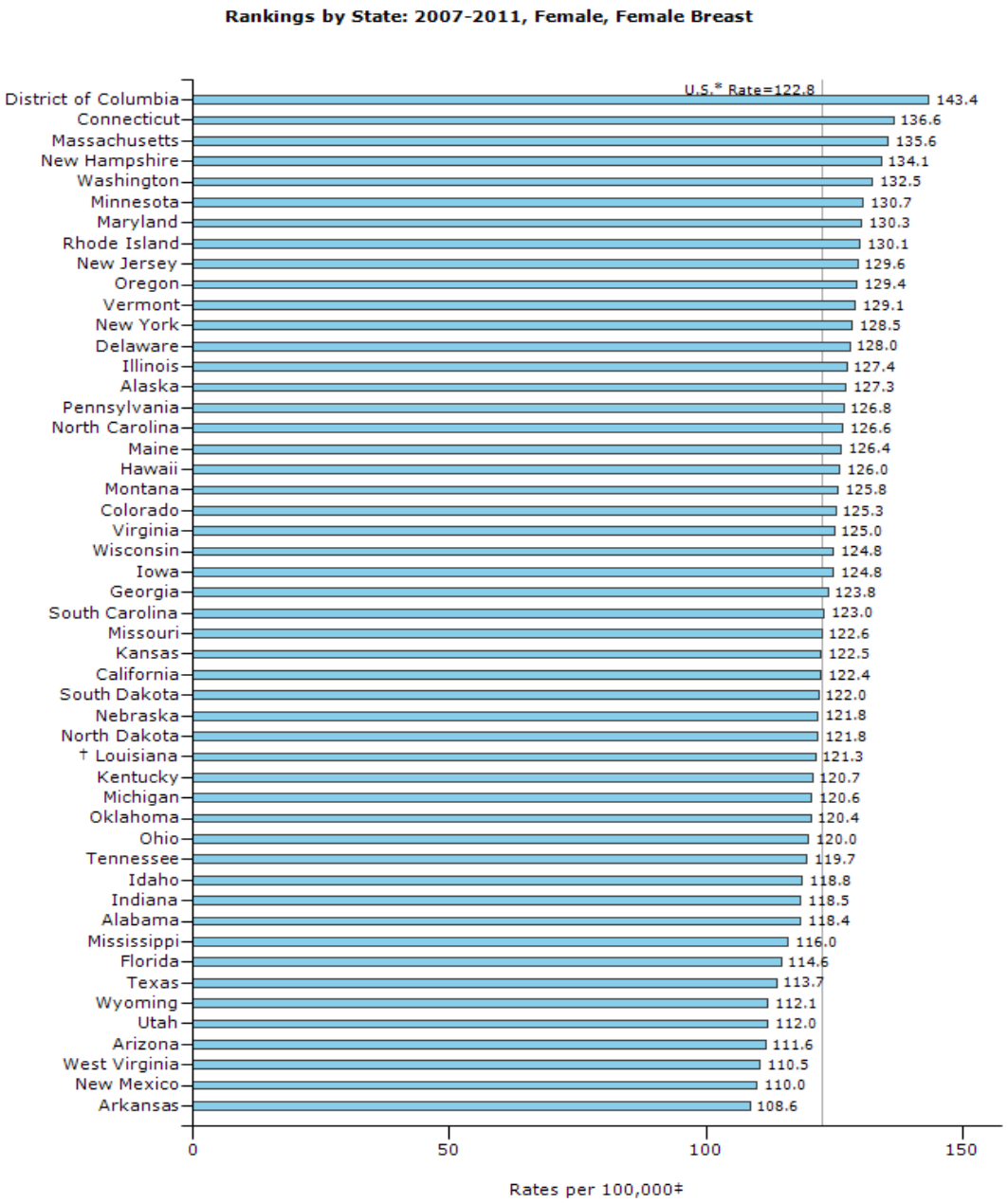

Figure 4. Rankings by state for female breast cancer, 2007-2011. Reprinted from the Centers for Disease control and Prevention, by U.S. Cancer Statistics Working Group, 2014. Retrieved from: http://nccd.cdc.gov/USCS/cancersrankedbystate.aspx. Copyright 2014 by the Centers for Disease Control. Reprinted with permission.

Though the above charts provides just one year for Europe and 4 years for U.S. of estimated breast cancer prevalence they reflect larger historic trends of highest breast cancer incidence among Northern and Western Europe, the U.S. (highest in Northeastern U.S.), and Australia/New Zealand (Bray et al., 2004; Jemal et al., 2011).

Methylmercury Exposure in Humans

Mercury is naturally occurring in soil, water, and air and is the only metal that is a liquid form at room temperature (United Nations Environment Programme Chemicals Branch, 2008). Elemental, particle-bound, and oxidized mercury is released regularly into earth's atmosphere by natural events (e.g. volcanoes) and industry (e.g. coal burning, mining, manufacturing) (EPA, 2013b).

Gross estimates figure 5,000 to 8,000 metric tons of mercury are released into earths atmosphere each year. Mercury then falls, often in precipitation, and is transformed into methylmercury (methylated) by soil bacteria before depositing into bodies of water. Aquatic plants absorb the methlymercury and are eaten by fish. Larger fish eat smaller fish and over time, methlymercury accumulates at higher and higher levels up the food chain, especially in the muscles of larger older predatory fish (United Nations Environment Programme Chemicals Branch, 2008; EPA, 2013b). It is not uncommon to find methlymercury levels in fish at more then a million times the concentration the water body they live in (Gochfeld, 2003).

Humans can only tolerate very low levels of methylmercury though naturally occurring in the environment (Mahalakshmi et al. 2011). Even slightly elevated concentrations and levels can be extremely toxic, and methylmercury is the most toxic

mercury form (Mahalakshmi et al., 2011). Of the methlymercury that accumulates in the human body, 95% is from the consumption of fish (Rahim et al, 2010). Methylmercury via fish consumption accounts for approximately 90% of all human mercury exposure (NJDEP, 2010). After methylmercury is consumed it is absorbed by the intestines (United Nations Environment Programme Chemicals Branch, 2008) then transferred from the blood to tissue throughout the human body (Hightower & Moore, 2003).

History

Although methlymercury was first used commercially as a fungicide around 1914 (Grandjean, Satoh & Eto, 2010) resulting in numerous poisoning outbreaks and deaths throughout the world (Bakir et al., 1973) human exposure to methylmercury was not widely identified as a health concern until the 1960's (NJDEP, 2010) following a methylmercury poisoning outbreak in Minamata bay of Japan (Chen & Williams, 2009). Between 1953 and 1965 consumption of methylmercury contaminated fish and shellfish in the Kumamoto area (including Minamata bay) of Japan, following industrial spill, led to 1,769 diagnosed human cases of methylmercury poisoning (acute neurotoxicity symptoms) (Eto, 1997) and deaths of people, cats, birds, and marine life (Grandjean et al., 2010). "Minamata disease" was named for methylmercury poisoning after the Japanese bay where the poisoning outbreak was first identified (Eto, 1997).

In 1964, eggs laid by hens fed with methylmercury treated seed grain were found to have high levels of methylmercury (5 mg/kg) in Sweden (Grandjean et al., 2010). This led some countries to ban Swedish egg imports and brought increased attention to the bioaccumulation of methlymercury in aquatic food chains (Grandjean et al., 2010).

Investigators found elevated levels of methylmercury in many of Sweden's water birds and fish, and in lake and river sediment located a non-impactful distance to any mercury discharging sites (e.g. industry) (Bakir et al., 1973).

A second Minamata disease outbreak occurred from consumption of methylmercury contaminated grain in Iraq in 1971-1972 (Chen & Williams, 2009) resulting in more than 6,500 hospitalizations and 450 deaths (Grandjean et al., 2010). In 1971, tests of swordfish finding high concentrations of methylmercury, far above the FDA suggested level of 0.5 parts per million wet ug/g (ppm), led the FDA to recommend avoidance of consuming swordfish resulting in collapse of the swordfish industry (Lipton, 1986). Still, it wasn't until the 1980's, in response to the growing body of evidence showing mercury contamination in water bodies and fish on a regional scale, that investigators began to focus on better understanding mercury's atmospheric disposition and bioaccumulation in the aquatic food chain (NJDEP, 2010).

After spending three years reviewing published research of environmental risk factors (indoor and outdoor) to children's health in the European Union (EU), the Policy Interpretation Network on Children's Health and Environment (PINCHE) network published their report and findings (Zuurbier et al., 2007). PINCHE was established to suggest policy to protect children, because they more susceptible to environmental exposures yet environmental policy are formed primarily on the experience of adults. The report found that many children in the EU were exposed to mercury in the form of methylmercury via consumption of fish at levels known to cause "serious health effects" (p. # 301). They concluded children in the EU were at highest risk from 5 environmental

risk factors, classified as “high priority” (p. # 301). They were nitrogen dioxide, benzene, tobacco smoke, allergens, and mercury (Zuurbier et al., 2007). In the U.S. approximately 7% of child bearing age females exceed recommended methylmercury levels of 0.1 ug/kg body weight/day (McElwee, Ho, Chou, Smith & Freedman, 2013).

Canned Tuna and Methylmercury Exposure

The widespread popularity of canned tuna fish has established tuna as the single most consumed fish in the world (Globefish Research Program, 2004; NJDEP, 2010). The highest levels of methylmercury have been found in swordfish, barracuda, marlin, scabbard, large tuna, shark, pike, and king mackerel (United Nations Environment Programme Chemicals Branch, 2008).

Because the majority of tuna canned are of the medium or small- sized varieties and believed to be of moderate to low methylmercury concentration (UNEP, 2002) its importance as a source of methylmercury exposure is often overlooked (Gerstenberger et al., 2010). Canned tuna is consumed far more widely and often then other high methlymercury containing fish and accounts for the single largest source of methylmercury exposure in humans (Ashraf, 2006; Boadi et al., 2011; Burger & Gochfeld, 2004; Burger, Stern & Gochfeld, 2005; Carrington & Bolger, 2002; Dabeka et al., 2004; FDA, 2002a; Globefish Research Program, 2004; Groth, 2010a; Hightower & Moore, 2003; Khansari et al., 2006; Ikem & Egiebor, 2005; Jaffry & Brown, 2008; Laxe & Gamallo, 2008; Moon et al., 2011; Rahimi et al., 2010; EPA, 1997c; Voegborlo et al., 1999; Xue et al., 2012). However, research of methylmercury exposure has predominantly focused on locally caught sporting fish, especially in water bodies near

mercury releasing industry, and types of commercial fish containing the highest methylmercury concentrations that are less frequently and less widely consumed than canned tuna (Gerstenberger et al., 2010).

In their review of methylmercury exposure literature, Burger and Gochfeld (2004) described that the vast majority of methylmercury data collection, research, and public safety focused on sporting fish caught in the wild, despite the fact that commercial fish comprised a "large majority of the fish most people eat" (p. # 1). Even of the commercial fish consumed, Burger and Gochfeld (2004) found that the single most commonly consumed fish, canned tuna received "little attention" (p. #1).

Canned Tuna and Geographic Methylmercury Biomarkers

Sheehan et al. (2014) and Mahaffey et al., (2009) describe that the body of methylmercury biomarker (MeHg levels in blood and hair) research is primarily composed of small studies of local populations. Sheehan et al. (2014) describes the purpose of their systematic review was to measure methylmercury exposure from consumption of fish and seafood globally for the first time. The authors describe biomarker research as based on the hypothesis that people living in coastal regions are at highest risk of methylmercury toxicity from consuming a lot of locally caught high methylmercury containing seafood. Sheehan et al. (2014) found that average MeHg biomarkers (which measure recent MeHg exposure) among "subsistence fishing communities that practice artisanal and small-scale gold mining" (p. # 257) and "Arctic peoples whose diet consists of apex marine predators such as the pilot whale" (p. # 257) were almost 4 times higher and the high median more than 10 times higher than the

reference levels applied by the WHO and FAO for high risk groups. Consumption of locally caught seafood is believed to be the primary source of high MeHg in these two populations. Overall, people living in coastal areas and especially in the Pacific and Mediterranean were found to have mean MeHg levels at 2-3 times higher rates than suggested by the WHO/FAO. Consumption of seafood "that is primarily commercially sourced" (p. # 258) is believed to be the primary source of high MeHg levels among coastal groups (Sheehan et al., 20014).

Of the 164 studies reviewed from 43 countries, Sheehan et al. (2014) describe that 78% used convenience sampling and 71% provided minimal specification about which types of seafood were consumed. Tuna and canned tuna fish were not mentioned, identified, or discussed. Sheehan et al. (2014) discussed numerous weaknesses. Among the studies reviewed there was a lack of information regarding what percentage of study participants did not consume any seafood, convenience samples may not be representative of the local population, possible harm of long term low level MeHg exposure was not addressed, and the biomarkers used only measured recent exposure to methylmercury. Though gold mining (which discharges mercury into local water bodies where fish is caught) is practiced in 70 countries only 6 had studies included in the review. Only 23 countries had studies of MeHg biomarkers from coastal areas. About 25% of studies measured MeHg biomarkers for people with mercury dental fillings, many of who did not regularly consume seafood. Only a few population-based studies were found from a few countries that are high income and considered low in seafood

consumption. Sheehan et al. (2014) concluded that increased monitoring of MeHg exposure and communication is needed to reduce risk of adverse health effects.

Mahaffey et al., (2009) completed a study that designated a combination of all types of tuna consumption (canned, fresh and frozen) in mapping mercury blood levels among women in the U.S. to compare with regional patterns of fish consumption. Data from the National Health and Nutrition Examination Survey, first collected MeHg biomarkers in 1999, was used for the study from 1999-2004. Mahaffey et al., (2009) describe that small local and international studies suggest differences of MeHg biomarkers by regions, with highest levels in coastal areas. Understanding which fish contribute to regional variances in MeHg levels is challenging because fish MeHg levels vary by more than 1000%. Adult women of childbearing age in the U.S. were found to have the highest MeHg levels in the northeast, second the west, third Midwest, and fourth the south. Below is a map from Mahaffey et al., (2009) of blood mercury levels [geometric mean (95% CI) (ug/L) and estimated 30-day dietary Hg intake [arithmetic mean (95% CI) ug/kg bw)] by U.S. Census region.

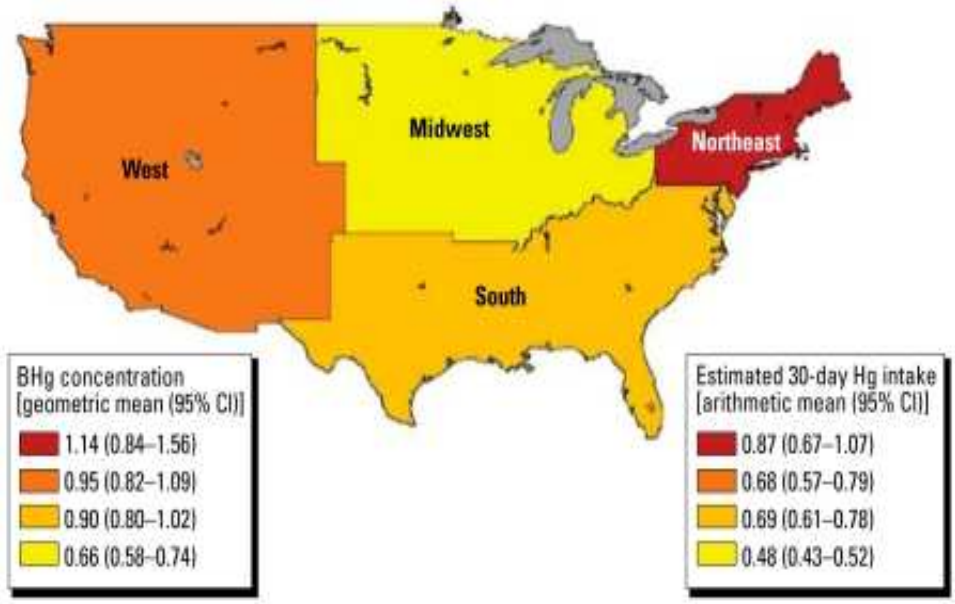

Figure 5. Blood mercury levels. Reprinted from " Adult Women's Blood Mercury Concentrations Vary Regionally in the United States: Association with Patterns of Fish Consumption (NHANES 1999-2004)" by K.R. Mahaffey, R.P. Clickner and R.A. Jeffries, 2009, Environmental Health Perspectives, 117. Copyright 2009 by Environmental Health Perspectives. Reprinted with permission.

Mahaffey et al. (2009) further regionalized data to separate coastal areas in addition to these four regions. Highest MeHg biomarker levels were found on the Eastern Coastline, followed by the Pacific Coastline, then the Gulf Coastline, followed by inland Northeast, then inland South, West, and Midwest. Figure 6 is a map from Mahaffey et al. of BHg (blood mercury) concentration (geometric mean [95% CI ug/L A] and estimated 30-day Hg intake [arithmetic mean 95% CI ug Hg/kg bw] (B) by coastal/inland regions.

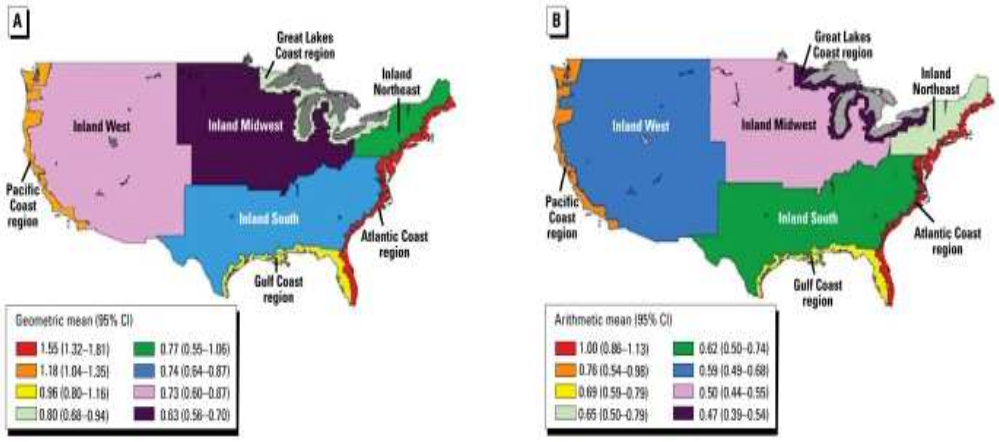

Figure 6. Blood mercury concentration. Reprinted from " Adult Women's Blood Mercury Concentrations Vary Regionally in the United States: Association with Patterns of Fish Consumption (NHANES 1999-2004)" by K.R. Mahaffey, R.P. Clickner and R.A. Jeffries, 2009, Environmental Health Perspectives, 117. Copyright 2009 by Environmental Health Perspectives. Reprinted with permission.

To compare with breast cancer geographic distribution, Figure 7 is a map from the National Cancer Institute Geographic Information Systems and Science GeoViewer application of age- adjusted breast cancer incidence rates by U.S. county from 2006 to 2010 (CDC, 2013g). Although the breast cancer map is by county and MeHg biomarker by coast and region, the geographic incidence appears to have some broad parallels. Both are highest in the Northeast and higher in coastal regions of the South, Gulf, and West. However, the inland Midwest shows elevated breast cancer incidence and low MeHg.

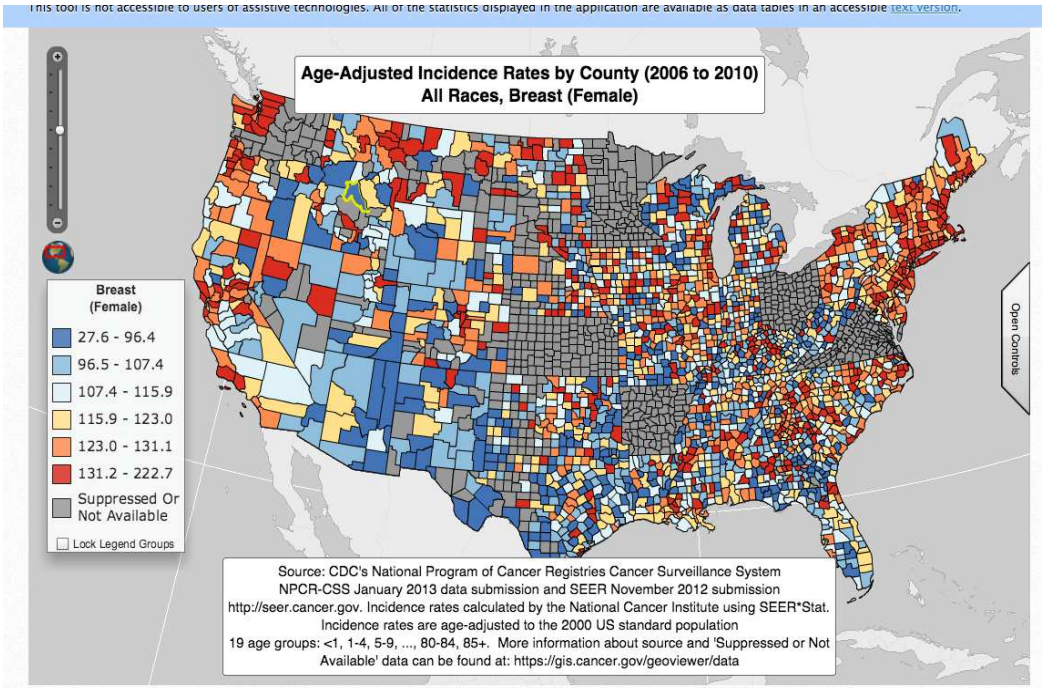

Figure 7. Age-adjusted incidence rates by county. Reprinted from the National Cancer Institute Geographic Information Systems and Science, 2015. Retrieved from: https://gis.cancer.gov/geoviewer/app/. Copyright 2015 by the National Cancer Institute. Reprinted with permission.

In the Mahaffey et al. (2009) study, canned, fresh, and frozen tuna was the most common fish eaten in all regions with the exception of the coastal gulf, which ate more shrimp. Highest total consumption of fish closely followed regional differences in MeHg biomarkers, led by the Atlantic coastline; then the Gulf and Pacific coastlines; and the inland South, West, Northeast, and Midwest. In Figure 8, Mahaffey et al. showed species and frequency of meals consumed by geographic residence.

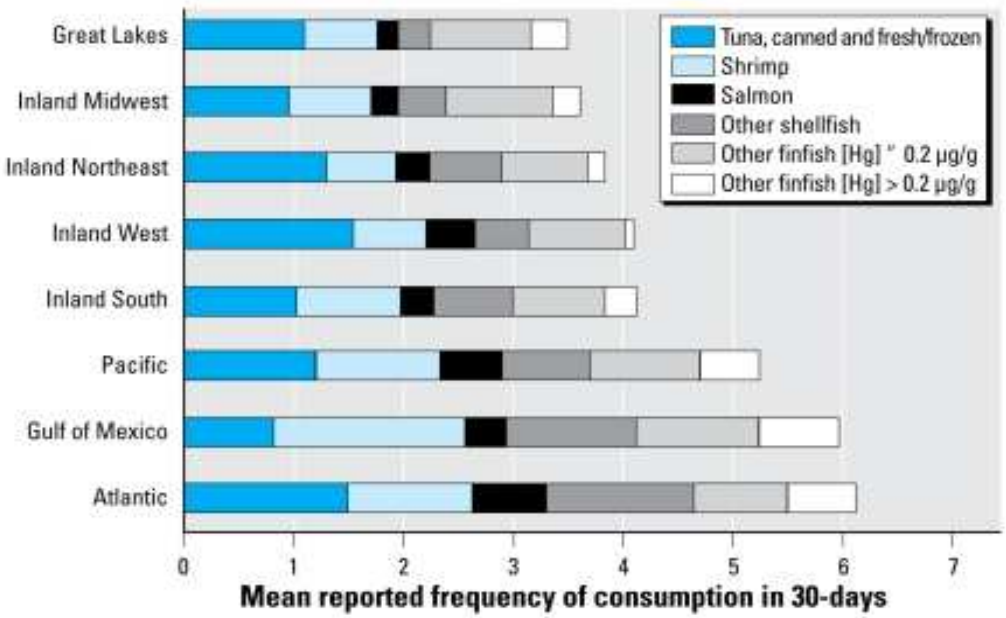

Figure 8. Mean reported frequency of consumption in 30 days. Reprinted from " Adult Women's Blood Mercury Concentrations Vary Regionally in the United States: Association with Patterns of Fish Consumption (NHANES 1999-2004)" by K.R. Mahaffey, R.P. Clickner and R.A. Jeffries, 2009, Environmental Health Perspectives, 117. Copyright 2009 by Environmental Health Perspectives. Reprinted with permission.

Figure 9 shows BHg concentrations (ug/L) by estimated frequency of total seafood consumption. The blue line is related to BHg levels found in female's cord blood, which Mahaffey et al. (2009) identified due to maternal and fetal risks of mercury toxicity.

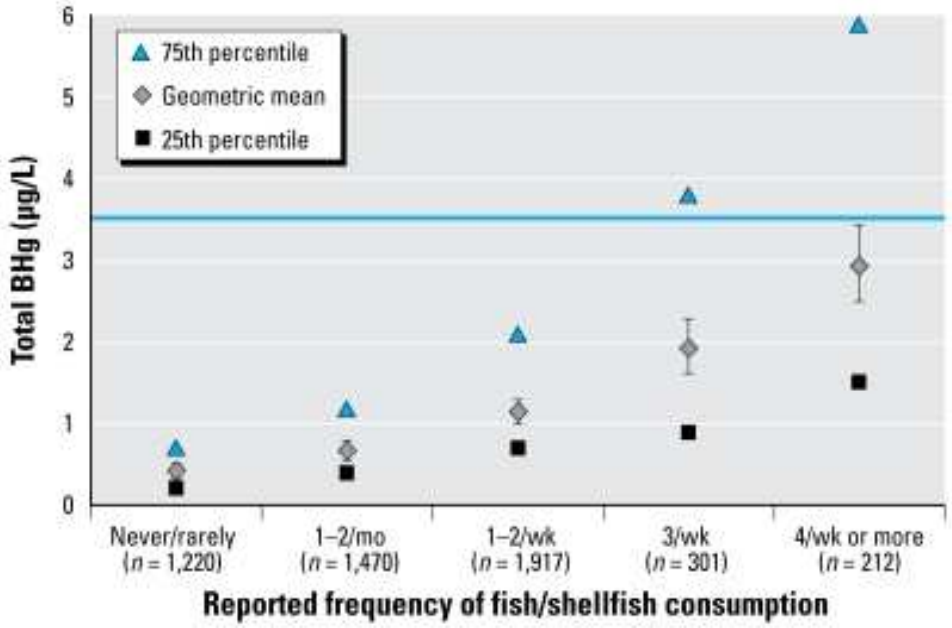

Figure 9. Reported frequency of fish/shellfish consumption. Reprinted from " Adult Women's Blood Mercury Concentrations Vary Regionally in the United States: Association with Patterns of Fish Consumption (NHANES 1999-2004)" by K.R. Mahaffey, R.P. Clickner and R.A. Jeffries, 2009, Environmental Health Perspectives, 117. Copyright 2009 by Environmental Health Perspectives. Reprinted with permission.

