

Health Consultation

Technical Support Document for a Methylmercury Reference Dose as a
Basis for Fish Consumption Screening Values (FCSVs)

**Prepared by the
Michigan Department of Community Health**

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Prepared under a Cooperative Agreement with the
U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
Agency for Toxic Substances and Disease Registry
Division of Health Assessment and Consultation
Atlanta, Georgia 30333

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Acronyms and Abbreviations

°C	degrees Celsius
°F	degrees Fahrenheit
/	per
±	plus or minus
µg	microgram
µg/kg/day	microgram per kilogram per day
µg/L	microgram/liter
µM	micromolar
8-OHdG	8-hydroxy-2'-deoxyguanosine
A	absorption factor
AC	Asian-Canadians
AOC	Area of Concern
ATSDR	Agency for Toxic Substances and Disease Registry
b	elimination constant
bw	body weight
CAS	Chemical Abstracts Service
CH ₃ Hg-Cys	methylmercury-cysteine complex
CHD	coronary heart disease
CI	confidence interval
CIA	Central Intelligence Agency
CVD	cardiovascular disease
CVLT	California Verbal Learning Test
DHA	docosahexaenoic acid
DNA	deoxyribonucleic acid
DPA	docosapentaenoic acid
<i>E. coli</i>	<i>Escherichia coli</i>
EC	Euro-Canadians
EC ₅₀	effective concentration for 50%
ELA	Experimental Lakes Area
EPA	eicosahexaenoic acid
f	fraction of daily intake in blood
FAO	Joint Food and Agricultural Organization
FAWCAC	Fish and Wildlife Contaminant Advisory Committee
FCSV	fish consumption screening value
FDA	Food and Drug Administration
g	gram
GCLC	glutamyl-cysteine ligase catalytic subunit
GCLM	glutamyl-cysteine ligase modifier subunit
GLFAW	Great Lakes Fish Advisory Workgroup
GSH	glutathione
GSTP1	glutathione S-transferase pi 1
HF	high frequency component
Hg	mercury

Hg ¹⁺	mercurous cation
Hg ²⁺	mercuric cation
HRV	heart rate variability
Hz	hertz
Ig	immunoglobulin
IMT	intima-media thickness
IOM	Institute of Medicine
JECFA	Joint FAO/WHO Expert Committee on Food Additives
kg	kilogram
KIHD	Kuopio Ischaemic Health Disease Risk Factor Study
L	liter
LC ₅₀	lethal concentration for 50%
LF	low frequency components
LOEL	lowest observed effect level
MBH	Maine Bureau of Health
MDCH	Michigan Department of Community Health
MDEQ	Michigan Department of Environmental Quality
MeHg	methylmercury
METAALICUS	Mercury Experiment to Assess Atmospheric Loading in Canada and the United States
MFCAP	Michigan Fish Consumption Advisory Program
MFCMP	Michigan Fish Contaminant Monitoring Program
mg	milligram
MoE	Ministry of the Environment
MRL	minimal risk level
MT	metallothionein
MTT	3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyltetrazolium bromide
NES	Neurobehavioral Evaluation System
ng	nanogram
ng/L	nanogram per liter
ng/m ²	nanogram per square meter
ng/m ³	nanograms per cubic meter
NHANES	National Health and Nutrition Examination Survey
NOAEL	no observed adverse effect level
NRC	National Research Council
PCBs	polychlorinated biphenyls
pg	picogram
PND	postnatal day
POUCH	Pregnancy Outcomes and Community Health
PP	pulse pressure
ppb	parts per billion
ppm	parts per million
pTDI	provisional tolerable daily intake
pTWI	provisional tolerable weekly intake
PUFAs	polyunsaturated fatty acids
R	ratio of cord blood to maternal blood

RfD	reference dose
RR	mean R-R intervals
SCDS	Seychelles Child Development Study
SRB	sulfate reducing bacteria
TDI	tolerable daily intake
U.S.	United States
U.S. EPA	United States Environmental Protection Agency
V	volume of blood in the body
WHO	World Health Organization
WRAVMA	Wide Range Assessment of Visual Motor Abilities

Disclaimer

This technical support document includes and relies on scientific information that was not available to the Agency for Toxic Substances and Disease Registry (ATSDR) when the Toxicological Profile for Mercury was finalized in 1999. This document is not intended to replace ATSDR minimal risk levels (MRLs) or recommendations.

Summary

Methylmercury is present in fish from the Great Lakes and other waterbodies in Michigan. Mercury is deposited on water and soil from the air and is converted to methylmercury by microorganisms. Fish that are older or that eat other fish can accumulate more methylmercury, which builds up in the fish meat. Blood mercury levels of sport fish eaters can increase with increasing amounts of fish consumption.

There are two historical instances of high methylmercury exposure. One was in Iraq, from eating grain, and the other was around Minamata Bay in Japan, from eating fish. Both groups developed symptoms from eating methylmercury. Symptom in the Iraqi children included slowed or slurred speech, and impairment of motor functions and mental development. People around Minamata Bay developed symptoms that included blurred vision, hearing impairment, clumsiness of hands, slurred speech, and mental retardation. It is unlikely that people in Michigan will eat enough fish to have a similarly high exposure. However, the data from these poisoning events suggest that unborn and young children are especially at risk of developing effects from methylmercury exposure.

Following these acute poisoning events, two long term studies were conducted on methylmercury exposed populations. One population was from Seychelles and the other was from the Faroe Islands, but both were exposed to lower levels of methylmercury than the people in Iraq and Minamata Bay. The Seychelles people eat methylmercury in fish and the Faroese eat methylmercury in both fish and pilot whale. In both these studies, mercury levels were measured at birth and the children were followed into their teens. Neurological and cardiovascular effects of the prenatal mercury exposure were observed in these children. Therefore, the Michigan Department of Community Health (MDCH) concludes that eating unlimited amounts of certain sport-caught fish from lakes in Michigan throughout the year could harm people's health.

Fish consumption advisories may be required for certain fish species at specific locations. The current screening values used by the State of Michigan for mercury are 0.5 parts per million (ppm), for restricted consumption, and 1.5 ppm, for no consumption. This document recommends use a new reference value to generate updated screening values for mercury in sport-caught fish from Michigan waters.

Purpose and Health Issues

The Michigan Department of Community Health (MDCH) is in the process of updating the fish consumption screening values (FCSVs) used in the Michigan Fish Consumption Advisory Program (MFCAP). All forms of mercury, including methylmercury in fish, can damage the nervous system. The purpose of this document is to review the recent literature on methylmercury and recommend changes in the MFCAP, if necessary, to ensure that the consumption advice remains protective of public health.

Background

Introduction

Mercury occurs naturally in the environment. The most common forms of mercury are metallic mercury, cinnabar ore, mercuric chloride, and methylmercury (ATSDR 1999). The Latin name for mercury is *hydrargyrum*, which translates to liquid silver (Clarkson et al. 2007).

Inorganic mercury, both as mercurous (Hg^{1+}) and mercuric (Hg^{2+}) cations, is bound to other elements and usually is a white powder or crystals. Cinnabar (mercuric sulfide), however, is red and can turn black after exposure to light (ATSDR 1999). Cinnabar contains mercuric sulfide, along with 25 trace elements. Cinnabar is a component of Chinese and Indian Ayurvedic medicines (Liu et al. 2008). Past uses of inorganic mercury compounds include components of fungicides, skin-lightening creams, topical antiseptics, and dyes (mercuric sulfide and mercuric oxide to color paint; mercuric sulfide as a red coloring agent in tattoo dyes) (ATSDR 1999).

Organic mercury, which includes methylmercuric chloride, dimethylmercury, phenylmercuric acetate, and methylmercury, is mercury bound to carbon. Two forms of organic mercury, methylmercury and ethylmercury, were used as fungicides on seed grains until the 1970s. In 1991, use of phenylmercuric compounds as antifungal agents in interior and exterior paints was banned due to the release of mercury vapors during use (ATSDR 1999).

Mercury release in the environment is due to both natural and human activities. Natural releases of mercury occur during volcanic eruptions, forest fires, erosion of mercury-bearing soils and rocks, and evaporation of mercury-containing water. In the United States (U.S.), the primary source of mercury due to human activity is combustion of coal, while in Canada smelting of non-ferrous metals is the primary source. The second largest source for both countries is incineration of municipal and medical wastes (Mohapatra et al. 2007). Approximately 80% of mercury released from human activities is as elemental mercury released to air. Standard levels of mercury in the outdoor air in urban settings are 10-20 nanograms per cubic meter (ng/m^3). Non-urban settings have mercury levels of approximately $6.0 \text{ ng}/\text{m}^3$ or less. Organic mercury compounds can be produced in the environment. Methylmercury is the most common organic mercury that microorganisms produce (ATSDR 1999).

Mercury concentrations are low in fruits and vegetables and mercury uptake by plants in the soil is low (Health Canada 2007). Between 5 and 135 nanograms per square meter (ng/m^2) per year of methylmercury is generated from mercury vapors that were absorbed by foliage (Miller et al.

2005). However, mushrooms can accumulate high levels if grown in contaminated soil and mercury accumulation is possible in the roots of certain plants (ATSDR 1999). For example, cattails have been used in phytoremediation of mercury-contaminated sites and efficiently removed the mercury from the soil (Shipp et al. 2000).

People commonly encounter mercury either by eating fish or marine mammals, which contain methylmercury in the muscle tissue, or possession of dental amalgam fillings containing mercury (ATSDR 1999). Measurement of blood levels of mercury can determine either recent or ongoing exposure (Risher and DeRosa 2007). The blood mercury levels of sport fish eaters are significantly associated with levels of fish consumption (Mohapatra et al. 2007).

Physical and Chemical Parameters

Mercury is a naturally occurring element and is present in the earth's crust at 0.5 parts per million (ppm), although the concentration varies based on location. It is usually found as mercuric sulfide. Algeria, China, Czech Republic (formerly part of Czechoslovakia), Finland, Kyrgyzstan, Mexico, Morocco, Russia, Slovakia, Slovenia, Spain, Turkey, and the Ukraine are major mercury producing countries (ATSDR 1999).

Metallic mercury is a liquid at room temperature with a melting point of around -38 °Fahrenheit (F), which is -38.87 °Celsius (C). Mercury and mercury compounds usually have no odor (ATSDR 1999). Detectable mercury vapor can form at temperatures as low as 47.3 °F (8.5 °C) (Asano et al. 2000) and the vapor is heavier than air (Cherry et al. 2002).

Mercury (Hg^{2+}) binds to human serum albumin. The primary binding site is the sulfur atoms in the number 34 cysteine. Secondary sites for organic mercury binding are the sulfur in disulfide bridges, negatively charged carboxylate oxygen, and the amide III nitrogen. The main secondary binding site is the negatively charged carboxylate oxygen (Li et al. 2007).

Appendix A provides names and Chemical Abstracts Service (CAS) numbers for several forms of mercury.

Analytical Methods

Mercury levels in fish collected in Michigan are measured by Atomic Absorption with a lower quantitation level of 0.001 ppm. Total mercury is measured, not methylmercury, in fish tissue samples because most of the mercury in fish is methylmercury (90-99% of the total mercury in biota [King et al. 2000]).

Fish Advisories

A majority (80%) of the fish consumption advisories in the U.S. are due to mercury contamination. The top 10 seafood species that comprise 80% of the U.S. seafood consumption are canned tuna, shrimp, pollock, salmon, cod, catfish, clams, flatfish, crabs, and scallops. These species generally have less than 0.2 ppm methylmercury. The Food and Drug Administration (FDA) recommends limited consumption of shark and swordfish (due to increased amounts of methylmercury in these fish). Consumption of no more than one meal (about 7 ounces) per week is recommended for the general population and no more than one meal per month is recommended for pregnant women and women of childbearing age (ATSDR 1999). The U.S. Environmental Protection Agency (U.S. EPA) recommends a level of less than 0.3 ppm of mercury in fish for safe consumption (Hammerschmidt and Fitzgerald 2006).

Ontario, Canada has fish consumption restrictions beginning at 0.26 ppm for women and children less than 15 years of age and recommends no consumption of fish with mercury levels greater than 0.52 ppm. Consumption restrictions recommendations for the general population begin at levels greater than 0.61 ppm and no consumption is recommended for fish with mercury levels greater than 1.84 ppm (MoE 2007). These mercury levels are based on the World Health Organization (WHO) provisional tolerable daily intake (pTDI) for mercury of 0.71 micrograms per kilogram per day ($\mu\text{g}/\text{kg}/\text{day}$). Since no more than two-thirds of that intake should be from methylmercury, daily intake of methylmercury for the general population should be no more than 0.47 $\mu\text{g}/\text{kg}/\text{day}$. For women of childbearing age and young children a pTDI for methylmercury was set at 0.20 $\mu\text{g}/\text{kg}/\text{day}$ (Health Canada 2007).

Fish consumption restrictions due to mercury dominate (85% of Ontario's total restrictions) the Canadian inland lakes restrictions. Fish consumption restrictions are driven by mercury to a lesser extent in Lake St Clair and the St Clair and Detroit Rivers (21% of Ontario's total restrictions). An even smaller percentage of Ontario, Canada's restrictions in the Great Lakes are due to mercury levels. Only 9% of the Lake Superior, 2% of the Lake Erie, 6% of the Lake Huron, and 8% of the Lake Ontario restrictions are due to mercury (MoE 2007).

MDCH currently (March 2009) advises a restricted consumption with median mercury fish tissue levels over 0.5 ppm and no consumption when median mercury fish tissue levels are greater than 1.5 ppm. Advisories are issued for fish of different lengths (MDEQ 2007).

Discussion

Environmental Contamination

Atmospheric transport and deposition

Approximately 50-75% of the atmospheric mercury emissions are from human-made sources (Landis et al. 2002). Elemental mercury can remain in the atmosphere from a half year to three years in several forms, including as a reactive gas or particulate mercury. Because of this, it has the potential for hemispheric transport (Perry et al. 2005). In addition, Vanarsdale et al. (2005) identified a seasonal deposition pattern from sampling done between 1996 and 2002. Increased

mercury deposition occurred during the summer (Vanarsdale et al. 2005). An explanation for this is that mercury deposition can occur in greater amounts when temperatures are warmer. Mercury is present in smaller amounts in snow or rain/snow mixes (less than 10 to 15 nanograms per liter [ng/L]) as compared to rain (greater than 15 ng/L) (Landis et al. 2002).

Global atmospheric emissions of mercury contribute to deposition in the Great Lakes basin, and other regions of both the U.S. and Canada. Modeling studies, using 1997 data, indicated an average annual deposition of approximately 2.5 tons over the Great Lakes basin. A majority of that mercury was from long-range atmospheric transport from Asia and Europe (61%). North American sources (21%) and other sources (18%) deposited lesser amounts of mercury over the Great Lakes Basin. The authors noted that a different report, from the International Joint Commission, concluded that only about 20% of the total mercury loading to the Great Lakes was from global sources outside of the U.S. and Canada (Mohapatra et al. 2007).

Total mercury was measured in Lake Superior water in both April and August of 2000. Total mercury levels were 0.57 ± 0.07 ng/L in April and 0.47 ± 0.03 ng/L in August. Both Lake Michigan and Lake Ontario waters had slightly lower levels of total mercury, 0.32 ng/L and 0.26 ng/L, respectively, as compared to Lake Superior water. The methylmercury level in Lake Superior was 5.0 ± 0.9 picogram per liter (pg/L), which was approximately 1.1% of the total mercury (Rolfhus et al. 2003). Rolfhus et al. (2003) determined that the watershed was contributing to the mercury levels in Lake Superior.

For Lakes Michigan and Superior, atmospheric deposition represents 75% of the overall mercury loading. Based on measurements taken in 1994-1995, Lake Michigan received 16% of the mercury loading from its tributaries, while Lake Superior received 27% of mercury loading from its tributaries (Cohen et al. 2004). This finding agrees with the above conclusion by Rolfhus et al. (2003) that the watershed contributed to mercury levels in Lake Superior.

Lakes Erie and Ontario acquired mercury from historical discharges, such as from chlor-alkali (chlorine and caustic soda) production. Technological advances provided alternatives to using mercury during this production. As mercury discharges to surface water are now substantially reduced, atmospheric loading is a more relevant pathway (Cohen et al. 2004).

An Experiment in Mercury Fate and Transport

The Mercury Experiment to Assess Atmospheric Loading in Canada and the U.S. (METAALICUS) spiked a lake and its watershed with mercury to determine the mobility of mercury. Lake 658, in the Experimental Lakes Area (ELA) in northwestern Ontario, Canada, had three different stable isotopes of mercury added to three separate areas. Aircraft deposited upland and wetland spikes once each year for three years (2001-2003). A boat was used to add the lake spike every two weeks during the open water season (Harris et al. 2007). Harris et al. (2007) followed the spiked mercury for three years (2001-2003).

After three years, the three different isotopes of mercury were being transported around the ELA. Amounts of the upland spike of mercury were greater in the soil versus the vegetation in comparison to the pre-existing (not spiked) mercury levels. Larger amounts of the wetland spike

of mercury were found in the vegetation as compared to the peat. Pre-existing mercury was greater in the peat as compared to the levels in the vegetation (Harris et al. 2007).

There were larger amounts of mercury, both pre-existing and spiked, in the lake sediments versus the water column. Most of the spiked mercury in the ELA was bound to vegetation and soil. Almost all (99%) of the mercury in runoff, from both the wetland and upland areas, was pre-existing mercury. Spiked mercury represented only a small fraction of the mercury transported to the lake during the experiment (0.1% in 2001, 0.3% in 2002, and 0.6% in 2003). Mercury directly spiked into the lake was the largest contributor of spiked mercury to the lake (Harris et al. 2007).

Spiking mercury into the area did not change the amount of upland and wetland mercury transported to the lake. The mercury spiked in the wetland was not detected in biota throughout the experiment, while mercury spiked in the upland was detected in the benthos (organisms that live on the bottom of the lake) and fish only in the third year (2003). The contribution of mercury spiked to the lake to methylmercury levels increased over the three years. Mercury spiked directly into the lake had the largest contribution to mercury in biota, such as fish. However, inorganic mercury present in the lake had been accumulating for longer than one year (Harris et al. 2007).

Harris et al. (2007) concluded from their study that a decrease in atmospheric deposition of mercury would lower fish concentrations. Lakes with mercury input primarily from atmospheric deposition would have decreased levels in fish within a decade, while lakes with multiple sources of mercury would have a rapid (within years) decline followed by a slower, centuries long, decline (Harris et al. 2007).

Methylation of mercury

Inorganic mercury is 90-99% of the total mercury in sediments, but less than 1% of the total mercury in biota. In contrast, methylmercury is 1-10% of the total mercury in sediments and 90-99% of the total mercury in biota (King et al. 2000). The amount of preformed methylmercury deposited in the watershed does not account for the amount that accumulates in biota or sediments. Rather, *in situ* formation of methylmercury increases the amount that reaches higher trophic levels (Ekstrom et al. 2003). Figure 1 present cycling in a lake and the surrounding watershed.

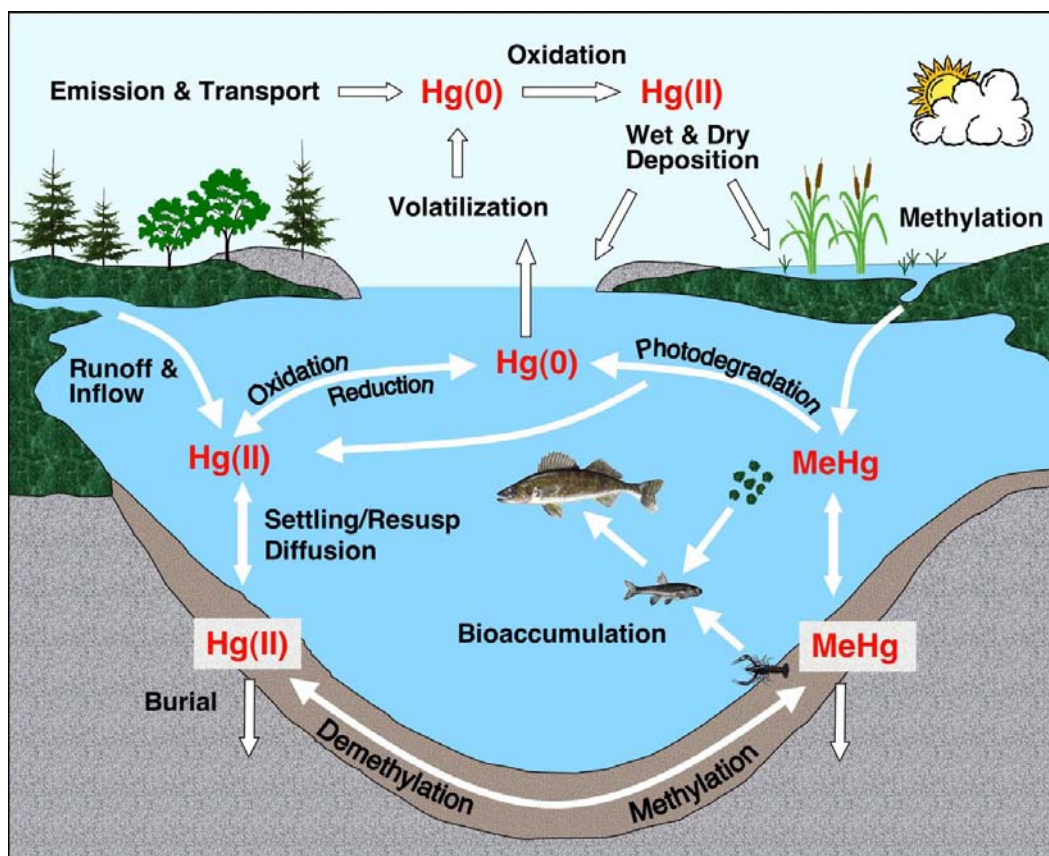


Figure 1: Diagram of mercury cycling in a lake and watershed. Taken from Engstrom (2007). Copyright 2007 National Academy of Sciences, United States of America.

Mercury methylation can be either an abiotic or biotic process. Microbial metabolism is primarily responsible for biotic methylation (Celo et al. 2006). Mercury respiration has been coupled to sulfate respiration (King et al. 2000). This has led researchers to identify sulfate reducing bacteria (SRB) as key mercury methylating organisms in the environment (Ekstrom et al. 2003).

Inorganic mercury, from atmospheric deposition, can be methylated in the water column or sediments (Celo et al. 2006). Mercury methylation does not occur as much in the water column due to lower amounts of nutrients and bacteria. The water column could potentially become more involved in mercury methylation if the volume of water containing the oxic/anoxic boundary was larger than the volume found in the surficial sediments. Methylmercury created in the water column may be more available for entry into the aquatic food web because it would not need to diffuse out of sediments (Eckley and Hintelmann 2006). Mercury methylation can also occur in periphyton biofilms (a grouping of algae and other microscopic organisms). When it does, a mix of algae, bacteria, fungi, and microinvertebrates methylate the mercury (Desrosiers et al. 2006).

Sport-caught Fish Tissue Concentrations

Methylmercury is a contaminant in both sport-caught and commercial fish around the world. Almost all of the Lakes Erie, Michigan, Huron, and Superior fish fillets sampled had detectable levels of total mercury. Table 1 presents the total mercury present in fish fillets sampled from 1986 to 2006 in Lake Erie.

Table 1: Total mercury levels (in ppm) in Lake Erie fish.

Fish species (number of fillets ¹ tested)	Year ²	Range (ppm)	Mean ± standard error (ppm)
Carp (55)	1986-2006	0.01-0.836	0.154 ± 0.020
Channel Catfish (30)	1986-2002	0.06-0.39	0.169 ± 0.017
Chinook Salmon (7)	1997	0.2-0.45	0.271 ± 0.033
Freshwater Drum (20)	1995-2006	0.12-0.61	0.278 ± 0.031
Lake Whitefish (9)	1997	0.02-0.06	0.038 ± 0.004
Largemouth Bass (10)	2006	0.036-0.23	0.091 ± 0.018
Rainbow Trout (10)	1997	0.08-0.21	0.119 ± 0.012
Smallmouth Bass (17)	1997-2006	0.11-0.38	0.203 ± 0.019
Walleye (76)	1986-2004	0.08-0.68	0.176 ± 0.012
White Bass (34)	1993-2006	0.05-0.463	0.180 ± 0.019
White Perch (20)	1995-2004	0.07-0.19	0.116 ± 0.007
Yellow Perch (40)	1993-2006	0.026-0.35	0.118 ± 0.013

¹ = Fillets are either skin-on or skin-off and vary by species

² = Data Source: Michigan Fish Contaminant Monitoring Program (MFCMP) database (2008)

The highest concentration reported by the Michigan Fish Contaminant Monitoring Program (MFCMP) for mercury from Lake Erie fillets was in a carp, however, the highest mean of the species tested was in freshwater drum. Lake whitefish had the lowest mean mercury of the species tested in Lake Erie. Most of the species have similar mean mercury levels. Table 2 presents total mercury in fish fillets from Lake Michigan fish.

Table 2: Total mercury (in ppm) in Lake Michigan fish.

Fish species (number of fillets ¹ tested)	Year ²	Range (ppm)	Mean ± standard error (ppm)
Brown Trout (82)	1986-2003	0.05-0.54	0.141 ± 0.009
Burbot (19)	1990-2001	0.19-0.52	0.364 ± 0.020
Carp (45)	1988-2004	0.1-0.47	0.262 ± 0.012
Chinook Salmon (48)	1986-1992	0.05-0.47	0.209 ± 0.012
Coho Salmon (15)	1994	0.14-0.2	0.160 ± 0.006
Lake Trout (124)	1986-1998	0.06-0.46	0.177 ± 0.007
Lake Whitefish (101)	1990-1999	0.02-0.14	0.058 ± 0.003
Longnose Sucker (31)	1988-2005	0.06-0.34	0.168 ± 0.014
Northern Pike (10)	1987	0.18-0.39	0.260 ± 0.020
Rainbow Trout (100)	1986-2004	0.03-0.4	0.125 ± 0.006
Redhorse Sucker (10)	2004	0.18-0.5	0.331 ± 0.032
Rock Bass (4)	2004	0.08-0.11	0.098 ± 0.006
Smallmouth Bass (29)	1992-2005	0.16-0.95	0.441 ± 0.039
Splake (7)	1992-1993	0.08-0.23	0.136 ± 0.019
Walleye (60)	1987-2005	0.1-1.15	0.478 ± 0.044
White Sucker (29)	1988-2005	0.04-0.3	0.147 ± 0.012
Yellow Perch (72)	1986-1997	0.05-0.39	0.126 ± 0.007

¹ = Fillets are either skin-on or skin-off and vary by species

² = Data Source: MFCMP database (2008)

The highest concentration reported by the MFCMP for Lake Michigan fillets was 1.15 ppm in a walleye. The mean mercury level (0.478 ppm) in walleye were the highest levels in all fish species tested from Lake Michigan. As seen with species tested in Lake Erie, lake whitefish had the lowest mean mercury level of all the Lake Michigan species tested. Except for a few species (carp, smallmouth bass, and walleye), levels of total mercury in fish from Lakes Erie and Michigan were similar. Table 3 presents total mercury levels in Lake Superior fish.

Table 3: Total mercury levels (in ppm) in Lake Superior fish.

Fish species (number of fillets ¹ tested)	Year ²	Range (ppm)	Mean \pm standard error (ppm)
Brown Trout (10)	1999	0.10-0.51	0.205 \pm 0.044
Burbot (14)	2006	0.044-0.671	0.228 \pm 0.042
Burbot liver (4)	2006	0.044-0.119	0.115 \pm 0.028
Chinook Salmon (27)	1988-2000	0.13-0.73	0.306 \pm 0.026
Coho Salmon (27)	1994-1997	0.05-0.23	0.111 \pm 0.010
Lake Herring (31)	1994-2007	0.03-0.219	0.088 \pm 0.009
Lake Sturgeon (3)	2000-2003	0.08-0.21	0.160 \pm 0.049
Lake Trout (148)	1984-2002	0.1-1.16	0.273 \pm 0.017
Lake Whitefish (96)	1984-2007	0.03-0.295	0.076 \pm 0.004
Longnose Sucker (10)	1998	0.07-0.31	0.167 \pm 0.029
Rainbow Trout (9)	2006	0.051-0.241	0.132 \pm 0.021
Siscowet (130)	1987-2007	0.02-1.0	0.358 \pm 0.017
Walleye (16)	2006	0.195-0.826	0.448 \pm 0.057
White Sucker (9)	1985	0.1-0.5	0.167 \pm 0.044
Yellow Perch (10)	1993	0.09-0.88	0.193 \pm 0.077

¹ = Fillets are either skin-on or skin-off and vary by species

² = Data Source: MFCMP database (2008)

The highest mercury concentration, 0.88 ppm, was in a yellow perch fillet. However, as seen with the species tested in Lake Michigan, Lake Superior walleye have the highest mean total mercury (0.448 ppm) of all the Lake Superior fish species tested. Lake Superior lake whitefish had the lowest mean mercury level. Overall, the levels in Lake Superior fish are similar to those in Lakes Erie and Michigan fish. Table 4 presents total mercury levels in Lake Huron fish.

Table 4: Total mercury levels (in ppm) in Lake Huron fish.

Fish species (number of fillets ¹ tested)	Year ²	Range (ppm)	Mean ± standard error (ppm)
Brown Trout (57)	1986-1993	0.06-0.29	0.132 ± 0.006
Burbot (4)	1990	0.04-0.19	0.105 ± 0.036
Carp (116)	1987-2004	0.04-0.33	0.134 ± 0.006
Channel Catfish (91)	1986-2004	0.03-0.56	0.146 ± 0.011
Chinook Salmon (35)	1986	0.1-0.55	0.299 ± 0.021
Chub (2)	1993	0.08	0.08 ± 0
Freshwater Drum (9)	2007	0.109-0.776	0.467 ± 0.079
Lake Trout (157)	1986-2004	0.09-0.52	0.176 ± 0.008
Lake Whitefish (75)	1992-2007	0.02-0.179	0.075 ± 0.004
Northern Pike (5)	1993	0.1-0.18	0.134 ± 0.015
Rainbow Smelt (10)	1993	0.04-0.05	0.044 ± 0.002
Rainbow Trout (20)	1991-1993	0.04-0.34	0.137 ± 0.016
Walleye (137)	1986-2004	0.05-0.56	0.183 ± 0.010
White Bass (21)	1993-2004	0.06-0.48	0.192 ± 0.022
White Perch (8)	1994	0.06-0.22	0.086 ± 0.019
White Sucker (40)	1991-2004	0.01-0.17	0.052 ± 0.006
Yellow Perch (71)	1987-2004	0.03-0.25	0.093 ± 0.004

¹ = Fillets are either skin-on or skin-off and vary by species

² = Data Source: MFCMP database (2008)

The highest concentration of mercury in a Lake Huron fillet was in a freshwater drum. Unlike the fish fillets tested from Lakes Michigan and Superior, walleye from Lake Huron do not have the highest total mercury levels. Instead, similar to data from Lake Erie fish, freshwater drum from Lake Huron have the highest mean mercury level (0.467 ppm). Also, unlike data from the other lakes, the lowest mean mercury level for Lake Huron was in white sucker. Although there are differences in the Lake Huron data, mercury levels are similar in all four lakes.

General advisories are given for sport-caught fish from inland lakes in Michigan. Women of child bearing age and children under 15 should not eat more than one meal/month of rock bass, yellow perch, or crappie over 9 inches in length and largemouth bass, smallmouth bass, walleye, northern pike, or muskellunge of any size. No one should eat more than one meal/week of rock bass, yellow perch, or crappie over 9 inches in length and largemouth bass, smallmouth bass, walleye, northern pike, or muskellunge of any size (MDEQ 2007).

Since methylmercury biomagnifies in the food web (Mason et al. 2006), carnivorous fish at the top of the food chain will have 10,000 to 100,000 times more mercury in their tissue than the surrounding water (ATSDR 1999). Biomagnification occurs when contaminant levels increase in fish as the trophic levels increase. Less of the total mercury is methylmercury in alewife (~84%), a fish in a lower trophic level, than in lobster, flounder, bluefish, and tautog, which are at higher trophic levels (~98% of the total mercury is methylmercury) (Hammerschmidt and Fitzgerald 2006).

Due to slower rates of elimination relative to the rate of dietary intake, bioaccumulation of methylmercury also occurs (Hammerschmidt and Fitzgerald 2006). Large or old saltwater fish will also bioaccumulate high levels of methylmercury (ATSDR 1999). Bioaccumulation and biomagnification resulted in fish methylmercury levels that were 1,000,000 to 10,000,000 (10^6 - 10^7) times greater than the levels found in surface water. There was 1,000,000 (10^6) times the level of methylmercury in alewife as in the water (Hammerschmidt and Fitzgerald 2006).

Kamman et al. (2005) reported on the Northeast States Research Consortium. The consortium included 24 different studies from northeastern Canada and the northeast U.S. Studies were excluded if the data was prior to 1980 or involved fish sampled from the Great Lakes or St Lawrence River. Measurement of mercury was from either fillet or whole body samples. Data was analyzed from 13 of 64 species included in this study (a total of 15,305 records). Muskellunge (0.98 ppm), walleye (0.76 ppm), white perch (0.72 ppm), and northern pike (0.64 ppm) had the highest mean mercury concentrations. Rainbow trout (0.09 ppm), sunfish species (0.16 ppm), bluegill (0.17 ppm), and brown bullhead (0.17 ppm) had the lowest mean mercury levels (Kamman et al. 2005).

The highest levels of mercury were in predatory fish at the top of the food web. Fish species that fed on invertebrates or plankton had the lowest levels of mercury. Variation in mercury levels found in specific waterbodies explained the variation in fish mercury concentrations for 12 of the 13 species analyzed. Total and dissolved organic carbon can act as a carrier for mercury. It will bind and sequester the mercury when carbon is present above a certain level (Kamman et al. 2005).

Gertenberger and Dellinger (2002) examined mercury in fish popular with the Ojibwa tribes in the upper Great Lakes. Members of the Ojibwa tribes collected whitefish, walleye, and lake trout and processed the samples as they would to eat them. Skin was removed from the walleye and lake trout fillets, while the whitefish had the scales removed but still had the skin. The walleye samples contained over 1.0 ppm mercury. Both lake trout and whitefish had lower levels of mercury than the walleye (Gerstenberger and Dellinger 2002).

Yellow perch take up methylmercury rapidly, but eliminate it slowly. Wild yellow perch were relocated from a lake with known spiked levels of methylmercury to a non-spiked lake. The relocated perch were contained in one section of the lake. The experimental perch grew less than perch native to the lake, possibly due to a greater difficulty in obtaining enough food while contained. One year after the transfer, 56% of the spiked methylmercury remained in the perch. In the confined perch, the elimination rate of the spiked methylmercury was 1.8 to 30 times slower than previously obtained laboratory rates using other small fish (Van Wallegghem et al. 2007).

Concentrations of mercury in northern pike from Isle Royale in Lake Superior declined from 2004 to 2006. Atmospheric deposition around Isle Royale in Lake Superior has occurred for a century. The reduction of methylmercury in the fish occurred without changing atmospheric deposition of mercury (Drevnick et al. 2007).

Exposure Pathways Analysis

An exposure pathway contains five elements: (1) the contaminant source, (2) contamination of environmental media, (3) an exposure point, (4) a human exposure route, and (5) potentially exposed populations. An exposure pathway is complete if there is a high probability or evidence that all five elements are present. Table 5 describes human exposure to methylmercury.

Table 5: Human exposure pathway for methylmercury.

Source	Environmental Medium	Exposure Point	Exposure Route	Exposed Population	Time Frame	Exposure
Atmospheric deposition of mercury and conversion in sediments to methylmercury	Fish (contact with sediments)	Sport-caught fish	Ingestion	Anyone who eats sport-caught fish (residents and tourists)	Past, Present, and Future	Complete
Natural and human-made emissions (inorganic mercury)	Air	Distribution to the gastrointestinal tract, then conversion of inorganic mercury to methylmercury by gut microflora	Inhalation	Global population	Past, Present, and Future	Complete

People ingest different amounts of methylmercury, and mercury, based on their choice of fish species. Concentrations of methylmercury in fish depend on species, age, size, and place in the food chain. Additionally, variation in people's internal conversion of mercury to methylmercury may expose people to differing amounts of methylmercury. Based on all of these factors, people's exposure to methylmercury may range from very low to very high.

Toxicological Evaluation

Reference Values or Regulatory Levels

The FDA action level for methylmercury in seafood is 1.0 ppm. The maximum contaminant level set by the U.S. EPA for mercury in drinking water is 2.0 parts per billion (ppb). Mercury has not been determined to be carcinogenic in people (ATSDR 1999), but the U.S. EPA lists it as a possible human carcinogen (U.S. EPA 2001A).

The U.S. EPA generated an updated reference dose (RfD) for methylmercury of 0.1 µg/kg/day in 2001. The RfD value is based on exposure estimations from levels of total mercury measured in cord blood, from a study of children (age seven) in the Faroe Islands (Grandjean et al. 1997). The critical effect was developmental neuropsychological impairment in children. This RfD utilized two uncertainty factors of 10^{0.5}, one to cover pharmacokinetic variability and uncertainty and

another to address pharmacodynamic uncertainty (U.S. EPA 2001A). Rice (2004) noted that this RfD only takes into account neurodevelopmental effects and does not address possible cardiovascular effects due to methylmercury exposure.

The Agency for Toxic Substances and Disease Registry (ATSDR) chronic minimal risk level (MRL) for ingestion of methylmercury is 0.3 µg/kg/day. This was based on an estimated no observed adverse effect level (NOAEL) of 1.3 µg/kg/day calculated from levels of methylmercury in hair. This estimated NOAEL was obtained from a Seychelles study examining the neurodevelopment of children at 66 months of age (Davidson et al. 1998; ATSDR 1999). An uncertainty factor was used for human pharmacokinetic and pharmacodynamic variability (3). A modifying factor (1.5) was also used to account for findings (lower test results in specific areas of functioning) in the Faroe studies.

Health Canada (2007) conducted a commercial fish consumption risk assessment on retail fish only. Levels of total mercury were approximately 0.5 ppm in all commercial fish sold except, shark, swordfish, and tuna. The Joint Food and Agricultural Organization (FAO)/WHO Expert Committee on Food Additives (JECFA), in 2003, set the provisional tolerable weekly intake (pTWI) for methylmercury as 1.6 µg/kg/week (equivalent to 0.23 µg/kg/day) in order to protect the developing fetus (Health Canada 2007).

Uncertainty in methylmercury effects

One caution about assessing prenatal mercury exposure in children is the possibility of latent effects becoming apparent several years after exposure. There have been several examples of latency in toxicity from methylmercury exposure, both from acute and chronic exposure (Weiss et al. 2002). Several studies carried out in non-human primates along with examples from the chronic Minamata exposure of humans have shown latent effects. Several reasons for the latency were proposed by Weiss et al. (2002). One was that the normal loss of cells during aging might be enhanced by a neurotoxic agent. Another reason could be that specific cells are more vulnerable to toxic effects and, once those cells die, the remaining cells may compensate. However, the compensating cells may eventually become “overworked” (Weiss et al. 2002). This raises the question of whether or not a mercury value deemed safe for children based on effects measured at age seven may cause effects later in life.

Along with neurological effects, there is increasing evidence that methylmercury can cause cardiovascular effects. See the Cardiovascular effects section below and the extended discussion in Appendix E. There have been several studies showing cardiovascular effects, including those seen in patients with Minamata disease and in the Faroe Islands and Seychelles cohorts. Based on the data already obtained, it appears that cardiovascular effects can occur at lower concentrations of mercury exposure than neurological effects.

Toxicokinetics

Approximately 70-80% of inhaled mercury vapors will be absorbed by the lungs and enter the bloodstream, while ingestion of metallic mercury results in less than 0.01% being absorbed by the stomach or intestines. Almost all (~95%) of the ingested methylmercury can be absorbed,

and later converted to inorganic mercury (ATSDR 1999). Organic mercury includes phenyl, methyl, and ethyl mercury compounds. Phenyl mercury rapidly degrades, while methyl and ethyl mercury compounds are stable in the body (Clarkson et al. 2007).

Methylmercury is absorbed well and spreads throughout the whole body (Castoldi et al. 2008). The gastrointestinal tract absorbs methylmercury and distributes it to all tissues, including crossing the blood/brain barrier and placenta. Methylmercury can also accumulate in scalp hair (Clarkson et al. 2007). Methylmercury, consumed in fish, distributes to tissues within 30-40 hours of a single meal of fish (Cernichiari et al. 2007). Distribution is complete within three days (Clarkson et al. 2007).

Additional discussion of mercury toxicokinetics is provided in Appendix B.