In Figure 10, Mahaffey et al. (2009) show the percentage of childbearing females, aged 16-49, who was found to have higher BHg concentrations than the EPA's reference mean MeHg. Approximately 7% to 14% had blood mercury levels equal to or greater than 3.5 ug/L and 2% to 7% equal to or greater than 5.8 ug/L.

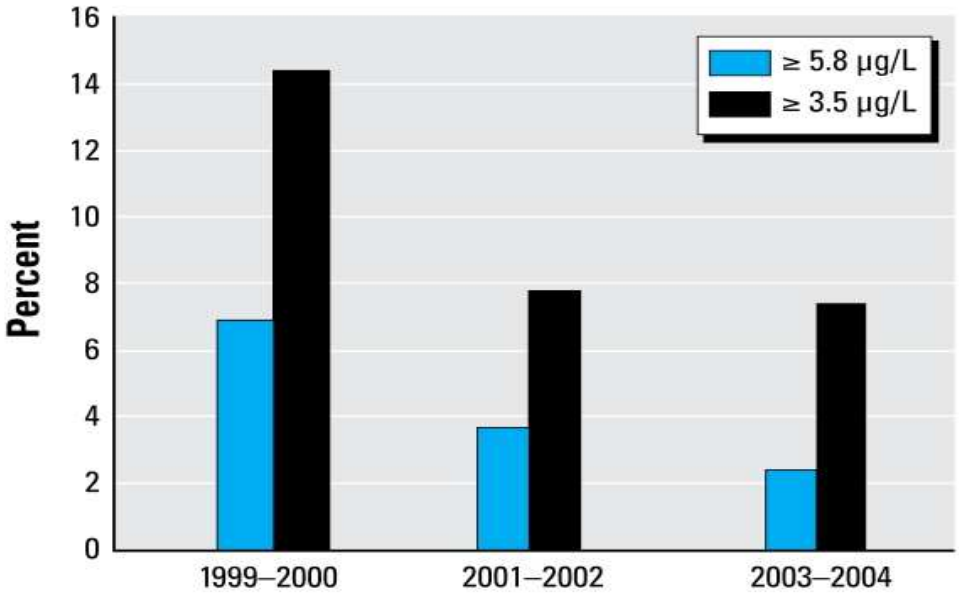

Figure 10. Rate of mercury in child bearing females. Reprinted from " Adult Women's Blood Mercury Concentrations Vary Regionally in the United States: Association with Patterns of Fish Consumption (NHANES 1999-2004)" by K.R. Mahaffey, R.P. Clickner and R.A. Jeffries, 2009, Environmental Health Perspectives, 117. Copyright 2009 by Environmental Health Perspectives. Reprinted with permission.

Unlike other exposures to environmental metals (e.g., lead in paint), BHg levels tend to increase with income (Mahaffey et al., 2009). In the highest income level, over 16% of females making $75,000 annual or more had BHg concentrations above 3.5 ug/L and 7.1% above 5.8 ug/L (Mahaffey et al., 2009). Table 1 is created from Mahaffey et al.'s (2009) table percentages of participants' BHg concentrations by annual income.

Table 1

Participant BHg Concentration by Income

BHg	All	$0- $9,999	$10,000-$19,999	$20,000-$34,999	$35,000-$54,999	$55,000-$74,999	$75,000 +
Percent > 3.5 ug/L	10.4	4.3	5.4	6.8	9.6	10.5	16.2
No. of women > 3.5 ug/L (millions)	6.92	0.18	0.40	0.80	1.11	0.92	2.72
Percent > 5.8 ug/L	4.7	1.0	1.7	2.9	5.3	6.0	7.1
No. of women > 5.8 ug/L (millions)	3.1	0.04	0.12	0.34	0.62	0.53	1.20

Table 1. Participant mercury levels by income. Adapted from " Adult Women's Blood Mercury Concentrations Vary Regionally in the United States: Association with Patterns of Fish Consumption (NHANES 1999-2004)" by K.R. Mahaffey, R.P. Clickner and R.A. Jeffries, 2009, Environmental Health Perspectives, 117. Copyright 2009 by Environmental Health Perspectives. Reprinted with permission.

Mahaffey et al. (2009) described coastal findings of highest fish consumption and MeHg levels as consistent with research throughout the world. Although federal and state MeHg advisories stress caution in consumption of high MeHg containing swordfish, king mackerel, shark, tilefish, and local game fish, Mahaffey et al. found these fish had little contribution to MeHg exposure. Overall, Mahaffey et al. claimed that exposure from highest MeHg fish (identified by local, state, and federal MeHg advisories) pale in comparison to MeHg exposure from the "most commonly consumed finfish in the United States" (p. # 52) of tuna. Although tuna consumption accounts for the largest MeHg

exposure in the United States, and albacore tuna's MeHg levels are multiple times higher than chunk light, fish questions on the NHANES does not differentiate types of tuna. Mahaffey et al. suggested that better choices of which fish to consume to decrease MeHg exposure is indicated.

Methylmercury Content of Canned Tuna Fish

MeHg concentrations in canned tuna are variable and dependent on where the tuna is caught, its size and age, the part of the tuna canned, and the type of tuna used (UNEP, 2002). However, this information remains unmonitored and unavailable to government or consumers (Gerstenberger et al., 2010; Sala-Vila & Calder, 2011). Though methlymercury accumulates in the human body over time, the FDA has demarked 0.5 parts per million wet, ug/g (ppm) and the EPA and other countries 0.3 ppm as the level of concern for fish and seafood consumption (Burger & Gochfeld, 2006; Karimi et al., 2012). The FDA has identified 1.0 ppm as the level of action for methlymercury content of fish (Burger & Gochfeld, 2006; Gerstenberger et al., 2010; Shim, Dorworth, Lasrado, & Santeere, 2004). Internationally, maximum-recommended MeHg levels in fish range from 0.1 ppm for freshwater fish in Slovakia to 1.5 ppm for predatory fish in Croatia, with the majority of countries using 0.05 ppm (UNEP, 2002).

The FDA (1991) conducted a non-comprehensive study and collected MeHg level data on 220 cans of tuna across the United States. The FDA found chunk white varieties mean MeHg at 0.31 ppm, chunk light at 0.10 ppm, and overall range of 0.1 to 0.75 ppm. The FDA used these results to establish a mean MeHg concentration for all canned tuna of 0.117 ppm that continues to be used for risk management estimates and public

education (Burger & Gochfeld, 2004; NJDEP, 2010). More recent testing by the FDA is represented on the FDA (2014c) website compiled from two previous reports in 1978 and 2010. For 451 total cans of albacore tuna tested from 1991-2010, the mean MeHg was measured at 0.350 ppm, median 0.338 ppm, standard deviation (SD) 0.128, with the highest level of 0.853 ppm. For canned light chunk tuna tested from 1991-2010, 551 cans had a mean MeHg of 0.128 ppm, median 0.078 ppm, SD 0.135, and highest level of 0.889 ppm (FDA, 2014c). Though the more recent results are significantly higher, the established mean of 0.117 ppm continues to be used by researchers, other government agencies, and the FDA in studies and advisories (NJDEP, 2010).

The NJDEP (2010, p. # 38) described significant limitations in both these FDA studies in accurately representing MeHg levels in canned tuna fish and concluded that

> There is a serious lack of current data on Hg (mercury) levels in commercial fish nationally and locally. Accurate characterization of exposure and risk from MeHg intake, as well as appropriate consumption guidance, requires the systematic and regular collection of such data.

Though comprehensive and systematic data on MeHg levels in commercial fish have not been collected (NJDEP, 2010), a body of small studies measuring MeHg levels in canned tuna does exist (Burger & Gochfeld, 2006).

Studies of Methylmercury Concentrations in Canned Tuna Fish

United States. Burger and Gochfeld (2004) tested total mercury in 168 cans of tuna obtained from a grocery store in New Jersey from 1998-2003 and found that at least 89% of all mercury present was in the form of methylmercury. Methylmercury levels

were compared for white versus chunk light variations and packed in water verses oil. Burger and Gochfeld found no significant differences in levels of MeHg between tuna packed in water versus oil or between tuna with all the water/oil drained out of the can or left in place. White canned tuna had significantly higher levels of MeHg (mean 0.407 ppm) than light (mean 0.118 ppm), which is consistent with the larger albacore tuna used for white and smaller skipjack tuna used for light. The white (albacore) solid canned tuna's mean MeHg was 0.429 ppm, SD 0.164, median 0.4 ppm, and range 0.018- 0.783 ppm. The white (albacore) chunk tuna's mean MeHg was 0.355 ppm, SD 0.166, median 0.315 ppm, and range 0.027- 0.997 ppm. Light (skipjack) canned tuna varieties (chunk and solid) had a combined mean MeHg of 0.118 ppm, SD 0.099, median 0.087 ppm, and range 0.015- 0.447 ppm. One in four cans of white (albacore) tuna exceeded the maximum allowable level of 0.5 ppm designated by the FDA. The FDA's established mean of 0.117 ppm MeHg in canned tuna is significantly lower than the levels found in white (albacore) tuna (mean 0.407 ppm) (Burger & Gochfeld, 2004).

In their study of mercury in commonly consumed canned seafood, Shim et al. (2004) tested 240 cans of tuna collected in 2003 from grocery stores in the Lafayette, Indiana area. Shim et al. found mean MeHg levels for light tuna at 0.54 ppm and white albacore tuna at 0.711 ppm. Shim et al. described these as falling below the FDA action level of 1.0 ppm.

In 1993, Yess published a study used by the U.S. FDA to establish the mean MeHg levels used for current calculations of 0.117 MeHg per can of tuna in advisories. Though only the abstract appears to be available, the study tested 220 cans of tuna in

1991 in the U.S. with selection focused on a diversity of types; tuna packed in water, brands most commonly consumed, and smaller cans. Yess (1993) found solid white (albacore) had significantly higher MeHg levels than chunk white and light and an overall mean MeHg concentration for all cans of 0.17 ppm with a range of 0.10- 0.75 ppm.

To assess possible canned tuna type variations in MeHg concentrations, not addressed in FDA MeHg research or recommendations to consumers, Burger and Gochfeld (2006) tested 20 light, 20 white, and 18 gourmet cans of tuna from markets near Chicago, IL. Overall, the authors found 64% of white tuna and 10% of light tuna to have mean MeHg concentration above 0.3 ppm, the highest level recommended by some states and countries, and 9% of canned white tuna exceeded the more commonly used 0.5 ppm recommended MeHg level. Burger and Gochfeld (2006) describe that lack of information about type of tuna canned (e.g. only 1/3 of gourmet tuna listed species of yellowfin and white is considered Albacore but not always specified) or where tuna are caught limits understanding of MeHg concentrations for research and health impacts for consumers.

In their study of heavy metal concentrations of randomly selected canned fish purchased in Montgomery, Alabama and Atlanta, Georgia, Ikem and Egiebor (2005) collected 29 cans of tuna, representing 9 brands for MeHg testing. The authors found significant variation in the concentrations of MeHg and that canned tuna had "unusually higher levels of mercury compared to any other brand of fish" (p. # 774) including seven times higher concentration than canned mackerel or pink salmon and four times higher than canned herring. Mean MeHg concentrations ranged from a high of 0.482 ppm for

Bumble Bee white tuna to a low of 0.082 for Blue bay tuna. Tuna's labeled white or Albacore had highest MeHg mean concentrations (0.482 ppm Bumble Bee white, 0.436 ppm Star-Kist white, 0.430 ppm Blue Bay white, and 0.424 ppm Star-Kist Albacore) and those labeled tuna or light/chunk light had lower concentrations, though with high variation (0.291 ppm Featherweight tuna, 0.288 ppm Bumble Bee light, 0.184 ppm Chicken of the Sea Chunk light, 0.110 ppm Chicken of the Sea tuna, and 0.082 ppm Blue Bay tuna). Ikem and Egiebor (2005) suggest moderate consumption of fish, especially by high-risk groups, and conclude that the widespread and high level of consumption of tuna fish may pose a significant health threat.

Gerstenberger et al., (2010) describe that methylmercury exposure via consumption of canned tuna is significantly understudied, and assumed to be of low MeHg concentration. To assess MeHg concentrations in canned tuna, the authors tested three brands and types of canned tuna collected from a grocery store in Las Vegas, Nevada monthly from 2005 to 2006. Significant differences in MeHg concentrations by brand and type were found, and 55% of the 155 cans of tuna had MeHg levels above the EPA recommended consumption level of 0.5 ppm and 5% had MeHg levels above the U.S. FDA action level of 1.0 ppm. Canned tuna from all three brands labeled solid white (49 cans) had a mean MeHg of 0.576 ppm (SD 0.178 ppm, Max 0.988 ppm), chunk white (48 cans) had a mean MeHg of 0.561 ppm (SD 0.212 ppm, Max 1.159 ppm) and chunk light (50 cans) a mean MeHg of 0.137 ppm (SD 0.063 ppm, Max 0.310 ppm). Canned tuna of all three types for Brand 1 (29 cans) had a mean MeHg of 0.541 ppm (SD 0.114 ppm, Max 0.869 ppm), Brand 2 MeHg mean 0.550 ppm (SD 0.199 ppm, Max 1.144

ppm), and Brand 3 MeHg mean was 0.714 ppm (SD 0.320 ppm, Max 1.666 ppm). No MeHg differences by temporal variation or type of packaging (oil versus water) were found. The authors suggest MeHg brand differences may be related to where the fish were caught, which is "confidential and not available to the consumer" (p. # 238) and the inclusion of different types (and sizes) of unidentified tuna used by different brands under the headings white, light, and chunk. To further test temporal trends, Gerstenberger et al., (2010) compare MeHg findings with four other studies spanning 1991 to 2006 and found that mean concentrations of MeHg in canned tuna appear to have increased moderately during this time period. The authors conclude that more information about where tuna is caught, which type of tuna was used, and more stringent regulations are needed to more accurately define methylmercury exposure and protect consumers in the U.S.

In his report regarding methylmercury exposure in school lunches, Groth (2012b) describes that U.S. children eat twice as much canned tuna as any other kind of fish, canned tuna is an integral part of school lunch programs, and describes being the first to directly test methylmercury levels in canned tuna used for school children. Groth (2012b) tested 59 cans of tuna from schools in 11 states and found that the 48 samples of light tuna had a mean MeHg level of 0.118 ug/g with range 0.020 to 0.640 ug/g and the 11 samples of albacore had a mean MeHg level of 0.560 ug/g with a range of 0.190 to 1.270 ug/g. Findings of light tuna were lower but similar to the mean used by the FDA (0.128) and albacore was significantly higher then the mean used by the FDA (0.350 ug/g). As a result, Groth (2012b) recommended that U.S. school children should not consume albacore tuna at all, small sized children should consume tuna once or less per month,

children who love eating tuna should be limited to two meals per month, subsidies for canned tuna in school lunch programs should be discontinued, methylmercury means and advisories should be updated and not identify light tuna as low mercury, and children who eat tuna once a week or more should undergo blood monitoring for methlymercury. Groth (2012b) also found that tuna canned in Latin America had the highest levels of methylmercury and suggests schools should purchase from suppliers in other areas.

A database created by Karimi et al., (2012) of all known mercury data of commercial fish in the U.S. examines the concentrations, exposure, and accuracy of public health warnings. The authors included data from small studies, monitoring programs, and the literature and describe their database as the largest and most complete to date. Karimi et al., (2012) found that mean MeHg concentration data on 1,362 cans of albacore tuna was 0.328 ppm (range 0.113- 0.955 ppm), and half of the samples exceeded the EPA recommended level of 0.3 ppm MeHg. Canned yellowfin tuna (298 cans) had mean MeHg concentration of 0.143 (range 0.029- 0.240 ppm) and canned light tuna a mean MeHg of 0.12 ppm (range 0.05- 0.40 ppm). The authors suggest mean MeHg levels used by the FDA to educate the public are based on small older studies and are too low. For fish eaten frequently (e.g. canned tuna) MeHg estimates and public health warnings should take into consideration the high variability of MeHg content and how often they are consumed. Lastly et al., (2012) suggest larger and more specific MeHg data sets of commercial fish are needed to accurately assess the exposure of the U.S. population.

Other countries. In their mercury assessment of commercial fish sold in Halifax, Toronto, and Vancouver Canada, Dabeka et al., (2004) tested 53 cans of tuna fish for

mercury content. Sixteen cans of white albacore tuna had the highest MeHg concentrations (mean of 0.26 ppm, range 0.19- 0.38 ppm), followed by 12 cans of yellow fin (mean 0.12, range .020- 0.59 ppm), then 7 cans of skipjack (mean .09 ppm, range .036- 0.17 ppm) and lowest in 5 cans of unidentified type of canned tuna (mean .047, range .025- .069 ppm). Overall, authors found that fresh predatory fish had the highest concentrations led by swordfish (mean 1.82 ppm, range 0.40- 3.85 ppm), then shark (mean 1.26 ppm, range 0.087- 2.73 ppm), then marlin (mean 1.43 ppm, range 0.34- 3.19 ppm) and then all types of tuna combined (fresh and frozen tuna mean MeHg 0.93 ppm, range 0.077- 2.12 ppm) (Dabeka et al., 2004).

In their study of mercury levels of farmed and imported fish in the United Kingdom (UK), Knowles, Farrington and Kestin (2003) tested 54 cans of tuna, collected from 2000-2001 for MeHg levels. The mean MeHg level found was 0.190 ppm with a range of 0.031-0.710 ppm. Although Knowles et al., (2003) describe canned tuna as accounting for at least half of all purchased canned fish/shellfish products in the UK, canned tuna and frequency of consumption as a contributor to human MeHg exposure is not mentioned specifically in their conclusions. The author's discussion focuses on the large fresh fish that exceeded recommended MeHg levels and conclude that the majority (including canned tuna) fall within recommended limits (Knowles et al., 2003).

To apply a new particle induced X-ray emission (PIXE) to study metals in canned tuna and assess for different metal concentrations based on brand and packaging, Boufleur et al. (2013) purchased 86 cans of tuna, randomly selected from a market in Porto Alegre, Brazil, representing three brands packed in brine and one brand packed in

oil. The largest Brazilian canned tuna brand, Gomes da Costa, is described as using skipjack and yellowfin in their canned tuna and 35 cans had a mean MeHg concentration of 0.142 ppm (range 0.070 - 0.199 ppm). For brand Pescador, described as using skipjack, albacore, yellowfin, blackfin, bigeye, southern bluefin, Atlantic bluefin and longtail in their canned tuna, 32 cans had a mean MeHg concentration of 0.202 ppm (range 0.1 - 0.290 ppm). The third brand, Coqueiro, described using skipjack, yellowfin and bigeye and being the only tested brand to pack in oil, had a mean MeHg of .106 ppm (range 0.066 - 0.0146 ppm). Boufleur et al. (2013) conclude that although the metal concentrations found in canned tuna were variable, the MeHg concentration found in Brazilian canned tuna is relatively low.

Voegborlo et al., (1999) describe canned tuna as being commonly eaten in Libya, which poses a health risk, though little information about metal concentration and exposure from canned tuna consumption exists. Twenty cans of tuna caught off the coast of Libya (in the Mediterranean ocean) and canned in Libya as chunks were tested for MeHg analysis. Mean MeHg concentrations were 0.29 ppm with a range of 0.20- 0.66 ppm. Only two samples tested above the recommended limit of 0.50 ppm by the Joint FAO/WHO. Compared with other geographic areas the authors describe MeHg levels as modest, though little information on MeHg levels in tuna canned from the Mediterranean existed at the time of the report (Voegborlo et al., 1999).

In their testing of 21 cans of tuna, caught in the Persian Gulf and canned as chunk tuna in Iran, Khansari et al., (2006) found a mean MeHg concentration of 0.117 ppm with a range of 0.043 to 0.235 ppm. Khansari et al., (2006) conclude MeHg levels within the

safe consumption recommendation range (under 0.5 ppm) are likely a result of Persian gulf waterways near Iran being less contaminated than other tuna fishery waters.

Rahimi et al. (2010) describes canned tuna as eaten often in Iran and studied 60 cans of tuna for metal content of mercury, cadmium and lead. Selection methods were not discussed and MeHg levels were combined and not separated by type (e.g. albacore, white, light, etc.). Rahimi et al. (2010) found mean MeHg levels of 0.125 ppm, SD 0.085, range 0.010- 0.401 ppm in canned tuna. The author compared results to 6 other studies of MeHg combined canned tuna range results, published between 1987 and 2007, from Australia (range 0.01- 0.89 ppm), Libya (range 0.20- 0.66 ppm), Malaysia (range 0.004- 0.500 ppm), Saudi Arabia (range 0.18- 0.86 ppm), U.S. (0.02- 0.76 ppm) and Iran (0.045- 0.253). Rationale for the studies chosen or comparison of range were not provided, though the author concluded MeHg canned tuna concentrations in Iran similar to other countries. Rahimi et al. (2010) concludes that some MeHg levels were above legal limits, more baseline data is needed, and suggests that the ocean area in which the canned tuna are caught as well as time of year caught should be included in future assessment of canned tuna MeHg concentrations.

To further assist in determining the baseline mercury content of fish consumed in Iran, Rahimi and Behzadnia (2011) tested 45 cans of tuna from Shiraz and Khuzestan and found only one can exceeded the recommended 0.5 mg Hg/kg MeHg limit. Types of tuna (albacore, chunk, light etc.) were not differentiated in the study. Mean MeHg were found at 0.146 ppm with a range of 0.023 to 0.529 ppm (Rahimi & Behzadnia, 2011).

Ashraf (2006) describes canned tuna as commonly consumed in the Kingdom of Saudi Arabia (KSA) and tested metal concentrations of 57 cans of tuna caught off the KSA coast and packaged as chunks in KSA. Mean MeHg concentration was 0.31 ppm (range 0.18 - 0. 86 ppm). Eleven cans of tuna exceeded MeHg 0.60 ppm identified by Ashraf (2006) as the FAO/WHO recommended limit. Ashraf (2006) suggests that mercury content of waters off KSA are likely low compared to other areas, nearer to naturally occurring mercury deposits where tuna are fished.

Because of the "scarcity of information about heavy metals in canned tuna fish", Mahalakshmi et al. (2011, p. # 43) tested 3 cans of tuna from Anchor, India and 3 cans of tuna from Grace, Canada to test for heavy metal content in those respective markets. Mahalakshmi et al. (2011) found mean MeHg concentrations of 0.62 ppm in canned tuna from India and 0.60 ppm from the Canadian canned tuna, significantly higher than the FDA's applied average of MeHg 0.1 – 0.2 ppm and recommended consumption level of below 0.5 ppm. Type of tuna (albacore, chunk, light etc.) was not specified in the study. Mahalakshmi et al. (2011) recommend further analysis of canned tuna samples in these markets.

Suppin et al., (2005) tested heavy metal content of 37 cans of Bluefin tuna from Austria and found mean MeHg concentrations of 0.11 ppm. The authors describe findings as representative of canned tuna's MeHg variability in the Austrian market but not market volume.

The UNEP (2002) references an unpublished report by Green and Lovell (1992) that tested an unidentified number of tuna cans from 1990-1992 in Fiji and found a MeHg concentration range of 0.01- 0.97 ppm.

Thompson and Lee (2009) describe that historically few fish and fish species (including canned tuna) imported into New Zealand have been tested for mercury content. To help fill knowledge gaps of dietary mercury in New Zealand, Thompson and Lee (2009) tested the mercury content of 40 types of canned tuna from a store in Wellington. All cans were imported from Thailand and the mean mercury levels for canned yellowfin was 0.029 ppm, range 0.007-0.112ppm, for canned skipjack was 0.074 ppm, range 0.018- 0.280 ppm and for unidentified canned tuna 0.082 ppm, range 0.007-0.387 ppm. Thompson and Lee (2009) describe these as low and safe levels of mercury and suggest further testing of canned tuna imported from other countries.

Though the body of knowledge of MeHg levels in canned tuna remains sparse (Burger & Gochfeld, 2006) albacore, solid and white canned tunas show highest mean methylmercury levels across studies (Groth, 2012b; Karimi et al., 2012). The highest canned tuna MeHg levels were found most often in the U.S. and overall MeHg concentrations show high variability (Karimi et al., 2012; Suppin et al., 2005; Thompson & Lee, 2009).

Canned Tuna Markets

Although a large body of knowledge regarding the history of global tuna fisheries has been compiled (FAO, 2014c), little public research of canned tuna demand and consumption has been conducted (Jaffry & Brown, 2008).

History. Though consumed since approximately 2000 BC, the development of the tuna fishery industry and large-scale production of canned tuna began in the 1940's (FAO, 2014c). The French are believed to be the first to can tuna fish in 1850 (Felando & Medina, 2012). In 1902 a sardine shortage led fisherman in Southern California to try canning tuna instead. Demand was intense and in 1903 the canned tuna industry was born. By 1918, a handful of tuna canning companies were established in the U.S. and Europe (Felando & Medina, 2012). By 1950, a global canned tuna market was established (Hamilton, Lewis, McCoy, Havice & Campling, 2011) and more then 400,000 metric tons of annual commercial tuna were caught and processed for consumption (FAO, 2004b). By 2002, world tuna captures had multiplied by more then ten (FAO, 2004b) consistently increasing to almost 6 million tonnes in 2005 (Miyake et al., 2010).

In the 1980's, overproduction of canned tuna caused a market contraction and consequential changes (Pacific Islands Forum Fisheries Agency [FFA], 1991). Canned tuna processing and exporting relocated from developed to developing countries (U.S. and Japan started importing canned tuna), leading to low inflation of price. Demand remained constant in the U.S., increased in Western Europe, and new markets in developing countries were established (FFA, 1991).

At the start of worldwide canned tuna production in the 1950's (Hamilton et al., 2011) about twice as many tuna were caught in the Pacific as the Atlantic Ocean (Miyake et al., 2010). By 2007, about 65% of the tuna used for canning were caught in the Pacific Oceans, 20% in the Indian Ocean, and 15% the Atlantic Ocean (Miyake et al., 2010;

Miyake, Miyabe & Nakano, 2004). The vast majority of albacore tuna is caught in the Atlantic Ocean (Miyake et al., 2010). From about 1950 until 1970, more albacore was caught and canned from the Atlantic Ocean than any other tuna, accounting for approximately 25% of all tuna canned. Since the mid 1970's, smaller tuna varieties of skipjack and yellowfin account for the vast majority of increased tuna catches in the Pacific, Atlantic and Indian Oceans. Tuna catches in the Atlantic peaked in the 1980's and have since declined, though the number of albacore has remained fairly consistent at almost 1000 thousand tonnes per year. The average size of caught tuna ranged from about 45kg to 60kg then peaked in the late 1980's at about 65kg and has declined significantly since the early 1990's, averaging about 50kg since 1997 (Miyake et al., 2010). The chart below shows world imports of all tuna, frozen, fresh, chilled and canned from 1974-2002 (FAO, 2004b).