Human Biomonitoring

Mercury can be detected in human hair, blood, milk, urine, and toenails. Blood and urine levels provide a measurement of recent exposure to mercury. Hair mercury levels reflect exposure, even long-term exposure, to methylmercury as levels do not change once they are in hair. Renal and neurological markers can also be used as biomarkers for mercury exposure (ATSDR 1999). Hair mercury levels, a reflection of blood mercury, may be altered by hair treatment, color, and exposure to mercury vapor. Methylmercury binds to hemoglobin and has a half-life in the body of one and one-half to two months. Methylmercury has a greater affinity for fetal hemoglobin (Budtz-Jorgensen et al. 2004A).

Prenatal exposure to methylmercury can be estimated from maternal hair and blood. Methylmercury levels in the fetal brain correlated to maternal blood, both measured after the death of the fetus. Cord blood samples, collected after delivery, can be unreliable, as they are a secondary priority after care of the mother and infant. This means the collection time and manner varies (Cernichiari et al. 2007).

There is an association between fish consumption and mercury concentrations in hair and blood. A majority (75-90%) of mercury in fish muscle is methylmercury (Morrisette et al. 2004). High fish consumption can lead to mercury blood levels of 200 microgram per liter ($\mu\text{g/L}$) (ATSDR 1999).

Total and inorganic mercury levels in whole blood from women (ages 16 to 49) and children (ages one to five) involved in the National Health and Nutrition Examination Survey (NHANES) from 1999 and 2000 were reported. Almost all of the women (97%) and children (99%) had inorganic mercury levels below the limit of detection. Total mercury was detected in almost all samples. Only 19% of the children and 6% of the women had total mercury levels below the limit of detection. The geometric mean for total mercury was 0.34 $\mu\text{g/L}$ in the children and 1.02 $\mu\text{g/L}$ in the women. Children's blood mercury doubled, if they ate fish within 30 days before the testing, as compared to blood mercury levels of children who ate no fish. Blood mercury levels, in women, increased with increasing seafood consumption (Schober et al. 2003).

Mahaffey et al. (2009) analyzed later NHANES data. Women's blood mercury levels were compared against two numbers. One, 5.8 µg/L, was obtained from dividing the cord blood value (58 µg/L) used to derive the U.S. EPA's RfD by 10, the total uncertainty factor used in the RfD. The second value, 3.5 µg/L, was selected as a value of concern because methylmercury bioconcentrates across the placenta (Mahaffey et al. 2009).

Increased blood mercury levels were present in women from coastal counties (16.3% of the women had greater than or equal to 3.5 µg/L and 8.1% had greater than or equal to 5.8 µg/L) as compared to blood mercury levels from women in non-coastal counties (6.0% and 2.1%, respectively). The Great Lakes coastal region had blood mercury levels with a geometric mean of 0.80 µg/L (95% Confidence Level [CI] = 0.68-0.94 µg/L), and women from this region had the lowest mean as compared to the other three coastal regions. The non-coastal areas of the Great Lakes region had a geometric mean of 0.63 µg/L (95% CI = 0.56-0.70 µg/L) (Mahaffey et al. 2009).

Women that self-identified as Asian, Native American, and Pacific and Caribbean Islanders had the highest mercury levels while women that self-identified as non-Hispanic black had the next highest levels. Mahaffey et al. (2009) also found that women with incomes of \$75,000 or over had statistically higher mercury levels than women with an income of \$55,000 and lower. Additional discussion of Mahaffey et al. (2009) is in Appendix C.

Knobeloch et al. (2007) investigated methylmercury exposure in people living in Wisconsin. Between January 2004 and May 2005, more than 2,000 volunteers completed fish consumption questionnaires and gave hair samples. The age range for both men (n = 978) and women (n = 1,050) was between 18 and 92. Mean fish consumption was 7.7 meals per month. Overall, 29% of the meals were commercial fish, 24% were tuna, 25% were in a restaurant, and 17% were sport-caught fish. Women ate more tuna as compared to men, but men ate more sport-caught fish (Knobeloch et al. 2007).

About half (52%) that ate sport-caught fish were a member of a household with a Wisconsin fishing license holder. Almost all (95%) knew to limit fish consumption due to mercury and over half (77%) followed or were aware of the Wisconsin sport-caught fish consumption advisory. Hair mercury levels were between 0.012 and 15.2 ppm with an average of 0.714 ppm. Highest levels of mercury were in people that were Hispanic, Asian, had less than 12 years of formal education, or had an income greater than \$75,000. Hair mercury levels positively correlated with monthly fish consumption estimates (Knobeloch et al. 2007).

In 2004, Dellinger studied fish consumption in the Upper Great Lakes. Several Ojibwe reservations participated, totaling 822 tribal members. The four major species of fish commonly consumed were walleye, lake trout, whitefish, and perch. Mercury bioaccumulation occurred according to fish species and size. Walleye tended to accumulate the most mercury, with an average mercury concentration of less than 0.5 ppm. Blood mercury correlated more closely to reported fish consumption as compared to hair mercury. Almost all groups, except males from other reservations (reservations outside of the primary study area) that eat large walleye, ingested mercury levels below the ATSDR MRL of 0.3 µg/kg/day (Dellinger 2004).

Methylmercury exposure in populations from the Faroe Islands is through consumption of pilot whale meat and blubber (Budtz-Jorgensen et al. 2004A). In the Faroe Islands, 996 cord blood samples were taken and had an average of 22.6 ng/L methylmercury. Maternal hair samples (n = 1,019) had had an average methylmercury concentration of 4.22 ppm. At seven years of age, whole blood methylmercury levels were 1.93 µg/L (n = 673). Levels at 14 years of age levels to 3.81 µg/L (n = 796) (Budtz-Jorgensen et al. 2004A).

Along with the risk of mercury ingestion, fish consumption can provide health benefits due to the presence of omega-3 fatty acids, including docosahexaenoic acid (DHA), in the fish. Fish and shellfish are almost the exclusive source of preformed DHA in the diet (Mahaffey et al. 2004). Consumption of omega-3 fatty acids found in fish reduces development of coronary artery disease (Foran et al. 2003). However, cardiovascular effects appear to be occurring at lower methylmercury exposure than levels currently associated with neurological and neurodevelopmental deficits (Mahaffey et al. 2004).

Different fish species can have different levels of mercury (e.g. swordfish and shark: 1 ppm; tuna, trout, pike, bass: 0.1-0.5 ppm; shellfish: concentrations lower than 0.1 ppm) (Foran et al. 2003). People, out of concern for the mercury levels in fish, may take fish oil supplements in order to benefit from omega-3 fatty acids without actually eating the fish. Foran et al. (2003) measured the amount of mercury in five brands of fish oil. Two of the five brands had mercury at levels between 10 and 12 ppm and the other three had less than 6.0 ppm, which was the detection limit. These levels of mercury are very low, as the mean daily intake for fish oil for a person in the U.S. is 3.5 µg, which would give a person a maximum mercury intake of 0.042 ng/day. A blood mercury level for people that do not consume fish is 2.0 µg/L, and those that eat two to four fish meals/week have a level of 8.4 µg/L, so fish oil ingestion without consumption of fish meat could result in a lower mercury intake (Foran et al. 2003).

Additional discussion of human biomonitoring is in Appendix C.

Genotoxicity

There is no evidence that exposure to mercury compounds, either by inhalation or ingestion, alter chromosomes in human somatic cells. There is some evidence, in laboratory rodents, that mercury can cause breaks in chromosomes, although the species and strain sensitivity might be different and the actual relevance to humans is unclear (ATSDR 1999).

Additional discussion of genotoxicity is in Appendix D along with discussion of mercury toxicity in cell culture models.

Toxicity in Humans

The nervous system is sensitive to all forms of mercury. Both methylmercury and metallic mercury vapors can reach the brain in larger relative amounts than inorganic mercury. Mercury exposure can cause permanent damage to the brain or the kidneys. Health effects of mercury exposure include irritability, shyness, tremors, changes in vision or hearing, memory problems, damage to the stomach and intestines, nausea, diarrhea, or severe ulcers, and a rapid heart rate

and increased blood pressure. There is a greater chance of a toxic effect from exposure to mercury if a person has a preexisting liver, kidney, lung, or nervous system condition (ATSDR 1999).

Mercury hypersensitivity (acrodynia or Pink disease) can also occur in adults and children. It causes symptoms that include itching, flushing, swelling, sloughing of the skin of the palms of the hands or soles of the feet, morbilliform (measles-like) rashes, excessive sweating, salivation, tachycardia, elevated blood pressure, insomnia, weakness, irritability, fretfulness, and peripheral sensory disturbances (ATSDR 1999).

Death is possible after ingestion of high levels of inorganic mercury or organic mercury. Most of the deaths from mercury exposure are due to neurotoxicity (ATSDR 1999).

Castoldi et al. (2008) reviewed methylmercury-induced developmental neurotoxicity in humans. Historically, there were two massive food poisonings with methylmercury. One was in Japan in the 1950s to 1960s where fish in Minamata Bay were heavily contaminated with methylmercury. Japanese women, who ate the fish, in the area had maternal hair mercury concentrations of 10 to 100 ppm. Cerebral total mercury in the children born from the exposed women ranged from 8.0 to 21 ppm and had a range of 2.4 to 8.4 ppm methylmercury (Castoldi et al. 2008). Some people, who ate the fish, had acute or subacute reactions to high doses of methylmercury and 157 people died (Oka et al. 2002).

Discussion of epidemiological studies concerning Minamata disease is in Appendix E.

The second poisoning was in Iraq in 1971-1972, from seed wheat treated with a fungicide that was used to make bread. At levels of 10 ppm in maternal hair, neurodevelopmental effects were observed in children exposed before birth. In both poisonings, neurodevelopmental toxicity occurred in offspring at levels of mercury that caused very few or no signs of toxicity in the mothers. Effects in the children, from both poisonings, included microcephaly, cerebral palsy, blindness, deafness, slowed or slurred speech (dysarthria), abnormal reflexes, and gross impairment of motor functions and mental development. Some children had improvements in motor function effects with time, but the cognitive effects did not improve (Castoldi et al. 2008).

Observational Epidemiology Studies

Faroe Islands cohort

The Faroe Islands are located between the Norwegian Sea and the North Atlantic Ocean (CIA 2009A), near Norway, Shetland, and Iceland. They are a Nordic fishing community and are primarily exposed to methylmercury through consumption of meat from pilot whale (Debes et al. 2006). Many of the people in the Faroe Islands are descended from Viking people, who arrived in the ninth century. Scandinavian is the only listed ethnic group and the languages spoken are Faroese and Danish (CIA 2009A).

Population sizes were estimated at 4,000 in the 1300s, 9,000 in the 1800s, and around 48,000 currently. The population may have been reduced in 1349, when the plague swept through, and

again in 1709, with a smallpox epidemic (Jorgensen et al. 2002). Due to the likely small population initially on the islands and events that lowered the population at different times, the Faroese people might have less genetic diversity as compared to the U.S. or other countries. In fact, analysis of Faroese mitochondrial DNA (passed from the maternal line) indicates that the Faroese population is the most homogenous and isolated in the North Atlantic region (Als et al. 2006).

Pilot whale is a commonly eaten marine mammal in the Faroe Islands. Pilot whale muscle tissue has an average mercury concentration of 3.3 ppm (Weihe et al. 1996). Polychlorinated biphenyls (PCBs) are present in pilot whale blubber, at an average of around 30 ppm. In 1986, the average daily consumption of both fish and pilot whale was assessed in adult Faroese. The adults consumed 72 grams (g) of fish, 12 g of whale muscle, and 7 g of blubber per day. Faroese had fish at 44% of the dinner meals and whale at 9.5% of the dinner meals. Total mercury was measured in whole blood from 53 adult women from Lørvik. The median was 12 µg/L and the range was 2.6 to 50 µg/L (Weihe et al. 1996).

The Faroe Islands cohort was set up in 1986-1987 and was comprised of 1,022 children (Grandjean et al. 1997). The children were followed for 14 years (Debes et al. 2006). Faroe Island children were primarily exposed to methylmercury through pilot whale meat. People from the Faroe Islands have a large variation in seafood consumption, but small social differences. Among the participating children, there were no obvious cases of methylmercury poisoning (Grandjean et al. 1997).

Over the course of the 14 years, various neuropsychological exams were used to determine if children in the cohort had effects due to methylmercury exposure. At age seven, results from nine out of 20 exams showed statistically significant mercury associated deficits (Grandjean et al. 1997). These deficits were associated with cord blood mercury levels, indicating prenatal mercury exposure was responsible (Grandjean et al. 1999).

Nervous system function was also assessed. Delays in measures of nervous system response (auditory evoked potentials) were associated with cord blood and maternal hair mercury levels at age seven (Murata et al. 1999). Auditory evoked potential delays were also associated with cord blood mercury at age 14 (Murata et al. 2004). Prenatal mercury exposure was associated with alterations of cardiac autonomic function at 7 years (Sorensen et al. 1999) and 14 years old (Grandjean et al. 2004).

Additional discussion of the Faroe Islands cohort is in Appendix E.

Seychelles Child Development Study

Seychelles is located in the Indian Ocean, northeast of Madagascar. Both France and Great Britain have each controlled the islands at different times until 1976, when they became independent. Ethnic groups present on the islands are French, African, Indian, Chinese, and Arab and languages spoken are Creole and English, among other languages (CIA 2009B). Seychelles islanders are exposed to methylmercury through consumption of deep sea and reef fish (Castoldi et al. 2008).

Two studies of Seychelles children have been carried out. One is the pilot cohort, started in 1987 with 789 children, and the second is the main cohort, started in 1989 with 778 children (Davidson et al. 2000). One purpose of the pilot cohort was to provide information for selection of parameters for the main cohort. Children involved in the main study cohort of the Seychelles Child Development Study (SCDS) have had more thorough testing as compared to children from the pilot cohort (Davidson et al. 2000).

Results from the primary analyses of the pilot cohort found no adverse effects of methylmercury exposure. In a secondary analysis, test scores from two tests had a significant adverse association with prenatal methylmercury exposure. After removal of data from three children, termed influential points (thought to have a large influence on the model), only one significant adverse association was found with a test measuring strategies and processes involved in learning and recalling verbal material (Davidson et al. 2000).

The main cohort of the SCDS was set up in 1989 and selected 779 mother-infant pairs, which represented 50% of the live births between February 1989 and January 1990 (Davidson et al. 2006A). Thirty-nine pairs were omitted, resulting in 740 remaining pairs for evaluation at six different ages. Children were evaluated at six (0.5 year), 19 (1.6 years), 29 (2.4 years), 66 (5.5 years), and 107 (9.0 years) months of age. Not all members of the cohort were evaluated at the different times (740 children at six months, 738 children at 19 months, 736 children at 29 months, 711 children at 66 months, and 643 children at 107 months). Global cognition endpoints were assessed at 19, 29, 66, and 107 months while cognitive function and developmental domains were assessed at 66 and 107 months. Mothers of the children ate an average of 12 fish meals per week during pregnancy (Davidson et al. 2006A).

Initial testing (ages 19 and 66 months) of the main cohort found no adverse effects of methylmercury exposure (Axtell et al. 1998; Davidson et al. 1998; Myers et al. 2000). Reanalysis of data collected at 66 months confirmed this finding (Palumbo et al. 2000; Axtell et al. 2000); although Palumbo et al. (2000) noted that the children may have been too young for effects to be apparent.

Davidson et al. (2006A) and Davidson et al. (2006B) reviewed data collected at different ages from the main cohort. No adverse effects were found in Davidson et al. (2006A) and only one adverse association, with a test of motor speed and coordination on the nondominant hand, was found with prenatal mercury exposure at 107 months of age in Davidson et al. (2006B).

Two reanalyses of the main cohort were carried out with different models. In the first reanalysis using a nonlinear model, one adverse association was identified with the timed test of manipulative dexterity. Huang et al. (2005) noted that possible adverse effects could occur in the uppermost range of maternal hair mercury (above 12 ppm). The second reanalysis was to determine if individual children would develop different effects at differing mercury exposure levels (nonhomogenous susceptibility). Huang et al. (2007) found an adverse association with a test previously found to have a beneficial mercury association. Additional associations were also seen, leading the authors to conclude that prenatal mercury exposure might result in different effects in different children (Huang et al. 2007).

At age 10.7 years, an adverse association was found between the Reproductions Task and prenatal methylmercury, but only when a data point the authors termed an outlier was included (Davidson et al. 2008). When the children were older (age 15), an association between increased diastolic blood pressure and increased prenatal mercury exposure was identified. This association was not apparent at age 12 (Thurston et al. 2007).

Two reports from the SCDS have stated that associations between prenatal mercury exposure and neurodevelopment might not have been identified. This might be because the associations were too subtle to measure with this number of study participants (Davidson et al. 2008) or might not be detectable until the children are older (Van Wijngaarden et al. 2006).

Additional discussion of the SCDS is in Appendix E.

Comparing Faroe and Seychelles studies

Nakai and Satoh (2002) discussed the differences in the Faroe and Seychelles studies. The major differences the authors noted were the age of the children at testing, tests that were given, the ethnic (genetic) background of the population, and potential differences in the timing, magnitude, and duration of the methylmercury exposure (Nakai and Satoh 2002).

Another author concluded that differences between the Faroe and Seychelles studies are unlikely to be due to using hair to measure mercury (Cernichiari et al. 2007). More likely differences are due to the different populations, differences in study design, and differences in methylmercury consumption patterns. In the Faroe Islands, people consume a larger amount of methylmercury in one sitting (1.6 µg mercury/g wet weight from whale) as compared to the consumption in Seychelles (0.31 µg mercury/g wet weight from ocean fish). This difference is because of the source of methylmercury exposure (Cernichiari et al. 2007).

Castoldi et al. (2008) expanded the discussion of consumption differences between Faroe and Seychelles studies. Both of the studies show *in utero* exposure to high methylmercury levels. At lower exposure, the association between methylmercury and adverse effects is weaker, and possibly dependent of the pattern of exposure. The Seychelles and Faroe populations have a different pattern of exposure. In the Seychelles Islands, people are typically exposed to 0.3 ppm daily from ocean fish. People from the Faroe Islands have about one exposure a month to 1.6 ppm mercury from pilot whales and may be further exposed from snacking on whale throughout the month. Additional contaminants, such as PCBs, are also present in the pilot whale, which are not present in the ocean fish that the Seychelles Island people eat (Castoldi et al. 2008).

Rice et al. (2003), in comparing the Faroe and Seychelles studies, focused on possible differences in genetic homogeneity in accounting for outcome differences between the studies. Both the Faroe and Seychelles populations were mentioned as having a more homogenous population than the U.S. The Faroe Islands is composed of a stable population with a Scandinavian background while Seychelles has a stable population of European and African backgrounds. Benchmark doses from the Faroe Islands are lower than benchmark doses derived from the Seychelles studies. However, derived benchmark doses, from a study including multiple ethnic groups from New Zealand, are even lower than those from the Faroe Islands studies (Rice et al. 2003).

Over the years, several opinion letters have been written about both the Faroe Islands study and the SCDS. One letter on the SCDS by Keiding (2003) discusses the possibility that the SCDS is victim to a type II statistical error, where a difference is not found when there really is one. This possibility was brought up because the SCDS is statistically comparable with the Faroe Islands study and null hypothesis. A potential reason for this is possible exposure misclassification due to maternal hair collection up to six months after birth (Keiding 2003). A second letter, by Lyketsos (2003), mentioned the possibility that the SCDS may not be large enough for adverse effects to be observable. However, the letter-writer concluded that if a study larger than the SCDS were needed to detect adverse effects, those effects would be “practically meaningless” (Lyketsos 2003).

Cardiovascular effects

Heart rate variability (HRV) is the beat-to-beat variability that occurs normally to allow people to respond to different circumstances. Certain parameters of HRV indicate parasympathetic nervous system functioning.

Oka et al. (2002) found parasympathetic nervous dysfunction in people diagnosed with Minamata disease. There were statistically significant differences in parameters measuring HRV in Minamata patients as compared to the control group. This could indicate that these people are more susceptible to cardiovascular disease later in life (This study is further discussed in the Appendix E Minamata disease section.)

Prenatal exposure to methylmercury might cause decreased HRV in boys seven years of age from the Faroe Islands (Sorensen et al. 1999). Sorensen et al. (1999) stated that, “prenatal exposure to MeHg (methylmercury) may affect the development of cardiovascular homeostasis.” These children might not have correct functioning of their cardiovascular systems due to changes in the autonomic nervous system controlling the heart (This study is further discussed in the Appendix E Faroe Islands section.)

In studies of Finnish men, higher hair mercury was associated with increased risk of acute coronary events (Rissanen et al. 2000; Virtanen et al. 2005). Guallar et al. (2002) also found an increased risk of myocardial infarction (heart attack) in men with elevated toenail mercury levels. However, no increases in coronary heart disease were associated with increased toenail mercury levels in a study of men conducted by Yoshizawa et al. (2002).