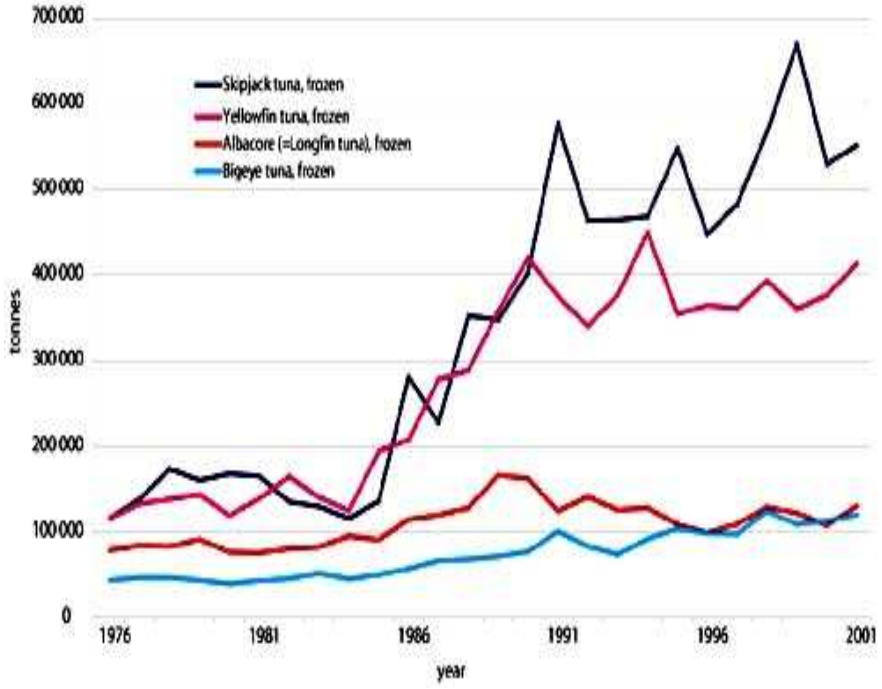

Figure 11. World imports from canned tuna. Reprinted from Globefish Research Programme, World Tuna Markets, Volume 74, FISHSTAT+, page 3. Copyright 2004 the Food and Agricultural Organization of the United Nations. Reprinted with permission.

Earliest consumers of canned tuna, the United States and Europe, would have consumed at least 1 out of every 4 cans of higher MeHg level albacore tuna compared with 1 in 10, and in most countries 1 in 20, today (FAO, 2004b; Miyake et al., 2010). As earliest consumers from 1950-1980, the United States and Europe would not have only consumed significantly higher frequency of albacore tuna, but also from older and larger fish (Boffetta et al., 1993; Karimi et al., 2012; Miyake et al., 2010).

Figure 12 below shows world imports of canned tuna in tons from 1976 to 2001 (FAO, 2004b).

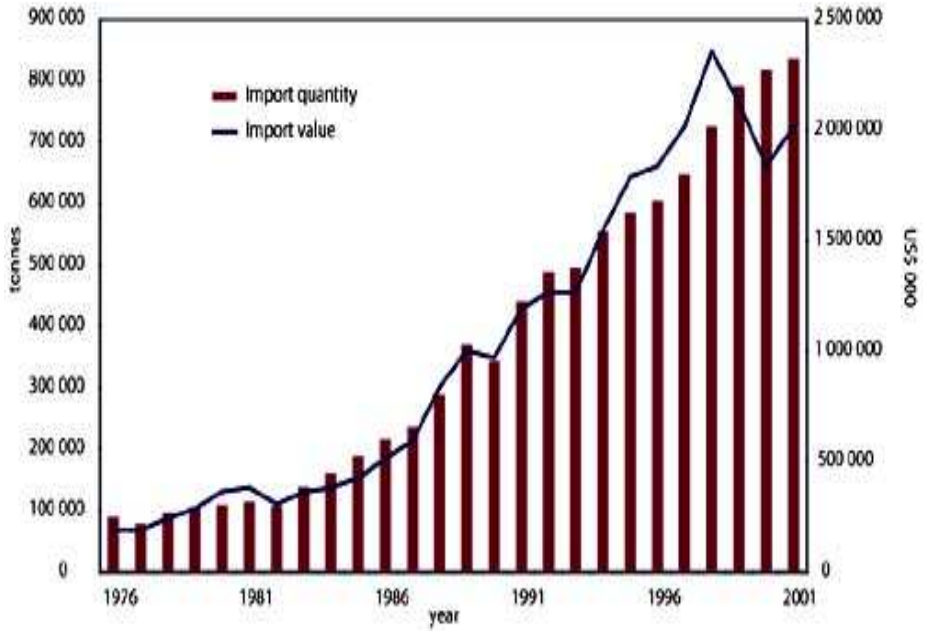

Figure 12. Imports of canned tuna from 1976 to 2001. Reprinted from Globefish Research Programme, World Tuna Markets, Volume 74, FISHSTAT+, page 18. Copyright 2004 the Food and Agricultural Organization of the United Nations. Reprinted with permission.

From approximately 1950 to the late 1980s, the United States was the largest consumer of canned tuna, accounting for about 30% of the world market (FAO, 1996a). Then U.S. tuna imports and consumption declined as a percentage of world consumption, accounting for approximately 20% of the world canned tuna market by 1995 (FAO, 1996a). In contrast, Europe's canned tuna consumption has increased steadily since the 1980s and has remained the highest consumer of canned tuna by volume (FAO, 1996a; Mongruel, Lemna, Mempes, & Mempes, 2010). By region and volume, the current largest consumers of canned tuna are Western Europe (accounting for 35% of the canned tuna market), the United States (accounting for 25% of the canned tuna market), Asia,

Latin America, the Middle East, Australia/New Zealand, Africa, and Eastern Europe (Hamilton et al., 2011; Mongruel et al., 2010). However, when regional volume is compared with population size, the highest canned tuna consumption per capita occurs in Western Europe, North America, and Australia/New Zealand (Hamilton et al., 2011; Mongruel et al., 2010; The World Bank, 2014). The IARC (2014b) designated these as high breast cancer incidence regions as well as Japan, which does not have the high breast cancer incidence of other developed areas.

Worldwide canned tuna consumption was estimated at 0.26 kg per person in 1990 and increased to 0.48 kg per person in 2002 (Project Fish, 2005). By volume and country in 2012, The PEW Charitable Trusts (2012) estimated that the largest consumers of canned tuna are the United States (24% of global market share), Japan (9.2%), the U.K. (9.2%), Spain (8.6%), Mexico (7.4%), Italy (7.1%), France (5.4%), Germany (5.2%), Netherlands (2.9%), Portugal (2.9 %), Canada (2.6%), Australia (2.5%), Egypt (2.4%), Belgium (0.7%), and the rest of the world 9.6%.

Though estimates of canned tuna consumption reflect the U.S., Northern and Western Europe, and Japan as the largest consumers by volume and also Australia/New Zealand per capita, my efforts to understand canned tuna consumption more specifically per capita, by type of canned tuna eaten (albacore, chunk, etc.), by smaller geographic areas (e.g. European country), since start of the canned tuna market (1950's) were partially successful. Results of this effort from a thorough literature search as described in the Literature Search Strategy section are included below.

Europe. The 27 countries of the European Union (EU) comprise the largest canned tuna market in the world (Hamilton et al., 2011). In 2010, Hamilton et al., (2011) estimated Spain was the largest canned tuna market by volume with 21% of the EU canned tuna market, followed by Italy at 20%, the UK and France at 19 % each, and Germany at 9%. However, Spain canned tuna consumption is often inflated because of its role as a processor and re-exporter of canned tuna to the E.U. (Hamilton et al., 2011).

As part of their report prepared for the Australian Center for International Agricultural Research regarding development of Papua New Guinea tuna fisheries, Campbell and Owen (1994) reviewed the data available on the largest canned tuna fish markets from 1982 to 1991. Campbell and Owen (1994) described European canned tuna markets as highly varied and consumption increasing significantly from 198.4 tons (.55 kg per person) in 1982 to 441.5 tons (1.22 kg per person) in 1991. Western and northern Europe had the highest consumption increases during this time period (increase of 112%), from .6 kg per person to 1.27 kg per person. The UK canned tuna market increased by 440% between 1982-1992. Historically, the UK market was comprised of mostly skipjack, which was changing in the 1980's towards lightmeat chunk. Germany's market increased almost 200% from 1982- 1991 and Germans were the most price conscious of the Western/Northern European countries, largely consuming cheapest brands labeled Bonito and solid chunk. Belgium and Luxemburg's market increased 161% and was considered a high value market, preferring solid packed yellowfin or chunk canned tuna (Campbell & Owen, 1994).

In the 1980's Italy had the largest canned tuna market by volume, comprised mainly of yellowfin with some slapjack (Campbell & Owen, 1994). France had the second largest canned tuna market by volume: canned tuna was present in almost half of all households, was becoming increasingly popular with the young, urban, and well off, and about 2/3 of the market was light meat chunk (less than 5% canned white albacore tuna) (Campbell & Owen, 1994). Sweden is a high fish-consuming country and its largest fish import is canned tuna (Fair Trade Center, 2007). Wu (2012) describes Sweden as being one of the highest fish consumption countries per capita in the world. Historically, the Swedish canned tuna market comprised a very high 20% white albacore market, which decreased slightly in the 1980's though overall canned tuna consumption increased by 87% (Campbell & Owen, 1994). Though Campbell and Owen (1994) do not provide specific details, they describe Switzerland as the highest quality fish market in Europe.

Hamilton et al. (2011) provided a review of major tuna markets with data from 2007- 2008 and described the French market as preferring canned skipjack and Italy and Spain preferring Yellowfin. For countries with more then 100,000 cans of tuna consumed annually, Hamilton et al. (2011) estimated per capita averages from 2000-2002 compared to 2008. Spain's estimated mean canned tuna per person increased from 2.22kg in 2001/2002 to 3.1 kg in 2008. Italy's from 2.11kg to 2.33 kg, U.K.'s 1.99 kg to 2.15 kg, France 1.92 to 1.93 kg and overall for 27 members of the E.U. was estimated based on 15 reporting members at 1.53kg for 15 in 2001/2002 and 1.38 kg for 2008. Types of tuna were not differentiated (Hamilton et al., 2011).

Livsmedelsverket (2004) completed an assessment report on dietary metal exposure to persons in the European Union. Information was gathered from single point estimates of consumption and estimated based on concentrations of methylmercury from samples. Overall, methylmercury intake level of concern is designated as 0.35 ppm per week for the average weighted adult. Livsmedelsverket (2004) found the average intake of methlymercury via fish consumption from the information submitted by EU member states was 0.387 ppm per week. Highest consumers of fish and fish products (including canned tuna) of the 11 countries that provided the information, by estimated consumption of MeHg per day, were in Northern Europe (Norway, Finland, Sweden), followed by Western Europe (France, Ireland, France) as shown in the chart below. The column for Mean level in Food (ug/g) identifies the base mercury level used to calculate intake per fish item consumed and is highly variable with Sweden using the lowest MeHg estimate at 0.02 ppm and Portugal the highest at 0.32 ppm.

Table 2

Mercury in Fish and Fish products, Including Mollusks, Crustaceans, and Echinoderms

Member State	Consumption (g/day) Mean Level	Consumption (g/day) High Level	Mean Level in Food (ug/g)	Intake (ug/day) Mean Level	Intake ug/day High Level	% of total dietary intake
Belgium	13.37	47.79	0.189	2.523	9.019	91
Denmark	23		0.0417	0.96		27.4
Finland	53		0.035-0.380	6.2		100
France	29.8	82.9-21.4	0.06-0.381	2.73	4.96-8.16	32.2
Germany	16.9	63.4	0.029-0.173	2.819	10.695	0.94
Greece	18	71	0.108-0.143	4.513		87.18
Ireland	23.31	74.86	0.07	0.96	3.5	
Italy			0.10-0.33	8.6		100
The Netherlands	10		0.019	0.19		11
Norway	70.4	237.6	0.005-1.082	3.3374	18.48	82.1
Portugal	40.5		0.32	13.10		
Sweden	30.1		0.02-0.23	2.7		100
U.K.	14		0.043	1.00		33

Note. Mercury in Fish and Fish products, Including Mollusks, Crustaceans, and Echinoderms. Adapted from Reports on tasks for scientific cooperation, Report of experts participating in Task 3.2.11, March 2004, Assessment of the dietary exposure to arsenic, cadmium, lead and mercury of the population of the EU member states, page 100-102. Retrieved from: http://ec.europa.eu/food/food/chemicalsafety/contaminants/scoop_3-2-11_heavy_metals_report_en.pdf.

By age group, Livsmedelsverket estimated mean population intake of mercury via fish and fish products among 11 European countries for 4- to 6-year-olds at 0.495 ppm/day and 10-to 12-year-olds at 0.673 ppm/day. Among 10- to 12- year-olds who consume fish, ppm/day intake of mercury is estimated at 0.964ppm.

Although Livsmedelsverket (2004) describes that fish and fish products make up more then 90% of human mercury exposure in the form of methlymercury the survey information for the study identified only fish and fish products as one of among 13 different categories of mercury containing food. Other categories included milk, meat, fats, eggs, beverages, etc. To calculate estimated MeHg levels in canned tuna, which appeared to be specified by only a few countries that provided information and estimated

under the fish and fish products category by an unidentified method, Livsmedelsverket (2004) uses one sampling of 13 cans of tuna in Belgium from 2002 which found a mean MeHg level of 0.16 mg/kg, two samplings from 1999-2002 in Greece of 28 fish canned in oil with mean MeHg 0.1020 mg/kg and 22 "fish canned in brine" with mean MeHg 0.0992 hg/kg, two samplings from 2001 in Ireland of one can of tuna with a mean MeHg of 0.0300 hg/kg, and 14 cans of tuna with a mean MeHg of 0.1071, and more than 7300 canned fish from 1990-2002 in Portugal which only identified a range (not mean) of less than 0.005 to 1.27 mg/kg.

In their assessment of mercury exposure via diet in the UK, the Food Standards Agency (FSA; 2002) identified canned tuna as being the largest contributor of MeHg to the British population. Using a mean of 0.19 MeHg per can of tuna based on average portion and body weight by age, FSA estimated of the mean hg/kg of MeHg per person per week from consuming canned tuna, reflected in Table 3 below.

Table 3

Mean MeHg per Person Per Week

UK Estimates 2002	Mean MeHg from Canned Tuna per person per week (based on 0.19 MeHg per can)	Mean MeHg per person per week for top 2.5% high consumers of Canned Tuna (based on 0.19 MeHg per can)
Infants	0.04 hg/kg	0.13 hg/kg
Toddlers	0.81 hg/kg	2.45 hg/kg
Age 4-6	0.53 hg/kg	1.61 hg/kg
Age 7-10	0.30 hg/kg	1.26 hg/kg
Age 11-14	0.32 hg/kg	0.98 hg/kg
Age 15-18	0.27 hg/kg	0.68 hg/kg
All Adults	0.25 hg/kg	0.62 hg/kg
Adult Women	0.27 hg/kg	0.62 hg/kg

Note. Table 3. Mean MeHg per Person Per Week. Adapted from the Statement on a survey of mercury in fish and shellfish, Food Services Agency Committee on Toxicity, 2002. Retrieved from: http://cot.food.gov.uk/cotstatements/cotstatementsyrs/cotstatements2003/cotmercurystatement. Copyright 2002 Food Standards Agency. Reprinted with permission.

Jaffry and Brown (2008) describe canned tuna as accounting for more than half of the total fish market in the UK. About 70% of canned tuna consumed in the UK is purchased in grocery stores and 30% prepared in restaurants, most commonly in sandwiches and salads. Jaffry and Brown (2008) stress that the average Briton does not differentiate between kinds of tuna, because almost the entire canned tuna market is skipjack, with yellowfin just being introduced in the years prior to 2008. Project Fish, European Commission, (2005) describes skipjack as the most important type of tuna for processing canned tuna, yellowfin as a higher quality and price sold often as sashimi and used in canned in oil and brine, pouched, and some luxury products, and the most expensive albacore maintains a niche market in the U.S., Spain and France.

Japan. Campbell and Owen (1994) describe Japan as largely consuming low priced canned tuna from Thailand. Hamilton et al. (2011) describe Japan's canned tuna consumption as remaining relatively stable at about 100,000 meter tons per year then declining 20% from 1995 to 2007. In 2007 the Japanese market comprised about 65% yellowfin, 27% skipjack, 6% albacore and 2% bigeye (Hamilton et al., 2011).

United States. In a study of 5,182 people in the U.S. using USDA nutritional survey data from 1994, 1995, 1996 and 1998, the EPA (2002a) found that among all fish species, tuna had the highest mean consumption level among those surveyed. For females under age 15 the estimated mean grams of uncooked tuna consumed per person per day was 1.973. For females aged 15 to 44, mean consumption per day was 3.572 grams, and for over age 45 the estimated mean grams of tuna consumed per day was 4.572 grams. The estimated mean uncooked tuna consumption was double to triple the amount of grams per day as the next most commonly consumed fish for each age group (shrimp, Cod and Salmon). Among all females, estimated mean tuna consumed per day was 3.564 grams, second was shrimp at 1.807 grams, then salmon at 1.312 grams. For males, estimated mean uncooked tuna consumed per person today was similar at 2.274 grams for those under age 15, for ages 15 to 44, 4.016 grams, 4.248 grams for those over age 45, and for all males 3.674 grams (EPA, 2002a).

The National Marine Fisheries Service (NMFS) (2012) calculated and estimated per capita fish consumption from 1910 to 2012 based on imports/exports divided by the U.S. population. Most data was collected from secondary sources, which may underrepresent consumption due to incomplete reporting. For all canned fish and

shellfish, which primarily represents canned tuna; each person in the U.S. is estimated to have consumed the following amount in pounds by year:

Figure 14 shows shellfish and canned fish consumed per year.

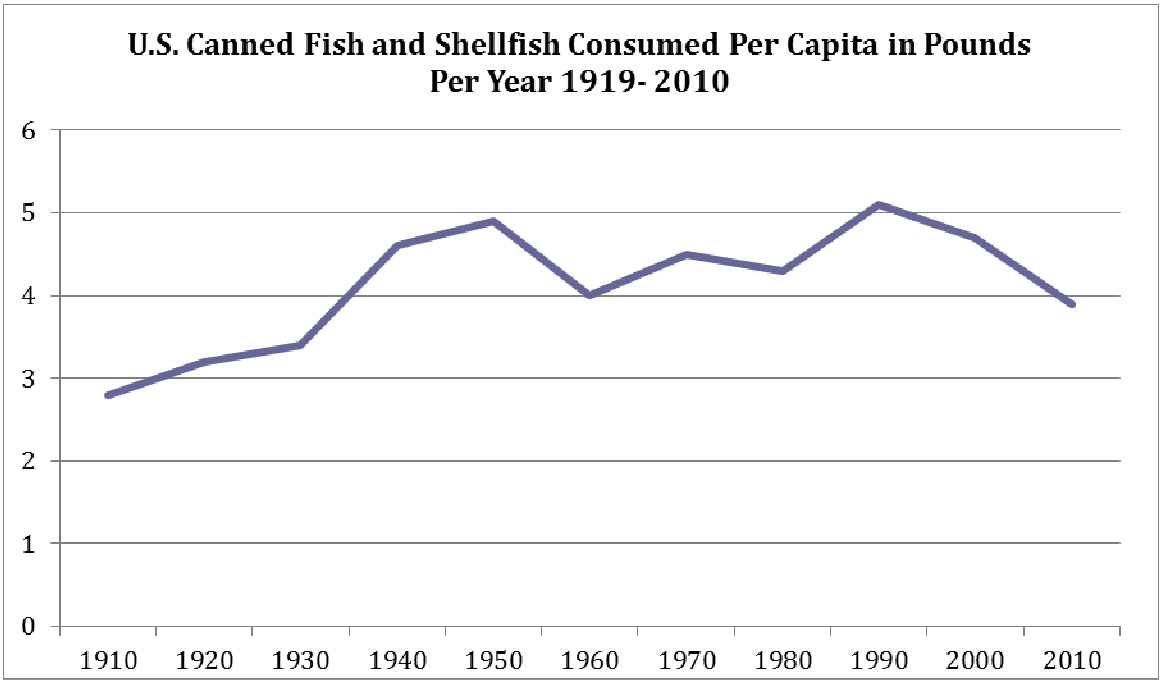

Figure 14. U. S. canned fish and shellfish consumed per capita. Adapted from the National Marine Fisheries Service, Per capita consumption by Fisheries of the United States, 2012. Retrieved from: http://www.st.nmfs.noaa.gov/Assets/commercial/fus/fus12/08_percapita2012.pdf/. Copyright 2012 Fisheries of the United States. Adapted with permission.

Per capita consumption of canned tuna is estimated only during the years from 1984 to 2012 and is listed below (NMFS, 2012) in Figure 15.

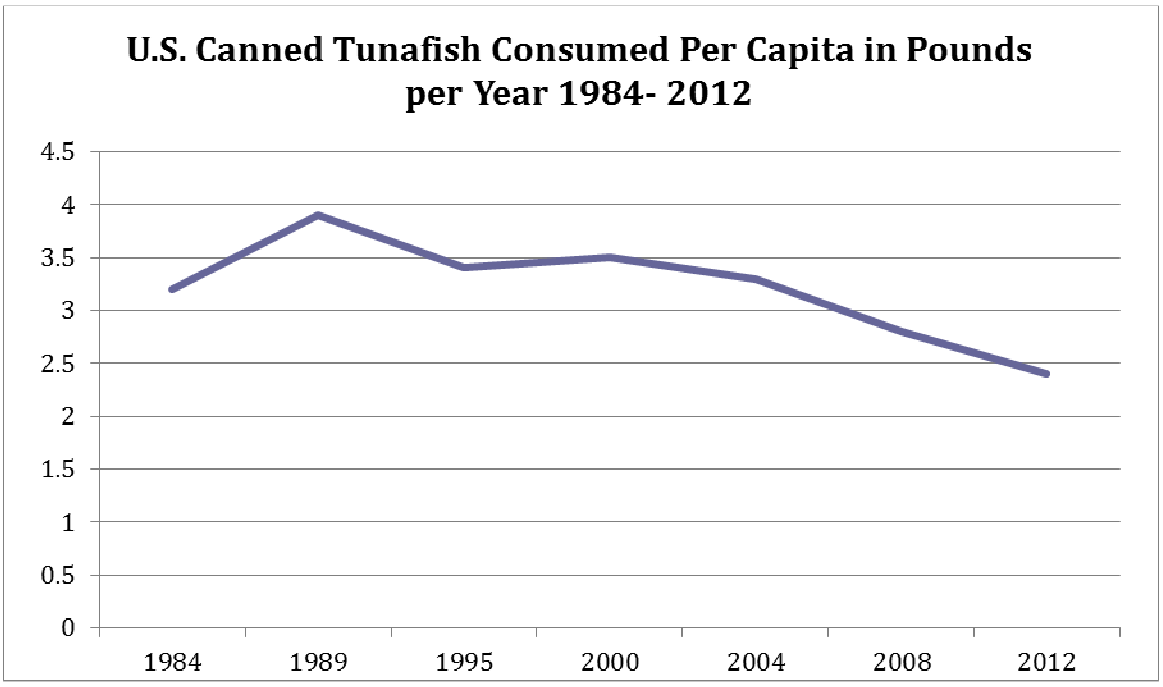

Figure 15. U. S. canned tuna fish consumed per capita. Adapted from the National Marine Fisheries Service, Per capita consumption by Fisheries of the United States, 2012. Retrieved from: http://www.st.nmfs.noaa.gov/Assets/commercial/fus/fus12/08_percapita2012.pdf/. Copyright 2012 Fisheries of the United States. Adapted with permission.

In their evaluation and overview of nationwide food consumption surveys dating back to the 1970's, the EPA (1997c) describes that consumption of fish and shellfish in the U.S. is highly varied by ethnicity, preferences, season, and geographic location, unlike other commonly consumed items (e.g. bread). Previous reports calculated per capita fish consumption rates using data from national surveys by dividing fish supply by the entire U.S. population and also per user fish consumption, which divides supply only among those in the population that report consuming the specific fish. Methylmercury estimates are also attempted, though geographic, temporal, and seasonal changes are vital

to accurately measuring methylmercury exposure variation in consumed fish, though not currently practiced and rarely viable. Long term records/surveys of individuals fish consumption are impractical and making long term estimates from short term data leads to mean estimates that are both higher and lower then the true average, resulting in overestimated standard deviations (EPA, 1997c).

Using data from the NHANES III survey administered in the 1990's, researchers from the EPA (1997c) found canned tuna was the most frequently eaten fish and shellfish among U.S. children, accounting for 33% of all fish/shellfish consumption among females aged one to five, 27% among males aged one to five, 26% among females aged six to eleven, 19% among males aged six to eleven, 28% among females aged twelve to fourteen, and 25% among males aged twelve to fourteen. The EPA (1997c) researchers describe that in a fish consumption survey of 1,856 persons from Oahu, Hawaii, almost 71% reported consuming canned tuna (type of tuna not identified), the most commonly consumed fish among participants, followed by almost 48% consuming shrimp and 42% consuming fresh tuna.

In what they describe as the first study to evaluate blood and hair mercury levels geographically, Mahaffey et al., (2009) examined mercury levels in the U.S. by region and compared to levels of fish consumption. Mahaffey et al., (2009) describe that blood and hair mercury levels are considered reliable indicators of the magnitude of methylmercury exposure, 90% of which is via fish/shellfish consumption. In their literature review of existing U.S. and international research, the authors concluded that local data suggests blood and hair mercury levels differ regionally and are higher in

coastal and other high fish/shellfish consuming areas. Data from the annual NHANES were used of fish/shellfish consumption from 1999-2004, blood mercury levels from 1999-2004, and hair mercury from 1999 among American women aged 16 to 49. By four major regions the highest mean blood mercury levels and estimated MeHg exposure by fish/shellfish consumption were found among adult women in the Northeast (BHg 1.14 ug/L, Hg intake 0.87 ug/kg), followed by those in the West (BHg 0.95 ug/L, Hg intake 0.68 ug/kg), then the South (BHg 0.90 ug/L, Hg intake 0.69 ug/kg) and Midwest (BHg 0.66 ug/L, Hg intake 0.48) (Mahaffey et al., 2009).

In their sample of 3,173 people that Thompson and Boekelheide (2013) describe as representative of 134.5 million American women of childbearing age, the body burden of lead, polychlorinated biphenyls (PCBs) and mercury blood levels were measured and compared with risk factors from 1999 to 2004. The authors found that levels of methylmercury increased significantly with age, was elevated above the median for women consuming any fish in the past 30 days, consumption of tuna (not specified by canned or fresh), salmon or haddock was significantly associated with higher mercury body burden, eating fish at all was significantly associated with increased mercury levels, and risk of elevated blood toxicity quadrupled for women who ate fish more than once a week (OR= 4.50, 95% CI, 2.49-8.12).