Increases in blood mercury were associated with changes in HRV and blood pressure in an Inuit population (Valera et al. 2008). However, a seasonal change in blood mercury levels in James Bay sports fishermen did not alter cardiovascular risk predictors, such as lipid and fatty acid profiles or blood antioxidant status (Belanger et al. 2008).

In Faroese whaling men, an increase in mercury levels, measured from blood and toenail, was associated with increases in blood pressure and increase in an indicator of carotid arteriosclerosis (Choi et al. 2009). Even though serum PCB levels increased with increases in mercury levels, they were not associated with the outcomes measured.

In children, an increase in prenatal mercury was associated with less parasympathetic nervous system control of the heart (Murata et al. 2006). This indicates that prenatal mercury exposure altered the normal cardiac autonomic function.

Additional discussion of cardiovascular effects is in Appendix E.

Additional human studies

Canadian fish consumers were assessed for blood mercury levels and thyroid hormone levels. In men, levels of thyroid stimulating hormone positively related to total, inorganic, and methylmercury in blood and total mercury in hair (Abdelouahab et al. 2008).

Massachusetts women with higher red blood cell mercury levels and lower fish consumption had children with lower scores on an assessment of visual motor abilities. However, if the women had higher fish consumption along with higher mercury levels, their children scored better on the assessment test. The authors suggested that the benefit of fish intake would be greater without mercury contamination (Oken et al. 2008)

Xue et al. (2007) determined that there was an association between women with maternal hair mercury greater than or equal to 0.55 ppm and preterm deliveries. Preterm deliveries were defined as less than 35 weeks.

People in the Baltimore Memory study were given tests to measure neurological function and had blood mercury measured (Weil et al. 2005). Increased mercury levels resulted in a better performance on one test and a worse performance on a different test. The authors speculated that, although there were mercury related differences, the changes in test score were so small that mercury may not have had a real effect (Weil et al. 2005).

Extended discussion of the above additional human studies is in Appendix E.
Animal toxicity studies are discussed in Appendix F.

Development of the MDCH Mercury Reference Dose (RfD)

RfDs were developed for calculation of FCSVs. The Boston Naming Test, given to the children at age seven in the Faroe Islands study, was selected by the National Research Council as an appropriate endpoint to derive an RfD. The U.S. EPA modeled a lower limit (95% confidence level) on a benchmark dose for mercury in cord blood as 58 µg/L, using results from the Boston Naming Test scores and cord blood mercury levels. The U.S. EPA then calculated a maternal intake from the lower limit on the benchmark dose. See Appendix G, Equation G-1 for the equation used.

MDCH also used the lower limit on the benchmark dose of 58 µg/L and the equation for calculating the maternal intake. However, several variables in the equation were different from those the U.S. EPA used. U.S. EPA reference dose assumed a cord to maternal blood ratio of 1.0, and asserted that any differences would be accounted for in the uncertainty factors (U.S. EPA 2001B). MDCH also used the cord to maternal blood ratio of 1.0.

MDCH used different variables than those used by the U.S. EPA for maternal weight and blood volume. Values used for variables in Equation G-1 are in Table G-1 in Appendix G, including the U.S. EPA's values. Maternal weight was calculated as 79.15 kg, which represents a maternal weight after total weight gain during pregnancy. The starting weight was 65.4 kg and an average of the recommended total weight gain (13.75 kg) for people of normal body weight was added (NRC and IOM 2007). Blood volume was calculated as 7.0% of the body weight (U.S. EPA 2001B). All other variables were the same as those used by the U.S. EPA. This resulted in a maternal intake of 1.0 µg/kg/day. The same uncertainty factors selected by the U.S. EPA were also used (a combined uncertainty factor of 10 for pharmacokinetic and pharmacodynamic variability), resulting in an RfD of 0.1 µg/kg/day. Appendix H, Table H-1 provides examples of FCSVs based on this RfD for both the general population (adult men and women over child-bearing age) and the sensitive population (children under 15 years of age and women of child-bearing age) due to potential cardiovascular effects as well as neurological effects.

Children's Health Considerations

Children could be at greater risk than are adults from certain kinds of exposure to hazardous substances. A child's lower body weight and higher intake rate results in a greater dose of hazardous substance per unit of body weight. If toxic exposure levels are high enough during critical growth stages, the developing body systems of children can sustain permanent damage.

Methylmercury targets the central nervous system, including the brain and both a developing fetus and child are particularly susceptible to this exposure (ATSDR 1999). Mercury easily crosses the placenta and both inorganic and organic mercury can be found in human breast milk. Additionally, maternal exposure to mercury levels that cause little or no signs of toxicity can result in severe neurotoxicity for a fetus (ATSDR 1999). Developing organ systems may have a reduced ability to excrete chemicals as compared to excretion in adult organ systems.

Prenatal exposure may result in subtle developmental alterations that will not show up for years. There have been several examples of latency in toxicity from methylmercury exposure, both from acute and chronic exposure (Weiss et al. 2002). Several studies carried out in non-human primates and rodents have observed effects only after the animals are older. This raises the question of whether a mercury value deemed safe for children could cause effects later in life.

Conclusions

MDCH concludes that eating unlimited amounts of certain sport-caught fish from lakes in Michigan throughout the year could harm people's health. This is a public health hazard. Fish consumption advisories may be required for certain fish species at specific locations.

Recommendations

Use the proposed RfD, protective of neurological effects, to develop updated mercury FCSVs and utilize these values to provide fish consumption advice in Michigan.

Continue monitoring of sport-caught fish in Michigan for mercury.

Provide the Fish and Wildlife Contaminant Advisory Committee (FAWCAC) and other relevant groups (Great Lakes Sport Fish Advisory Task Force and Great Lakes Human Health Network) with a copy of this document.

Public Health Action Plan

1. MDCH will issue advisories in the Michigan Family Fish Consumption Guide using new mercury FCSVs (see Appendix H for sample FCSVs).
2. The MDCH Analytical Chemistry Laboratory will continue to screen fish, collected for the Michigan Fish Contaminant Monitoring Program (MFCMP) (administered by the Department of Environmental Quality and Department of Natural Resources).
3. MDCH will share a copy of this document so that FAWCAC and other relevant groups will have this information.

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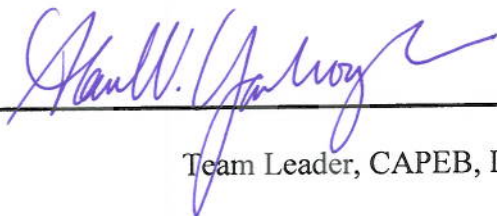
Certification

This Health Consultation was prepared by the Michigan Department of Community Health under a cooperative agreement with the Agency for Toxic Substances and Disease Registry (ATSDR). It is in accordance with approved methodology and procedures. Editorial review was completed by the cooperative agreement partner.



Technical Project Officer, Cooperative Agreement Program Evaluation Branch (CAPEB), Division of Health Assessment and Consultation (DHAC), ATSDR

The Division of Health Assessment and Consultation, ATSDR, has reviewed this public health consultation and concurs with the findings.



Team Leader, CAPEB, DHAC, ATSDR

Appendix A: Various Forms of Mercury and the Corresponding CAS Numbers

Metallic	Inorganic	Organic
Mercury (7439-97-6)	Mercuric (II) chloride (7487-94-7)	Mercuric (II) acetate (1600-27-7)
	Mercuric (II) sulfide (1344-48-5)	Methylmercuric chloride (115-09-3)
	Mercurous (I) chloride (10112-91-1)	Dimethyl mercury (593-74-8)
		Phenylmercuric acetate (62-38-4)

Appendix B: Extended Discussion of Toxicokinetics

Retention of methylmercury in the body occurs by associating with thiol (sulfhydryl group) containing proteins (ATSDR 1999). The amino acid cysteine contains a thiol. Only 1% of methylmercury in the body is complexed with cysteine ($\text{CH}_3\text{Hg-Cys}$). The methylmercury-cysteine complex is similar to methionine, a neutral amino acid (Cernichiari et al. 2007). Neutral amino acid carriers transport methylmercury, bound to thiol containing proteins, across cell membranes due to the resemblance to methionine (Clarkson et al. 2007). The methylmercury complex also travels across the placenta on the neutral amino acid carriers. Other ligands that can bind to methylmercury are reduced glutathione (GSH), albumins, hemoglobins, keratins, and tubulins (Cernichiari et al. 2007).

The methylmercury concentration is five times higher in the brain than in the blood (Castoldi et al. 2008). Methylmercury has a whole body half-life of around 70 days, resulting in a loss of 1% of the body burden every 24 hours (Cernichiari et al. 2007). Methylmercury will accumulate in the body if the rate of intake is greater than the rate of excretion (Knobeloch et al. 2007). After one year of ingestion of methylmercury, it is at steady state in the body. For most studies, the assumption is that populations are in steady state (Cernichiari et al. 2007).

Metabolism of all forms of mercury is similar for humans and other animals. Absorbed metallic and inorganic mercury can enter an oxidation-reduction cycle. Unoxidized mercury can travel to the brain, be oxidized to the inorganic divalent cation (Hg^{2+}) by the hydrogen peroxidase-catalase pathway, and be trapped there. The rate of oxidation depends on the catalase in tissue, the endogenous production of hydrogen peroxide, and the mercury vapor at the oxidation sites (ATSDR 1999).

Conversion of mercury, including the conversion of organic mercury to inorganic mercury, can occur in the bodies of humans and other animals. The liver metabolizes and excretes organic mercury, but reabsorption can occur in the gastrointestinal tract (ATSDR 1999). The kidneys accumulate a majority of the total body burden of inorganic mercury (Clarkson et al. 2007) as the methylmercury-cysteine complex can be absorbed by kidney tubules (Cernichiari et al. 2007). Urinary levels of mercury reflect kidney levels of mercury and are a direct result of mercury released from the kidneys (Clarkson et al. 2007).

Studies have reported the mercury hair to blood concentration ratios as 250:1, although ratios identified from studies have been between 140:1 and 416:1. Some differences in the reported ratios may be explained by sampling differences, such as hair location and distance from the scalp (ATSDR 1999). Table B-1 provides levels of mercury in the body, relative to the amount in plasma, and the proteins that mercury is likely to be bound to in that component of the body.

Table B-1: Methylmercury levels in the body (relative to amount in plasma) (Clarkson et al. 2007)

Component	Relative amount (to plasma)	Protein bound to methylmercury in component
Plasma	1	Albumin (about 99%)
Hair	2,250	Keratins
Red blood cells	20	Hemoglobin
Brain	50	Tubulins

Cernichiari et al. (2007), in another study reporting on methylmercury ratios in the body, agreed with the red blood cells to plasma ratio of 20:1, but reported a range for methylmercury brain to whole blood ratios as 5 to 10:1. This is a different comparison than the brain to plasma ratio in Clarkson et al. (2007), as almost all (95%) of the methylmercury in whole blood is inside red blood cells (Cernichiari et al. 2007). The remaining methylmercury in whole blood is in the plasma (5% as a thiol ligand to mercaptalbumin) and bound to cysteine (~1%). Blood to brain ratios of mercury are different in different species. Rats have a methylmercury blood to brain ratio of 0.06:1, mice have a ratio of 1.2:1, and squirrel monkeys and humans have a ratio of 6:1 (Cernichiari et al. 2007).

Around 90% of methylmercury is excreted in the feces and less than 10% is excreted in the urine (Cernichiari et al. 2007). Methylmercury is eliminated in the feces via secretion in the bile. However, mercury secreted in the bile can be reabsorbed in the gallbladder and gastrointestinal tract. People's intestinal microflora are capable of converting some methylmercury to inorganic mercury, which can alter levels of excretion. Diet can influence this conversion and excretion. Increases in fecal excretion can occur with a high fiber diet, while antibiotic treatment can decrease fecal excretion (Clarkson et al. 2007).

In the blood, the half-life of methylmercury is approximately 60 days (Knobeloch et al. 2007) and may have a 44 day average for adults (Cernichiari et al. 2007). Mercury excretion has two phases, a fast initial excretion and a slower second excretion. Males tend to excrete mercury faster initially, during the fast initial excretion, but females excrete mercury more rapidly during the slower second excretion (ATSDR 1999).

Carrier et al. (2001A) developed a model for distribution and elimination of methylmercury. Time profiles of blood and tissue mercury concentrations and cumulative excretion in rats were utilized for development of the model. The model was validated with two other sets of rat data, but the authors planned to adapt the model for use in people (Carrier et al. 2001A).

Carrier et al. (2001B) adjusted the methylmercury distribution and elimination rat model for use with human data. The model predicted a smaller amount of organic mercury in feces, hair, and urine in humans as compared to rats. The transfer of the whole body burden to feces was estimated as a hundred times lower in humans as it was in rats, and humans had a negligible transfer of mercury to urine. The transfer of whole body burden to hair was forty times less in humans. The metabolism of mercury was 3.0 to 3.5 times lower, on average, in humans than in rats. In humans, the transfer of inorganic mercury from blood to hair was five times lower than it

was in rats. Retention of mercury in human kidneys was approximately 19 times higher as compared to retention in rat kidneys. However, the mercury transfer from the human kidney to urine was estimated as two times greater than that of the transfer in rats. The methylmercury half-life in humans was variable in the model, extending from 35 to 120 days. Most measured half-lives have been reported as 45 to 55 days. The hair to blood concentration obtained from this model was 333:1, which was similar to the published ranges of 200 to 300:1 (Carrier et al. 2001B).

Schlawicke Engstrom et al. (2008) investigated whether differences in methylmercury metabolism could be due to the presence or absence of variants in genes for GSH-synthesis (glutamyl-cysteine ligase modifier subunit [GCLM]-588 and glutamyl-cysteine ligase catalytic subunit [GCLC]-129) or GSH-conjugation (glutathione *S*-transferase pi 1 [GSTP1]-105 and GSTP1-114). People possessing the two variants of GSTP1 had a different mercury amount in red blood cells at higher methylmercury exposures as compared to people without the two gene variants. People homozygous for (with two copies of) the GCLM-588 gene variant had higher levels of red blood cell mercury (Schlawicke Engstrom et al. 2008).

Appendix C: Extended Discussion of Human Biomonitoring

Mahaffey et al. (2009) used National Health and Nutrition Examination Survey (NHANES) data to compare women's blood mercury levels against two levels. One, 5.8 $\mu\text{g/L}$, was obtained from dividing the cord blood value (58 $\mu\text{g/L}$) used to derive the U.S. EPA's RfD by 10, the total uncertainty factor used in the RfD. The second value, 3.5 $\mu\text{g/L}$, was selected as a value of concern as methylmercury bioconcentrates across the placenta (Mahaffey et al. 2009).

Nationwide, 10.4% of the women (around 6.92 million women) in the study had blood mercury levels greater than or equal to 3.5 $\mu\text{g/L}$. Almost 5% of the women (approximately 3.1 million women) had blood mercury levels greater than or equal to 5.8 $\mu\text{g/L}$. These national numbers were divided into coastal and non-coastal counties. Coastal counties were those along the Great Lakes, Atlantic Ocean, Gulf of Mexico, and Pacific Ocean. Non-coastal counties were those without shoreline or with the county center more than 25 miles away from an ocean or the Great Lakes. Population estimates could not be calculated for these divisions, as the populations were calculated from the four major Census regions (Northeast, Midwest, South, and West). A larger percentage of women in the coastal counties had blood mercury levels greater than or equal to 3.5 $\mu\text{g/L}$ (16.3% of the women) and greater than or equal to 5.8 $\mu\text{g/L}$ (8.1% of the women) as compared to women in the non-coastal counties, 6.0% and 2.1% respectively (Mahaffey et al. 2009).

The coastal region was further broken down into Great Lakes, Gulf of Mexico, Atlantic, and Pacific Coasts regions. The Great Lakes coastal region had blood mercury levels with a geometric mean of 0.80 $\mu\text{g/L}$ (95% CI = 0.68-0.94 $\mu\text{g/L}$), which was the lowest of the coastal regions. The Inland Midwest region, containing the non-coastal areas of the Great Lakes region, had a geometric mean of 0.63 $\mu\text{g/L}$ (95% CI = 0.56-0.70 $\mu\text{g/L}$) and was the lowest group overall. The 30 day estimated mercury intake, based on 30 day dietary recording, had an arithmetic mean of 0.47 $\mu\text{g/kg}$ (95% CI = 0.39-0.54 $\mu\text{g/kg}$) for the Great Lakes coast region. Women in the Inland Midwest region had an estimated consumption of 0.50 $\mu\text{g/kg}$ (95% CI = 0.44-0.55 $\mu\text{g/kg}$). The estimated consumption of mercury was the lowest for the Great Lakes Coast Region as compared to the other coast regions (Mahaffey et al. 2009).

The survey respondents were also divided into ethnic groups. Those women in the "Other" category for ethnic groups had the highest percentages greater than or equal to 3.5 $\mu\text{g/L}$ (27.4%) and 5.8 $\mu\text{g/L}$ (15.7%). The "Other" category included Asians, Native Americans, and Pacific and Caribbean Islanders. The women with the next highest mercury levels were those that self-identified as non-Hispanic black, followed by non-Hispanic white, and Other Hispanic. Mexican Americans had the lowest percentages of mercury levels over 3.5 $\mu\text{g/L}$ and 5.8 $\mu\text{g/L}$ (Mahaffey et al. 2009).

Mahaffey et al. (2009) also evaluated income. There were increasing percentages of women with greater than or equal to 3.5 $\mu\text{g/L}$ and 5.8 $\mu\text{g/L}$ blood mercury levels as the income levels increased. Those with an income of \$75,000 or over had the highest percentage of women (16.2%) with blood mercury levels greater than or equal to 3.5 $\mu\text{g/L}$ compared to women in lower income groups. Women in the highest income bracket also had the highest percentage of women (7.1%) with blood mercury levels greater than or equal to 5.8 $\mu\text{g/L}$. The authors found

statistically higher blood mercury levels in the income group of \$75,000 or over as compared to the income groups of \$55,000 and lower (Mahaffey et al. 2009).

Blood mercury levels were significantly associated with age and estimated 30 day mercury intake. With an increase in the amount of seafood (fish or shellfish) a woman consumed, there was an increase in levels of total blood mercury. Only 513 of the survey participants (5,120 women total) ate meals of seafood three times a week or more. A majority of the survey participants rarely or never consumed seafood or only had one to two meals a month (n = 2,690). The remaining survey participants (n = 1,917) ate seafood only one to two times a week. Overall, the blood mercury levels, equal to or greater than 3.5 or 5.8 µg/L decreased from the years 1999-2000 to 2001-2002 with another slight decrease during 2003-2004 in woman ages 16 to 49. The study authors attributed this to a shift to consumption of seafood with lower mercury in the highest seafood consumers rather than a reduction in consumption (Mahaffey et al. 2009).

Morrisette et al. (2004) studied the mercury variation in women, from southwest Quebec, during pregnancy and the relationship of fish choices with mercury levels in cord and maternal blood. Exposure levels were below the U.S. EPA and Health Canada recommendations of 1.5 and 6.0 µg/g in hair, respectively. The authors mentioned that pregnant women have a temporal variation of mercury during pregnancy. Blood data was collected during the second trimester (n = 147) and blood, both maternal (n = 101) and cord (n = 92), was also collected at delivery. Approximately 43% of the women smoked prior to pregnancy and 31% smoked throughout pregnancy. More than 80% of the women ate one fish meal per month both before and during pregnancy. A majority of them (n = 106) reduced or maintained their fish consumption, while 25 women increased their consumption during pregnancy (Morrisette et al. 2004).

Of the 43 women that ate St Lawrence River fish, 95% ate perch, 33% ate walleye, 19% ate smallmouth bass, and 16% ate pike. Total mercury in maternal blood (n = 101) at delivery averaged 0.61 µg/L, with cord blood (n = 92) averaging 0.62 µg/L. Organic mercury levels averaged 0.26 µg/L in maternal blood at delivery and 0.45 µg/L in cord blood. Inorganic mercury levels averaged 0.35 µg/L in maternal and 0.24 µg/L in cord blood at delivery (Morrisette et al. 2004).

Maternal hair to blood ratio varied with trimester. A hair to blood ratio of 190:1 was calculated in the 1st trimester, 203:1 in the 2nd trimester, and 213:1 in the 3rd trimester. Maternal hair levels of mercury highly correlated with mercury levels in maternal blood. The frequency of fish consumption during pregnancy significantly and positively correlated to organic mercury levels in maternal and cord blood during pregnancy. Consumption of market and canned fish, not sport-caught, significantly correlated with blood and hair mercury levels (Morrisette et al. 2004).

In 2002, a study assessed women's consumption of sport fish from the St Lawrence River. Nadon et al. (2002) reanalyzed data from Montreal-area sport fishers (Canada, 1995-1996). The data included information from 205 women. Approximately half of the women (n = 100) were less than 45 years old. Six of the women were identified as having a potentially high level of exposure. Three of those high level exposure women were assessed for fish consumption and blood mercury levels. These women consumed 138.1 fish meals per year (81.4 g fish/day) and had an average blood mercury level of 5.60 µg/L (Nadon et al. 2002).

Eleven of the original 205 women were identified as having a lower level of exposure and eight were further screened. These eight women ate 33.25 fish meals per year (27.3 g/day) and had an average blood mercury level of 1.45 µg/L. Just under half of the low exposure women (45%) ate less than one meal per month and only 18% ate one to two sport fish meals per week. Perch was the sport fish most often eaten, by all of the study participants, followed by walleye, pike, and bass (Nadon et al. 2002).

In the winter and late summer/fall of 1996, 1,118 on-site interviews were conducted with individuals who ate St Lawrence River fish (Kosatsky et al. 2000). Of the people interviewed, 132 participated in an end of season interview and biomarker assessment. Frequent fish consumers (geometric mean of 2.3 sport fish meals/week) were more likely to eat waterfowl. Infrequent consumers ate 0.4 fish meals/week (geometric mean). Those that ate greater or equal to one fish meal/week had hair mercury levels ranging from below the level of detection to 6.59 ppm and blood mercury between 0.20 and 21.0 µg/L. Individuals that consumed less than one fish meal/week had hair mercury ranging from below the level of detection to 3.38 ppm and blood mercury ranging from 0.2 to 10.2 µg/L. The hair to blood ratio from this study was 292:1, within the range of the other ratios (275-325:1) cited by Kosatsky et al. (2000). People who also ate commercial fish had lower levels of mercury, which the authors attributed to altered reporting of sport fish consumption (Kosatsky et al. 2000).

Cole et al. (2004) compiled data from multiple Canadian surveys to assess blood mercury levels of fish eaters. Two groups of Ontario anglers, one from Cornwall and the other from Mississauga were included as well as Great Lakes' Areas of Concern (AOC) fish eaters, which included Chinese/Vietnamese communities of Metro Toronto and Hamilton. Also included were participants of the Sport Fish and Wildlife Consumption Study (shore survey), and family members, friends, or acquaintances of potential recruits (Cole et al. 2004).

Mississauga anglers consumed 11 meals of sport-caught fish/year. Most of the fish (76%) were from inland lakes and rivers and the major species were small or largemouth bass, walleye, northern pike, and yellow perch. Additional fish were from Lake Ontario (40%) and the Georgian Bay (32%). Cornwall anglers ate 29 meals of sport-caught fish/year. Almost all (96%) of the fish were from the St Lawrence River and the major species consumed were yellow perch, small and largemouth bass, and walleye. Additional fish (28%) were from other inland lakes and rivers (Cole et al. 2004).

Total fish consumption, including both sport-caught and commercial fish, was 26.5 meals/year for Cornwall women and 82 meals/year for Mississauga men. Total blood mercury was above the detection limit for 87% of the samples from the anglers and all were less than 20 µg/L, which is the upper limit of the acceptable range set by Health Canada. Inorganic mercury had a range of less than 1.1 to 3.15 µg/L from 58 samples. Organic mercury was about 62% of total mercury and had a range of 0 to 15.8 µg/L. There were no differences in the mercury levels between the two angler groups (Cole et al. 2004).

The Great Lakes' AOC survey participants caught fish from 81 different inland lakes and Great Lakes locations. These participants were broken down into two groups: Asian-Canadians (AC)

and Euro-Canadians (EC). The AC subgroup consumed a median of 213 fish meals/year. The three most frequent species of sport-caught fish were rock, largemouth, and white bass. The AC subgroup ate more commercial fish during the year as compared to the EC subgroup. The EC subgroups ate a median of 169 fish meals/year and the most frequent species of fish consumed were yellow perch, walleye, and smallmouth bass (Cole et al. 2004).

All of the AOC survey participants had detectable levels of blood mercury. Two samples were above Health Canada's upper limit of 20 µg/L, with the highest sample measuring 26.0 µg/L. The AC subgroup had the highest levels of total mercury. The EC subgroups' and both angler survey groups' mercury levels were one-third of the AC subgroup. Levels of total mercury from the AOC survey participants were comparable to levels obtained from a previous Quebec Inuit survey (Cole et al. 2004).

Fish consumption and mercury levels in hair were compared from sport and subsistence fishers in three regions in Canada (Canuel et al. 2006). In 2002, 146 individuals from Abitibi (Caucasian origin), 130 individuals from Lake St Pierre (Caucasian origin), and 118 individuals from the First Nations people Innu community in Labrador (non-Caucasian origin) had fish intake and mercury levels monitored during the three month spring season. This season is the "camp" season, when there is the closest adherence to the traditional way of life (Canuel et al. 2006).

Based on fish intake, the authors obtained modeled values for mercury levels in hair, along with direct mercury measurements from hair samples. The measured mercury levels in hair for study participants were 0.83 ppm for those from Lake St Pierre, 1.2 ppm for those from Abitibi, and 0.4 ppm for those from Labrador. Modeled levels for mercury in hair were close to measured mercury levels for Lake St Pierre (1.2 ppm) and Abitibi (2.3 ppm) individuals. For the Innu individuals from Labrador, the actual mercury hair levels (0.4 ppm) did not match the modeled hair mercury levels calculated from fish intake (5.7 ppm). The authors speculated that the difference could be because of metabolism or excretion differences in the individuals from Labrador as compared to the individuals from Abitibi and Lake St Pierre (Canuel et al. 2006).

The Inuit traditional diet includes a large amount of tissue from marine mammals, fish, and terrestrial wild game (Fontaine et al. 2008). Blood mercury levels in Inuit adults (209 male and 183 female) from Nunavik had a mean of 21.8 µg/L mercury and a range of 0.8 to 112 µg/L in 1992 (Dewailly et al. 2001). Plasma omega-3 polyunsaturated fatty acid (PUFA) levels correlated with mercury levels. Total mercury levels were related to age of the individual and consumption of seal and beluga whale (Dewailly et al. 2001).

Blood mercury levels, among other contaminants, were measured in pregnant Inuit women from Nunavik, Canada from November 1995 to March 2001 (Muckle et al. 2001). Ninety percent of the women smoked during pregnancy. Cord blood, maternal blood, and maternal hair were collected. The average mercury in cord blood was 22.7 µg/L (n = 95) and the average in maternal blood was 12.6 µg/L (n = 130). The average mercury in maternal hair was 4.5 ppm (n = 123), and different segments of hair were evaluated to identify mercury amounts specific to different trimesters. The average mercury in maternal hair in the first trimester was 4.4 ppm (n = 124), the average for the second was 4.6 ppm (n = 124), and the third was 4.4 ppm (n = 125) (Muckle et al. 2001).

Fontaine et al. (2008) reevaluated blood mercury levels in Inuit populations, from Nunavik. Two populations, both between the ages of 18 and 74, were compared, one in 1992 consisting of 492 people and one in 2004 with 917 people. Both of the Inuit study populations had similar characteristics. For both the 1992 and 2004 populations, the average blood mercury was statistically higher in women (geometric mean of 15.98 µg/L in 1992 and a geometric mean of 11.52 µg/L in 2004) as compared to men (geometric mean of 14.06 µg/L in 1992 and a geometric mean of 9.16 µg/L in 2004). Blood mercury levels were also statistically higher in older adults (ages 45-74; geometric mean of 27.18 µg/L in 1992 and a geometric mean of 21.32 µg/L in 2004) as compared to younger adults (ages 25-44; geometric mean of 13.84 µg/L in 1992 and a geometric mean of 8.86 µg/L in 2004). Blood mercury had a statistically significant decrease in 2004 from levels obtained in the 1992 survey. Geometric mean blood mercury was higher in people from Nunavik (10.24 µg/L) than in the general population of Quebec City (0.74 µg/L, n = 470). Although both of these populations had mean mercury levels lower than Health Canada's acceptable mercury level for the general population (19.94 µg/L), the maximum blood mercury concentration was 240 µg/L. Twenty-eight percent of the population from Nunavik and 72% of women of child-bearing age had blood mercury concentrations above the maximum recommended levels. Overall, age was most highly associated with blood mercury concentration, followed by marine mammal meat consumption (Fontaine et al. 2008).

Yasutake et al. (2003) collected hair from five districts in Japan (Minamata, Kumamoto, Tottori, Wakayama, and Chiba) and measured hair mercury. Hair samples (total of 3,686 with 2,020 males and 1,666 females) were collected and questionnaires were completed. The geometric mean for the male hair samples was 2.55 ppm and the female hair samples had a geometric mean of 1.43 ppm. The authors observed that hair treatment (dyeing and a permanent wave) decreased hair mercury levels. Mercury levels were different in the five districts tested. Only 0.4% of the females tested had hair mercury levels above 10 ppm, a level above which effects may occur to a fetus *in utero* (Yasutake et al. 2003).

Sakamoto et al. (2004) measured mercury and PUFAs in maternal and fetal red blood cells from 63 pregnant Japanese women ages 21 to 41 (average age 29.6 ± 4.9 years). Fetal red blood cells were collected from umbilical cord blood. Maternal red blood cell mercury had an average of 9.12 ng/g ± 3.67 ng/g (geometric average of 8.41 ng/g) and a range of 3.76 to 19.1 ng/g. Fetal red blood cell mercury had an average of 14.7 ng/g ± 6.37 ng/g (geometric average of 13.4 ng/g), and a range of 4.92 to 35.4 ng/g. In all 63 pairs, fetal red blood cell mercury was higher than maternal red blood cell mercury levels. This indicates that mercury is actively transferred to the fetus from across the placenta. The authors found a significant correlation between methylmercury and omega-3 PUFA levels, especially docosahexaenoic acid (DHA) and eicosahexaenoic acid (EPA) (Sakamoto et al. 2004).

Fresh cord tissue was collected from 116 Japanese women, ages 19 to 41 years (average age 30 ± 5 years) (Sakamoto et al. 2007). Study participants were from Tsushima Islands (n = 30), Fukuoka City (n = 68), or Katsushika (n = 18). There were significant correlations between total mercury in cord blood and maternal blood, cord tissue methylmercury and cord tissue total mercury, and cord blood methylmercury and maternal hair total mercury. Cord tissue was found to be an appropriate sample for measurement of mercury due to the significant correlation.

Additionally, brain growth occurs rapidly in the third trimester and the umbilical cord also grows during this time (Sakamoto et al. 2007).

In a study by Ohno et al. (2007), levels of mercury in hair, toenails, and urine were correlated to daily mercury intake. A food frequency questionnaire estimated daily mercury intake for residents of Akita, Japan. Based on the responses, daily mercury intake was estimated as $9.15 \pm 7.84 \mu\text{g/day}$ ($0.175 \pm 0.130 \mu\text{g/kg/day}$). The estimated daily mercury intake significantly correlated with the hair, toenail, and urine mercury levels. Hair, toenail, and urine mercury levels also correlated with each other. The mean hair mercury was $1.51 \pm 0.91 \mu\text{g/g}$, and all samples were below the safe limit for hair mercury of $10 \mu\text{g/g}$, as set by the WHO. Toenail mercury levels averaged $0.59 \pm 0.32 \mu\text{g/g}$, and urine mercury levels averaged $0.86 \pm 0.66 \mu\text{g/g creatinine}$ (Ohno et al. 2007).

Appendix D: Extended Discussion of Genotoxicity and Toxicity in Cell Culture Models

Extended Discussion of Genotoxicity

Human peripheral blood lymphocyte cultures treated with methylmercuric chloride and mercuric chloride had an increase in chromosomal aberrations and polyploid cells (cells with extra chromosomes) after a 48 hour exposure to methylmercuric chloride (0.1 to 1,000 $\mu\text{g/L}$). Methylmercuric chloride was observed to be both genotoxic and cytotoxic (Silva-Pereira et al. 2005).

Urinary levels of 8-hydroxy-2'-deoxyguanosine (8-OHdG) have been shown to increase in response to systemic oxidative deoxyribonucleic acid (DNA) damage or repair. Jin et al. (2008) examined mice fed different dietary fats to determine if certain fats would alter methylmercury induced DNA damage. Increased levels (two fold) of urinary 8-OHdG were detected from mice on the lard diet + methylmercury (3.0 mg/kg) after 14 days of treatment, but not in methylmercury containing diets with soy oil, DHA, seal oil, or fish oil (Jin et al. 2008).

Hempel et al. (1995) investigated the genotoxicity of organic mercury compounds in nematodes and *Escherichia coli*. Methylmercury was genotoxic to nematodes at levels equal to or greater than 5.0 $\mu\text{g/L}$, and the effective concentration for 50% (EC_{50}) genotoxicity is 15 $\mu\text{g/L}$. DNA damage in *E. coli* was assessed using the SOS Chromotest, which checks for direct acting mutagenicity. If the *E. coli* had DNA damage (mutations), an enzyme, beta-galactosidase, would not be active. Methylmercury concentrations greater than 50 $\mu\text{g/L}$ were found to be mutagenic (Hempel et al. 1995).

Toxicity in Cell Culture Models

A study was conducted with primary human neurons and astrocytes, from fetal brain tissue, and SH-SY5Y human neuroblastoma cells. All three of these cell types were treated with methylmercury, in the form of a chloride salt. The authors observed statistically significant toxicity after one to two days at methylmercury concentrations of 5.0 micromolar (μM) in primary neurons and SH-SY5Y human neuroblastoma cells and 10.0 μM in the primary astrocytes. Longer exposure times resulted in reduced survival. When the primary and SH-SY5Y cells were treated with glutathione (GSH), cysteine, selenite, vitamin E, or catalase, the authors observed slight protection against the neurotoxicity of methylmercury (Sanfeliu et al. 2001).

The effects of methylmercury were assessed on primary rat sensory neurons, from fetal rats, and the PC-12 rat neuronal cell line (Wilke et al. 2003). In non-neuronal cell lines, methylmercury has been reported to damage DNA. Primary rat neurons had necrosis at levels higher than 1.0 μM methylmercury. PC-12 neuronal cells had an altered pattern of gene expression at 1.0 μM methylmercury. Of the 1,032 genes that had altered expression, 189 had a greater than two-fold expression change. Fifty-eight of the 189 genes (around 32%) were components of previously characterized antioxidant defense mechanisms. The EC_{50} for alteration of gene expression in PC-12 cells was 0.7 μM methylmercury. The authors reported that serum methylmercury levels in people affected by sensory problems were 0.1 to 0.5 μM , similar to the range of concentrations used in this study (Wilke et al. 2003).

In primary rat cerebellar neurons and astrocytes, methylmercury was determined to be the most toxic of the organic mercury compounds, except for dialkyl organomercurials (Morken et al. 2005). The mechanism for methylmercury toxicity in cells is unknown. Cytotoxicity occurred at methylmercury levels less than 1.0 μM . Cerebellar neurons accumulated more methylmercury quicker than astrocytes. Cerebellar neurons were more sensitive to methylmercury compared to astrocytes, as measured by a viability (3-[4, 5-dimethylthiazol-2-yl]-2, 5 diphenyltetrazolium bromide [MTT]) assay. Leakage of lactate dehydrogenase also occurred out of the cerebellar neurons, indicating cell damage (Morken et al. 2005).

Appendix E: Extended Discussion of Observational Epidemiology Studies

Minamata disease

Methylmercury chloride, a byproduct from an aldehyde plant, was released into Minamata Bay in Japan. The factory continued to release methylmercury into the sea until 1968, contaminating fish in the area (Ekino et al. 2007). In 1968, the factory responsible for the mercury contamination of the bay discontinued use of mercury and some of the bay was covered and other areas were dredged (Harada et al. 1998). The duration of the exposure was about 20 years, from 1950 until 1968 (Ekino et al. 2007). Measurements of mercury levels in fish were over 10 ppm in 1961, but had decreased to an average of 0.5 ppm in 1969 (Eto 1997).

As early as 1953, there were reports of cats dying of “dancing disease” (Grandjean and Choi 2008). Acute poisoning, in humans, occurred in the late 1950s (Ekino et al. 2007). In 1956, the first cases of Minamata disease were diagnosed in Japan (Grandjean and Choi 2008). In 1960, methylmercury was first linked to the disease, but in 1971, the potentially responsible party suggested that the disease was caused by a toxin in rotting fish (scombrototoxin), not mercury contamination of the water by the factory (Grandjean and Choi 2008).

Minamata disease is a chronic neurologic syndrome with symptoms that include tunnel vision, ataxia (poor muscle coordination), dysarthria (slowed or slurred speech), and paresthesia (abnormal neurological sensations) (Grandjean and Choi 2008). The core symptoms of Minamata disease include “glove-and-stockings type” sensory disturbances, ataxia, concentric constriction of visual fields, and hearing impairment (Uchino et al. 2005). There are several types of Minamata disease, primarily adult or fetal. Additional categories classify patients as acute onset with short-term survival, acute onset with long-term survival, and chronic onset (Eto et al. 1997).

Adult cases of Minamata disease were due to acute methylmercury poisoning. Symptoms included blurred vision, hearing impairment, olfactory and gustatory disturbances, ataxic gait, clumsiness of hands, dysarthria, and somatosensory and psychiatric disorders (Ekino et al. 2007). Symptoms of fetal Minamata disease are similar to adult cases, but include mental retardation, symmetrical motor disturbance, and parasympathetic hypoactivity (measured by lower values for the high frequency component [HF] of heart rate variability [HRV]) (Murata et al. 2006).

Patients with chronic onset of Minamata disease are thought to have been exposed to small amounts of methylmercury (Eto et al. 1997). A majority of the patients were diagnosed with chronic Minamata disease, which had an “insidious onset” after 1960 with mild or vague neurological manifestations (Uchino et al. 2005). Chronic methylmercury poisoning was not well-acknowledged until 1995. The main complaint was paresthesia (burning or prickling sensation) at the distal parts of the extremities (fingers and toes) along with sensory reduction (Ekino et al. 2007).

Over 100 patients were diagnosed between 1953 and 1962 with Minamata disease (Uchino et al. 2005). The number of diagnosed Minamata patients reached 2,264 in 2000, although there were

200,000 suspected cases in the population in coastal areas in 1960 (Ekino et al. 2007). Of the patients verified to have Minamata disease, two-thirds had died by 2005 (Uchino et al. 2005).

Yorifuji et al. (2007) examined the population around the Minamata region for an elevated incidence of cancer. There was a negative association of methylmercury and gastric cancer, possibly due to lower salt intake. However, the authors found a positive association of methylmercury and leukemia. Yorifuji et al. (2007) found no confounding variables that might explain the positive association of methylmercury intake with leukemia (Yorifuji et al. 2007).

Patients with fetal type Minamata disease were examined for a difference in cardiovascular autonomic nervous function (Oka et al. 2002). Sixty children were born with fetal type Minamata disease between 1945 and 1965. Symptoms in these children resembled those observed in cerebral palsy, including ataxia (poor muscle coordination), strabismus (eyes that cross), hypersalivation, convulsions, and mental retardation. As of 2003, those born with fetal type Minamata disease were over 40 years of age. Of the 1,000 patients that were alive in 2003, 40 lived in Meisuien, the Minamata municipal welfare institute (Oka et al. 2002).

Nine patients (five male and four female), diagnosed with fetal type Minamata disease, were examined for difference in their cardiovascular autonomic nervous function as compared to 13 age matched control participants (four males and nine females) (Oka et al. 2002). Oka et al. (2002) found statistically significant differences in mean R-R intervals (RR), high HF, and pulse pressure (PP) as compared to the control group. Those with fetal type Minamata disease had an increased heart rate as compared to the control group, as measured by the mean RR. HF, an indicator of parasympathetic nervous activity, was lower in patients with fetal type Minamata disease. PP, the difference between the systolic and diastolic pressure, was significantly lower in the group with fetal type Minamata disease as compared to the age-matched controls. Oka et al. (2002) speculated that, based on these results, prenatal exposure to lower levels of methylmercury is a risk factor for parasympathetic nervous dysfunctions later in life.