Australia/New Zealand. For the year 2008/2009, the Australian Fisheries Research and Development Corporation (2011) found that among all seafood imported into Australia (approximately 70-80% of all seafood consumed), canned fish, comprised of about 70% tuna, had the highest volume at 54,132 tons. In Australia, canned tuna is

packaged in jars of glass, packages, and cans in different sizes (Australian Fisheries Research and Development Corporation, 2011). In their research by phone interview of a representative sample of 692 Australians, the Australia Fisheries Research and Development Corporation (2006) found 54% reported that they consumed seafood at least once a week, 12% twice a week, and 12% at least three times a week. Forty-one percent of respondents said they usually purchase canned seafood and 77% report they usually purchase fresh seafood (Australian Fisheries Research and Development Corporation, 2006).

Historically, the largest imports of canned fish into Australia and New Zealand were from North America (Nelson, 1989), whose Atlantic waters were the primary source of albacore canned tuna and comprised 1 out of every 4 cans until the 1980's (Miyake et al., 2010). But since the 1990's most of Australia's canned tuna has been imported from Thailand (Australian Fisheries Research and Development Corporation, 2011). Though overall consumption of seafood is fairly low in Australia, canned tuna fish has become increasingly popular and now accounts for over 33.3% of all seafood purchased in Australia's largest cities (Australian Fisheries Research and Development Corporation, 2011). In Melbourne and Sydney, nutrition survey results showed canned tuna was the most purchased seafood item for in home consumption (Australian Fisheries Research and Development Corporation, 2011). A 14-year report of retail sales (from 1985 to 1999) showed about 1/3 of fresh fish markets in Melbourne closed while retail sales of fish in supermarkets (primarily canned) increased by approximately 400% (Fisheries Research and Development Corporation, 2006). An online food industry article,

published in 2003, reported that Australian's had increased their canned tuna fish consumption by almost 60% since 1998, and 13% in just the previous year (2002) (Just-Food, April 10, 2003).

The average per capital consumption of all seafood among Australians was estimated at 12.3 grams per day in the late 1950's, increasing to 15.3 in the late 1960's, 17.5 in the late 1970's, then increasing more substantially to 22.7 in 1988-1989 and 29.6 in 1998-1999 (Australian Institute of Health and Welfare, 2006). Karatela, Paterson, Schluter and Anstiss (2011) describe that in 2007, 45% of New Zealanders ate fish at least once a week.

Summary. Studies of canned tuna fish in the U.S. show consistently higher levels of MeHg than other countries, though with so few studies true comparisons are limited (Berger & Gochfeld, 2004; Gerstenberger et al., 2010; Knowles et al., 2003; Thompson & Lee, 2009). Evidence is consistent that albacore has the highest mean MeHg levels of all canned tuna (Burger & Gochfeld, 2004; Dabeka et al., 2004; Groth, 2012b). Though the mean MeHg of other canned tuna are considerably lower, the range of these other types shows extreme variation. In some cases upper ranges are even higher than albacore (Dabeka et al., 2004). Overall, methylmercury levels found in canned tuna are highly varied and inconsistent (Groth, 2012b; Khansari et al., 2006; Knowles et al., 2003). Without better information about which types of tuna are canned and where they are caught, there is no way to assess expected methylmercury levels of non-albacore canned tuna fish. Given the high degree of variation found in all types of canned tuna fish it is possible that the fairly small number of studies and total number of cans tested for MeHg

mean estimates (used for advisories and research baselines) do not accurately reflect MeHg exposure. Lastly, evidence points to trends that in many countries the MeHg levels of canned tuna are higher than mean levels used for government advisories and research baselines, and that overall populations consume far more methylmercury from canned tuna than estimated (Burger & Gochfeld, 2006; Groth, 2012b; Karimi, Fitzgerald & Fisher, 2012).

Parallels Between Breast Cancer Incidence and Canned Tuna Consumption

What is known about the geographic consumption levels of canned tuna has some historic, social and geographic parallels to female breast cancer prevalence (Bray et al., 2004; Campbell & Owen, 1994; Cancer Research UK, 2013; FAO, 2004b; Globefish, 2004). In the 20th century environmental methylmercury levels were the highest in recorded history and accumulated in the bodies of large tuna fish (Boffetta et al., 1993; Rahimi et al., 2010; WHO, 2008c, p. 29-30). Starting in the 1950s large and long living varieties of tuna fish were canned, and became the most commonly consumed fish item in the United States and Europe, and the primary source of human methylmercury exposure (FOA, 1996; Hamilton et al., 2011; Mongruel et al., 2010; NJDEP, 2010). Starting in the 1950's, the U.S. and Europe experienced significant geographically demarked elevations in breast cancer prevalence (Cancer Research UK, 2011; Yaghoubi & Barlow, 2007).

Since widespread introduction of canned tuna to the marketplace in the 1950's, the U.S. and Europe have been the primary consumers of canned tuna, comprising greater than 60% of the worldwide canned tuna market (FOA, 1996. P8; Hamilton, Lewis,

McCoy, Havice & Campling, 2011; Mongruel et al., 2010). Specifically, it appears the canned tuna with the highest methylmercury levels, albacore, may have been preferred and consumed most often by highest breast cancer incidence area's in the Northeastern U.S., Western and Northern Europe, and possibly Australia/New Zealand (Bray et al., 2004; Campbell & Owen, 1994; Cancer Research UK, 2011; GLOBEFISH, 2004; Groth, 2012b; Jemal et al., 2010; Livsmedelsverket, 2004; Nelson, 1989; EPA, 1997c; USDA, 2012).

Another identified parallel between canned tuna fish consumption and breast cancer incidence is sociodemographic (Daniel et al., 2011; Beiki et al., 2012; EPA, 1997c). Studies have shown that consumption of canned tuna fish is highest for individual Caucasian women of higher income and education. Geographic areas of predominantly high income and high education level Caucasian women are also highest canned tuna fish consumers (Daniel et al., 2011; Beiki et al., 2012; EPA, 1997c).

Mercury, Hormones, and Breast Cancer

Follow-up studies from the Minamata mercury outbreak in Japan found that those with acute leukemia had significantly higher hair mercury content (1.24 ppm) than those without (0.49 ppm) and higher incidence of renal tumors (Crespo-Lopez et al., 2009). These findings led to the first identification of mercury as a potential carcinogen by the IARC and U.S. EPA less than twenty years ago in 1997 (CDC, 2013d; EPA, 2014e).

Exposure to mercury is known to cause many ill health effects, including to the nervous and cardiovascular systems, skin, brain, and organs (EPA, 2014e). It crosses the blood brain barrier, is passed from mother to fetus, to infants through breast milk and is

especially harmful to fetuses, infants, and children (EPA, 2014e). The EPA (2014e) cancer guidelines were last updated in 2005 and using data from a 1997 mercury report, concluded that human studies of mercury exposure and cancer were too limited to ascertain mercury's carcinogenic effects (EPA, 2014e). In 1997 the IARC classified methylmercury as a possible human carcinogen and inorganic and elemental mercury as unclassifiable (CDC, 2013d; WHO, 1997d). However, more recent evidence from population-based, animal, and laboratory research literature increasingly supports human exposure to methylmercury as having a significant and plausible pathway relationship to female breast cancer (Florea & Busselberg, 2011; Settle & Patterson, 1980; Yaghoubi & Barlow, 2007; Voegborlo et al., 1999). In their review of metals and breast cancer, Byrne et al., (2013) describe mercury classification as a probable carcinogen.

The Breast Cancer and Environmental Research Act of 2008 was created to reduce the impact of breast cancer in the U.S. This act established the U.S Interagency Breast Cancer and Environmental Research Coordinating Committee (IBCERCC) (2013). Researchers from the IBCERCC (2013) suggested better identification and understanding of environmental risk factors as vital to future prevention. Although genetic factors are related to breast cancer etiology, the majority of breast cancer cases have no family history of the disease, insinuating that environmental factors are likely primary to breast cancer etiology. Significantly understudied are endocrine disruptors and how environmental exposure effects breast cancer. Recent advances in understanding include timing of environmental exposures during mammary gland development (IBCERCC, 2013).

Hormone, Mercury, and Breast Cancer Research

Exposure to heavy metals, including mercury, has a significant though not well understood relationship to breast cancer (Mohammadi et al., 2014). In studies of metal exposure and breast cancer, researchers have suggested numerous pathways related to how mercury exposure activates and mimics estrogen in the body, resulting in increased risk of breast cancer (Mohammadi et al., 2014). Scholars have found mercury and other heavy metals present in benign and malignant breast tissues at significantly higher concentrations than healthy tissue (Mohammadi et al., 2014). Though exactly how hormones affect breast cancer is not fully understood, estrogen and progesterone are fundamental to breast cancer etiology (Hiatt, Haslam, & Osuch, 2009). For example, Cummings et al. (2009) found an overall increased breast cancer risk of 2.0-2.2 for postmenopausal women with the highest levels of progesterone and estrogen in a review of 15 studies.

In their study of data from the Surveillance, Epidemiology, and End Results Program, St-Hilaire, Mandal, Commendador, Mannel and Derryberry (2011) developed a model to assess environmental exposure by U.S. county with breast cancer incidence and found significant positive association between estrogen positive (ER+) breast cancers and environmental risk factors (including mercury exposure). Though an association between breast cancer and estrogen has been clearly established the cellular response differs based on estrogen receptor status. Often in treatment, ER+ breast cancers are responsive to hormone blockers and estrogen negative ER- breast cancers are not. St-Hilaire et al. (2011) suggest that future breast cancer studies of environmental risk factors (including

mercury exposure) differentiate between estrogen positive (ER+) and negative (ER-) breast cancer types.

Byrne et al. (2013, p. # 68) describe in vitro studies have found that exposure to mercury "induce the proliferation of estrogen dependent breast cancer cells, increase the transcription and expression of estrogen regulated genes, and activate Era in transfection assays supporting the estrogen-like effects of these bivalent cationic metals in vitro." Occupational and population-based studies have found an association between mercury exposure and lung, glioma, stomach, prostate, bladder, and cervix cancers (Byrne et al., 2013). In animal studies mercury exposure has induced cancers of the pancreas, lungs, liver, kidneys, breast and in tissue at the site the mercury was injected. Estimates place the daily average mercury intake at 0.28 ug to 25 ug per person. Byrne et al. (2013) concludes that significant increases of environmental metal exposure in the last 5-6 decades, it's known activation of estrogen in the body and accumulation in breast tissue may increase risk of breast cancer, though better understanding of metals' carcinogenic etiology is needed.

The Endogenous Hormones and Breast Cancer Collaborative Group (2013) was created to evaluate studies of the relationship between sex hormones and risk of female breast cancer. They state, "There are now sufficient data from studies of hormones and breast cancer risk in postmenopausal women to show that risk is positively associated with circulating concentrations of oestrogens and androgens" (p. # 1010). In their analysis of seven studies they found evidence that circulating oestrogens and androgens were also significantly associated with breast cancer in premenopausal women. Although

studies show that oestrogens and breast cancer risk are clearly related, the relationship is not well understood (The Endogenous Hormones and Breast Cancer Collaborative Group, 2013).

In their review of what is currently known about how metals act as endocrine disruptors, Iavicoli et al., (2009) confirm that the primary exposure of mercury in the population is through fish in the form of methylmercury. The authors describe results of some specific animal studies that found mercury exposure altered testosterone and estradiol hormone levels in fish and rats and correlated with dose-related reproductive problems in monkeys (including low birth weight and spontaneous abortion). In humans, Iavicoli et al., (2009) describe that numerous studies have found significant association of elevated mercury blood levels and mercury exposure with elevated estradiol hormone levels. Evidence also exists that mercury exposure may alter thyroid metabolism and sperm development. Iavicoli et al., (2009) express concern that endocrine studies examine exposure to one metal when humans are often exposed to metals simultaneously throughout their lifespan.

Brophy et al. (2012) conducted a large Canadian occupational case-control study of 1,005 breast cancer cases with 1,146 controls and found women working in environments with significant exposure to endocrine disruptors (chemicals that disrupt hormones) had increased risk of breast cancer. Exposures were summarized by occupation and not specified by type (e.g. metal or mercury) (Brophy et al., 2012).

Mercury and Cancer Population-Based Studies

Although methylmercury via fish consumption accounts for approximately 90% of all human mercury exposure (NJDEP, 2010) and the EPA and IARC have recommended further study of mercury and cancer (Crespo-Lopez et al., 2009), the few population-based studies that have examined this association have focused on occupational settings with mercury chloride (not methylmercury) as the primary mercury exposure (WHO, 1997d).

Studies prior to the year 2000. One of the earlier studies of mercury exposure and cancer, published in 1990 by Barregard, Sallsten and Jarvholm, tested mercury levels in chloralkali workers at intervals between 1946 and 1984 and monitored adverse health outcomes. Of these 1,190 male workers from eight chloralkali plants in Sweden, about ¼ had biological mercury levels exceeding the human average by 1000 years. Approximately half of the participants were also exposed to asbestos, a significant study confounder that may explain findings of OR 2.0 (95% CI 1.0- 3.8) for the association between mercury exposure and lung cancer. Barregard et al., (1990) found a higher incidence of kidney (OR 1.6, 95% CI, 0.3-4.7) and brain tumors (OR 2.7, 95% CI 0.5-7.7) then were expected, but small sample size did not provide enough power to confidently measure these associations. Ellingsen, Thomassen, Langard and Kjuus, (1993) conducted a cancer mortality study of 674 chloralkali plant workers in Norway and found a small increase in lung cancer mortality compared to the country's mean (OR 1.66, 95% CI 1.00-2.59) which could be explained by smoking and asbestos exposure.

In their study's literature review, published in 1996, Kinjo et al. describe that autopsies in Japan have shown increased rates of prostate and thyroid cancer in methylmercury polluted areas and that male mice contracted kidney cancer in experimental mercury studies. Kinjo et al. (1996) conducted a historical nested cohort study from 1970/1971 to 1981/1984 of 1,351 coastal Japanese residents with mercury poisoning disease (Minamata disease) and compared their cancer mortality and fish consumption with over 5,500 residents as controls. The most significant finding was increased leukemia mortality (RR 8.35, 95% CI 1.61-43.3), which the authors anticipate is caused by an unknown variable unrelated to mercury exposure. Overall, no significant increase in cancer incidence or mortality was found between participants and controls though persons with Minamata disease showed a small decrease in stomach cancer mortality (RR 0.49, 95% CI 0.26-0.94) (Kinjo et al., 1996). One weakness of the study is that participants and controls all came from the same coastal geographic areas in one of the highest seafood consumption regions of the world. Though controls suffered a disease related to mercury exposure, whether a significant difference in lifetime mercury exposure levels between participants and controls existed remains unclear (Kinjo et al., 1996).

Boffetta et al. (1998) conducted a cohort study of 6,784 male and 265 female mercury workers from four mill/mine sites in the Ukraine, Italy, Spain and Slovenia and compared mortality to each countries mean rates. Mercury exposure information was estimated based on occupation category and duration, as well as biological testing on collected specimens at three of the four sites. Workers in the four plants were followed

for mercury exposure and cancer mortality from 1950 to 1995. Exposure confounders of radon and crystalline silica at mercury mining sites may explain findings of increased lung cancer mortality (standardized mortality ratio (SMR) 1.19, 95% CI 1.03-1.38). Increased mortality was also found for liver cancer (SMR 1.64, 95% CI 1.18-2.22). Bofetta et al. (1998) describes that three deaths from ovarian cancer of female workers at one mine in the Ukraine significantly exceeds expected incidence, though no statistical description of this relationship is provided. Bofetta et al. (1998) concludes that these study results provide evidence that mercury is a likely cause of cancer, and suggests additional research of ovarian and liver cancers and mercury exposure.

A study of 1,146 Tuscany, Italy fur hat workers who suffered and were compensated for occupational mercury intoxication were studied from 1950 to 1992 for mortality by Merler et al. (1994). The largest study of female occupational mercury exposure to date, over 70% or 820 of the hat workers were women. After controlling for smoking, results showed significant increased lung cancer mortality among female hat workers (SMR 2.10, 95% CI 1.05-3.76), which Merler et al. (1994) explained could be related to an unknown confounder. Significant mortality from stomach cancer was found in males (SMR 1.83, 95% CI 1.05-2.98) and females (2.14, 95% CI 1.34-3.25), which may be explained by elevated stomach cancer rates in the Tuscany region. Merler et al. (1994) found no association for breast cancer mortality and did not provide statistical outcomes of this finding. Merler et al. (1994) concludes that the primary findings of this research was higher then expected overall cancer mortality and elevated lung cancer

rates, especially among women who did not smoke, unrelated to known confounders or study bias.

Studies after the year 2000. Zadnick and Pompe-Kirn (2007) studied cancer incidence in a heavy mercury mining area in Idrija, Slovenia. The area is deemed a mercury pollution area due to 500 years of mercury milling and mining. Slovenia cancer registry incidence and mortality from 1961 to 2000 were evaluated by residence in polluted (568 male and 598 female cases with cancer) and non-polluted (571 male and 490 female controls with cancer) surrounding area's, as well as among 1,589 area mercury workers from 1950 to 2000. Among women, significant increased incidence of all cancers was found in mercury-polluted areas (standardized incidence ratio (SIR) 1.34, 95% CI 1.24-1.45), including cancer of the gallbladder (SIR 1.74, 95% CI 1.03-2.75) and cancer of the breast (SIR 1.27, 95% CI 1.06-1.25). Among men, significant increased incidence of all cancers was found in mercury-polluted areas (SIR 1.29, 95% CI 1.18-1.40), including lung cancer (SIR 1.29, 95% CI 1.10-1.52) and bladder cancer (SIR 1.50, 95% CI 1.01-2.14). Miners had significantly higher incidence of lung cancer (SIR 1.36, 95% CI 1.03-1.77) and kidney cancer (SIR 3.23, 95% CI 1.18-7.02) though Zadnick and Pompe-Kirn (2007) describe study weaknesses (e.g. non-miners confounders ignored) and confounders (miner's have high rates of obesity, smoking and alcohol consumption) that weaken findings. Numerous other cancers were found to have higher then expected incidence but did not show a statistically significant relationship. Zadnick and Pompe-Kirn (2007) focus their conclusions on the need for health promotion programs for this highly exposed area population.

In their case-control study of the occupational exposures of 793 Swedish persons with a benign brain tumor called acoustic neuroma and over 100,000 controls, Prochazka et al. (2010) found that occupational exposure to mercury ten years prior to diagnosis was significantly associated with increased risk of tumor development (OR 2.9, 95% CI 1.2-6.8).

The cancer mortality of Japanese residents, from 1988 to 1997, living in a highly mercury polluted coastal area were compared to residents of surrounding areas, to follow-up on previous studies of this cohort of over 90,000 cases and 150,000 controls followed since 1960 (Yorifuji et al. 2007). Yorifuji et al. (2007) findings reflected previous studies where mercury exposure showed a decrease in gastric cancer mortality and increased mortality by leukemia (SMR 2.00, 95% CI 1.69-2.37). Though a plausible pathway for mercury exposure and leukemia has not been researched, Yorifuji et al. (2007) suggest consistent findings of elevated leukemia mortality and mercury exposure should be investigated further in this region on an individual level.

In their effort to better understand the role of mining and industry releases and the high incidence of bladder cancer in Spain, Lopez-Abente et al. (2006) applied a Bayesian spatial model to map the entire country. The authors found that area's with heavy mining, industry, and heavy fish and shellfish consumption had the highest incidence of bladder cancer. However, Lopez-Abente et al. (2006) warn that spatial analysis of bladder cancer was very similar to lung cancer, which may reflect cigarette-smoking rates instead of environmental exposure. Gomez et al. (2007) studied the cancer mortality of over 3,000 Spanish mercury miners followed from 1895 to 1994 and found elevated rates

of liver cancer mortality (20 deaths and 17 were expected). Overall cancer mortality, mostly from reduced bladder and colon cancer rates, was lower than expected. A detailed analysis of Gomez et al.'s (2007) research is not available because the full publication is only available in Spanish.

Fish, Canned Tuna, and Breast Cancer

Romeiu (2011) described that diet was an important consideration in understanding geographic differences in breast cancer incidence. Processes involved with the etiology of breast cancer, for example hormone or inflammation, can be triggered by dietary variables. The regional differences in breast cancer incidence may be related to differences in consumption of foods involved with breast cancer etiology processes (Romeiu, 2011).

The most common source of human mercury exposure, canned tuna fish, is typically absent or poorly delineated in the current body of fish and breast cancer research (Florea & Busselberg, 2011; McElroy et al., 2004; Yaghoubi & Barlow, 2007; Zadnick & Poompe-Kirn, 2007). Holmes, James and Levy (2009) describe the body of mercury exposure and health outcome research as limited and conclude that the evidence suggests that populations exposed to low levels of methylmercury via seafood are at highest risk of adverse health outcomes.

Current research of fish consumption variables and breast cancer regularly combine fish by type, preparation method, or group into levels based on fat content (Engeset et al., 2006; EPIC, 2012a). It is extremely rare for nutritional surveys, registries, or research studies on cancer to specify canned tuna in questions related to fish

(Engeset et al., 2006; EPIC, 2012a). Overall, findings of association between fish consumption and breast cancer are highly varied, and include evidence of both a positive and protective relationship (Engeset et al., 2006; Franceschi et al., 2006; Kim et al., 2009; McElroy et al., 2004; Stripp et al., 2003; Terry et al., 2003; Vatten et al., 2006; Yuan, Wang et al., 1995).

Florea and Busselberg (2011, p. # 1) describe that current evidence shows both nutrition and environmental exposures have "a decisive role in breast carcinogenesis". In more developed countries sex hormone related cancers (breast and prostate) occur at significantly higher rates than less developed countries (Sala-Vila & Calder, 2011). However, as less developed countries increasingly participate in the developed world economy, taking on similar lifestyle practices including diet, their rates of sex hormone related cancers increase, paralleling those of developed countries. After smoking, diet is the considered the most important modifiable factor to prevent breast cancer. Though it is clear that diet has a significant relationship to breast cancer etiology, fish consumption's association to breast cancer in epidemiological studies is highly varied (Sala-Vila & Calder, 2011).

Cottet et al. (2009) studied and followed 2,381 postmenopausal women with breast cancer in France from 1993 to 2005 to examine dietary factors. The authors found an increased risk of breast cancer (hazard ratio = 1.20, 95% CI: 1.03, 1.38, p= 0.007 for linear trend) for women who ate a western dietary pattern that included canned fish, starches, deserts and fatty condiments. When comparing women eating this western dietary pattern (that included canned fish) between the highest and lowest quartiles, a

statistically significant association was found for ER+ positive tumors and not ER-negative (hazard ratio= 1.33, 95% CI: 1.07, 1.65, p= 0.0005 for linear trend) (Cottet et al., 2009).

Terry et al., (2003) reviewed 7 prospective cohort and 19 case control studies of fish intake and hormone-related cancers for evidence of a protective carcinogenic relationship of marine fatty acids. Results were mixed. The authors found commonly cited case-control study results showing a protective role for marine fatty acids in fish and cancer studies were weakened by participation bias and recall bias. Though cohort studies findings help mitigate the bias of case-control design, they are vulnerable to other weaknesses resulting from diet misclassifications over or under weighted as exposures (Terry et al., 2003).

Though the American Cancer Society has for years advised for people to eat more fish to decrease cancer risk, Daniel et al., (2011) describe a dearth of research of fish's relationship to cancer. They describe that the American Institute for Cancer and World Cancer Research Fund deemed current evidence as too limited and inadequate to make conclusions about fish and poultry's relationship to cancer. The anticarcinogenic and anti-inflammatory properties of long-chain n-3 fatty acids found in fish are thought beneficial to cancer prevention. However, studies do not account for potential confounding of the most commonly eaten seafood in the U.S., canned tuna, due to possible carcinogenic effects of mercury (Daniel et al., 2011).

In what they describe as "the largest U.S. prospective investigation of white meat and cancer risk to date", Daniel et al. (2011, p. # 1910) studied the diet questionnaires of

74,418 people with cancer followed for nine years. The diet questionnaire grouped fish consumed as total fish (including canned tuna) with some differentiation of canned tuna. For those that reported canned tuna consumption it accounted for 1/3 of total fish eaten. Melanoma was found associated to total fish intake, mostly due to consumption of canned tuna [HR and 95% for fifth versus first quintile, 1.2 (1.16-1.46), $p < .0001$). Canned tuna consumption also showed increased risk of bladder cancer [1.13 (0.99-1.28) p trend = 0.04] and ovarian cancer [1.28 (1.02-1.61), p trend = 0.05]. Overall findings for fish intake and cancer were mixed with little evidence of a preventative relationship to cancer. Daniel et al. (2011) describes this as consistent with previous studies and surprising due to the expected protective roll of long-chain n-3 fatty acids (Daniel, 2011). Ovarian cancer is also a hormone related cancer and higher levels of estrogen increase risk (Yokoyama & Mizunuma, 2013). Daniel et al. (2011) describes that poor consumption specificity and lack of risk factor adjustment may explain these findings via unidentified confounders. Participants who ate canned tuna were more likely to have graduated college, exercise more, and smoke less often than the baseline of participants (Daniel et al., 2011).

In their literature review of the body of knowledge on the relationship between breast, colorectal, and prostate cancers and fish consumption, Sala-Vila and Calder (2011) reviewed 106 studies. Studies were excluded if they reported only intake of fish oils or n-3 fatty acids or if their study design excluded measures of effect. Overall, 53 out of 273 measured associations found a decreased risk of cancer from fish consumption and 12 out of 273 found increased risk. Twelve prospective cohort and one nested case-

control study specific to breast cancer ranged in year from 1987 to 2006 and included the U.S., Norway, Japan, Singapore, Denmark, and ten European countries. Exposures were grouped primarily as total fish, but also poached, dried, lean, fatty, salmon, and fish plus shellfish. Sala-Vila and Calder (2011) found that the majority of studies found no association or increased risk of breast cancer when type of fish, disease stage, and pre or post menopause variables were not specified. The authors also reviewed studies by design. Sala-Vila and Calder (2011) reviewed twenty-two case-control studies of fish intake and breast cancer. They ranged in year from 1981 to 2003 and included populations in China, Finland, U.S., Italy, Uruguay, Spain, Japan, Switzerland, Singapore, Russia, Canada and Argentina. Fish exposures were measured most often as total fish, but also total seafood, lean, fatty, freshwater, marine, preserved, boiled, broiled, cooked, and dried. Findings related to specific exposures (total fish, salted, etc.) and outcomes (breast cancer, premenopause, postmenopause etc.) were inconsistent with the majority showing no association, one to two showing decreased risk and a one to two showing increased risk (Sala-Vila & Calder, 2011).