Methylmercury poisoning caused Minamata disease, however, the actual methylmercury exposure dose was unknown (Akagi et al. 1998). Since families in the area have followed local custom and have kept a dried piece of the umbilical cord, collected at birth and stored in a wooden box, exposure amounts can be estimated from the amounts in the cord tissue. Samples (n = 176) were collected from births between 1950 and 1973. Samples were donated to the study and may not represent a complete background population. Children were diagnosed as having no disease (control group, n = 132), congenital Minamata disease (n = 21), infantile Minamata disease from postnatal exposure (n = 3), mental retardation (n = 11), cerebral palsy (n = 7), or other (n = 2) before any cord methylmercury levels were considered. Cord methylmercury levels ranged from 0.00 to 2.7 ppm in children diagnosed with no disease, cerebral palsy or other (n = 125); 0.20-2.5 ppm in children with mental deficiency (n = 11); and 0.15 to 4.7 ppm in children with Minamata disease (n = 23). Methylmercury levels differed significantly between the groups with Minamata disease and the control group and between the control groups and the groups with mental deficiency (Akagi et al. 1998).

Using the cord tissue methylmercury levels, Akagi et al. (1998) estimated the cord blood and maternal hair methylmercury amounts for those diagnosed with congenital or infantile Minamata

disease (n = 24). Cord tissue methylmercury ranged from 0.15-5.3 ppm, while cord blood was estimated as ranging from 20 to 699 µg/L and maternal hair range estimations were 3.8 to 133 ppm. The authors speculate that about one in four children with Minamata disease developed this condition without maternal hair mercury exceeding 10-20 ppm or the mother's methylmercury consumption being more than 29 µg/day. Akagi et al. (1998) recommend lowering the methylmercury levels considered safe.

Yorifuji et al. (2008) compared neurologic signs in people from three areas of Japan. Participants in an investigation of Minamata disease in 1971 were screened for neurologic signs. People from three areas of interest were selected from the 1971 study population. Three villages around the Minamata Bay (Tsukinoura, Dezuki, and Yudo) were designated as the Minamata area and had 833 participants. Three villages near Goshonoura (Arakuchi, Koshiji, and Hokabira) were designated as the Goshonoura area and had 1,450 participants. Three villages near Ariake (Akazaki, Suji, and Oura) were designated the Ariake area and had 755 study participants. Participants from the Minamata area had the highest prevalence of all neurologic signs (Yorifuji et al. 2008).

Recently, levels of total mercury in hair, from 1960, were compared to neurologic alterations, assessed in 1971, from the population in the Minamata area of Japan (Yorifuji et al. 2009). Residents from Minamata and Goshonoura (n = 120), who ate methylmercury contaminated fish, were involved in two studies, one in 1960 and the other in 1971. Residents from Ariake (n = 730), not considered an exposed population, were selected from the 1971 study. Total hair mercury from residents in Ariake was assumed to be similar to those in Kumamoto City, which was also thought to be unexposed. Median total hair mercury was 2.1 ppm. Minamata and Goshonoura residents were categorized into four groups based on hair mercury levels, 0 to 10 (n = 43), greater than 10 to 20 (n = 32), greater than 20 to 50 (n = 33), and greater than 50 ppm (n = 12). Total mercury was significantly associated with a trend in patients with perioral (around the mouth) sensory loss (Yorifuji et al. 2009).

Ninomiya et al. (1995) examined data, from 1975 to 1979, describing people from Oura, who ate polluted fish, and people from Ichiburi, who ate unpolluted coastal fish. There were over 250 total study participants, 121 from Oura and 142 from Ichiburi. Participants were tested for five neurological signs. In the participants from Oura, 56.2% had more than one of the five signs and 15.7% had more than three of the neurological signs. Of the participants from Ichiburi, only 9.2% had one of the five signs and none of them had more than three (Ninomiya et al. 1995).

Dried umbilical cords (n = 151), collected from births in the Minamata area from 1950 to 1969, had been wrapped in gauze and kept in a wooden box according to local custom (Harada et al. 1999). The children were divided into groups: congenital Minamata disease (those with the disease at birth; n = 25), acquired Minamata disease (those that acquired symptoms after birth; n = 13), mental retardation (n = 20), others (n = 16), and those with no symptoms (n = 77). The cords from the children diagnosed with congenital Minamata disease had the highest methylmercury values. Methylmercury levels from those with acquired Minamata disease or mental retardation were significantly higher than in those with no symptoms (Harada et al. 1999).

In 2001, Sakamoto et al. examined the birth ratio, using birth certificates, in and around Minamata City from 1950 to 1969. Over 20,000 children were born between 1950 and 1969 in Minamata City. When methylmercury pollution was the most severe, lower numbers of male offspring were born in the city. In a control population, male stillbirths occurred 1.2 times more than female stillbirths. There was 1.7 times the number of male stillbirths as compared to female stillbirths between 1950 and 1969 in Minamata City. The ratio of male to female stillbirths was significantly different in Minamata City as compared to the control population (Sakamoto et al. 2001).

Dried umbilical cords were collected from 12 patients with congenital Minamata disease, 16 with other mental disturbances, and 64 control people (Dalgard et al. 1994). Dalgard et al. (1994) found increased mercury levels in the dried cord from Minamata patients compared to controls. Mercury amounts in dried cord from those with Minamata disease and other mental disturbances overlapped (Dalgard et al. 1994). Using a model created with Faroe Islands data, which had a significant correlation between umbilical cord tissue, cord blood, and maternal hair, the mercury levels from the dried cord were used to calculate mercury in maternal hair and cord blood along with daily mercury intake (Dalgard et al. 1994). The range for the maternal mercury intake was calculated as 1.4 to 671 $\mu\text{g}/\text{day}$ (Dalgard et al. 1994).

In August of 1995, scalp hair was collected from 191 fishermen and their families and measured for total mercury and methylmercury (Harada et al. 1998). Only six samples of hair were above a total mercury level of 10 ppm (considered the upper limit of normal), the rest (185 samples) were below 10 ppm. However, of 188 individuals surveyed for objective disturbances, 75% (141) had glove and stocking type sensory disturbances. Although mercury levels in fish from the bay were greatly reduced by this time, these families had lived in the area for 32 to 82 years (Harada et al. 1998).

Faroe Islands cohort

The Faroe Islands cohort was set up in 1986-1987 and was comprised of 1,022 children (Grandjean et al. 1997). The children were followed for 14 years (Debes et al. 2006). Faroe Island children were primarily exposed to methylmercury through pilot whale meat. Residents of the Faroe Islands have a large variation in seafood consumption, but small social differences. Among the participating children, there were no obvious cases of methylmercury poisoning (Grandjean et al. 1997).

At the age of seven, 917 children (from the Faroe Island cohort) participated in 20 neuropsychological exams (Grandjean et al. 1997). Nine out of 20 exams had statistically significant mercury associated deficits. Cord blood mercury, from 894 children, had a geometric average of 22.9 $\mu\text{g}/\text{L}$ and an interquartile range of 13.4 to 41.3 $\mu\text{g}/\text{L}$. Maternal hair was taken at the children's birth ($n = 914$) and had a geometric average of 4.27 ppm and an interquartile range of 2.6 to 7.7 ppm. Mercury levels in cord blood varied based on alcohol consumption, current residence, if the mother was Faroese or unskilled, the use of daycare, and the presence of older siblings. Alcohol was discounted as an influence on mercury levels as the authors attributed that difference on location in the country. Use of alcohol was low in an area that also had low levels of methylmercury exposure, due to little availability of alcohol and pilot whale meat. The authors

concluded that prenatal mercury exposure had widespread brain function effects. They also stated that, “a discernible, insidious effect” seems to be present when maternal hair mercury is below 10 ppm (Grandjean et al. 1997).

Grandjean et al. (2005) also measured methylmercury in the cord tissue. The geometric mean for cord blood methylmercury was 22.35 $\mu\text{g/L}$ and had a total range of 0.90 to 351 $\mu\text{g/L}$ ($n = 996$). Cord tissue was reported as either dry or wet weight. Cord tissue methylmercury had a geometric mean of 0.210 $\mu\text{g/g}$ dry weight and a range of 0.000 to 1.28 ($n = 447$). Cord tissue methylmercury, reported as wet weight, had a geometric mean of 0.0249 $\mu\text{g/g}$ wet weight and a range of 0.0024 to 0.23 $\mu\text{g/g}$ wet weight ($n = 422$). Grandjean et al. (2005) noted that cord tissue mercury represented an average of mercury during the third trimester, due to development of the umbilical cord.

Grandjean et al. (1999) investigated whether deficits observed in the neuropsychological tests given at age seven ($n = 917$) were due to prenatal or postnatal mercury exposure. Prenatal mercury exposure was measured in cord blood and maternal hair, collected at birth. Cord blood mercury had a geometric mean of 22.9 $\mu\text{g/L}$ ($n = 894$). Maternal hair mercury levels had a geometric mean of 4.27 ppm ($n = 914$). Postnatal mercury exposure was measured using the children’s hair, collected at ages one and seven years, and blood collected at age seven. Children’s hair mercury levels had a geometric mean of 1.12 ppm ($n = 527$) at age one and a geometric mean of 2.99 ppm ($n = 903$) at age seven. Blood mercury levels, at age seven, had a geometric mean ($n = 672$) of 8.82 $\mu\text{g/L}$ and in interquartile range of 4.8 to 18.2 $\mu\text{g/L}$. The study authors only found a significant association with cord blood mercury and tests measuring language, attention, and memory (Neurobehavioral Evaluation System [NES] finger tapping – preferred hand, NES continuous performance test – missed responses and reaction time, Wechsler Intelligence Scale – digit spans, Boston Naming Test – no cues and with cues, California Verbal Learning Test – immediate recall and delayed recall) (Grandjean et al. 1999).

The Faroe Island cohort was also analyzed for cardiovascular effects at seven years of age (Sorensen et al. 1999). Children were grouped into two groups, one with a birth weight lower than 3,700 g and the other with a birth weight of over 3,700 g. (Infants born in the Faroe Islands have a higher average birth weight than those born in the U.S.) In children with a lower birth weight ($n = 83$), an increase in cord blood mercury from 1.0 to 10 $\mu\text{g/L}$ was associated with an increase in blood pressure, both systolic and diastolic. Children with a higher birth weight had increased blood pressure ($n = 62$). HRV decreased slightly with increasing concentrations of cord blood mercury, especially in boys, indicating that the parasympathetic nervous system may be altered. Results were in the normal range for children of this age; however, the authors stated that lower birth weight might act as an effect modifier (Sorensen et al. 1999).

Murata et al. (1999) reported the auditory evoked potentials of children, age seven, in the Faroe Islands cohort. Evoked potentials provide a measurement of the nervous system’s response (electrical signals) to a sensory stimuli. Due to technical difficulties with the measurement equipment, auditory evoked potential latencies were only analyzed from seven year olds measured in 1993 ($n = 388$). Cord blood and maternal hair mercury levels were predictors for auditory evoked potential latencies, but mercury was not associated with visual evoked potential

latencies. Polychlorinated biphenyl (PCB) levels, measured in cord blood, were not significantly associated with evoked potential latencies (Murata et al. 1999).

Grandjean et al. (1998) subdivided children of the Faroe Islands cohort, at age seven, into a control and case group. The control group (n = 112, 47 girls and 65 boys) had maternal hair mercury below 3.0 ppm. The case group (n = 112, 47 girls and 65 boys) had maternal hair mercury between 10 and 20 ppm. Comparisons of test scores for these two groups of children were carried out. This comparison was done because maternal hair mercury of 10 to 20 ppm was usually thought to be safe for the fetus. Overall, test scores of the case group were “less satisfactory” than the control group. The control groups performed better on all tests. For six out of 18 tests, the lower results obtained by the case group were significant. Boys had poorer results (case versus control groups) compared to the girls (case versus control groups). Cord blood PCB concentrations were only associated with one of the 18 tests. Based on the results, the authors concluded that the 10 to 20 ppm range for maternal hair mercury was not appropriate for a no observed adverse effects level (NOAEL) (Grandjean et al. 1998).

At 14 years of age, 878 of the original cohort were still living (86%) (Debes et al. 2006). The average age was 13.83 years, and the cohort had 438 boys and 440 girls. Cord blood mercury levels had a geometric mean of 22.5 µg/L (n = 838). Blood mercury levels at age seven were lower, with a geometric mean of 9.00 µg/L (n = 606), but blood mercury levels at age 14 were even lower (geometric mean of 4.08 µg/L, n = 779). Higher prenatal methylmercury exposure was associated with lower finger tapping scores, increased reaction time, and lower cued naming scores. Higher levels of prenatal methylmercury were associated with better scores on Wechsler Memory Scale-III Spatial Span. For most of the outcomes tested, prenatal methylmercury exposure only slightly changed test scores (Debes et al. 2006).

Cardiac parameters were assessed at 14 years of age in children from the Faroe Islands (Grandjean et al. 2004). Over 800 children were involved, 424 boys and 433 girls. Both the HF and low frequency (LF) components (indicators of cardiac autonomic function) were affected, but no effect was seen on blood pressure. Imprecision in methylmercury exposure, methylmercury was only measured at three timepoints, may have resulted in underestimation of effects in the children (Grandjean et al. 2004).

Over 800 children (n = 878) from the Faroe Islands cohort were assessed for auditory evoked potential at age 14 (Murata et al. 2004). Of the group tested, 835 had cord blood mercury measured. This group had a geometric mean of 22.6 µg/L and an interquartile range of 13.2 to 40.8 µg/L. Cord blood mercury was associated with certain auditory potentials (20 hertz [Hz] III, V and 40 Hz III, V). The authors concluded, “developmental vulnerability to methylmercury neurotoxicity is likely to extend into the teenage period” (Murata et al. 2004).

Budtz-Jorgensen et al. (2007) provided a commentary on the risk and benefit of seafood consumption using the Faroe Island study data at ages seven and 14. At ages seven and 14 approximately 90% of the 1,022 study enrollees participated. The log transformed frequency of fish dinners statistically significantly correlated with mercury in cord blood and maternal hair. The authors spent some time discussing the fact that the exposure to the toxicant, methylmercury, occurred from a food source that has beneficial components, and that the

beneficial components of the food and the toxicant affect the same epidemiological outcome. The authors also mentioned that the U.S. EPA's benchmark dose calculation may be less protective than is thought, as data obtained may underestimate the effects of mercury exposure. This issue was previously discussed in another paper, Budtz-Jorgensen et al. (2004B). The authors proposed a lower limit of a benchmark dose to account for exposure errors. Different models were utilized to account for exposure measurement error. The U.S. EPA's derived RfD is based on a lower limit of a benchmark dose of 58 µg/L. Using the model and exposure adjustments proposed to account for exposure measurement error, the lower limit on the benchmark dose would be either 44.0 or 45.5 µg/L (Budtz-Jorgensen et al. 2004B).

Grandjean et al. (2001) assessed Faroese birth weight in 182 infants born in Torshavn from 1994 to 1995. The mean birth weight \pm standard deviation for boys was 3,801 g \pm 469 g and was 3,537 g \pm 463 g for girls. Half of the mothers ate fish at least three times a week and around half had whale meat (muscle) while pregnant. Slightly more of the mothers (60%) had whale blubber while pregnant. Most (82.6%) did not change their dietary habits during their pregnancy. Cord blood mercury had a geometric mean of 20.34 µg/L. Cord blood mercury had no effect on gestational length, birth weight, or placental weight. Different fatty acids and PCBs each had significant effects on one or more of the parameters measured (gestational length, birth weight, or placental weight) (Grandjean et al. 2001).

Seychelles Child Development Study (SCDS)

Two studies of Seychelles children have been carried out. One is the pilot cohort, started in 1987 with 789 children, and the second is the main cohort, started in 1989 with 778 children (Davidson et al. 2000). One purpose of the pilot cohort was to provide information for selection of parameters for the main cohort. Children involved in the main study cohort of the SCDS have had more thorough testing as compared to children from the pilot cohort (Davidson et al. 2000).

Children (n = 87) from the pilot cohort were given: five of 13 subtests of the Wechsler Intelligence Test for Children-III (knowledge of common events, spatial reasoning, word knowledge, short-term memory for numeric sequences, reproduction of visual symbol sequences), the California Verbal Learning Test (strategies and processes involved in learning and recalling verbal material), Boston Naming Test (ability to name pictures), Beery-Buktenica Developmental Test of Visual Motor Integration (copying simple to complex geometric figures), design memory subtest of the Wide Range Assessment of Memory and Learning (draw geometric designs from memory), Grooved Pegboard (timed test of manipulative dexterity), Trailmaking (speed and visual search, attention, mental flexibility, and motor function), and the Finger Tapping Test (motor speed). These tests were given in the same order by the team that administered the tests to children in the main SCDS cohort (Davidson et al. 2000).

The median maternal hair methylmercury exposure for the pilot cohort was 7.8 ppm and the mean was 9.4 ppm \pm 6.9 ppm (Davidson et al. 2000). The range was 0.6 to 35.4 ppm. The children scored similarly to nine year old children from the U.S. on the California Verbal Learning Test (CVLT), error scores for the Trailmaking test, the Finger Tapping Test, and the Beery-Buktenica Developmental Test of Visual Motor Integration. However, the children scored lower than scores expected from U.S. children, reported previously, on the Finger Tapping,

Grooved Pegboard, and Trailmaking tests. Scores on the five subtests of the Wechsler Intelligence Test for Children-III were also lower for children than would be expected for U.S. children, reported previously, but the distribution of the scores was similar. No reduction of scores due to methylmercury exposure occurred, and in some instances, with increased methylmercury exposures, scores increased (Davidson et al. 2000).

The primary analysis, which examined the association between prenatal methylmercury exposure and neurodevelopmental outcomes, did not identify any adverse effects of methylmercury exposure (Davidson et al. 2000). The second analysis accounted for both prenatal and postnatal methylmercury exposure. Two significant adverse associations were identified in the secondary analysis. One was with the CVLT Short Delay subtest and the other was with the CVLT Long Delay subtest. The authors stated that three influential points (data from three children) were identified in the analysis. These three children had been identified as having mild mental retardation. Prenatal methylmercury levels (maternal hair mercury) for these three ranged from 5 to 23 ppm and postnatal methylmercury levels (children's hair mercury collected at age 108 months) ranged from 3 to 32 ppm. When the authors removed these three, a significant adverse association was only with the CVLT Short Delay subtest (Davidson et al. 2000).

The main cohort of the SCDS was set up in 1989 and selected 779 mother-infant pairs, which represented 50% of the live births between February 1989 and January 1990 (Davidson et al. 2006A). Thirty-nine pairs were omitted, resulting in 740 remaining pairs for evaluation at six different ages. Children were evaluated at 6.0 (0.5 year), 19 (1.6 years), 29 (2.4 years), 66 (5.5 years), and 107 (9.0 years) months of age. Not all members of the cohort were evaluated at the different times (740 children at 6.0 months, 738 children at 19 months, 736 children at 29 months, 711 children at 66 months, and 643 children at 107 months). Global cognition endpoints were assessed at 19, 29, 66, and 107 months while cognitive function and developmental domains were assessed at 66 and 107 months. Mothers of the children ate an average of 12 fish meals per week during pregnancy (Davidson et al. 2006A).

Seychellois children (n = 738) from the main study cohort of the SCDS were evaluated at 19 months of age (Axtell et al. 1998). Total mercury was measured in segments of maternal hair grown during pregnancy. Interviews with the child's parent or caregiver provided the age that the child walked without support and talked (using words other than "mama" and "dada"). There were no differences in either age of walking or talking among the mercury exposed groups (Axtell et al. 1998).

Children from the SCDS (n = 711) were assessed at 66 months (Davidson et al. 1998). The children were tested with six primary measures: the General Cognitive Index of the McCarthy Scales of Children's Abilities (cognitive ability), the Preschool Language Scale Total score (receptive and expressive language), the Letter and Word Recognition and the Applied Problems subtests of the Woodcock-Johnson Tests of Achievement (reading and math readiness), the total error score from the Bender Gestalt test (visual-spatial ability), and the total T score from the Child Behavior Checklist (social and adaptive behavior). Pure tone hearing threshold, tested using a portable audiometer, and caregiver IQ were tested. Samples (greater than or equal to five samples for each species) of different species of fish commonly caught and eaten in the Seychelles were tested for mercury. Total mercury, in 350 fish samples, ranged from 0.004 ppm

to 0.75 ppm. Medians of mercury in the fish were between 0.05 and 0.25 ppm. PCBs were measured in the serum of 49 children. PCB was not detectable in the serum of any children (detection limit = 0.2 ng/ml) (Davidson et al. 1998).

In the primary analysis of children tested at 66 months, girls typically scored better than boys. The results were similar to those observed with U.S. children. Mercury levels did not alter their test results. A secondary analysis was carried out, comparing children with methylmercury exposure levels, measured in hair, of 3 ppm or less to children with exposure levels greater than 12 ppm. Test results were not different between these two groups of children (Davidson et al. 1998).