Sala-Vila and Calder (2011) propose numerous reasons for the high variability and likely confounding in findings of fish consumption and breast, prostate, and colorectal cancer research. The preponderance of exposures measured as “total fish intake” greatly limits and understates the differences in fish and their plausible carcinogenic or protective pathways. For example, the fatty acids n-3 LC-PUFA’s have been found to protect against inflammation, and are therefore hypothesized to also protect against cancer. These fatty acids are found in vastly different amounts depending on the

type of fish and how much of each specific fish is consumed. Another concern is a lack of sensitivity in questions about how frequently fish is consumed. For example, many studies designate fish consumption by eating more than once a week compared to hardly ever/never eat. Fatty acids are found to be protective against inflammation, depending on the type of fish, at much greater frequency than once a week. Sensitivity to this frequency is not captured in current studies. Sala-Vila and Calder (2011) conclude that the body of epidemiological literature does not support fish consumption as having a protective effect against the risk of breast cancer and suggest more specific measures are needed for type of fish, cooking method, and frequency as well as cancer stage and menopausal status.

In an older literature review, published in 2003, of the body of epidemiological studies of breast, colorectal, and prostate cancer and fish consumption Hjartaker summarized results as highly varied and pointing to no association or an inverse relationship. Although most studies published since the 1960's suggest fish may be associated with increased incidence and mortality from breast cancer, Hjartaker (2003) found differentiation based on location. Most case-control studies finding a significant association were of populations in North America and southern Europe and those finding no association were primarily among populations in the Far East. Overall cohort studies found no association between fish consumption and breast cancer. Hjartaker (2003) described numerous methodical weaknesses including: findings of a protective relationship of fish to cancer could be explained by reduction in less healthy meats (e.g. red meat), almost all studies combined fish under one or two variables instead of specifying by possible carcinogenic factors (e.g. fat, preparation, mercury content, etc.),

some studies include chicken and fish together, and most studies do not measure the actual amounts consumed or frequency.

Romieu (2011) identifies methodological weaknesses of the current body of fish and cancer literature. The variety of diet geographically may not allow for comparison and nutrition questionnaires are vulnerable to recall bias and underestimation. Current methods do not capture the interaction of foods with each other or genetic mechanisms. Also, the timing of questions may be vital to capture important developmental stages (e.g. exposure windows) for breast cancer (Romieu, 2011).

There appears to be evidence of significant confounding in fish and breast cancer research which could be related to lack of differentiation of the canned tuna variable and lack of specification of types and mercury levels of canned tuna (Daniel et al., 2011; Hjartaker, 2003; Karimi, Fitzgerald & Fisher, 2012; Sala-Vila & Calder, 2011).

Conclusions

Conclusions from the literature review to be applied in this study include:

- Methylmercury is classified as a probable human carcinogen (Byrne et al., 2013).
- There is significant evidence of relationship and plausible pathway of methylmercury exposure (as an estrogen activator) to breast cancer etiology (Byrne et al., 2013; Florea & Busselberg, 2001; Settle & Patterson, 1980; Sukocheva, Yang, Gierthy & Seegal, 2005; Yaghoubi & Barlow, 2007; Voegborlo et al., 1999).

- Canned tuna consumption accounts for the single largest source of methylmercury exposure in humans (Ashraf, 2006; Boadi et al., 2011; Burger & Gochfeld, 2004; Burger et al., 2005; Carrington & Bolger, 2002; Dabeka et al., 2004; FDA, 2002a; Globefish Research Program, 2004; Groth, 2010a; Hightower & Moore, 2003; Khansari et al., 2006; Ikem & Egiebor, 2005; Jaffry & Brown, 2008; Laxe & Gamallo, 2008; Moon et al., 2011; Rahimi et al., 2010; EPA, 1997c; Voegborlo et al., 1999; Xue et al., 2012).
- Methylmercury levels in canned tuna fish are highly varied and often higher than means suggested by government agencies (Gerstenberger et al., 2010; Groth, 2012b).
- Canned tuna consumption and breast cancer prevalence share some geographic, historic, racial/ethnic, educational, and socioeconomic parallels (Beiki et al., 2012; Bray et al., 2004; Campbell & Owen, 1994; Cancer Research UK, 2013; Daniel et al., 2011; FAO, 2004b; Globefish, 2004).
- Current epidemiological studies of fish and breast cancer consistently group canned tuna fish with other fish variables (Sala-Vila & Calder, 2011).
- The body of epidemiological literature of fish (including canned tuna) and breast cancer show highly varied results, sometimes supporting a

protective role, sometimes showing evidence of increased risk relationship, or reflecting no association (Sala-Vila & Calder, 2011).

- Lack of specification and delineation of the canned tuna variable may explain some of the apparent confounding in fish and breast cancer research (Hjartaker, 2003; Karimi et al., 2012; Sala-Vila & Calder, 2011).
- Though canned tuna is the single most consumed fish in the world, it's cumulative role as a source of methylmercury exposure is overlooked and it's relationship to breast cancer understudied (Florea & Busselberg, 2011; Gerstenberger et al., 2010; IBCERCC, 2013).

Summary

A substantial body of evidence supports the probable and plausible role of methylmercury exposure via canned tuna in breast cancer etiology (Brophy et al., 2012; Byrne et al., 2013; Iavicoli, et al., 2009). Though current evidence points to increased risk of breast cancer as a result of increased estrogen exposure via the metalloestrogen methylmercury, the mechanisms involved in this relationship remains unclear and association understudied (Byrne et al., 2013). The historic, social, and geographic parallels between canned tuna consumption and high breast cancer incidence regions are exploratory and suggestive. More in-depth data and evidence is needed to confirm if these parallels are meaningful. The exposure of methylmercury to populations via consumption of canned tuna fish is clearly underrepresented, underestimated, and under prioritized in current literature (Ashraf, 2006; Boadi et al., 2011; Burger & Gochfeld, 2004; Jaffry & Brown, 2008; NJDEP, 2010). The research undertaken in this dissertation

can make meaningful contributions to better understanding canned tuna's role as a vehicle of methylmercury exposure.

There is evidence of significant confounding in fish and breast cancer research (Daniel et al, 2011; Hjartaker, 2003; Sala-Vila & Calder, 2011). It appears that lack of individuation of the canned tuna variable (including specification of canned tuna types by methylmercury levels) due to it's potential role as a metalloestrogen is a fairly new and unexamined aspect of fish and breast cancer research (Daniel et al., 2011; Hjartaker, 2003; Karimi, et al., 2012; Sala-Vila & Calder, 2011). The research undertaken in this dissertation contributes to current understanding of canned tuna as a potential confounding variable. The specification of the canned tuna fish variable and risk of breast cancer in this study contributes to the methylmercury, metalloestrogen, environmental and fish and breast cancer bodies of research, all of which are in need of additional study. In Chapter 3, the methodology of the study will be covered.

Chapter 3: Research Method

Introduction

The purpose of this population-based, case control study was to measure methylmercury exposure via canned tuna fish consumption and to examine its association to breast cancer in the 2004-2004 and 2005-2006 NHANES. This study had two research questions: one on the association between canned tuna fish consumption frequency and breast cancer and another between total blood mercury levels, used as a proxy for methylmercury level, and breast cancer. A secondary goal was to evaluate the descriptive statistics of the social determinants of canned tuna fish consumption. In this chapter, I will present the research design and rationale, methodology (including population, recruitment, sampling, questionnaire validity, data access procedures, and data analysis), threats to validity, ethical considerations, and a summary.

Research Design and Rationale

A population-based, case control study design was applied to secondary data from the NHANES 2003-2006 survey. The primary study purpose of exploring the association between canned tuna consumption, total blood mercury levels, and breast cancer was based on carcinogenic theory, which describes estrogen as primary to breast cell development and postulates that cumulative exposure to estrogen increases breast cancer risk (Henderson et al., 1988). Canned tuna fish is the primary route of methlymercury exposure in humans (Gerstenberger et al., 2010). Methlymercury accumulates in the body over time and activates estrogen as a metalloestrogen (Byrne et al., 2013; Georescu, Georgescu, Daraban, Bouaru, & Pascalau, 2011). The dependent variable was breast

cancer diagnosis or lack of diagnosis. The independent variables were the frequency of canned tuna fish consumption reported in the food frequency questionnaire and total blood mercury levels used as a proxy for MeHg (Sheehan et al., 2014).

Covariate factors designated as having adequate evidence of confounding and were available in the NHANES 2003-2006 dataset were examined and included race/ethnicity, annual household income, education level, age of menarche, hormone therapy, obesity, age, alcohol consumption, age when breast cancer first diagnosed, age at first full-term pregnancy, parity, breast feeding, smoking cigarettes, personal history of cancer, early age at menopause, and diabetes (National Cancer Institute 2014a; NHANES, 2014c). As described in Chapter 4, some covariates were inappropriate for inclusion in the final model due to question design or collinearity.

The secondary study purpose was to evaluate the descriptive frequencies of the social determinants of canned tuna fish consumption. This was based on the theory of medical geography, which is used to describe aspects of the cultural, social, and geographic environment to understand spatial differences and etiology of disease (Paul, 1985). The goal was to examine descriptive statistics of the social determinants of canned tuna fish consumption and to assess if they parallel those known of breast cancer. The dependent variable was frequency of canned tuna fish consumption reported in the food frequency questionnaire. Race/ethnicity, age, education level, and annual household income were used as independent variables.

Case control studies are routinely used in epidemiological research on the association between diet and breast cancer and environmental risk factors and breast

cancer (Brennan, Cantwell, Cardwell, Velentzis, & Woodside; Cancer Research UK, 2014; Hopper et al., 2005). Because breast cancer is a disease with long latency periods between exposure and diagnosis, identification of risk factors are often assessed retrospectively after a person is diagnosed (Song & Chung, 2010). Population-based case control analysis is inherent to the NHANES survey, which is designed to increase understanding of the nutrition and health of the nonnstitutionalized U.S. population (Hopper et al., 2005; NHANES, 2014c).

Though some breast cancer registries and studies include canned tuna fish in nutritional questionnaires, the association between methylmercury exposure via canned tuna fish consumption and breast cancer remains largely absent from consideration in the literature (Florea & Busselberg, 2011; Gerstenberger et al., 2010; IBCERCC, 2013; Sala-Vila & Calder, 2011). In exploring questions related to this premise, four secondary data sets that individuated the canned tuna fish variable and included women with breast cancer were considered. A full application process was completed and submitted to the Breast Cancer Family Registry; I was denied due to a lack of an available and interested research collaborator (E., John, personal communication, August 17, 2015). A submission of this study's rationale to the Multiethnic Cohort study for secondary data resulted in no response. Data from the European Prospective Investigation into Cancer and Nutrition (EPIC, 2015b) was also considered. However, communication from an EPIC researcher discouraged further pursuit, describing the process of obtaining the data as lengthy, difficult, and not possible (G., Nicolas, personal communication May 24, 2012). Finally, NHANES was identified as a viable option for reasons described below.

In the survey periods of 2003-2004 and 2005-2006, NHANES individuated canned tuna fish and women with breast cancer (NHANES, 2014c). The NHANES years of 2003-2006 also included laboratory testing results of total blood mercury (which include both MeHg and inorganic mercury). Starting in the survey period of 2011-2012 with data up to 2013-2014, NHANES laboratory results specified blood MeHg levels, but did not individuate canned tuna fish. Because the premise of this study was of canned tuna fish as the primary vehicle of MeHg exposure, use of the canned tuna fish variable during the survey years of 2003-2006 was deemed to be more applicable to the primary research relationship than blood methylmercury level absent canned tuna fish during the survey years of 2011-2014. Total blood mercury is more representative of recent and acute mercury exposure but has been validated as a MeHg biomarker (Sheehan et al., 2014) and can be used as a proxy for MeHg during the survey years 2003-2006.

For the secondary research purpose related to medical geography, comparing canned tuna fish consumption by a geographic indicator (e.g., country of birth) was considered. However, concerns included the generalizability of lifetime canned tuna consumption to a geographic area for reasons related to migration and diet representativeness. Instead of comparing canned tuna consumption to compare geographic locations, medical geography was examined by looking more in depth at the social determinants of participants.

Methodology

The target population for this study was adult women, representative of the U.S. population, with and without breast cancer who completed the NHANES survey from

2003 to 2006. It was anticipated that raw data from approximately 170 women with breast cancer and 10,000 women without breast cancer were available from the 2003-2006 NHANES survey, though application of NHANES weights increased these numbers significantly (NHANES, 2008a; NHANES, 2008b).

Population, Sampling, Recruitment, and Data Collection

NHANES was established in the 1960s to survey and collect nutrition and health data representative of the population of the United States (NHANES, 2014c). Annually since 1999 about 5,000 people throughout the country have been sampled to ensure they are representative of the population. The sampling design used is described by NHANES as a "complex, multistage, probability sampling design" (CDC, 2013i, p. #1). First, a complex method is used to identify sample levels based on minority groups and geographic areas to define primary sampling units. Then these units are further segregated into local neighborhoods. Within each neighborhood, homes are chosen randomly, being careful to properly over or under sample to maintain representativeness. Lastly, individual participants within households are randomly selected within sociodemographic categories (CDC, 2013i).

NHANES participants complete most survey questions in person via in-home interviews by trained NHANES staff (CDC, 2013i). More personal questions are answered by in-person interview and private computer-assisted interviews at mobile examination centers. Health evaluations were also conducted at mobile examination centers. Questions for participants included demographics, medical conditions, and food/nutrition (CDC, 2013f). A high degree of consistency and rigor in executing all

aspects of data collection is well documented and integrated into staff training. All data are collected in software programs on laptops (for the in-home interviews) or by computer (CDC, 2013f)

To determine statistical power, Research Questions 1 and 2 were used to search and identify a total of raw data for 170 breast cancer cases and 10,250 available controls in the NHANES 2003-2006 survey period. For the purpose of this study, all cases and controls were included. This provided a raw proportion of approximately 1 case to 60 controls. The G Power 3.1 program was used to estimate the power of 170 raw cases using multiple regression analysis (Faul, Erdefelder, Buchner, & Lang, 2009). For a two-sided test and an alpha of 0.05 in a sample size of 170, 80% statistical power was able to detect as significant an odds ratio of 1.45 or greater. The application of NHANES weights significantly increased statistical power.

Procedure for Data Access

NHANES is a publically available data set available for download for the purposes of health analysis (CDC, 2012b). Any other use of the data or efforts to identify any participants from the data is prohibited and against the law (CDC, 2012b). Access to the data for the purposes of this study was available and the Walden IRB approval # 07-16-15-0058453 was secured.

Questionnaires

NHANES food frequency questionnaire (FFQ) was first used by NHANES in 2003-2004 and adapted by the National Cancer Institute from their validated Diet History Questionnaire (NHANES, 2008a). The Diet History Questionnaire was first validated in

1992 and was then improved and validated again in 1997-1998 and 2001 (National Cancer Institute, 2014d). The Diet History Questionnaire was created to measure the frequency of food items consumed by adults and was adjusted throughout the 1990s to incorporate children. It was developed based on active research of each item's validity, questionnaire usability, and was compared with other surveys (National Health and Nutrition Examination Survey). For other questionnaire portions of the NHANES 2003-2006 survey, thorough testing and quality assurance methods were conducted before, during, and after use to increase consistency, accuracy, and execution competence (CDC, 2013f).

Research Questions and Operationalization of Variables

To examine the association between canned tuna fish consumption, total blood mercury level, and breast cancer incidence in the NHANES 2003-2006 survey and to examine social determinants of canned tuna consumption, the following Research Questions and hypotheses were used.

1. Is there a relationship between consumption of canned tuna fish and breast cancer?

$H_0$1: There is no relationship between consumption of canned tuna fish and breast cancer.

$H_1$1: There is a relationship between consumption of canned tuna fish and breast cancer.

2. Is there a relationship between total blood mercury level and breast cancer?

$H_0$2: There is no relationship between total blood mercury level and breast cancer.

$H_1$2: There is a relationship between total blood mercury level and breast cancer.

3. What is the frequency of women's canned tuna fish consumption for different age groups, race/ethnicities, annual household income, and education level?

(This question was addressed with descriptive statistics [i.e., no specific hypotheses were tested]).

The variables used to answer the above research questions were operationalized prior to analysis. The final operationalization of variables is reflected in Table 14. Variable Transformation Table in Chapter 4.

Unfortunately, the primary risk factors BRCA gene and family history of breast cancer were not captured in the NHANES survey (NHANES, 2014c). Additional risk factors of breast density, radiation exposure, and working shifts at night were not measured by NHANES and were unavailable as covariates (Cancer Research UK, 2015; NHANES, 2014c). There is mixed evidence that consumption of dietary fat increases breast cancer risk (Cancer Research UK, 2015; UCSF, 2015). Measuring dietary fat requires a large number of nutritional items. It was not included because its complexity is beyond the scope of this study's analysis and was unlikely to be a significant confounder. Zhang et al. (2013) found that total dietary energy intake was associated with increased risk of breast cancer; however, more studies are needed to confirm this as a risk (Cancer Research UK, 2015). The complexity of measuring total energy intake was beyond the scope of this study, is not yet established in research, and was not included in analysis.

As described in Chapter 4, some covariates were inappropriate for inclusion in the final model due to question design or collinearity.

Data Analysis Plan

For data analysis, the SPSS 21 statistical software was used. Data were cleaned and recoded as recommended by NHANES (CDC, 2013e). NHANES recommended first identifying missing values, checking for patterns, assessing outliers and distributions, and then recoding of variables (CDC, 2013e). Weight adjustments for each variable, as suggested by NHANES, were fully incorporated into the analysis. An unmatched full logistic regression model, including all cases and controls, was used. The NHANES complex sampling procedures, multiple probability levels, and application of weights resulted in significantly different representative values in the final data set. The fairly small raw sample size of approximately 170 cases was compared to a large sample of 10,000+ controls. Matching at a rate of 1:3 or 1:4 would have caused a significant decrease in the number of controls and power to address research questions and control for covariates. For expediency, clarity, and to maintain as many accurate population-based values as possible, matching was not used.

First, univariate analysis was used to examine and describe variables in the dataset to ensure model fit. For Research Question 1, the relationship between canned tuna consumption and breast cancer was analyzed using a logistic regression model to test the binary outcome of breast cancer *yes/no* while controlling for known covariates. In developing the logistic regression model, two-tailed bivariate analysis was applied to examine relationships between breast cancer and covariates. All appropriate covariates

were included in the multivariable logistic regression model to ensure as many clinically relevant risk factors were controlled for as possible. The Hosmer-Lemeshow statistic was used to compare the expected and observed probability to test for goodness of fit (Hosmer & Lemeshow, 1980). Results for Research Question 1 were interpreted using odds ratios with corresponding 95% confidence interval limits, and *p* value of significance at $p<0.05$.

For Research Question 2 the relationship between total blood mercury level and breast cancer was analyzed using a logistic regression model to test the binary outcome of breast cancer while controlling for known covariates. In developing the logistic regression model, a two-tailed bivariate correlation was applied to examine relationships between mercury level and covariates. All appropriate covariates were included in the multivariable logistic model to ensure as many clinically relevant risk factors were controlled for as possible. The Hosmer-Lemeshow statistic was used to compare the expected and observed probability to test for goodness of fit (Hosmer & Lemeshow, 1980). Results for Research Question 2 were interpreted using Odds Ratios with corresponding 95% confidence interval limits, and *p* value of significance at $p<0.05$.

The covariates examined in Research Questions 1 and 2 included race/ethnicity, annual household income, education level, age of menarche, hormone therapy, obesity, age, alcohol consumption, age when breast cancer first diagnosed, age at first full term pregnancy, parity, breast-feeding, smoking cigarettes, personal history of cancer, early age at menopause and diabetes. (National Cancer Institute, 2014a; NHANES, 2014c). For both Research Questions 1 and 2 the pairwise relationships between covariates were

monitored for potential signs of multicollinearity. As described in chapter 4 some covariates were inappropriate for inclusion in the final model due to question design or collinearity.

Research Question 3 was analyzed using descriptive statistics examining social determinants and canned tuna fish consumption trends. Null and alternative hypotheses and hypothesis testing were not used. Frequencies for the social determinant variables of age, education level, annual household income, and race/ethnicity were examined with canned tuna consumption levels.

Threats to Validity

Threats to the content validity of measuring methylmercury exposure via canned tuna fish consumption were probably the most significant in this proposed study. Because the amount of methylmercury found in canned tuna are highly variable and not specifically measured in this sample and assumed lifetime methylmercury exposure via canned tuna fish limited to reported frequency in a long questionnaire, a considerable amount of methylmercury and metalloestrogen attributes are assumed or unaccounted for in Research Question 1. However, it is clear that canned tuna is the largest single source of methylmercury exposure in humans (Rahimi et al., 2010). The assumption that methylmercury exposure increases by consuming canned tuna fish more often is supported by this studies literature review findings and the U.S. FDA and EPA (U.S. FDA, 2014c; EPA, 2013d). Strong evidence of a plausible causal relationship between methylmercury exposure and breast cancer also exists (Florea & Busselberg, 2011), all supporting the face validity of this understudied association. Results from the

measurement of total blood mercury level in Research Question 2 provide insight. If results of association from the general category of canned tuna frequency found parallel evidence of association based on total blood mercury level, this would have implied that greater sensitivity (specific MeHg levels in canned tuna) was not warranted to address Research Question 1 and canned tuna fish consumption can represent MeHg exposure. If evidence of association was found between total blood mercury level and breast cancer but not canned tuna fish frequency and breast cancer, this would have implied that greater sensitivity in MeHg levels in canned tuna was warranted to accurately measure exposures and generally defined canned tuna fish does not accurately reflect MeHg exposure levels. If canned tuna fish was found to have significant association to breast cancer but not blood mercury level and breast cancer, this would have implied that canned tuna fish is a better representative of long term MeHg levels than blood mercury level.

The construct validity concerning whether the results can be inferred to the study purpose within the theoretical constructs is twofold. Within the framework of medical geography investigation is new and speculative. Results from Research Question 3 were limited to suggesting further investigation of canned tuna consumption from a medical geographic perspective.

Positive findings from Research Questions 1 and 2 regarding methylmercury exposure via canned tuna consumption based on carcinogenic theory have stronger inference due to a plausible causal relationship. Given the impact of breast cancer and efforts to reduce incidence, evidence supporting carcinogenic theory, and body of current exploratory research, significant and consistent positive results from Research Questions

1 and/or 2 could infer a convincing case that MeHg be taken much more seriously as an estrogen related risk factor in future breast cancer research. If total blood mercury and not canned tuna fish showed evidence of relationship this would provide support for short term or acute MeHg exposure as a breast cancer risk factor understood through carcinogenic theory and to represent problems with the construct validity of canned tuna fish as representative of MeHg exposure. If canned tuna fish showed evidence of relationship to breast cancer absent findings for total blood mercury, this could have represented problems with the construct validity of blood mercury level (which is known to be more representative of short term or acute MeHg exposure) (Sheehan et al., 2014) and point to a possible role for canned tuna fish as an effective proxy for chronic and/or low level MeHg exposure.

Additional anticipated threats to validity included being unable to control for the covariates of BRCA gene and family history of breast cancer. Possible minor threats included being unable to control for the covariates of breast density, working shifts at night, dietary fat, exercise, unknown confounders, and memory and survey fatigue in completing the Food Frequency Questionnaire. It was also possible that women who were diagnosed with breast cancer may have answered questions in the diet questionnaire differently than those who did not have breast cancer.

Strengths that compensate for threats to validity included a representative population-based cross-section sample design, the ability to control for known confounders, a widely used and calibrated Food Frequency questionnaire, a canned tuna fish consumption variable with a good level of detail, two separate research questions to

provide evidence related to the study premise, and a large, established, evaluated, validated, and widely published data set.

Ethical Considerations

NHANES established its own Institutional Review Board and was renamed the NHCS Research Ethics Review Board (ERB) in 2003 (CDC, 2012c). NHANES has a long history and has received many reviews of its ethical practices and standards. An informed consent process ensures participants are knowledgably enrolled and all information related to participants is maintained confidentiality. All data is unidentifiable. Downloading data is only allowed for health analysis and any attempt to use data for other purposes or to identify participants is against the law (CDC, 2012c). IRB approval for the 2003-2006 data set was secured by NHANES ERB under Protocol #98-12 and Protocol #2005-06 (CDC, 2012c). Walden IRB approval #07-16-15-0058453 was secured prior to accessing NHANES data and all NHANES ethical and privacy policies for data use were reviewed and respected.

Summary

This population-based case control study measured canned tuna fish consumption and total blood mercury level (as a proxy for MeHg) (Sheehan et al., 2014) and examined results of their association to breast cancer in the 2003-2006 NHANES survey. The descriptive statistics of the social determinants of canned tuna consumption was also explored. Testing of two hypotheses of the association between canned tuna fish consumption with breast cancer and total blood mercury level with breast cancer was measured using a logistic regression model while controlling for confounders. A third

research question used results from descriptive statistics to explore the social determinants of age, education, annual household income, and race/ethnicity with canned tuna fish consumption.

The major threat to validity in this proposal was the content validity of methlymercury exposure via canned tuna fish in Research Question 1. Strong face validity and results from Research Question 2 helped to minimize this threat. Positive results for Research Questions 1 or 2 provide support for strong inference within carcinogenic theory. Results related to Research Question 3 based on medical geography were new and inference was speculative. NHANES appeared to apply rigorous methods to ensure highest ethical and confidential standards for participants and data. This representative population-based dataset had many design strengths that further contributed to research design, methodology, and ethical treatment of participants. In Chapter 4 data was analyzed using the methods described in this chapter.

Chapter 4: Results

Introduction

The purpose of this population-based case control study was to measure methylmercury exposure via canned tuna fish consumption and to examine the results of its association to breast cancer in the 2004-2004 and 2005-2006 NHANES. This purpose is reflected in two research questions (RQ).

1. Is there a relationship between consumption of canned tuna fish and breast cancer?

$H_0$1: There is no relationship between consumption of canned tuna fish and breast cancer.

$H_1$1: There is a relationship between consumption of canned tuna fish and breast cancer.

2. Is there a relationship between total blood mercury level and breast cancer?

$H_0$2: Null: There is no relationship between total blood mercury level and breast cancer.

*H1*2: There is a relationship between total blood mercury level and breast cancer.

A secondary goal was to evaluate the descriptive statistics of the social determinants of canned tuna fish consumption reflected in Research Question 3.

3. What is the frequency of women's canned tuna fish consumption for different age groups, race/ethnicities, annual household income, and education level?

The sections of this chapter include data collection, research question sample characteristics and results, and summary. Under data collection the secondary sections of missing values, variable recoding, baseline descriptive statistics, bivariate analysis, and variable correlation adjustments are covered. Under research question sample characteristics and results, the secondary sections of Research Question 1 and 2 sample characteristics, Research Question 1, Research Question 2, and secondary analysis of Research Question 3 is covered.

Data Collection

Each year since 1999, the NHANES has administered yearly in-home surveys and health exams at mobile examination centers (MEC) throughout the United States (NHANES, 2014c). Sampling of participants is done using a complicated and multistage process to ensure representativeness of the general population (CDC, 2013i). To account for different probabilities used in NHANES sampling, subpopulation weights were applied for data analysis (CDC, 2013j). Data from adult females surveyed between 2003-2006 were downloaded to the SPSS 21 for analysis.