Discrete measures of behavior were examined in children 66 months of age from the SCDS (Myers et al. 2000). The mean maternal hair mercury (n = 708) during pregnancy was 6.8 ppm and the range was 0.5 to 26.7 ppm. The mean hair mercury for the 66 month old children was 6.5 ppm (n = 708) and the range was 0.9 to 25.8 ppm. Achenbach Child Behavior Checklist scores were similar to scores obtained from the U.S. population. No adverse effects were found in children with prenatal exposure to methylmercury and no consistent trends were identified with increasing exposure (Myers et al. 2000).

Palumbo et al. (2000) reanalyzed data from the main cohort of the SCDS obtained when the children were 66 months. Over 700 children (n = 711) were evaluated using the McCarthy Scales of Children's Abilities by Functional Domain. No adverse effects were identified from mercury exposure in this reanalysis, but it was noted that the children may not have been old enough for effects to be observable (Palumbo et al. 2000).

Children from the SCDS were evaluated at 66 months (5.5 years of age) and no adverse effects were found. Axtell et al. (2000) reanalyzed the data to determine if there was a nonlinear relationship between mercury levels and effects. Total mercury levels were determined from maternal hair (prenatal exposure) and the child's hair (postnatal exposure). Six developmental endpoints were evaluated. The reanalysis found two beneficial effects and one adverse effect with mercury exposure. The authors noted that less data was available for hair mercury levels above 10 ppm. The authors concluded that, overall, there was no "clear evidence" of adverse effects of mercury, over the entire exposure range, on the six endpoints (Axtell et al. 2000).

Davidson et al. (2006A) examined testing at multiple ages to determine if adverse effects of methylmercury exposure were developing over time. Total mercury in maternal hair was assessed to measure prenatal exposure. The mean mercury level in maternal hair was 6.8 ppm \pm 4.5 ppm and the range was 0.5 to 26.7 ppm. The children's hair was measured at 66 and 107 months to assess postnatal mercury exposure. Total mercury in hair had a mean of 6.5 ppm \pm 3.3 ppm and a range of 0.9 to 25.8 ppm at 66 months. At 107 months, the total mercury hair levels had a mean of 6.1 ppm \pm 3.5 ppm and a range of 0.5 to 24.8 ppm. However, at 107 months, 143 males out of the 643 cohort children had shaved their heads (as a style choice). Missing data was replaced with hair samples collected at 66 months for 129 subjects. Prenatal and postnatal total mercury in hair did not correlate. No adverse effects were identified for exposure to mercury. However, Davidson et al. (2006A) did note that SCDS children would be evaluated at age 15.

Davidson et al. (2006B) reviewed the data from the main cohort of the SCDS. Of the primary analyses done of the data, only one adverse association with prenatal mercury exposure was identified. The adverse effects were identified in the test of motor speed and coordination on the nondominant hand when the children were nine years old. Adverse effects were observed at maternal hair mercury levels of 10 to 12 ppm. A reanalysis might indicate that adverse effects may be present in the study population. (Two reanalyses are discussed below.) Davidson et al. (2006B) stated that the SCDS results may be due to delayed or latent neurotoxicity, similar to what is observed in adults after acute exposure to high levels of mercury.

Two reanalyses were done on the main cohort of the SCDS results obtained when the children were nine years old. The first was Huang et al. (2005). A nonlinear model was used to model data from 643 children, age 107 months (nine years), for six endpoints. There was evidence that a nonlinear relationship occurred for one of the six endpoints, although the only significant associations were the same as the primary (linear) analysis. The authors stated that the adverse effect of mercury, found in the Grooved Pegboard test for the nondominant hand in boys, and the beneficial effect of mercury, found on the Connors Teacher Rating scale, were probably due to chance. Using the nonlinear model, the authors speculated that possible adverse effects could occur in the uppermost range of mercury (above mercury levels of 12 ppm in maternal hair) (Huang et al. 2005).

The second reanalysis was to examine the possibility that different children would develop effects at different mercury exposure levels (nonhomogenous susceptibility) (Huang et al. 2007). Twenty-one endpoints were evaluated, taking into account the child's home environment among other factors. The child's home environment was evaluated because it can improve a child's IQ above the level biologically inherited from the parents. The authors found an adverse association of Connors Teacher Rating Scale scores and postnatal mercury exposure. Associations of mercury exposure, using this model, were observed that were not present with the previous two analyses. Because of this, exposure and outcome relationships may not be homogenous to the study population. This could mean that not all of the children would respond the same to equivalent mercury exposures. Huang et al. (2007) concluded that the effects of prenatal mercury exposure might not be the same between children with different backgrounds.

van Wijngaarden et al. (2006) developed a benchmark dose from the data obtained from the main cohort in the SCDS at nine years of age. Close to 650 children (n = 643) were tested for 26 endpoints. This data was modeled with the k-power model and a 95% lower confidence limit on the benchmark dose was determined to be 20.4 ppm (range 17.9 to 23.0 ppm). The lower limit on the benchmark dose for the nine year old children's data was 20% less than the value obtained using data from 66 months of age. The authors suggested that as the children mature, associations between methylmercury and neurodevelopment are detectable at lower exposure levels (van Wijngaarden et al. 2006).

Davidson et al. (2008) conducted a study of prenatal methylmercury exposure and visuospatial ability of children from the SCDS at 10.7 years. This age was an addition to the original plan of the SCDS in order to provide test results that could be compared to the test results obtained from the Faroe Islands study. The authors found no association between prenatal methylmercury and the Bender Copying Task, as was seen in studies with Faroe Islands children. There was a

significant adverse association between prenatal methylmercury and the Reproductions Task, when including a single data point the authors termed as an outlier. The authors noted that the study may be underpowered and therefore would not be able to measure subtle effects (Davidson et al. 2008).

Children from the main cohort of the SCDS had mercury effects on blood pressure evaluated at ages 12 and 15 (Thurston et al. 2007). Data was obtained as part of normal school medical exams from 644 12-year-olds (313 boys and 331 girls) and 559 15-year-olds (267 boys and 292 girls). Increased prenatal mercury exposure was associated with an increase of diastolic blood pressure at age 15, but not at 12 years (Thurston et al. 2007).

Cardiovascular effects

Heart rate variability (HRV) is the beat-to-beat variability that occurs normally to allow people to respond to different circumstances. Certain parameters of HRV indicate parasympathetic nervous system functioning. Sandercock et al. (2005) recommended that reliability coefficients always be checked when measuring HRV. Leicht and Allen (2008) assessed the reliability of HRV data using children and discussed the reliability of adult HRV data. (Neither the children or adults had measurements of mercury.) For the adults, HRV data was reliable over one year, however, data produced from one study may not be reproducible (Leicht and Allen 2008).

The children in Leicht and Allen's (2008) study were group (n = 10 [six boys and four girls]) of non-exercising children (seven to 12 years old) who had HRV measured eight weeks apart. HRV was measured resting and during three exercise trials. The heart rate and HRV was not significantly altered over eight weeks either for the resting or during light to moderate exercise. However, the authors found large intra-individual variation. The variation in the children's HRV might be due to their developing and maturing autonomic nervous system. The authors noted that this study was not conclusive and an expanded study was necessary (Leicht and Allen 2008).

The Kuopio Ischaemic Health Disease Risk Factor Study (KIHD) reported results from 1,871 men, ages 42 to 60, after 10 years as study participants (Rissanen et al. 2000). The purpose of this study was to assess the effects of docosahexaenoic acid (DHA) + docosapentaenoic acid (DPA) on the risk of acute coronary events. Mercury was also measured to determine if it altered the effects of DHA + DPA. The men had a mean hair mercury of 1.91 ppm and a range of 0 to 15.67 ppm. The authors found an increased relative risk of acute coronary events in men with lower serum DHA + DPA and higher (greater than 2.0 ppm) hair mercury (Rissanen et al. 2000).

The KIHD continued to follow men (n = 1,871) in eastern Finland to determine if methylmercury exposure had cardiovascular effects (Virtanen et al. 2005). The men were followed for an average of 13.9 years and ranged in age from 42 to 60 at enrollment. The mean hair mercury was 1.9 ± 1.9 ppm (range 0 to 15.7 ppm). Men were divided into three groups based on hair mercury levels, less than 0.84 (n = 624), between 0.84 and 2.03 (n = 625), and greater than or equal to 2.03 ppm (n = 622). Men with low hair mercury and high serum DHA + DPA had a decreased risk of cardiovascular events or death. High hair mercury (highest of the three groups) was significantly associated with an increased risk of coronary events, such as cardiovascular disease (CVD), coronary heart disease (CHD), and all-cause mortality compared to the other two groups

combined. The authors calculated that for each microgram of mercury in hair, the risk of acute coronary event, CVD death, CHD death, and any death increased by approximately 11%, 10%, 13%, and 5%, respectively. For these study participants, it appeared that beneficial effects of fish oil, measured as serum DHA + DPA, were reduced with higher hair mercury levels (Virtanen et al. 2005). This study is used for the calculation of an RfD based on cardiovascular effects at the end of this section.

The Health Professionals Follow-up Study was composed of 51,529 men, ages 40 to 75 at enrollment in 1986 (Yoshizawa et al. 2002). These men were dentists, veterinarians, pharmacists, optometrists, osteopathic physicians, and podiatrists. Four hundred and forty-two participants with CHD were matched to a study participant without CHD. Toenail mercury levels in the control group ranged from 0.03 to 14.56 ppm. Dentists in the control group had a mean toenail mercury of 0.91 ± 1.47 ppm and nondentists, also in the control group, had a mean toenail mercury of 0.45 ± 0.40 ppm. Toenail mercury increased with increasing fish consumption. The mean toenail mercury for the control group was 0.72 ± 1.40 ppm and for the patients with CHD was 0.74 ± 1.21 ppm. The authors did not find any alteration of CHD risk with an increase in toenail mercury. Omega-3 fatty acid intake from fish also did not change the risk of CHD. If dentists were excluded from the analysis, a positive association, but not statistically significant, was observed between mercury levels and CHD risk (Yoshizawa et al. 2002).

Toenail mercury levels in male patients who had a myocardial infarction ($n = 684$) were compared to control men ($n = 724$) (Guallar et al. 2002). All men were age 70 or younger and were from Finland, Germany, Israel, the Netherlands, Norway, Russia, the United Kingdom, Spain, and Switzerland. Toenail mercury averaged 0.25 ppm in the control groups and 0.27 ppm in the patient group. After adjustments for age, location, and DHA levels, patients had higher toenail mercury as compared to the control group. The authors found an increased risk for myocardial infarction with increased toenail mercury. Higher DHA levels were associated with a lower risk of myocardial infarction, after adjusting for mercury level. The authors cautioned that there may be errors in measurement of mercury and DHA due to only one sample collection and the low levels of mercury and DHA in the samples. Associations between myocardial infarction and mercury and DHA may have been underestimated as a result (Guallar et al. 2002).

Murata et al. (2006) conducted a study to assess prenatal methylmercury exposure and cardiac effects. Study participants were mothers and children in first grade from 28 schools in the Akita and Tottori Prefectures, Japan ($n = 136$). Methylmercury was measured from dried cord tissue, which was saved according to local custom. Levels of methylmercury in the dried cord tissue ($n = 136$) were 0.017 to 0.367 ppm. These levels were lower than methylmercury levels in patients with Minamata disease (0.15 to 4.7 ppm), and even lower than levels from the Faroe and Seychelles studies. At the time of the study, mercury in the children's hair was between 0.43 and 6.32 ppm. Methylmercury in cord tissue correlated negatively with indicators of parasympathetic components of cardiac autonomic function measured in the children (Murata et al. 2006). Cord tissue methylmercury positively correlated with LF/HF ratio and percent LF, which together represent a sympathovagal balance. This indicates that cardiac autonomic function was controlled by the sympathetic nervous system because of vagal hypoactivity (less parasympathetic nervous system control) due to increased mercury exposures (Murata et al. 2006).

Valera et al. (2008) examined the Inuit at Nunavik for cardiac effects due to mercury exposure. Historical levels of mean blood mercury were 48 µg/L from a 1977-1982 study and 21.8 µg/L from a 1992 study. These levels were higher as compared to the mean blood mercury levels from people from Southern Quebec (0.74 µg/L) and Nunavik residents of Caucasian origin (3.72 µg/L). The study conducted by Valera et al. (2008) resulted in mean blood mercury (n = 205) of 26.64 µg/L with a range of 0.48-152 µg/L. Holter Monitor data was obtained for 280 adults. Due to technical difficulties, only data from 205 adults were used (85 men and 120 women with a mean age of 52.1 years). Changes in the cardiovascular system, blood pressure, and HRV occurred with increased blood mercury levels. Possible methylmercury cardiovascular effects included increased myocardial infarction, high blood pressure, and reduced HRV (Valera et al. 2008).

Belanger et al. (2008) investigated the connection between methylmercury exposure and cardiovascular risk predictors. Men (n = 31), ages 22-61 with a mean age of 46.7, were all James Bay sport fishers. Twelve were current smokers. Men taking lipid altering medications were excluded. Blood samples were taken before and after the fishing season, and the exposure of these men was described as “moderate seasonal exposure.” Blood mercury levels were measured along with lipid and fatty acid profiles, low density lipoprotein oxidation, and blood antioxidant status. Blood mercury levels had a mean of 4.38 ± 0.74 µg/L before the fishing season and a mean of 7.12 ± 1.04 µg/L after the fishing season. Blood mercury levels increased by 63% by the end of the fishing season. The mean hair mercury levels were 1.4 ± 0.3 ppm before the fishing season and 2.8 ± 0.4 ppm after the fishing season. Hair mercury levels increased by 100% by the end of the fishing season. Due to the fish consumption, the men had increased high density lipoprotein, by 5%, and decreased very low density lipoprotein cholesterol and triacylglycerols, by 8 to 9%. The changes in blood lipids and other predictors of cardiovascular risk were not associated with mercury levels. The authors found no indication that increased mercury would adversely affect cardiovascular health (Belanger et al. 2008).

Choi et al. (2009) investigated cardiovascular effects of methylmercury exposure in Faroese whaling men. The authors collected detailed whale consumption data on the 41 men, ages 30 to 70, involved in the study. Twenty-six of the men had greater than or equal to three meals of whale a month. The intima-media thickness (IMT) of the carotid arteries was measured as an indicator of carotid arteriosclerosis. Seven years before the more recent samples (hair, blood, and toenails), mercury was measured in hair. Mercury hair levels from seven years ago had a geometric mean of 13.9 ppm and a range of 4.80 to 43.7 ppm. More recent hair mercury levels were lower and had a geometric mean of 7.31 ppm and a range of 0.92 to 46.0 ppm. Blood mercury had a geometric mean of 29.5 µg/L and a range of 5.19 to 128.4 µg/L. Toenail mercury had a geometric mean of 2.04 ppm and a range of 0.14 to 8.26 ppm. Serum PCBs were also measured and positively correlated to mercury levels. Elevated methylmercury levels were not associated with age. Increases in blood pressure and IMT were observed in men with elevated mercury levels. Brainstem auditory evoked potential latencies had slight delays with increased mercury. Serum PCB levels had no statistically significant association with the outcomes measured. Choi et al. (2009) concluded that methylmercury may aid in development of cardiovascular disease.

The recent study of cardiovascular events in 1,871 Finnish men (Virtanen et al. 2005), discussed above, was selected to provide a point of departure for an RfD for mercury-related cardiovascular effects. Men with hair mercury above 2.03 ppm had an increased risk for cardiovascular events and death. A NOAEL value of 2.0 ppm hair mercury was selected and converted to 8.0 ppb blood mercury using Equation G-2 in Appendix G. Intake was calculated from the 8.0 ppb blood value using Equation G-1 in Appendix G with variables from Table G-1. An RfD of 0.014 µg/kg/day results from the intake (0.14 µg/kg/day) divided by an uncertainty factor of 10 (for pharmacokinetic and pharmacodynamic variability).

Table E-1 lists fish concentrations for different numbers of fish meals using an RfD of 0.014 µg/kg/day based on cardiovascular effects. This RfD is lower than the one for neurological effects and is used for both the general and sensitive populations, as this RfD is also protective of neurological effects. At this time, the uncertainty of methylmercury causing cardiovascular effects precludes recommendation of using this RfD as a basis for FCSVs. Cardiovascular effects that are not due to an alteration of cardiac autonomic function were primarily measured in adult men. It is unclear as to whether the outcomes measured were due to current methylmercury exposure or latent effects of alterations in neurologic function, including cardiac autonomic function. The purpose in presenting this was to provide an example of FCSVs protective of cardiovascular effects, based on data from currently published studies.

Table E-1: FCSVs based on an RfD protective for cardiovascular effects.

Population	Fish Concentration (ppm)	Fish Meals
General Population	Less than 0.011	Unrestricted
	Greater than 0.011 to 0.034	One meal/week
	Greater than 0.034 to 0.148	One meal/month
	Greater than 0.148 to 0.296	Six meals/year
	Greater than 0.296	Do not eat
Sensitive Population (women of childbearing age and children under 15)	Less than 0.009	Unrestricted
	Greater than 0.009 to 0.029	One meal/week
	Greater than 0.029 to 0.124	One meal/month
	Greater than 0.124 to 0.247	Six meals/year
	Greater than 0.247	Do not eat

- All calculated values rounded to 3 decimal places
- Adapting U.S. EPA's equation for consumption limit of fish (contaminant amount [mg/kg] = (RfD*bw)/Fish consumption [kg/day] [mg/kg = ppm]) (U.S. EPA 2000)
 - RfD = 0.000014 mg/kg/day
 - Body weight (U.S. EPA 1997)
 - General population body weight (bw) = 78.1 kg
 - Sensitive population bw (female of child-bearing age) = 65.4 kg
 - Fish Consumption (0.227 kg fish/meal):
 - Unrestricted = 156 meals/year (97 g fish/day)
 - One meal/week = 52 meals/year (32 g fish/day)
 - One meal/month = 12 meals/year (7.4 g fish/day)
 - Six meals/year = 3.7 g fish/day

Additional Human Studies

Abdelouahab et al. (2008) studied freshwater fish consumers, 124 men and 87 women, between the ages of 18 and 74, from two communities in Canada to determine if there were relationships between gender, thyroid hormone levels, and contaminants in fish, including mercury. The two communities surveyed were from the Lake St Pierre area and Northeastern Quebec. The Lake St Pierre group was surveyed in March/April 2003 and Northeastern Quebec group was surveyed in July/August 2003. No gender differences were found between bioindicators of mercury or blood selenium. Total blood mercury had an interquartile range of 0.72 to 6.02 µg/L for men and 0.60 to 4.90 µg/L for women. The maximum total blood mercury was 26.90 µg/L for men and 15.7 µg/L for women. The interquartile range of hair mercury was 0.22 to 1.37 ppm for men and 0.15 to 1.15 ppm for women. Maximum hair mercury was 8.16 ppm for men and 4.96 ppm for women. Concentrations of total blood mercury and hair mercury increased with age. Freshwater fish consumption was positively related to total blood mercury, blood methylmercury, and hair mercury. These relationships did not occur with market fish consumption. The thyroid stimulating hormone level positively related to total, inorganic, and methylmercury in blood and total mercury in hair in men only (Abdelouahab et al. 2008).

Oken et al. (2008) looked into maternal fish intake and mercury levels in 341 Massachusetts women. The mean maternal fish intake was 1.5 servings/week from a range of 0-7.5 servings/week. Twelve percent of the women (40) consumed more than two servings/week, and 14% (47) never consumed fish. A maternal consumption of greater than two servings/week was associated with higher scores on the children's cognitive tests. The red blood cell total mercury had a mean of 3.8 ng/g and a range of 0.03-21.9 ng/g. Thirty-five of the women had levels above the 90th percentile (9.1 ng/g). Maternal fish intake correlated with red blood cell total mercury. Higher maternal red blood cell mercury levels were associated with poorer child test performance (Oken et al. 2008).

Maternal red blood cell total mercury greater than 9.1 ng/g resulted in different test performances in children depending on maternal fish intake. Consumption of less than or equal to two servings of fish/week with high red blood cell total mercury levels (greater than 9.1 ng/g) resulted in lower scoring by the children on the Wide Range Assessment of Visual Motor Abilities (WRAVMA) total score (Oken et al. 2008). Consumption of greater than two servings of fish/week with high red blood cell total mercury levels (greater than 9.1 ng/g) resulted in scores higher than the group of children eating less than or equal to two servings on the WRAVMA total score. The greater the maternal fish consumption, the better the scores of the children at three years of age in tests of language and visual motor skills. Greater than 90% of the total blood mercury was in the red blood cells and approximately 95% of that mercury was methylmercury. This study did not measure overall intelligence. The authors concluded that the benefit of fish intake increased with adjustments for mercury levels. The authors further suggested that if mercury contamination were not present there would be greater cognitive benefits of fish intake (Oken et al. 2008).