The respondent sequence number was downloaded as the unique identifier for all participants. Raw (unweighted) frequencies for the variable for age range at last menstrual period had 139 responses. An alternative variable, age at last menstrual period, had 2,035 responses and was used instead. The variable age when first menstrual period occurred (6,484 responses) was also used to replace the variable age range at first menstrual period (69 responses).

During the survey periods from 2003-2006, female in-home interviews were completed with an unweighted response rate of 80- 81%, and females participating in the health examination had an unweighted response rate of 76-78% (NHANES, 2015d). Weighted final totals reflect 138,747,398 adult female participants used for this study, which is representative of the adult female population of the United States NHANES is a free publically available dataset and was downloaded in accordance with NHANES Data User Agreement (2009).

The smallest NHANES subpopulation used in this study was women who completed reproductive questions in the computer-assisted, personal interview in the MEC. As directed by the NHANES codebook (NHANES, 2008b) weights from the MEC full sample 2-year interview from 2003-2004 and 2005-2006 were merged and applied to the dataset for analysis. To accurately accommodate the complex sampling and weight procedures of NHANES, SPSS procedure for complex sample weight was completed as described by IBM (n.d.). The combination of weights from two survey periods provides representativeness to the U.S. population at the survey range midpoint (CDC, 2013h).

Missing Values

NHANES surveys are broken down into different sections so participants may complete some but not all survey content areas. Some of the variables used in this data set are from skip questions. This means that a person only would have answered the question if his or her response was positive.

In contrast to system-missing values, participant missing values represent participants who completed a questionnaire and did not answer an applicable question or

the answer was input incorrectly. Three participants were identified as having individual missing values. The missing value for age of first period for Participant 24,140 was reported incorrectly as 0, a value not attributed to the variable. The value of 0 was replaced with a value identified as missing consistent with other missing data. A missing value for Participant 40,219 for canned tuna frequency was answered as 7 but recognized as a missing value within the data system. This value of 7 was replaced with the value of 7.

One missing proportion for Participant 39,966 was the answer of number of years smoked. Participant 39,966 was diagnosed with breast cancer at age 48 and reported smoking 20 cigarettes per day but quit and had left blank number of years smoked this amount. This participant reported having her first birth at age 27, a Body Mass Index (BMI) of 21.43, no diabetes, answered 3 to annual household income ($ 10000-14999), education level 5 (college graduate or above), race/ethnicity 3 (non-Hispanic White), and was aged 52 at the time of the screening. To estimate what the number of years smoked value should be for Participant 39,966 two means procedures of smoking related variables were analyzed. First the mean for all participants for number of cigarettes per day when quit, education level, income, and years smoked were used as dependent variables and age of screening as an independent variable. Results for participants aged 50-55 showed a mean range of 3.31-3.77 (high school grad to some college) education level, a mean range of 7.52-8.21 ($35,000-54,999) income level, and mean range of 15.27 to 25 years smoked. A second means analysis compared race/ethnicity as an independent variable with dependent variables of age at screening and age smoked. These results

reflected that for non-Hispanic White women the mean age at screening for those who reported years smoked was 50.39 and mean years smoked was 8.35. The lowest estimated mean of 8 years rounded down from 8.35, from the second mean comparison, was taken as the most conservative estimate of Participant 39, 966 years of smoking. The number 8 was manually entered for this value.

For NHANES, system-missing values represent participants who did not complete all sections of the 2003-2006 NHANES surveys, including skip questions.

Table 4

Raw Data Univariate Statistics

Univariate Statistics

	N	Mean	Std. Deviation	Missing		No. of Extremes[a]	
				Count	Percent	Low	High
Mercury	8556	1.1113	1.55981	1864	17.9	0	306
Age Breast CA	145	748.64	8299.547	10275	98.6	0	1
Age screening	10420	29.17	24.613	0	.0	0	543
BMI	9026	25.4415	7.76437	1394	13.4	0	357
Age 1st birth	2792	27.04	73.938	7628	73.2	0	16
# cigs day quit	956	19.05	72.278	9464	90.8	0	5
# cigs day now	791	16.38	36.471	9629	92.4	0	2
Years smoked	791	20.65	79.242	9629	92.4	0	5
Age 1st period	6534	22.82	101.620	3886	37.3	0	70
Age last period	2175	105.70	234.153	8245	79.1	0	140
Ever cancer	5214			5206	50.0		
Kind of cancer	493			9927	95.3		
Kind of cancer	43			10377	99.6		
Race/ethnicity	10420			0	.0		
Education	5215			5205	50.0		
Annual income	10028			392	3.8		
Diabetes	9961			459	4.4		
Ever Pregnant	6401			4019	38.6		
Breastfed	3568			6852	65.8		
Birth control	6401			4019	38.6		
Hormones	4479			5941	57.0		
Canned tuna	6629			3791	36.4		
Alcohol	4483			5937	57.0		

a. Number of cases outside the range (Mean - 2*SD, Mean + 2*SD).

Table 4 presents the univariate statistics for the raw sample data. The number of cases (N) for skip questions is significantly smaller then other variables. It also reflects approximate size of groups that completed portions of the survey that included skip

questions. For example, the skipped question variables of breast cancer (*n*=145) and age when breast cancer first diagnosed (*n*=145) was part of the same medical questionnaire used to follow up after a positive answer for the question ever cancer (*n*= 5214).

Table 5

Missing Patterns Part 1

Number of Cases	AGE SCREEN1	Race Ethnicity	DIABETES 1	INCOME 1	BMI	EVER PREGGO1	BIRTH CONTROL1	AGE 1ST PERIOD1	ALCOHOL1
0									
120									
127									
61									
497									
567									
231									
114									
180									
94									
93									
87									
344									
209									
60									
78									
116									
106									X
245									X
97						X	X	X	X
108						X	X	X	X
133					X	X	X	X	X

Table 6

Missing Patterns Part 2

HORMONES 1	EDUCATION 1	EVER CANCER1	MERCURY 1	CANNED TUNA 1	BREASTFED 1	AGE BIRTH1	AGE LAST PERIOD 1
							X
					X	X	X
							X
				X			
				X			X
							X
						X	
					X	X	
					X	X	X
						X	X
				X			X
				X		X	X
				X	X	X	X
X	X	X		X	X	X	X
X	X	X			X	X	X
X					X	X	X
X				X	X	X	X
X			X	X	X	X	X

Table 7

Missing Patterns Part 3

BREAST CA1	AGE BREAST 1	YEARS SMOKE 1	CIGS DAY NOW 1	CIGS DAY QUIT 1	Complete if ...[b] 0
X	X			X	146
X	X			X	281
X	X			X	438
X	X	X	X	X	1853
X	X	X	X	X	1099
X	X	X	X		296
X	X	X	X	X	1303
X	X	X	X	X	2334
X	X	X	X		394
X	X	X	X	X	1296
X	X	X	X	X	1467
X	X	X	X	X	3036
X	X	X	X	X	2354
X	X			X	375
X	X	X	X	X	2969
X	X	X	X	X	3871
X	X	X	X	X	4319
X	X	X	X	X	3348
X	X	X	X	X	3213
X	X	X	X	X	4241
X	X	X	X	X	4920

Tables 5, 6, and 7 represent the missing value patterns by variable in the sample. The trends in missing values from the tabulated patterns above show increasingly missing data for skip questions. For example, age at screening and other general demographic questions on the left have few missing data, followed by questions from the reproductive survey section, then mercury from those who had laboratory draws at an MEC, and canned tuna, from the food frequency questionnaire. Then are questions specific to those who reported being diagnosed with breast cancer and having ever smoked cigarettes.

Missing system values for the skipped question and dependent variable of breast cancer were recoded as having answered no. The logic behind this change is that women who skipped this question on the survey did not have a breast cancer diagnosis.

For the skipped question of ever having breastfed your children all missing values were recoded as no, not having breastfed. This logic is that only women who had children

would have answered this question yes or no, so those who did not answer clearly did not breastfeed. All missing values for the variables of # of cigarettes smoked per day now, # of cigarettes smoked per day when quit and # years smoked this amount was recoded as having smoked 0 cigarettes. The logic to this change is that women who skipped this question were not ever smokers, so the value of 0 is an accurate representation of those who skipped these questions.

There are three skipped questions that did not have a logical value for those who didn't answer to compare with those that did; age when breast cancer first diagnosed, age at first live birth, and age of last menstrual period. These three questions were excluded from the final logistic regression model analysis. Additional effort was made to try and locate additional NHANES variables to replace or compute age of last menstrual period because it is an established estrogen related risk factor. Unfortunately no alternative or combination of values could be ascertained to measure age of menopause in the sample.

For system-missing data that were not skipped questions, all missing values were considered missing completely at random and excluded list wise. The exclusion of missing values for all non-skipped variable questions was expected to reduce the participant size to those who completed all the same NHANES subsections included in this data set. This reduction should account for participants who reviewed and chose to skip the skip questions or were not asked questions because their value for the question was negative or did not apply.

Variable Recoding

The answers “don’t know” and “refused” were coded as missing in order to be excluded from logistic regression model analysis. The answers of “below limits of first and second detection” (meaning blood mercury level could not be detected) for total blood mercury were excluded from analysis. The variable history of cancer (ever had cancer) includes women with and without breast cancer and is too closely associated with the dependent variable. It was removed from logistic regression model analysis. The variables # of cigarettes smoked when quit, # of cigarettes smoked now, and # of years smoked this amount are highly related to one another. A new variable combining these variables to reflect pack years was created.

To further clean the variables to prepare for logistic regression model analysis the answer # 3 for diabetes, “borderline”, was combined with the answer of “yes”. Less than 1% of respondents (.9%) responded “borderline”. Given that diabetes is a progressive disease and is included in this analysis to control for confounding, it was collapsed into “yes” because those who identify as borderline are more likely to develop the disease and it is better to include them to control for confounding. The answer of “above and below $20000” for annual household income was collapsed into income level categories; “below $20,000” into “$15000 to $19999” and “above $20000” collapsed into “$20000-$24999”. During the survey respondents who refused or did not know their annual household income level were then given the choice of answering “above or below $20,000”. The logic is to include these respondents into categories closest to the $20,000 threshold of the question and closest to the participant mean. The recoding of variables is

reflected in Table 11, Final Variable Transformation Table found in the Variable Correlations Adjustments section of this chapter.

Baseline Descriptive Statistics

The dependent variable for RQ 1 and 2 are breast cancer diagnosis. Canned tuna consumption frequency is the independent variable for RQ1 and blood mercury level is the independent variable for RQ 2. Covariates for both RQ 1 and RQ 2 include race/ethnicity, annual household income, education level, age of menarche, hormone therapy, obesity, age at screening, alcohol consumption, age when breast cancer first diagnosed, age at first full term pregnancy, parity, breast feeding, smoking, personal history of cancer, early age at menopause and diabetes.

In this representative (weighted) total sample of 138,747,398 adult females, 3% or 4,153,240 women reported being diagnosed with breast cancer and 97% or 1,334,594,157 women did not. Please note that the numbers of women reported being diagnosed with breast cancer and those women who did not report diagnosis do not sum to the total sample due to NHANES complex weights. The mean age at screening was 48.33 years.

Table 8

Race/Ethnicity of Sample

Race/Ethnicity	Mexican American	Other Hispanic	Non-Hispanic White	Non-Hispanic Black	Other race or Multiracial
Percentage	6%	3.5%	74.8%	10.7%	5%

Table 9

Education Level of Sample

Education Level	Less than 9th grade	9 to 11th grade	High School Grad or GED	Some college or AA	College grad or higher
Percentage	4.6%	10%	25.7%	32.4%	27.3%

Table 10

Income Level of Sample

Income Level	Percentage
$ 0-4999	1.1%
$ 5000-9999	3.3%
$ 10000-14999	6.2%
$ 15000-19999	6.1%
$ 20000-24999	8.7%
$ 25000-34999	12.1%
$ 35000-44999	10.2%
$ 45000-54999	10.5%
$ 55000-64999	8.2%
$ 65000 74999	5.7%
$ 75000 and over	27.8%

Table 11

Canned Tuna Frequency Characteristics of Sample

Canned Tuna Frequency	Percentage
Never	13.9%
1-6 times per year	20.6%
7-11 times per year	15.4%
1 time per month	14.1%
2-3 times per month	20.4%
1 time per week	8.4%
2 times per week	4.8%
3-4 times per week	1.9%
5-6 times per week	.2%
1 time per day	.2%
2 or more times per day	.1%

Table 12

Additional Characteristics of Sample

Variable	% Yes	% No	Mean (if applicable)
Breast Cancer	3%	97%	
Age at Screening			48.33
Had at least 12 Alcohol drinks in year	83.2%	36.1%	
Ever Pregnant	83.2%	16.8%	
Ever taken birth control	72.2%	27.8%	
Ever taken hormones	29.7%	70.3%	
Age 1st live birth			23.85
Age breast cancer diagnosed			59.27
Diabetes	9.2%	90.8%	
BMI			28.5164
Blood Mercury			1.6424
Age 1st period			12.68
Pack years smoking cigarettes			3.06
Age at last menstrual period			44.02
Ever diagnosed with cancer	10.1%	89.9%	

Tables 8 through 12 reflect the race/ethnicity, educational level, income, canned tuna frequency and additional characteristics of the sample.

Bivariate Analysis

To better understand the relationship between variables in order to build a logistic regression model two-tailed bivariate correlation analysis was conducted. The Pearson's *r*

statistic was used for variables that met assumptions of interval or ratio scale, were of approximate normal distribution and without significant outliers. The variables age of screening, blood mercury level BMI and age at 1st period met these assumptions and was tested with Pearson's *r*. The variables BMI (figure 19) age of screening (figure 21) and age of 1st period (figure 24) reflect approximately normal distribution. Smoking pack years in figure 30 did not have a normal distribution and was not tested with Pearson's *r*.

Spearman's *rho* correlations were used for other continuous and ordinal variables that did not meet Pearson's r assumptions. Variables tested using Spearman's *rho* include canned tuna, education, income, and smoking pack years. Both Pearson's and Spearman's Correlation values span from -1 to 1 with values both positive and negative between .1-.29 interpreted as indicating a small relationship, .3-.49 a medium relationship and .50-1 a strong relationship (LAERD Statistics, 2013).

For correlations of nominal variables of breast cancer, breastfed, alcohol, diabetes, ever pregnant, ever birth control, ever hormones and one categorical variable race/ethnicity, a chi-square test interpreting the Cramer's *V* effect size was applied. Cramer's *V* values span from -1 to 1, values both positive and negative between .1-.29 were interpreted as indicating a small effect size, .3-.49 a medium effect size and .50-1 a large effect size (with 1 degree of freedom) (United States Geological Survey, Statistical Interpretation, 2015).

The results of Pearson's, Spearman's and chi-square tests showed all variable relationships to be significant at the $p < .05$ levels.

All values greater than .2 are represented in Table 13 below in order of strength:

Table 13

Variable Correlations greater than .2

Variables	Test value
Hormones and age of screening	*rho*= .441
Breastfed and ever pregnant	Cramers *V*= .421
Income and education	*rho*= .412
Ever pregnant and age of screening	*rho*= .294
Birth control and age of screening	*rho*= .289
Blood Mercury and Canned Tuna	*rho*= .278
Income and birth control	Cramers *V*= *,257*
Diabetes and BMI	*rho* =.226
Alcohol and birth control	Cramers *V*= .224
Blood Mercury and Income	*rho*= .217
Birth Control and Education	Cramers *V* .202
Blood Mercury and Education	rho= .201

Variable Correlation Adjustments

For the correlation between diabetes and BMI, the diabetes variable was excluded from the final regression model. BMI is a stronger predictor of breast cancer then diabetes and diabetes and BMI are closely related. For the correlation between income and education, the variable of income was excluded from the final regression model. Education is regularly used in epidemiological research and has been validated as an indicator of overall socioeconomic status. Education is a more reliable and valid measure then the closely related income variable. Excluding the income variable also addresses the correlation between income and birth control and blood mercury.

For the correlation between breastfed and ever pregnant both were included in the final regression model. Each are primary reproductive breast cancer risk factors and

regularly controlled for the in the body of breast cancer research. There appears to be stronger evidence in the body of research of an independent relationship between not having children and breast cancer then not having breastfed and breast cancer, so if further evidence of collinearity were evident breastfed would have been considered for exclusion.

Blood mercury and canned tuna are independent research question variables and not covariates; therefore no changes were made. No adjustments were made for correlations between birth control and alcohol and birth control and education, but evidence of collinearity was monitored in the final model.

The correlations between both hormones and age of screening and birth control and age of screening were anticipated. Younger women of childbearing age are more likely to take birth control pills and older women in or approaching menopause are more likely to take hormones. Birth control pills, hormones, ever pregnant and age are all known to have independent relationships to breast cancer and are regularly controlled for in the body of breast cancer research. Each of these variables was kept in the logistic regression model. However, in response to the correlation of age of screening with numerous variables (hormones, birth control and ever pregnant) the age variable was transformed into categories to attempt to minimize it's residual affect. Five age categories were created from ages 18-29, 30-42, 43-57, 58-71 and 72-85+. To test if correlations decreased after transforming the age of screening variable into 5 intervals, correlations were again run and reviewed. The correlations for the new age of screening ordinal variable with both hormones and birth control showed very small Pearson's rho and

Cramers *V* value decreases and age of screening and ever pregnant showed very small increases. Since transformation of the continuous variable of age of screening to an ordinal variable did not significantly decrease the correlations, the continuous age of screening variable was used in the final regression model. These variable changes are reflected in the following Table 14 Variable Transformation Table.

Table 14

Variable Transformation Table

Variable and Question	NHANES survey Values	1st Variable recode	2nd Variable recode	3rd Variable recode	Final Model
Alcohol: Had at least 12 drinks/1yr?	1= yes 2= no 7= refused 9= don't know . = Missing	1= yes 2= no . = Missing (includes don't know, refused)	1= yes 0= no	1= yes 0= no	1= yes 0= no
Total Blood Mercury, total (ug/L)	0.20 to 33.20 = range of values 0.10 = below first limit of detection 0.14 = below second limit of detection . = Missing	0.20 to 33.20 = range of values . = Missing (includes 0.1 and 0.14)	0.20 to 33.20 = range of values	0.20 to 33.20 = range of values	0.20 to 33.20 = range of values
Ever told you had cancer or malignancy?	1= yes 2= no 7= refused 9= Don't know . = Missing	1= yes 2= no . = Missing (includes don't know, refused)	Excluded	Excluded	Excluded
Age when breast cancer first diagnosed	4 to 84= range of values 85= 85 or older 777777 =Refused 99999 =Don't Know	18 to 84= range of value . = Missing (includes age 0-17, refused and don't know)	Excluded	Excluded	Excluded
Age at Screening	0 to 84= range of values 85= 85 or older . = Missing	18 to 85= range of value . = Missing (includes age 0-17, refused and don't know)	18 to 85= range of value	1= 18-29 2= 30-42 3= 43-57 4= 58-71 5= 72-85+	18 to 85= range of value
Race/Ethnicity	1= Mexican American 2= Other Hispanic 3= Non-Hispanic white 4= Non-Hispanic Black 5. Other race-including multi- racial . = Missing	1= Mexican American 2= Other Hispanic 3= Non-Hispanic white 4= Non-Hispanic Black 5. Other race-including multi-racial			1= Mexican American 2= Other Hispanic 3= Non-Hispanic white 4= Non-Hispanic Black 5. Other race-including multi-racial
Education Level Adults 20+	1= less than 9th grade 2= 9-11th grade 3= high school grad/GED or equivalent 4= Some College or AA degree 5= College graduate or above 7= Refused 9= Don't Know . = Missing	1= less than 9th grade 2= 9-11th grade 3= high school grad/GED or equivalent 4= Some College or AA degree 5= College graduate or above . = Missing	1= less than 9th grade 2= 9-11th grade 3= high school grad/GED or equivalent 4= Some College or AA degree 5= College graduate or above	Will be used to represent Socioeconomic Status	1= less than 9th grade 2= 9-11th grade 3= high school grad/GED or equivalent 4= Some College or AA degree 5= College graduate or above

		(includes refused and don't know)			
Annual Household Income	1= $0-4999 2= $5000-9999 3=$10000-14999 4= $ 15000-19999 5= $ 20000-24999 6= $25000-34999 7= $35000-44999 8= $45000-54999 9= $55000-64999 10= $65000=74999 11= $75000 and over 12= Over $20000 13= Under $20000 77= Refused 99= Don't Know . = Missing	1= $0-4999 2= $5000-9999 3=$10000-14999 4= $ 15000-19999 5= $ 20000-24999 6= $25000-34999 7= $35000-44999 8= $45000-54999 9= $55000-64999 10= $65000=74999 11= $75000 and over 12= Over $20000 13= Under $20000 . = Missing (includes refused and don't know)	1= $0-4999 2= $5000-9999 3=$10000-14999 4= $ 15000-19999 5= $ 20000-24999 6= $25000-34999 7= $35000-44999 8= $45000-54999 9= $55000-64999 10= $65000=74999 11= $75000 and over	Excluded	Excluded
Doctor told you have diabetes	1= Yes 2= no 3= Borderline 7= Refused 9= Don't Know . = Missing	(FYI .9% answered 3= borderline) 1= Yes 2= no 3= Borderline . = Missing (includes refused and don't know)	1= Yes and borderline 0= no	Excluded	Excluded
Body Mass Index (kb/m**2)	In 2003-2004: 12.4 to 64.97= Range of Values . = Missing In 2005-2006- 11.74= 130.21 range of values . = Missing	11.74-130-21= range of values			11.74-130-21= range of values
Ever been pregnant	1= yes 2= no 7= refused 9= don't know . = Missing	1= yes 2= no . = Missing (includes refused and don't know)	1= yes 0= no		1= yes 0= no
Age at first live birth	2003-2004 12 to 54= range of valued 777= refused 999= don't know . = Missing 2005-2006 15-38 =Range of Values 14= 14 years or under 45= 45 years or older	12-54 =range of values . = Missing (includes don't know and missing)	Excluded	Excluded	Excluded

	777= refused 999= don't know . = Missing			
Breastfed any of your children	1= yes 2= no 7= refused 9= don't know . = Missing	1= yes 2= no . = Missing (includes refused and don't know)	1= yes 0= no	1= yes 0= no
Ever taken birth control pills	1= yes 2= no 7= refused 9= don't know . = Missing	1= yes 2= no . = Missing (includes refused and don't know)	1= yes 0= no	1= yes 0= no
Ever use female hormones	1= yes 2= no 7= refused 9= don't know . = Missing	1= yes 2= no . = Missing (includes refused and don't know)	1= yes 2= no	1= yes 2= no
# Cigarettes smoked per day when quit	1 to 95= range of values 777= refused 999= don't know . = Missing	1 to 95= range of values . = Missing (includes refused and don't know)	0 to 95= range of values then Recoded into Pack year variable	Transformed into pack year
# Cigarettes smoked per day now	2003-2004 1 to 70= Range of values 95= 95 cigarettes or more 777= refused 999= don't know . = Missing 2005-2006 2 to 80= range of values 1= 1 cigarette or less 95= 95 cigarettes or more 777= refused 999= don't know . = Missing	1-95 = range of values . = Missing (including refused and don't know)	0 to 95= range of values then Recoded into Pack year variable	Transformed into pack year
How many years smoked this amount	2003-2004 1 to 72= range of values 777= refused 999= don't know . = Missing 2005-2006 2 to 70= range of values 1= 1 year or less 777= refused 999= don't know . = Missing	1-72 = range of values . = Missing (including refused and don't know)	0 to 72= range of values then Recoded into Pack year variable	Transformed into pack year
Did you eat canned tuna	1= never 2= 1-6 times per year 3= 7-11 times/year 4= 1 time per month	1= never 2= 1-6 times per year 3= 7-11	1= never 2= 1-6 times per year 3= 7-11	1= never 2= 1-6 times per year 3= 7-11

	5= 2-3 times per month 6= 1 time per week 7= 2 times per week 8= 3-4 times per week 9= 5-6 times per week 10= 1 time per day 11= 2 or more times per day 88= blank 99= error	times/year 4= 1 time per month 5= 2-3 times per month 6= 1 time per week 7= 2 times per week 8= 3-4 times per week 9= 5-6 times per week 10= 1 time per day 11= 2 or more times per day . = Missing (includes blank and error)	times/year 4= 1 time per month 5= 2-3 times per month 6= 1 time per week 7= 2 times per week 8= 3-4 times per week 9= 5-6 times per week 10= 1 time per day 11= 2 or more times per day	times/year 4= 1 time per month 5= 2-3 times per month 6= 1 time per week 7= 2 times per week 8= 3-4 times per week 9= 5-6 times per week 10= 1 time per day 11= 2 or more times per day
Age when first menstrual period occurred	2003-2004 0 to 21= range of values 777= refused 999 = don't know . = Missing 2005- 2006 0-20 = Range of values 777= refused 999= don't know . = Missing	0 to 21= range of values . = Missing (includes refused and don't know)	0 to 21= range of values	0 to 21= range of values
Age at last Menstrual period	2003-2004 13 to 67= range of values 777= refused 999= don't know . = Missing 2005-2006 13 to 67= range in values 777= refused 999= don't know . = Missing	13 to 67= range of values . = Missing (includes don't know and missing)	Excluded	Excluded
What kind of cancer	10-39= types of cancer 14= Breast Cancer 66= more than 3 kinds 77= Refused 99= Don't Know . = Missing	Transformed into Breast Cancer variable below		
Breast Cancer	Transformed from type of cancer: 10-39= types of cancer 14= Breast Cancer 66= more than 3 kinds 77= Refused 99= Don't Know . = Missing	0= All other types of cancer (including answer 66) 1= Breast Cancer 99= missing, Don't know, Refused	0= Missing, don't know, refused, all other types of cancer 1= breast cancer	0= Missing, don't know, refused, all other types of cancer 1= breast cancer
Pack Years	First the # of cigs per day when quit and # of cigs per day now were	Then the # of packs per day was recoded into	0= non smokers 1-114 = range of values	0= non smokers 1-114 = range of values reflecting

added together. Then the number of cigs per day was recoded to reflect packs per day at: 0=0 1-20= 1 21=40= 2 41-60 = 3 61-80= 4 81- 95+ = 5	a new variable that multiplied packs per day by # of years smoked to reflect the number of pack years. 0= non smokers 1-114 = range of values reflecting the number of packs per year.	reflecting the number of packs per year.	the number of packs per year.

Research Question Sample Characteristics and Results

Both Research Questions #1 and #2 were addressed using the same dependent variable (breast cancer) and covariates. Therefore logistic regression modeling and most descriptive statistic analysis were combined for both research questions. This section begins with sample characteristics for both RQ1 and RQ2, then results specific to RQ 1, and RQ2, and lastly RQ 3.

Research Questions 1 and 2 Sample Characteristics

Table 15

Race/Ethnicity of Breast Cancer vs. Controls

Race/Ethnicity	Mexican American	Other Hispanic	Non-Hispanic White	Non-Hispanic Black	Other race or Multiracial
Percentage with Breast Cancer	2.1%	0.5%	88.4%	7.4%	1.6%
Percentage Without Breast cancer	6.1%	3.6%	74.4%	10.8%	5.1%

Table 16

Education Level of Breast Cancer

Education Level	Less than 9th grade	9 to 11th grade	High School Grad or GED	Some college or AA	College grad or higher
Percentage with Breast cancer	8.6%	15%	30.6%	28.5%	17.4%
Percentage without breast cancer	4.5%	9.9%	25.5%	32.5%	27.6%

Table 17

Income Level of Breast Cancer vs. Controls

Income Level	Percentage with Breast cancer	Percentage without Breast Cancer
$ 0-4999	0	1.1%
$ 5000-9999	2.3%	3.6%
$ 10000-14999	10.7%	6.1%
$ 15000-19999	10.1%	6.0%
$ 20000-24999	13.7%	8.5%
$ 25000-34999	12.6%	12%
$ 35000-44999	13.7%	10.1%
$ 45000-54999	12.5%	10.4%
$ 55000-64999	6.9%	8.2%
$ 65000 74999	1.7%	5.8%
$ 75000 and over	15.7%	28.2%

Table 18

Canned Tuna Frequency Characteristics of Breast Cancer vs. Controls

Canned Tuna Frequency	Percentage with Breast cancer	Percentage without Breast cancer
Never	15%	13.8%
1-6 times per year	19.8%	20.6%
7-11 times per year	8.8%	15.6%
1 time per month	16.8%	14$
2-3 times per month	19.9%	20.4%
1 time per week	9.5%	8.3%
2 times per week	5.3%	4.7%
3-4 times per week	4.3%	1.9%
5-6 times per week	0	0.3%
1 time per day	0.6%	0.2%
2 or more times per day	0	0.1%

Table 19

Additional Characteristics of Breast Cancer vs. Controls

Variable	With Breast Cancer % Yes	With Breast Cancer % No	Without Breast Cancer % Yes	Without Breast Cancer % No
Breast Cancer	3%		97%	
Had at least 12 Alcohol drinks in year	63.9%	36.1%	64.1%	35.9%
Ever Pregnant	90.7%	9.3%	83%	17%
Ever taken birth control	53.8%	46.2%	72.8%	27.2%
Ever taken hormones	35.8%	64.2%	29.5%	70.5%
Diabetes	18.3%	81.7%	8.9%	91.1%
Ever diagnosed with cancer	100%		7.3%	92.7%

Table 20

Canned Tuna Fish and Blood Mercury

Total Blood Mercury Level	Canned tuna level **Low** (Never to 7-11 times per year)	Canned tuna level **Medium** (1x per month to twice per week)	Canned tuna level **High** (1x per week to 2x per day)
Mean	1.3434	1.8873	2.9533
Median	.8200	1.3000	1.79000
Minimum and Maximum	.20 – 33.20	.20- 16.30	.23- 10.90
Range	33	16.10	10.67

Though fish consumption and blood mercury level is established in the literature the direct relationship between canned tuna consumption and blood mercury level is rarely specified. Table 20 above shows significant increases in mean blood mercury levels as canned tuna consumption was increased.

Research Question 1

The first research question for this study was: Is there a relationship between consumption of canned tuna fish and breast cancer? Ho: There is no relationship between consumption of canned tuna fish and breast cancer. H1: There is a relationship between consumption of canned tuna fish and breast cancer.

Characteristics of the sample in many ways reflect anticipated differences between those diagnosed with breast cancer and the general female population. Women with breast cancer are older, more non-Hispanic white, and a higher percentage report taking hormones. Evidence of differences in canned tuna consumption between those

with and without breast cancer was also reflected in descriptive analysis. A higher percentage of women with breast cancer reported eating canned tuna more frequently.

To test the Null hypothesis a binary logistic regression entry model, which included all independent and covariate variables at the same time, with the dependent variable of breast cancer was run for analysis. As identified in the Variable Transformation Table, the final covariates included in the logistic regression model were alcohol, age at screening, race/ethnicity, education level, BMI, ever pregnant, ever breastfed, ever taken birth control pills, ever taken female hormones, pack years of smoking, and age at 1st menstrual period.

Table 21

Logistic Regression P Value Odds Ratio and CI

Variable	P Value	Odds Ratio	95% Confidence Interval Lower	95% Confidence Interval Upper
Mexican-American (group membership)	.000	1.064	1.053	1.075
Other Hispanic (group membership)	.000	.354	.349	.360
Other Hispanic (Mexican- American as reference)	.000	.333	.328	.338
Non-Hispanic White (group membership)	.000	2.276	2.258	2.294
Non-Hispanic White (Mexican-American as reference)	.000	2.140	2.125	2.155
Non-Hispanic Black (group membership)	.000	1.787	1.772	1.802
Non-Hispanic Black (Mexican-American as reference)	.000	1.680	1.667	1.693
Other Race/Multiracial (Mexican-American as reference)	.000	.940	.930	.950
BMI	.000	.963	.963	.963
Blood Mercury Level	.000	1.002	1.002	1.003
Canned Tuna Fish Frequency	**.000**	**1.068**	**1.067**	**1.069**
Age of 1st Menstrual Period	.000	1.470	1.469	1.471
Education Level	.000	.863	.862	.863
Had 12 alcohol drinks in 1 year	.000	.995	.993	.997
Ever Pregnant	.000	1.050	1.046	1.054
Ever Taken Birth Control	.000	1.510	1.506	1.0514
Ever Taken Female Hormones	.000	1.409	1.407	1.410
Pack Years Smoked Cigarettes	.000	.988	.988	.988
Age of Screening	.000	1.074	1.074	1.074

Canned tuna fish frequency was found significant *p*=. 000 with an OR of 1.068, 95% CI 1.067-1.069. Women who reported eating canned tuna at one level of increased frequency had 6.8% greater odds of being diagnosed with breast cancer then those that reported eating less frequently. The 11 levels of canned tuna frequency were:

1. = Never
2. = 1-6 times per year
3. = 7-11 times/year
4. = 1 time per month
5. = 2-3 times per month
6. = 1 time per week
7. = 2 times per week
8. = 3-4 times per week
9. = 5-6 times per week
10. = 1 time per day
11. = 2 or more times per day

Therefore the Null hypothesis for RQ1, Ho: There is no relationship between consumption of canned tuna fish and breast cancer, was rejected and the alternative hypothesis, H1: There is a relationship between consumption of canned tuna fish and breast cancer, accepted.

The covariates of non-Hispanic white, non-Hispanic black, ever being pregnant, ever taking birth control, ever taking female hormones, age of first menstrual period, and age at screening resulted in increased odds of being diagnosed with breast cancer. The

covariates of Mexican American, other Hispanic, Other Race/Multiracial, smoking pack years, BMI, drinking 12 alcoholic drinks in last year, and education level resulted in decreased odds of being diagnosed with breast cancer.

Model fit. Results from the binary logistic regression reflected mixed results of model fit. The Hosmer Lemeshow statistic (H-L) was statistically significant, the -2 Log Likelihood high and the classification table prediction did not increase after adding all the variables. These are evidence of poor model fit. However, in the Variables in the Equation table, all variables show significance at $p \leq .05$, with odds ratio's (OR) under Exp (B) and confidence interval's (CI's) that reflect great precision and a clinically sound model. The evidence of a clinically sound model (OR's and CI's) likely reflects the power of the large sample size and appropriate inclusion of predictor variables. The evidence of poor model fit (H-L, -2 Log likelihood, classification table prediction) likely reflects an imbalance between cases and an extremely large group of controls. It is expected this lack of model predictability results from a high proportion of controls to cases.

Research Question 2

The second research question for this study was: Is there a relationship between total blood mercury level and breast cancer? Ho: There is no relationship between total blood mercury level and breast cancer. H1: There is a relationship between total blood mercury level and breast cancer.

As described for RQ1, characteristics of the sample in many ways reflect anticipated differences between those diagnosed with breast cancer and the general

female population. Women with breast cancer are older, more non-Hispanic white, and more report taking hormones. Evidence of differences in blood mercury level between those with and without breast cancer was also reflected in descriptive analysis.

To test the Null hypothesis a binary logistic regression entry model, which included all independent and covariate variables at the same time, with the dependent variable of breast cancer was run for analysis. As identified in the Table 11 Variable Transformation Table, the final covariates included in the logistic regression model were alcohol, age at screening, race/ethnicity, education level, BMI, ever pregnant, ever breastfed, ever taken birth control pills, ever taken female hormones, pack years of smoking, and age at 1st menstrual period.

Table 22

Logistic Regression P Value Odds Ratio and CI

Variable	P Value	Odds Ratio	95% Confidence Interval Lower	95% Confidence Interval Upper
Mexican-American (group membership)	.000	1.064	1.053	1.075
Other Hispanic (group membership)	.000	.354	.349	.360
Other Hispanic (Mexican- American as reference)	.000	.333	.328	.338
Non-Hispanic White (group membership)	.000	2.276	2.258	2.294
Non-Hispanic White (Mexican-American as reference)	.000	2.140	2.125	2.155
Non-Hispanic Black (group membership)	.000	1.787	1.772	1.802
Non-Hispanic Black (Mexican-American as reference)	.000	1.680	1.667	1.693
Other Race/Multiracial (Mexican-American as reference)	.000	.940	.930	.950
BMI	.000	.963	.963	.963
Blood Mercury Level	**.000**	**1.002**	**1.002**	**1.003**
Canned Tuna Fish Frequency	.000	1.068	1.067	1.069
Age of 1st Menstrual Period	.000	1.470	1.469	1.471
Education Level	.000	.863	.862	.863
Had 12 alcohol drinks in 1 year	.000	.995	.993	.997
Ever Pregnant	.000	1.050	1.046	1.054
Ever Taken Birth Control	.000	1.510	1.506	1.0514
Ever Taken Female Hormones	.000	1.409	1.407	1.410
Pack Years Smoked Cigarettes	.000	.988	.988	.988
Age of Screening	.000	1.074	1.074	1.074

Blood mercury level was found significant *p*=. 000 with an OR of 1.002, 95% CI 1.002-1.003. Women with a .01 increase of micrograms per liter (Ug/L) total blood mercury level had 0.2% increased odds of being diagnosed with breast cancer. Therefore the Null hypothesis for RQ2, Ho: There is no relationship between total blood mercury level and breast cancer, was rejected and the alternative hypothesis, H1: There is a relationship between total blood mercury level and breast cancer, accepted.

The covariates of non-Hispanic white, non-Hispanic black, ever being pregnant, ever taking birth control, ever taking female hormones, age of first menstrual period, and age at screening resulted in increased odds of being diagnosed with breast cancer. The covariates of Mexican American, other Hispanic, Other Race/Multiracial, smoking pack years, BMI, drinking 12 alcoholic drinks in last year, and education level resulted in decreased odds of being diagnosed with breast cancer.

Model fit. Results from the binary logistic regression reflected mixed results of model fit. The Hosmer Lemeshow statistic (H-L) was statistically significant, the -2 Log Likelihood high and the classification table prediction did not increase after adding all the variables. These are evidence of poor model fit. However, in the Variables in the Equation table, all variables show significance at $p \leq .05$, with odds ratio's (OR) under Exp(B) and confidence intervals that reflect great precision and a clinically sound model. The evidence of a clinically sound model (OR's and CI's) likely reflects the power of the large sample size and appropriate inclusion of predictor variables. The evidence of poor model fit (H-L, -2 Log likelihood, classification table prediction) likely reflects an

imbalance between cases and an extremely large group of controls. It is expected this lack of model predictability results from a high proportion of controls to cases.

Secondary Analysis Research Question 3

The secondary analysis for this research is reflected in the 3rd research question: What is the frequency of women's canned tuna fish consumption for different age groups, race/ethnicities, annual household income, and education level? This question was addressed using descriptive statistics from the cleaned and weighted NHANES data.

Table 23

Canned Tuna and Income Level

Canned Tuna Level	Low Income $0-$24999	Working Income $25000 - $44999	Middle Income $45000-$74999	High Income $75000+
Income Level Baseline Sample Percentages	25.7%	22.3%	24.4%	27.8%
Low (Never to 7-11 times per year)	25.7%	23.2%	24.2%	26.8%
Difference from Baseline	0	+. 9%	-.2%	-1%
Medium (1x per month to twice per week)	25.7%	21.2%	24.4%	28.8%
Difference from Baseline	0	-1.1%	0	+1%
High (1x per week to 2x per day)	25.4%	21.4%	24.1%	29.2%
Difference from Baseline	-.3%	-.9%	-.3%	+1.4%

Table 23 reflects the percentage of the sample that reported each income level and the percentage within each income level that reported low, medium, and high canned tuna

consumption frequency. The rows, difference from baseline, reflect the increase or decrease in the percentage of canned tuna frequency from the percentage of participants within each income level. The highest annual household income group, $75000+, were the most frequent medium consumers of canned tuna at 1% greater than baseline and the most frequent high level consumers of canned tuna at 1.4% over baseline.

Table 24

Canned Tuna and Education

Canned Tuna Level	Less than 9th grade education	9-11th grade education	High school graduate or GED education	Some college or AA education	College graduate or above education
Education Level Baseline Percentages	4.6%	10%	25.7%	32.4%	27.3%
Low (Never to 7-11 times per year)	5.8%	10.4%	25.4%	32.1%	26.4%
Difference from Baseline	+1.2%	+. 4%	-.3%	-.3%	-.9%
Medium (1x per month to twice per week)	3.4%	9.7%	26%	33.2%	27.8%
Difference from Baseline	-1.2%	-.3%	+. 3%	+. 8%	+. 5%
High (1x per week to 2x per day)	3.6%	10.4%	25.7%	24.6%	35.6%
Difference from Baseline	-1%	+. 4%	0	-7.8%	+8.3%

Table 24 reflects the percentage of the sample that reported each educational level and the percentage within each educational level that reported low, medium, and high canned tuna consumption frequency. The rows, difference from baseline, reflect the increase or decrease in the percentage of canned tuna frequency from the percentage of participants within each educational level. Results from this table show a trend of

increasing level of canned tuna consumption paralleling increasing levels of educational attainment. Those from the educational level of some college/ AA were the most frequent medium consumers of canned tuna at .8% over baseline. Those from the educational level of college graduate or above were the most frequent high-level consumers of canned tuna at a very high 8.3% greater than baseline.

Table 25

Canned Tuna and Race/Ethnicity

Canned Tuna Level	Mexican American	Other Hispanic	Non-Hispanic White	Non-Hispanic Black	Other Race/ Multiracial
Race/Ethnicity Baseline Percentages	6%	3.5%	74.8%	10.7%	5%
Low (Never to 7-11 times per year)	5.3%	2.9%	73.7%	11.4%	6.7%
Difference from baseline	-.7%	-.6%	-1.1%	+. 7%	+1.7%
Medium (1x per month to twice per week)	6.7%	4 %	76.2%	9.6%	3.4%
Difference from baseline	+. 7%	+. 5%	+1.4%	-.4%	-1.6%
High (1x per week to 2x per day)	7.3%	6.5%	69.5%	16.4%	0.4%
Difference from baseline	+1.3%	+3%	-5.3%	+5.7%	-4.6%

Table 25 reflects the percentage of the sample that reported each race/ethnicity and the percentage within each race/ethnicity that reported low, medium, and high canned tuna consumption frequency. The rows, difference from baseline, reflect the increase or decrease in the percentage of canned tuna frequency from the percentage of participants within each race/ethnicity. The race/ethnicity non-Hispanic was the most frequent medium level consumers of canned tuna consumption at 1.4% above baseline. The

race/ethnicity of non-Hispanic black were the most frequent high-level consumers of canned tuna at a high 5.7% above baseline.

Table 26

Canned Tuna and Age at Screening

Canned Tuna Level	Age 18-29	Age 30-42	Age 43-57	Age 58-71	Age 72-85+
Age Level Baseline Percentages	15.3%	25.2%	30%	18.1%	11.5%
Low (Never to 7-11 times per year)	16.8%	26%	28.2%	17.5%	11.4%
Difference from Baseline	+1.5%	+. 8%	-1.8%	-.6%	-.1%
Medium (1x per month to twice per week)	13.5%	24.5%	31.4%	18.7%	11.9%
Difference from Baseline	-1.8%	-.7%	+1.4%	+. 6%	+. 4%
High (1x per week to 2x per day)	16.6%	23.1%	38.1%	17.3%	4.9%
Difference from Baseline	+1.3%	-2.1%	+8.1%	-.8%	-6.6%

Table 26 reflects the percentage of the sample by age at screening level and the percentage within each age level that reported low, medium, and high canned tuna consumption frequency. The rows, difference from baseline, reflect the increase or decrease in the percentage of canned tuna frequency from the percentage of participants within each age at screening level. Those in the age group 43-57 were the most frequent medium and high consumers of canned tuna with medium level 1.4% above baseline and high consumers at a very high 8.1% above baseline.

In completing the analysis the relationship between blood mercury level and canned tuna fish consumption was overlooked. Though fish consumption and blood mercury level is established in the literature the direct relationship between canned tuna consumption and blood mercury level is rarely specified. To examine possible parallels

descriptive statistics were run to examine canned tuna fish consumption level and blood mercury level.

Table 27

Canned Tuna Fish and Blood Mercury

Total Blood Mercury Level	Canned tuna level **Low** (Never to 7-11 times per year)	Canned tuna level **Medium** (1x per month to twice per week)	Canned tuna level **High** (1x per week to 2x per day)
Mean	1.3434	1.8873	2.9533
Median	.8200	1.3000	1.79000
Minimum and Maximum	.20 – 33.20	.20- 16.30	.23- 10.90
Range	33	16.10	10.67

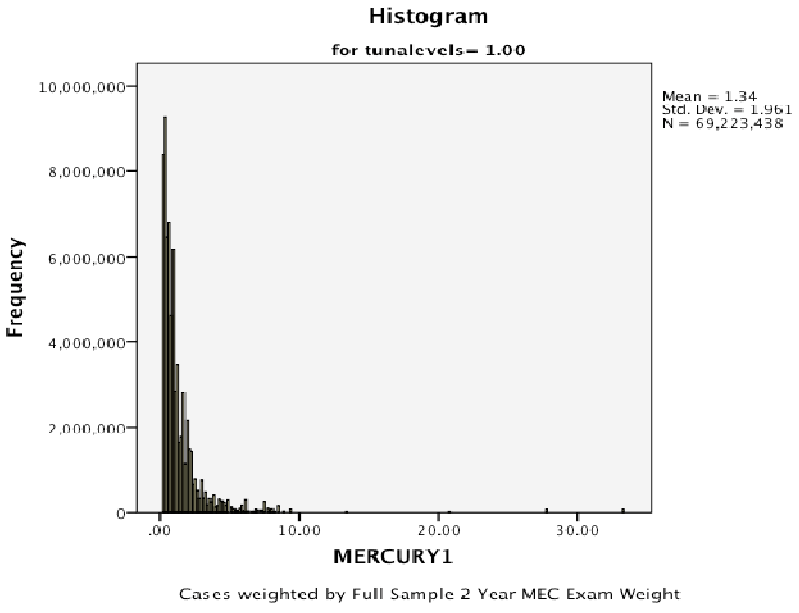

Figure 27. Blood mercury histogram for low level canned tuna

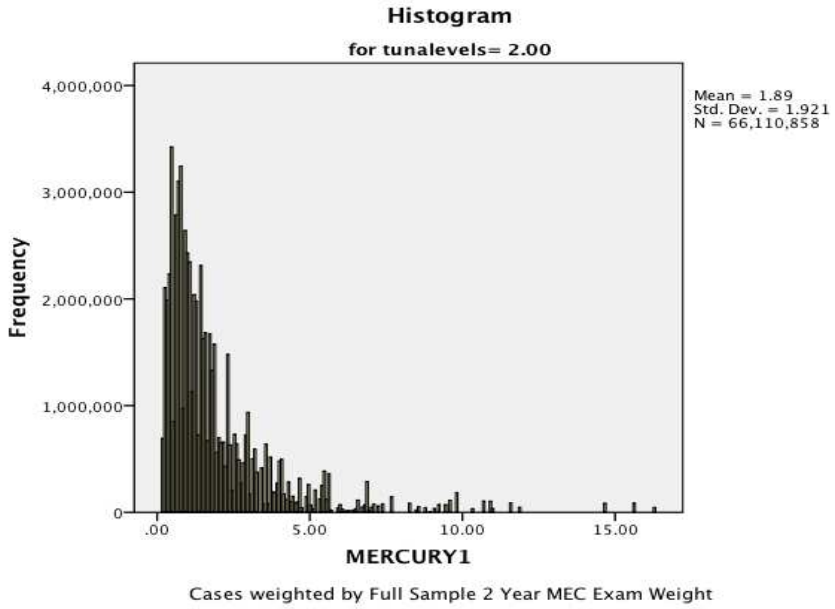

Figure 28. Blood mercury histogram for medium level canned tuna

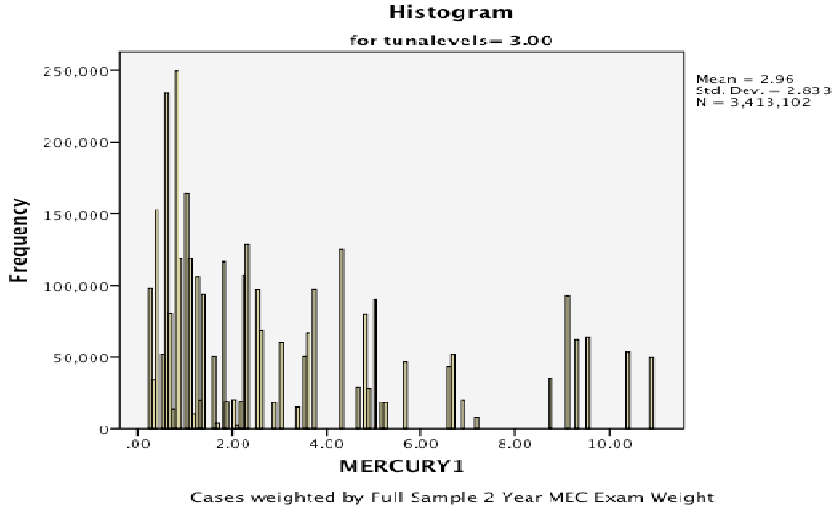

Figure 29. Blood mercury histogram for high level canned tuna

Summary

The purpose of this population-based, case-control study was to measure methylmercury exposure via canned tuna fish consumption and examine results of its association to breast cancer in 2004-2004 and 2005-2006 NHANES. A secondary goal was to measure the social determinants of canned tuna fish consumption.

For RQ1 a binary logistic regression model was run controlling for covariates. Results showed canned tuna fish consumption frequency to have a relationship to breast cancer, p =. 000 OR 1.068 and 95% CI 1.067-1.069. Women who reported eating canned tuna at one level of increased frequency had 6.8% greater odds of being diagnosed with breast cancer then those that reported eating less frequently. Therefore Ho: There is no relationship between consumption of canned tuna fish was rejected and H1: There is a relationship between consumption of canned tuna fish and breast cancer, was accepted.

For RQ2 a binary logistic regression model was run controlling for covariates. Results showed blood mercury level to have a relationship to breast cancer, p =. 000 OR 1.002 and 95% CI 1.002-1.003. Women with a .01 Ug/L increase in total blood mercury level had 0.2% greater odds of being diagnosed with breast cancer. Therefore Ho: There is no relationship between total blood mercury level and breast cancer was rejected and H1: There is a relationship between total blood mercury level and breast cancer accepted.

The secondary goal to evaluate the descriptive statistics of the social determinants of canned tuna fish consumption was reflected in the research question, what is the frequency of women's canned tuna fish consumption for different age groups, race/ethnicities, annual household income, and education level? Descriptive statistic

frequencies were run to compare canned tuna consumption levels with the differences in social variable baseline sample percentages. Results showed that high annual household income of $75000+ per year, higher educational level, the race/ethnicity of non-Hispanic white and non-Hispanic Black, and the age group of 43- 57 were the most frequent canned tuna consumers. The strongest trends were reflected in the variable of education. As educational level increased parallel increases in the percentage of canned tuna consumption levels over baseline were observed. Those in the highest educational category of college graduates and above were the most frequent high-level canned tuna consumers with a 8.3% above baseline.

In chapter 5 these findings were disseminated for interpretation, limitations, recommendations and implications.

Chapter 5: Discussion, Conclusions, and Recommendations

Introduction

Consumption of canned tuna fish is the primary source of human exposure to methylmercury (Iavicoli et al., 2009), a metalloestrogen that accumulates in the body over time and is presumed to contribute to breast carcinogenesis by activating estrogen (Byrne et al., 2013). Canned tuna fish has been consumed regularly since the 1950s in countries that have experienced the highest rates of breast cancer incidence, and its relationship to breast cancer has been unrecognized and understudied (Byrne et al., 2013; FAO, 2014c; Gerstenberger et al., 2010; Globefish Research Program, 2004; Sala-Vila & Calder, 2011).

The purpose of this population-based, case-control study was to measure methlymercury exposure via canned tuna fish consumption and to examine its association to breast cancer in 2003-2004 and 2005-2006 NHANES surveys. The primary goal was to measure evidence of the association between canned tuna fish and breast cancer and blood mercury level and breast cancer. A secondary goal was to examine the social determinants of canned tuna consumption. In this representative (weighted) population-based sample of 138,747,398 adult females, 3% or 4,153,240 women reported being diagnosed with breast cancer and 97% or 1,334,594,157 women did not. Please note the number of women with and without breast cancer did not sum to the total due to NHANES complex weights.

A logistic regression model controlling for covariates alcohol, age at screening, race/ethnicity, education level, BMI, ever pregnant, ever breastfed, ever taken birth control pills, ever taken female hormones, pack years of smoking, and age at first menstrual period resulted in women who reported eating canned tuna at one level of increased frequency (of 11 frequencies) having a 6.8% increased odds of being diagnosed with breast cancer (p =. 000 OR 1.068 and 95% CI 1.067-1.069). Logistic regression analysis also resulted in women with a .01 Ug/L increase in total blood mercury level having a 0.2% increased odds of being diagnosed with breast cancer (p =. 000 OR 1.002 and 95% CI 1.002-1.003). To help put the sensitivity of blood mercury findings in perspective, the mean total blood mercury level for participants was 1.64 Ug/L, SD 1.99, mode .23, with range of 33, minimum of .20 and maximum of 33.20.

In an examination of descriptive statistics, I found high annual household income of $75000+ USD per year, higher educational level, the race/ethnicity of non-Hispanic White and non-Hispanic Black, and the third highest of five adult age groups, 43- 57 years, were most frequent canned tuna consumers. The strongest trends were reflected in the variable of education. As educational level increased, so did the percentage of canned tuna consumption levels over baseline. Those in the highest educational category of college graduates and above were the most frequent high-level canned tuna consumers with a 8.3% above baseline.