Xue et al. (2007) investigated 52 prenatal clinics in five Michigan communities during the Pregnancy Outcomes and Community Health (POUCH) study. The POUCH study involved 1,024 women and examined the relationship of maternal mercury and preterm deliveries. The

women ate an average of 19.6 ± 28.2 meals of fish (canned, bought, sport-caught, and other fish and shellfish) during the first six months of pregnancy. Hair mercury ranged from 0.01 to 2.50 ppm. A majority (70-90%) of the mercury in hair was methylmercury. An association was found between preterm deliveries, defined as less than 35 weeks, and maternal hair mercury levels greater or equal to 0.55 ppm (Xue et al. 2007).

Weil et al. (2005) investigated whether methylmercury affected memory in aging people. Close to 500 people ($n = 474$, ages 50 to 70, 325 women) in the Baltimore Memory Study were given tests to measure neurological function and a questionnaire regarding their food intake over the past year. The mean blood mercury was 2.76 ± 2.35 $\mu\text{g/L}$. In the final model developed by the authors, an increase in mercury resulted in a 3% decline in test scores for the Rey complex figure-delayed recall test, which measures visual memory. However, increased mercury also resulted in a 2% increase in test scores for both dominant and non-dominant hand finger tapping, which measures motor and manual dexterity. Due to the small amount of increase and decrease in tests scores, the authors speculated that mercury might have no effect on the participant's test results (Weil et al. 2005).

Appendix F: Discussion of Animal Toxicity Studies

Cats and monkeys are more sensitive than rodents to inorganic and organic mercury toxicity. However, some strains of rats and mice have an autoimmune response when exposed to low levels of mercury vapor or mercuric chloride. This can lead to kidney damage (ATSDR 1999). Certain rodent strains are more susceptible to mercury induced autoimmunity than others (Hultman and Nielsen 1998).

Cynomolgus monkeys (*Macaca fascicularis*, n = 5) were treated with methylmercury, 50 µg/kg/day, orally from birth until three to four years of age (Rice and Gilbert 1982). Blood mercury levels peaked at 1.2 to 1.4 ppm and then declined to 0.6 to 0.9 ppm. At this treatment level, the monkeys did not have any overt signs of toxicity. Food intake and weight gain were similar between control and treated monkeys. When tested at three to four years of age, the methylmercury treated monkeys had impaired spatial vision compared to the controls (Rice and Gilbert 1982).

Monkeys (*Macaca fascicularis*) were gavaged orally with methylmercury at birth and then at one, two, and three weeks of age (Burbacher et al. 2005). No difference in weight and brain weight was seen between treated and untreated monkeys. Data of total blood mercury, measured over time, fit a one compartment model. The authors estimated that one twentieth of the mercury body burden is in the blood. Two days after the first mercury dose, blood levels were 8.0 to 18 ng/ml. Peak blood mercury levels, after all four doses, were 30 to 46 ng/ml. The half-life of mercury in the blood was calculated as 19.1 ± 5.1 days, while half-life in the brain was 59.5 ± 24.1 days. The concentration of total mercury in the brain was 2.5 ± 0.3 times higher than the concentration of mercury in the blood (Burbacher et al. 2005).

Adult male marmosets (n = 6, four years old) were given methylmercury (5.0 ppm) in drinking water for different times (Eto et al. 2002). Animals were divided into three groups of two. For the first set of two (animals A and B), each was treated for a different time (A was treated for 70 days and B was treated for 90 days) to model an acute exposure with symptoms of methylmercury poisoning. The second set (animals C and D) was also treated for different times (C for 24 days and B for 31 days), but both were sacrificed 38 days after the exposure began to model an acute subclinical methylmercury poisoning. Animals E and F, the third set, were both treated for 21 days and then observed for 2.5 years to model a transient subclinical methylmercury exposure (total lifespan is between 10 and 15 years). Control animals (n = 4) were between two and six years of age (Eto et al. 2002).

For animals A and B, blood levels of mercury increased quickly over three weeks, to mercury levels above 8,000 µg/L. Body weight decreased during the methylmercury treatment for animals A and B. Examination of the brains of animals A and B revealed severe neuronal loss and alterations of cell numbers. The exposure stopped for animals C and D before significant toxic symptoms were observed. However, the exposure for each animal was only discontinued after blood mercury exceeded 6,000 µg/L. Blood mercury levels for animals E and F declined to control levels by 400 days. On day 180, after discontinuation of mercury treatment, animal E developed slight limb ataxia and visual impairment. Animal F was only noted as developing irritability or anxiety (Eto et al. 2002).

Satoh (2003) reviewed many rodent experiments with mercury exposure and found that the timing of the exposure affected the exhibition of health effects. The effects fit within the category of behavioral teratology (postnatal effects of prenatal exposure). Effects included abnormal development, behavioral deviation, neurological disorder, immunological deficiency, generalized debilitation, premature death, reproductive debility, and birth defects. Offspring of rats given mercury, as low as 0.16 milligram (mg)/kg, had neurological effects.

Methylmercury-induced autoimmune syndrome occurs only in genetically susceptible mice (Havarinasab et al. 2007). Most strains of mice have methylmercury-induced neurotoxicity, but a genetic susceptibility is required for immunotoxicity. A reported lowest observed effect level (LOEL) for immune effects resulting from methylmercury exposure is 0.3 mg/kg/day, while a LOEL for methylmercury-induced neurotoxicity is lower at 0.2 mg/kg/day. Mice were treated for 30 days with methylmercury, in the form of methylmercuric chloride (4.2 mg/L) in drinking water (420 µg/kg/day). After 30 days of treatment, the kidney mercury level was 42 ppm. Eighty percent of the total mercury was methylmercury. The mercury in lymphoid tissue was 7.4 ppm and 77% of the total mercury was methylmercury. A 70% reduction in kidney and lymphoid tissue mercury levels occurred two weeks after stopping the treatment. The authors calculated a seven day half-life for mercury in the lymphoid tissue and an eight day half-life for mercury in the kidneys. Inorganic mercury had a half-life of 19 days in the lymph nodes and 22 days in the kidneys (Havarinasab et al. 2007).

Mice (OLA129/C57BL/6J mice [wild type] and metallothionein [MT] I/II-knockout mice [MT-null]) were exposed perinatally to low levels of cadmium or methylmercury to determine if developmental effects would occur (Mori et al. 2006). Methylmercury has been linked to neurological abnormalities, and cadmium has been linked to renal dysfunction. Methylmercury was fed to the mice at 5.0 ppm in chow. The methylmercury fed mice had no change in litter size or birth weight. There also was no difference in liver type 1 deiodinase or brain type 2 deiodinase activities. Thyroxine levels were higher in methylmercury treated MT-null mice as compared to the wild-type methylmercury treated mice. Brain type 3 deiodinase activity changed in both wild-type untreated and methylmercury treated groups. Overall, there were changes in brain deiodinase activities changed due to methylmercury treatment (Mori et al. 2006).

Mice (OLA129/C57BL/6J mice [wild type] and metallothionein [MT] I/II-knockout mice [MT-null]) were fed methylmercury, 5.0 ppm, starting on the first day of gestation and going through early lactation, to postnatal day (PND) 10 (Yoshida et al. 2008). MT is a protein that protects cells from heavy metals. On PND 10, the treated male offspring had brain mercury levels of 384 ± 146 ng/g, for wild-type, and 486 ± 120 ng/g, for the MT-null, while all control mice had levels of 2 ± 1 ng/g. Three months after birth all mice, both male and female control and treated, had brain mercury levels of 4 to 7 ± 1 ng/g. The offspring had no statistically significant behavioral changes at 12 to 13 weeks of age. However, at 52 to 53 weeks of age certain groups of offspring treated with mercury had statistically significant differences in performance on the tests as compared to controls. For the test that measures distance traveled in a cage in 10 minutes, mercury treated male and female wild-type mice traveled a statistically significant reduced distance while male and female MT-null mice traveled a statistically significant greater difference as compared to the control mice (Yoshida et al. 2008).

Mice (2 types of knock-out and wild-type mice) were treated perinatally with 1 μ M methylmercury, at 0.2 mg/L in drinking water (Bjorklund et al. 2007). Only results from wild-type mice are discussed here. (Knock-out mice were not discussed because brain mercury levels were not measured for those mice.) Higher levels of total mercury were found in the brains of treated wild-type mice (males 11.07 ± 0.713 ng/g, females 11.90 ± 0.693 ng/g) as compared to the brains of control wild-type mice (males 6.07 ± 0.318 ng/g, females 5.97 ± 0.203 ng/g). No difference between total mercury levels in male and female mice was observed. However, adolescent males exposed to methylmercury had a significant reduction of horizontal activity and rearing as compared to the untreated males and all females (Bjorklund et al. 2007).

Bjorklund et al. (2007) also noted that mice may be a more appropriate research model than rats as mice have a blood to brain ratio close to 1: 1, which is closer to the ratio for primates (2.6-3.8: 1) than the blood to brain ratio in rats (0.06: 1). Mercury levels found in the treated mice were within the range found in adult human brains from normal populations (mercury levels ranging from around 10 to 100 ng/g). Although females have a faster whole-body clearance than males, there was no difference in total mercury in male and female brains (Bjorklund et al. 2007).

Female Wistar rats were fed 5.0 ppm methylmercury for eight weeks before mating (Sakamoto et al. 2002). This allowed the blood levels of methylmercury to plateau before gestation. Pregnant rats were fed 5.0 ppm methylmercury throughout gestation and lactation and then the offspring were fed 5.0 ppm methylmercury after weaning, on PND 30, for 25 additional days. These offspring had no signs of mercury poisoning. However, motor control was different in methylmercury treated rats as compared to control. Two rats were selected from four different litters (8 control rats and 8 methylmercury treated rats). Only 12.5% of the methylmercury treated rats were able to stay balanced on a rod for 60 seconds while 87.5% of the control did. This was a statistically significant difference. In the passive avoidance test, with electric shocks in a dark compartment, 100% of the methylmercury treated rats went to the dark compartment while only 12.5% of the control rats did. There was no difference in performance of the water maze test between control and methylmercury treated rats. Offspring from all four of the litters were selected for visual analysis of the brains. Abnormalities were observed in 50% (3 of the 6) of the methylmercury treated rats and none of the control rats (0 of 6) (Sakamoto et al. 2002).

Male Wistar rats were chronically exposed to methylmercury, as methylmercuric chloride, at 0, 1.0, and 5.0 ppm (Yasutake et al. 1997). Total mercury was measured at 3.0 ppm in the brain of rats given 5.0 ppm methylmercuric chloride after two years of exposure. The kidney mercury level of rats in the 5.0 ppm group was almost 100 ppm by the second year of treatment. Toxicity of the kidney was observed after two years of exposure to 5.0 ppm. The authors concluded that mercury levels were too low for neurological effects, but 5.0 ppm was high enough for kidney toxicity. By 30 months, rats in all three groups were observed to have hind limb-crossing, which was attributed to aging. After 32 months, 50% of the rats given 5.0 ppm died. After 34 months, 50% of the rats given 0 or 1 ppm died (Yasutake et al. 1997).

There have been reports that selenium, zinc, cysteine, protein, fats, vitamins, and fiber are able to modulate mercury toxicity (Jin et al. 2007). A previous study found that coconut oil increased the whole body retention of mercury, while cod liver oil did not. To investigate this further, rats were fed fish oil, lard, soy oil, seal oil, or docosahexaenoic acid (DHA) for 26 days before

methylmercury (0, 1, or 3 ppm) treatment. Rats were chosen because they bind more mercury in the blood than mice or guinea pigs. Rats also have a higher blood to brain ratio as compared to mice (Jin et al. 2007).

Jin et al. (2007) treated male Sprague-Dawley rats orally for 14 days with methylmercury chloride while feeding with different dietary fats. Methylmercury alone had a significant effect on relative liver, spleen, testis, and epididymis weight. Methylmercury treatment altered monocyte counts and had significant effects on white blood cell counts and neutrophil counts. The authors found a significant effect of diet on relative liver, spleen, and epididymis weight. Relative heart weight was also significantly altered by diet. Diet had significant effects on white blood cell and neutrophil counts and also altered IgM levels. Both diet and methylmercury altered immunoglobulin (Ig) G levels. A significant effect of diet and methylmercury on the relative weight of the thymus was identified. Diet and methylmercury interacted significantly to alter the relative thymus weight. All livers, from rats in all treatment groups had background levels of inflammation possibly indicating a non-study related problem. These results suggest that the target organ for methylmercury toxicity changes with the dietary fat (Jin et al. 2007).

Neurotoxicity of methylmercury and mercuric sulfide was measured in male Sprague-Dawley rats (Chuu et al. 2007). Mercuric sulfide is toxic between 0.1-1.0 g/kg/day, but not toxic at 0.01 g/kg/day. Methylmercury toxicity is thought to be approximately 1,000 times more potent than mercuric sulfide. In this study, rodents were treated with mercuric sulfide (1.0 g/kg/day) and methylmercury (0.002 g/kg/day). Methylmercury treatment resulted in lower body weight, reduced motor equilibrium, and changed both nerve conduction and velocity increased tail flick latency. Mercury accumulated in the liver, kidney, and cerebral cortex. A greater accumulation occurred with methylmercury exposure as compared to mercuric sulfide exposure (Chuu et al. 2007).

Devlin (2006) examined methylmercury toxicity in fathead minnows. Methylmercury was only a fraction of the mercury in the water column, and has been measured to be around 15% of total mercury in freshwater systems in Eastern North America. Acute exposure typically occurs at higher concentrations, in the $\mu\text{g/L}$ range, and the usual environmental range is ng/L . Fathead minnow embryos exposed to methylmercury displayed unusual behaviors. They were less active swimmers and had occasional erratic twitching movements as compared to the controls. Methylmercury treated embryos also had circulatory system abnormalities and an irregular heartbeat. The lethal concentration producing 50% mortality (LC_{50}) was 221 (one day of exposure), 71 (two days of exposure), 42 (three days of exposure), and 39 (four days of exposure) $\mu\text{g/L}$ (Devlin 2006).

Appendix G: Equations for Conversion of Mercury in Hair or Blood to Intake

Blood mercury values, either calculated from hair or measured directly from blood, can be converted to mercury intake. Equation G-1 is the estimation of mercury intake from blood mercury levels (Stern 2003).

Equation G-1: Calculation of mercury intake (in $\mu\text{g}/\text{kg}/\text{day}$) from blood mercury (in $\mu\text{g}/\text{L}$ or ppb).

$$(\text{dose ingested}) \mu\text{g} / \text{kg} / \text{day} = \frac{(\text{bloodHg}) \cdot 1 / R \cdot b \cdot V}{A \cdot f \cdot \text{bw}}$$

maternal dose ingested = maternal dietary intake = $\mu\text{g}/\text{kg}/\text{day}$

cord blood mercury (Hg) in $\mu\text{g}/\text{L}$ (ppb)

R = ratio of cord blood to maternal blood

b = elimination constant (days^{-1})

V = volume of blood in the body (L)

A = absorption factor (no units)

f = fraction of daily intake in blood (no units)

bw = body weight in kg

Values used for the variables in Equation G-1 are presented in Table G-1, including all of the values used by the U.S. EPA.

Table G-1: Values for variables in the equation calculating mercury intake from blood mercury.

Symbol for variable	Variable	U.S. EPA's value ^a	Modified values for neurological effects	Modified values for cardiovascular effects
Not applicable	Blood mercury (Hg) in $\mu\text{g/L}$ (ppb)	58 (cord blood)	Same as U.S. EPA	8.0 (based on 2.0 $\mu\text{g/g}$ hair mercury)
R	Ratio of cord to maternal blood (no units)	1.0	Same as U.S. EPA	Not applicable
b	Elimination constant in days^{-1}	0.014	Same as U.S. EPA	Same as U.S. EPA
V	Volume of blood in the body in L	5.0	5.6 ^b	5.5 ^b
A	Absorption factor (no units)	0.95	Same as U.S. EPA	Same as U.S. EPA
f	Fraction of the daily intake in blood (no units)	0.059	Same as U.S. EPA	Same as U.S. EPA
bw	Body weight in kg	67.0	79.15 ^c	78.1 ^d

a = values are taken from the methylmercury IRIS webpage (U.S. EPA 2001A)

b = blood volume is calculated as seven percent of the body weight (U.S. EPA 2001B)

c = body weight of 79.15 kg represents an average body weight of 65.4 kg (U.S. EPA 1997) plus an average pregnancy weight gain of 13.75 kg (NRC and IOM 2007)

d = body weight of 78.1 kg represents average male body weight (U.S. EPA 1997)

In converting hair mercury levels to blood mercury levels, the assumption is that the ratio of hair to blood mercury is 250:1. In previously published reports this ratio has ranged from 140:1 to 416:1 (ATSDR 1999). Differences in the reported ratios may be due to sampling differences, such as hair location and distance from the scalp, or population differences, such as metabolism differences because of different genetic backgrounds. Equation G-2 converts hair mercury to blood mercury (U.S. EPA 2001B). If population specific data is known, the hair to blood mercury ratio could be altered to account for population specific differences.

Equation G-2: Converting hair mercury (in ppm or mg/kg) to blood mercury (in ppm or mg/L).

$$(bloodHg)mg / L = \frac{(hairHg)mg / kg}{250}$$

Hg = mercury

mg/L is the same as ppm

mg/kg is the same as ppm

Appendix H: Development of FCSVs

Table H-1 lists fish concentrations for different numbers of fish meals using an RfD of 0.1 µg/kg/day derived from a lower limit on a benchmark dose based on neurological effects. The same RfD is used for both the general population and a sensitive population due to provide a measure of protection against methylmercury related cardiovascular effects.

Table H-1: FCSVs based on an RfD protective for neurological effects.

Population	Fish Concentration (ppm)	Fish Meals
General Population	Less than 0.08	Unrestricted
	Greater than 0.08 to 0.24	One meal/week
	Greater than 0.24 to 1.06	One meal/month
	Greater than 1.06 to 2.11	Six meals/year
	Greater than 2.11	Do not eat
Sensitive Population (women of childbearing age and children under 15)	Less than 0.07	Unrestricted
	Greater than 0.07 to 0.20	One meal/week
	Greater than 0.20 to 0.88	One meal/month
	Greater than 0.88 to 1.77	Six meals/year
	Greater than 1.77	Do not eat

- All calculated values rounded to 2 decimal places
- Adapting U.S. EPA’s equation for consumption limit of fish (contaminant amount [mg/kg] = (RfD*bw)/Fish consumption [kg/day] [mg/kg = ppm]) (U.S. EPA 2000)
 - RfD = 0.0001 mg/kg/day
 - Body weight (U.S. EPA 1997)
 - General population body weight (bw) = 78.1 kg
 - Sensitive population bw (female of child-bearing age) = 65.4 kg
 - Fish Consumption (0.227 kg fish/meal):
 - Unrestricted = 156 meals/year (97 g fish/day)
 - One meal/week = 52 meals/year (32 g fish/day)
 - One meal/month = 12 meals/year (7.4 g fish/day)
 - Six meals/year = 3.7 g fish/day

To allow for comparison to other states’ fish screening values, including Great Lakes states, levels on mercury for meal advisories for the sensitive population are included in Table H-2.

Table H-2: Fish screening levels for sensitive populations in seven different states.

State	Unrestricted	1 meal per week	No consumption
Maine ^a	NA	0.2 ppm (8 ounces)	NA
Pennsylvania ^b	Up to 0.12 ppm	0.13 to 0.25 ppm	Greater than 1.9 ppm
Indiana	NA	Less than 0.16	Greater than 0.65 ppm
Illinois	Less than or equal to 0.06 ppm	Greater than 0.06 to 0.23 ppm	Greater than 1.89 ppm
Wisconsin	NA	0.05 to 0.22 ppm	Greater than 1.0 ppm
Minnesota	Less than or equal to 0.05 ppm	Greater than 0.05 to 0.20 ppm	Greater than 1.0 ppm
Ohio	NA	0.05 to 0.219 ppm	Greater than 2.0 ppm

NA = not available

a = Maine information taken from MBH (2001)

b = Pennsylvania, Indiana, Illinois, Wisconsin, Minnesota, and Ohio information taken from GLFAW (2007)