Interpretation of Findings

Findings of a 6.8% increased odds of breast cancer for each reported increase in frequency level of canned tuna consumption (of 11) (p =. 000 OR 1.068 and 95% CI

1.067-1.069) indicate an association between methylmercury exposure via canned tuna fish and breast cancer. Findings that women with only a .01 increase in Ug/L total blood mercury level had a 0.2% increased odds of being diagnosed with breast cancer (p =. 000 OR 1.002 and 95% CI 1.002-1.003) also indicates an association between total blood mercury level and breast cancer.

The covariates of non-Hispanic white (p = .000 OR 2.276, 95% CI 2.258- 2.294), non-Hispanic black (p = .000 OR 1.787, 95% CI 1.772- 1.802), ever being pregnant (p = .000 OR 1.050, 95% CI 1.046- 1.054), ever taking birth control (p = .000 OR 1.510, 95% CI 1.506- 1.0514), ever taking female hormones (p = .000 OR 1.409, 95% CI 1.407- 1.410), age of first menstrual period (p = .000 OR 1.470, 95% CI 1.469- 1.471), and age at screening (p = .000 OR 1.074, 95% CI 1.074- 1.074) resulted in increased odds of being diagnosed with breast cancer. The covariates of Mexican American (p = .000 OR 0.940, 95% CI 0.930– 0.950), other Hispanic (p = .000 OR 0.354, 95% CI 0.328- 0.338), Other Race/Multiracial (p = .000 OR 0.940, 95% CI 0.930– 0.950), smoking pack years (p = .000 OR 0.988, 95% CI 0.988- 0.988), BMI (p = .000 OR 0.963, 95% CI 0.963- 0.963), drinking 12 alcoholic drinks in last year (p= .000 OR 0.995, 95% CI 0.993- 0.997), and education level (p= .000 OR 0.863, 95% CI 0.862- 0.863) resulted in decreased odds of being diagnosed with breast cancer.

The majority of covariate findings are consistent with other breast cancer studies. DeSantis et al.'s (2014b) summarized breast cancer statistics in the U.S. and found highest breast cancer incidence among non-Hispanic Whites then non-Hispanic Blacks. The risk factors of ever being pregnant, taking birth control, taking female hormones,

earlier age of first menstrual period and age are established breast cancer risk factors (University of California San Francisco Medical Center, 2015). Studies of the covariate BMI have shown a small decrease in breast cancer risk for those who are overweight or obese prior to menopause and a small increase in breast cancer risk for those who are overweight or obese following menopause (Nelson et al., 2012; University of California San Francisco Medical Center, 2015). The larger proportion of participants of pre menopausal age and lack of specification of the BMI variable by pre or post menopausal status would explain the decreased odds of breast cancer with increase in BMI among participants.

The covariate of education is often used in breast cancer studies as a proxy or as part of measuring socioeconomic status (Herndon, Kornblith, Holland, & Paskett, 2013). However, this association is related to other factors that are more common among those of higher education/ socioeconomic status, for example age at first birth, number of births and BMI (Heck & Pamuk, 1997). In their study of the relationship between education and breast cancer Heck & Pamuk (1997) found that after they adjusted for reproductive covariates and height in their analysis, the direct relationship between education and breast cancer was reduced from a "direct dose-response association" (p. # 1) to one that was no longer statistically significant. This studies findings of decreased odds of breast cancer as education level is likely related to the oversampling of lower income white Americans by NHANES. Descriptive statistics show that almost 10% more controls then women with breast cancer had a college degree or higher then and 13% more controls then women with breast cancer had incomes at 75,000 or more. Findings that one

increase in smoking pack years resulted in slightly decreased odds of breast cancer is consistent with the weak and mixed results of this association within the body of cigarette smoking and breast cancer research (Gaudet et al., 2013). Studies of alcohol consumption and breast cancer have found evidence of association for those that consume 2 to 3 or more alcoholic beverages per day (University of California San Francisco Medical Center, Breast Cancer Risk Factors, 2015). The alcohol variable used for this study was limited to two categories of those that drank 12 or more drinks per year and those that did not. It is likely the inability to categorize and compare frequent alcohol consumers resulted in inadequate measurement for this covariate.

Mercury and Cancer

Although methylmercury via fish consumption accounts for approximately 90% of all human mercury exposure (NJDEP, 2010) and the EPA and IARC have recommended further study of mercury and cancer (Crespo-Lopez et al., 2009), the few population-based studies on this association have focused on occupational settings with mercury chloride (not methylmercury) as the primary mercury exposure (WHO, 1997d). Results from this study support the EPA and IARC recommendations. However, positive results from this large, representative, population-based study should be interpreted to recommend that future studies specify methylmercury exposures with the outcome of breast cancer.

Fish and Methylmercury Exposure

The widespread popularity of canned tuna fish has established tuna as the single most consumed fish in the world (Globefish Research Program, 2004; NJDEP, 2010).

Because the majority of tuna canned are of the medium or small sized varieties and are believed to be of moderate to low methylmercury concentration (UNEP, 2002) its importance as a source of methylmercury exposure is often overlooked (Gerstenberger et al., 2010). Although MeHg accumulates over time in the body, research of methylmercury exposure via fish has predominantly focused on locally caught sporting fish, especially in water bodies near mercury releasing industry, and types of commercial fish containing the highest methylmercury concentrations that are less frequently and less widely consumed than canned tuna (Gerstenberger et al. 2010). The significance of the association from this study's analysis, widespread regular consumption of canned tuna fish and elevated levels of MeHg described in my literature review, provide further evidence that canned tuna fish is the primary vehicle of MeHg exposure in the human population.

Methylmercury Content of Canned Tuna Fish

MeHg concentrations in canned tuna fish are variable and dependent on where the tuna is caught, its size and age, the part of the tuna canned, and the type of tuna used (UNEP, 2002). However, this information remains unmonitored and unavailable to government or consumers (Gerstenberger et al., 2010; Sala-Vila & Calder, 2011). The FDA has demarked 0.5 parts per million wet, ug/g (ppm) and the EPA and other countries 0.3 ppm as the level of concern for MeHg in fish and seafood consumption (Burger & Gochfeld, 2006; Karimi et al., 2012). The FDA has identified 1.0 ppm as the level of action for MeHg content of fish (Burger & Gochfeld, 2006; Gerstenberger et al., 2010; Shim et al., 2004). The FDA uses a mean estimate of 0.117 ppm MeHg for each

can of tuna fish for calculating public health exposure advisories and estimates (Burger & Gochfeld, 2004). Though the body of knowledge of MeHg levels in canned tuna remains sparse (Burger & Gochfeld, 2006), results from my literature review were concerning. Below is a summary of findings from some large more recent studies conducted in the U.S.

Burger and Gochfeld (2004) tested total mercury in 168 cans of tuna obtained from a grocery store in New Jersey from 1998-2003. White canned tuna had significantly higher levels of MeHg (mean 0.407 ppm) than light (mean 0.118 ppm). The white (albacore) solid canned tuna's mean MeHg was 0.429 ppm, SD 0.164, median 0.4 ppm, and range 0.018- 0.783 ppm. The white (albacore) chunk tuna's mean MeHg was 0.355 ppm, SD 0.166, median 0.315 ppm, and range 0.027- 0.997 ppm. Light (skipjack) canned tuna varieties (chunk and solid) had a combined mean MeHg of 0.118 ppm, SD 0.099, median 0.087 ppm, and range 0.015- 0.447 ppm. One in four cans of white (albacore) tuna exceeded the maximum allowable level of 0.5 ppm designated by the FDA. The FDA mean of 0.117 ppm MeHg in canned tuna is significantly lower than the levels found in white (albacore) tuna (mean 0.407 ppm) by Burger and Gochfeld, 2004.

In their study of mercury in commonly consumed canned seafood, Shim et al. (2004) tested 240 cans of tuna collected in 2003 from grocery stores in the Lafayette, Indiana area. Shim et al. found mean MeHg levels for light tuna at 0.54 ppm and white albacore tuna at 0.711 ppm. In their study of heavy metal concentrations of randomly selected canned fish purchased in Montgomery, Alabama and Atlanta, Georgia et al., (2005) collected 29 cans of tuna, representing 9 brands. The authors found significant

variation in the concentrations of MeHg and that canned tuna had "unusually higher levels of mercury compared to any other brand of fish" (page number) including seven times higher concentration than canned mackerel or pink salmon and four times higher than canned herring. Mean MeHg concentrations ranged from a high of 0.482 ppm for Bumble Bee white tuna to a low of 0.082 for Blue bay tuna. Tuna's labeled white or Albacore had highest MeHg mean concentrations (0.482 ppm Bumble Bee white, 0.436 ppm Star-Kist white, 0.430 ppm Blue Bay white, and 0.424 ppm Star-Kist Albacore) and those labeled tuna or light/chunk light had lower concentrations, though with high variation (0.291 ppm Featherweight tuna, 0.288 ppm Bumble Bee light, 0.184 ppm Chicken of the Sea Chunk light, 0.110 ppm Chicken of the Sea tuna, and 0.082 ppm Blue Bay tuna). Georgia et al., (2005) suggest moderate consumption of fish, especially by high-risk groups, and conclude that the widespread and high level of consumption of tuna fish may pose a significant health threat.

Gerstenberger et al., (2010) describe that methylmercury exposure via consumption of canned tuna is significantly understudied and assumed to be of low MeHg concentration. The authors tested three brands and types of canned tuna collected from a grocery store in Las Vegas, Nevada monthly from 2005 to 2006. Significant differences in MeHg concentrations by brand and type were found and 55% of the 155 cans of tuna had MeHg levels above the EPA recommended consumption level of 0.5 ppm and 5% had MeHg levels above the FDA action level of 1.0 ppm. The authors suggest MeHg brand differences may be related to where the fish were caught, which is "confidential and not available to the consumer" and the inclusion of different types (and

sizes) of unidentified tuna used by different brands under the headings white, light, and chunk. Gerstenberger et al., (2010) conclude that more information about where tuna is caught, which type of tuna was used, and more stringent regulations are needed to more accurately define methylmercury exposure and protect consumers in the U.S.

In his report regarding methylmercury exposure in school lunches, Groth (2012b) describes that U.S. children eat twice as much canned tuna as any other kind of fish, canned tuna is an integral part of school lunch programs, and describes being the first to directly test methylmercury levels in canned tuna used for school children. Groth (2012b) tested 59 cans of tuna from schools in 11 states and found that the 48 samples of light tuna had a mean MeHg level of 0.118 ug/g with range 0.020 to 0.640 ug/g and the 11 samples of albacore had a mean MeHg level of 0.560 ug/g with a range of 0.190 to 1.270 ug/g. Findings of light tuna were similar to the mean estimate used by the FDA (0.128 ug/g) and albacore was significantly higher then the mean used by the FDA (0.350 ug/g). As a result, Groth (2012b) recommended that U.S. school children should not consume albacore tuna at all, small sized children should consume tuna once or less per month, children who love eating tuna should be limited to two meals per month, subsidies for canned tuna in school lunch programs should be discontinued, methylmercury means and advisories should be updated and not identify light tuna as low mercury, and children who eat tuna once a week or more should undergo blood monitoring for methlymercury.

A database created by Karimi et al., (2012) of all known mercury data of commercial fish in the U.S. examined the concentrations, exposure, and accuracy of public health warnings. The authors included data from small studies, monitoring

programs, and the literature and describe their database as the largest and most complete to date. The authors found that mean MeHg concentration data on 1,362 cans of albacore tuna was 0.328 ppm (range 0.113- 0.955 ppm), and half of the samples exceeded the EPA recommended level of 0.3 ppm MeHg. The authors suggest mean MeHg levels used by the FDA to educate the public are based on small older studies and are too low. For fish eaten frequently (e.g. canned tuna) MeHg estimates and public health warnings should take into consideration the high variability of MeHg content and how often they are consumed (Karimi et al., 2012).

Results from the literature review and logistic regression analysis of this study should provides evidence that MeHg exposure from canned tuna fish is likely significantly higher, and therefore a far more substantial threat to public health than currently advised or previously understood. Consumers should be accurately informed.

Fish, Canned Tuna, and Breast Cancer

Romeiu (2011) described that diet was an important consideration in understanding geographic differences in breast cancer incidence. Processes involved with the etiology of breast cancer, for example hormone or inflammation, can be triggered by dietary variables. The regional differences in breast cancer incidence may be related to differences in consumption of foods involved with breast cancer etiology processes (Romeiu, 2011).

The most common source of human mercury exposure, canned tuna fish, is typically absent or poorly delineated in the current body of fish and breast cancer research (Florea & Busselberg, 2011; Hjartaker, 2003; Karimi et al., 2012; McElroy et

al., 2004; Yaghoubi & Barlow, 2007; Zadnick & Poompe-Kirn, 2007). The majority of fish and breast cancer research tests fish consumption for its protective role in breast carcinogenesis (Florea & Busselberg, 2011; Hjartaker, 2003; Karimi et al., 2012; McElroy et al., 2004; Yaghoubi & Barlow, 2007; Zadnick & Poompe-Kirn, 2007). Holmes, James and Levy (2009) described the body of mercury exposure and health outcome research as limited and concluded that the evidence suggests that populations exposed to low levels of methylmercury via seafood are at highest risk of adverse health outcomes.

In their literature review of the body of knowledge on the relationship between breast, colorectal, and prostate cancers and fish consumption, Sala-Vila and Calder (2011) reviewed 106 studies. Overall, 53 out of 273 measured associations found a decreased risk of cancer from fish consumption and 12 out of 273 found increased risk. Exposures were grouped primarily as total fish, but also poached, dried, lean, fatty, salmon, and fish plus shellfish. Sala-Vila and Calder (2011) found that the majority of studies found no association or increased risk of breast cancer when type of fish, disease stage, and pre or post menopause variables were not specified. Sala-Vila and Calder (2011) propose numerous reasons for the high variability and likely confounding in findings of fish consumption and breast, prostate, and colorectal cancer research. The preponderance of exposures measured as total fish intake greatly limits and understates the differences in fish and their plausible carcinogenic or protective pathways (Sala-Vila & Calder, 2011).

It is plausible that lack of differentiation of the canned tuna variable for its probable role as a breast carcinogen may explain some of the unidentified confounding present in fish and breast cancer research (Daniel et al., 2011; Hjartaker, 2003; Karimi, et al., 2012; Sala-Vila & Calder, 2011). This study's findings of the positive association between canned tuna consumption, total blood mercury, and breast cancer should serve as a catalyst to increase concern that canned tuna fish may have a major role as a confounder in the body of fish and breast cancer literature.

Although the findings of association in this large population-based study are of strong significance, evidence was not be interpreted to advocate women eat less canned tuna fish to reduce risk of breast cancer. Additional studies confirming this association are needed and suggested to establish canned tuna fish and total blood mercury level as breast cancer risk factors.

Social Determinant Parallels between Canned Tuna Fish and Breast Cancer

Prevalence of breast cancer by country and region varies by more than 500 percent and closely reflects geographic differences in mortality (Jemal et al., 2010). North America, Australia, New Zealand, Northern Europe and Western Europe have the highest breast cancer incidence (Jemal et al., 2011). In 2004, Bray et al. described a marked variance in worldwide breast cancer incidence attributable to differences in reproductive, nutritional, and environmental factors. Studies of lower incidence populations (e.g. Asian and southern European) migrating to higher incidence populations (e.g. Australia and the United States) showed that within a generation, breast cancer risk increased significantly to parallel incidence rates of the migrants new higher

risk country. Female breast cancer incidence increases were especially marked for those who relocated from low-risk to high-risk areas in childhood (Bray et al., 2004).

The widespread introduction of the canned tuna fish industry in the 1950's and high consumption levels of Europe and the U.S. provide an interesting and unexamined parallel to historic and geographic differences in breast cancer prevalence (Boffetta et al., 1993; Cancer Research UK, 2013; FAO, 1996a; FAO, 2004b; Karimi et al., 2012; Miyake et al., 2010). In the 20th century environmental methylmercury levels were the highest in recorded history and accumulated in the bodies of large tuna fish (Boffetta et al., 1993; Rahimi et al., 2010; WHO, 2008c, p. 29-30). Starting in the 1950s large and long living varieties of tuna fish were canned, and became the most commonly consumed fish item in the United States and Europe, and the primary source of human methylmercury exposure (FOA, 1996; Hamilton, et al., 2011; Mongruel et al., 2010; NJDEP, 2010). Also starting in the 1950's, the U.S. and Europe experienced significant geographically demarked elevations in breast cancer prevalence (Cancer Research UK, 2011; Yaghoubi & Barlow, 2007).

Since widespread introduction of canned tuna to the marketplace the U.S. and Europe have comprised greater than 60% of the worldwide canned tuna market (FOA, 1996; Hamilton et al., 2011; Mongruel et al., 2010). Specifically, it appears the canned tuna with the highest methylmercury levels, albacore, may have been preferred and consumed most often by highest breast cancer incidence areas in the Northeastern U.S., Western and Northern Europe, and possibly Australia/New Zealand (Bray et al., 2004; Campbell & Owen, 1994; Cancer Research UK, 2011; GLOBEFISH, 2004; Groth,

2012b; Jemal et al., 2010; Livsmedelsverket, 2004; Nelson, 1989; EPA, 1997c; USDA, 2012).

Another parallel between canned tuna fish consumption and breast cancer incidence is sociodemographic (Daniel et al., 2011; Beiki et al., 2012; EPA, 1997c). Previous studies have shown that consumption of canned tuna fish is highest among Caucasian women of higher income and education levels. Higher income and education level Caucasian women also experience the highest incidence of breast cancer (Beiki et al., 2012; Daniel et al., 2011; EPA, 1997c).

The findings from descriptive statistics of this study confirm social determinant parallels between canned tuna fish consumption and breast cancer incidence. Both occur more frequently by the factors of race/ethnicity (both highest in non-Hispanic white and non-Hispanic black) (DeSantis, Ma, Bryan & Jemal, 2014b), higher income (DeSantis, Ma, Bryan & Jemal, 2013a), and higher educational levels (Goldberg, et al., 2015). The social determinant of age provides knowledge that the age group of 43-57 consumes the most canned tuna and was not expected to parallel breast cancer incidence because methylmercury accumulates in the body over time and the incubation period to breast cancer is unknown.

Carcinogenesis Theory

Carcinogenesis theory is a conceptual framework based on evidence that estrogen is primary to breast cell development and cumulative exposure to estrogen increases breast cancer risk (Henderson et al., 1988). In 1998, Henderson et al., (1998, p. # 248) described estrogen as the “primary stimulant for breast cell proliferation”. They explain

that increased risk among most known risk factors, including age of menarche, not breast-feeding, hormone therapy, and advanced pregnancy age increase the number of years a woman is exposed to higher levels of estrogen. Henderson et al., (1998, p. # 248) hypothesized that "cumulative exposure of breast tissue to bioavailable estrogens" is what determines breast cancer risk.

In 1993, Davis et al. published a medical hypothesis that exposure to natural or synthetic compounds which effect estrogen may be a significant cause of geographic differences and increased incidence in breast cancer. In their review of current evidence of the mechanisms by which environmental metals activate estrogen (metalloestrogens) in breast cancer, Byrne et al. (2013) describe that current understanding suggests that the high incidence of breast cancer is caused to some degree by exposure to environmental estrogens.

The positive findings of strong association for research questions 1 and 2 should be interpreted to support the assumptions underlying carcinogenesis theory applied in this study, and most specifically that exposure to the metalloestrogen methylmercury increases the risk of breast cancer (Byrne et al., 2013).

Medical Geography Theory

Medical geography is a concept that focuses on investigation of the cultural, social and geographic environment to understand spatial differences and etiology of disease (Paul, 1985). The seeds of medical geography can be traced back to the Greek physician Hippocrates who identified the importance of environment to human health (Harvard University Library, Contagion, Historical View of Diseases and Epidemics,

2014). Considered the father of modern Epidemiology, John Snow, applied the concept of medical geography in the efforts to map and understand the spatial environment surrounding the Cholera outbreak in London in 1854 (McLeod, 2000). Findings from this study's exploration of the social determinants of canned tuna fish consumption validate the preliminary historic, social, and geographic parallels between canned tuna consumption and high breast cancer incidence regions identified and explored in the literature review from a medical geography perspective.

Limitations of the Study

Limitations of this study related to breast cancer include the inability to control for the major breast cancer risk factors of BRCA genes, family history of breast cancer, age of menopause, diabetes, alcohol, and personal history of breast cancer and numerous additional minor breast cancer risk factors, for example radiation exposure and breast density. Though consumption of canned tuna fish has been established as the primary source of methylmercury exposure in humans (Iavicoli et al., 2009) the inability to precisely and cumulatively measure or estimate MeHg exposure from canned tuna fish is a primary limitation of this study. Because of the strength of association specific to canned tuna frequency and breast cancer, and blood mercury level and breast cancer, confounding from other sources of MeHg does not appear present but is certainly possible.

Additional potential limitations reflect larger questions within the body of nutritional questionnaire research and NHANES. Archer, Hand and Blair (2013, p. # 1) reported on significant methodological limitations of NHANES after finding that the

validity of reported caloric intakes from 1971-2010 for more then 55% of men and 65% of women "were not physiologically plausible" due to underreporting. Archer, Pavela and Lavie (2015) questioned the efficacy and accuracy of memory based food questionnaires widely used in nutrition research. The authors explain that years of numerous changes to dietary guidelines based on evidence from memory based food questionnaires, may have in hindsight caused population based harm. For example, advice to decrease fat contributed to an increase in obesity. Archer, Pavela and Lavie (2015) describe evidence that fundamental flaws exist in memory based surveys throughout the literature, but what and where these flaws lie are unknown.

Recommendations for Action

As a result of this study I have two recommendations for action. First, results from this study's literature review provide consistent and concerning evidence that long term MeHg exposure from canned tuna fish is likely significantly higher and therefore a more dangerous public health concern then reflected in current advisories by the FDA and EPA. Efforts to understand and advise the public on MeHg exposure should be reprioritized to reflect the role of canned tuna fish as the primary vehicle of MeHg exposure in the human population. Public advisories of MeHg should be updated to accurately reflect findings from the body of MeHg research. Second, given the strength of findings of the association between canned tuna fish frequency, total blood mercury level, and breast cancer in this large population and demographically representative sample, the individuation of canned tuna fish in future nutritional, breast cancer, and MeHg research is imperative. Future individuation of the canned tuna fish variable is

critical to accurately assess methylmercury exposure levels and the impacts of these exposures to human health.

Recommendations for Future Research

Based on this study's results, additional population-based studies of blood mercury level; canned tuna fish consumption and the outcome of breast cancer from a carcinogenic theory perspective are recommended. Studies of canned tuna fish consumption and/or blood mercury level and the outcomes of estrogen receptor positive or negative breast cancer are also recommended to provide an additional level of specificity to support or refute this association via carcinogenesis theory.

Because of this study's findings of the positive association between canned tuna consumption and breast cancer and the literature review it is recommended that canned tuna fish be more robustly considered as a possible major confounder in fish and breast cancer research.

It is understood that this study is the first to identify and examine the historic, geographic, and social/cultural relationship between canned tuna fish consumption and breast cancer from a medical geography perspective. Additional inquiry into these parallels from a medical geography perspective may provide new insights into the geographic differences in breast cancer incidence and is recommended.

Results from the literature review summarizing MeHg levels in canned tuna fish reflects a small body of literature with significant variability and significantly higher MeHg exposure then assumed in government advisories. Additional studies of MeHg

levels in specific types of canned tuna fish are recommended to better understand exposure levels and provide more accurate recommendations for the public.

However, the individuation of canned tuna fish in future nutritional, breast cancer, and MeHg research is the most essential research recommendation based on findings from this studies analysis and literature review. Future specification of the canned tuna fish variable is the only way to accurately assess health outcomes associated with its role as the most commonly eaten fish and primary route of MeHg exposure in humans.

Social Change Implications

This research contributes to positive social change by providing support for better understanding and specification of canned tuna fish in future nutrition, fish, and breast cancer research and better identification of methylmercury levels in canned tuna fish for public knowledge. These findings contribute insight to current understanding of canned tuna as a potential confounding variable in fish and breast cancer research. Findings of significant association between canned tuna fish consumption frequency, blood mercury level, and breast cancer provides evidence supporting the role of methylmercury as a metalloestrogen in breast cancer etiology via carcinogenic theory. The parallels of the educational, income, and race/ethnicities of canned tuna fish consumption and breast cancer incidence found in this study suggests further examination of social and geographic differences from a medical geographic perspective is appropriate. Lastly, findings from the literature review and research questions provide strong support for additional research of canned tuna fish related to the methylmercury, metalloestrogen,

environmental, and fish and breast cancer bodies of research, all of which currently grossly underrepresent the canned tuna fish.

Conclusion

Breast cancer is the leading cause of female cancer death and the most frequently diagnosed cancer in the world (Jemal et al., 2010). Incidence continues to increase worldwide (WHO, 2013a). Prevalence of breast cancer by country and region varies by more than 500 percent and closely reflects geographic differences in mortality (Jemal et al., 2010). Environmental (including nutrition) factors are believed to explain the dramatic geographic differences in breast cancer prevalence and account for up to half of breast cancer incidence throughout the world (California Breast Cancer Research Program, 2013). The National Cancer Institute (2013c) estimates that up to 67% of all cancer cases are affected by environmental factors.

Consumption of canned tuna fish is the primary source of human exposure to methylmercury (Iavicoli et al., 2009), a metalloestrogen that accumulates in the body over time and is presumed to contribute to breast carcinogenesis by activating estrogen (Byrne, et al., 2013). Canned tuna fish has been consumed regularly since the 1950's in high breast cancer incidence countries and its plausible relationship to breast cancer incidence has been largely unrecognized and understudied (Byrne et al., 2013; FAO, 2014c; Gerstenberger et al., 2010; Globefish Research Program, 2004; Sala-Vila & Calder, 2011).

In this population-based study representing 138,747,398 U.S. adult females, one increased level of canned tuna consumption frequency, out of 11, was associated with a

6.8% increase in odds of having breast cancer (p =. 000 OR 1.068 and 95% CI 1.067-1.069). An increase of only .01 Ug/L in total blood mercury level resulted in a 0.2% increased odds of having breast cancer (p =. 000 OR 1.002 and 95% CI 1.002-1.003).

These study results and the literature review provide strong evidence to support the likelihood that MeHg exposure from canned tuna is significantly higher then reflected in current advisories, that canned tuna fish be considered more robustly as a confounder in fish and breast cancer research, that additional population-based studies of blood mercury level, canned tuna fish and breast cancer be undertaken, and further exploration of the geographic differences in canned tuna consumption and breast cancer from a medical geography perspective are warranted. However, the most vital suggestion resulting from the evidence of this study is future individuation of the canned tuna fish variable in public health research questions and analysis. Current research of human exposure to MeHg, and the body of nutrition, fish, mercury and breast cancer literature customarily ignores the role of the primary MeHg exposure in humans: consumption of canned tuna fish. Future individuation of canned tuna fish is imperative to accurately assess MeHg exposure levels and the impacts of these exposures to human health.

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