ASSESSMENT OF TOTAL MERCURY AND METHYL MERCURY IN SELECTED
SUBSISTENCE FISH IN WESTERN ALASKA

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SUBSISTENCE FISH IN WESTERN ALASKA

A

THESIS

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By

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Abstract

Total Hg (THg) and methylmercury (MeHg) were examined in muscle and liver samples of salmon species (Chinook: *Oncorhynchus tshawytscha*; Chum: *O. keta*; Sockeye: *O. nerka*; Coho: *O. kisutch*) and freshwater fish species (Pike: *Esox lucius*; Grayling: *Thymallus arcticus*; Whitefish: *Coregonus nelsoni*) collected in 1999 and 2000 from the Western Alaska rivers (Yukon, Kuskokwim, Nushagak and Kvichak). The THg in salmon muscles has a mean value of 62 ng/g (ww). In Pike muscles, THg has a mean value of 879 ng/g. The mean concentrations of THg in Grayling and Whitefish muscle are 153 ng/g and 32 ng/g respectively. In salmon muscle and liver the MeHg levels constitute 77% and 62% of the THg levels, respectively. In Pike muscle the MeHg levels constitute 100% of the THg levels. A significant correlation between Hg levels and fish length was found. Calculated consumption limits indicate that children may consume 0.05-1.5 kg of fish per month, depending on the species consumed. This study suggests that, from 1979 to 1998, nearly 21 kg of MeHg was transported by Sockeye salmon to the Alaskan rivers of the Bering Sea east coast.
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Chapter 1

Introduction

Mercury (Hg) is a naturally occurring element that can become a contaminant of concern in Alaska ecosystems. In the past, high mercury levels were reported in subsistence fish in Yukon-Kuskokwim delta areas in Alaska (Duffy, 1999), Alaskan polar bear (Lentfer and Galster, 1987), Beluga Whale and Ringed Seal (Becker et al., 1995) and greenwinged teal of the Aleutian area (BSEPR, 1998). Increased mercury levels in red king crabs from offshore of Nome, North Bering Sea were also reported subsequent to successive dredging operations (Jewett, 1999; Jewett and Naidu, 2000).

Under natural geological processes, the fluxes of metals through the environment are relatively slow so animals and plants can adapt to increased concentration of mercury by biochemical or behavioral modification. However, as input of metals from human activities usually occur over a much shorter time scale, biological adaptation may not be able to keep pace with the rapid environmental changes. Thus, a rapid increase in toxic metals can threaten the physical health of plant and animal species, as well as human populations that depend on these wildlife for their subsistence (Usher, 1992; Van Oostdam et al., 1999; Wheatley and Parades, 1996).

In Alaska, mercury can accumulate in sediments and biota from a combination of sources such as atmospheric transport of industrial emissions, natural erosion of
cinnabar deposits, and biological transport by returning salmon. Because industrial activities in Alaska are limited, the major anthropogenic input is believed to be derived from atmospheric deposition of mercury transported from distant areas (Mason et al., 1994; Hudson et al., 1995; Fitzgerald et al., 1998). Central and western Asia are considered the principal source area of contaminants to arctic Alaska, with only a small fraction of the pollutants in Alaska originating from southern Canada and the United States (Rahn and Lowenthal, 1986). Due to the increased global energy consumption including coal burning and waste combustion, mercury transported to the Arctic areas may be an increasing problem (Nriagu and Pacyna, 1988).

Mercury has several chemical forms: elemental Hg, inorganic Hg and organic Hg. The elemental Hg has relatively low toxicity while inorganic Hg is more of a problem because mercuric chloride can cause serious liver, kidney and digestive tract damage (Hammond, 1971). Methylmercury (MeHg) is one of the most common and toxic Hg species. MeHg is a lipid-soluble molecule that easily passes through cell membranes and biomagnifies along the food web (AMAP, 1997). MeHg is more readily absorbed from gastrointestinal tract than inorganic Hg (Wolfe, 1998) and readily penetrates the blood-brain barrier, causing neurological responses (Kerper, 1992). Minamata disease is one of the first and most serious cases of toxicity resulting from MeHg poisoning that occurred in humans who ingested fish and shellfish contaminated by extremely high levels of MeHg. Typical symptoms of MeHg poisoning include sensory disturbances, ataxia,
dysarthria, constriction of the visual field and auditory disturbances. Elevated values of Hg in mother’s blood can lead to neurological disorders in child development.

Discharges of mercury to the environment from anthropogenic and natural sources are predominately in the form of elemental and inorganic mercury ($\text{Hg}^0$, $\text{Hg}^{2+}$). However, a large proportion of total Hg in river and marine organisms, particularly fish, is MeHg (Westöö, 1967; Bishop and Neary, 1974; Kendall, 1978; Grieb et al., 1990; Bloom, 1992), despite the fact that less than 10% of THg in water is in the methyl form in most ecosystems (Kelly et al., 1995). Bacterial metabolism results in the biological transformation of inorganic Hg to MeHg (Bijer et al., 1979) (Figure 1.1). Methylation of inorganic Hg by bacteria was first demonstrated by Jensen and Jernelöv (1969) in aquaria sludge and by Wood et al., (1968) in extracts from a methanogenic bacterium. Sulfate reducing bacteria (SRB) have been identified as effective methylators of Hg (Compeau and Bartha, 1984; 1985). Methylcobalamin, a Vitamin B$_{12}$ analogue, has an important role in the final step of Hg methylation. It has been proposed that any organism capable of B$_{12}$ synthesis is capable of MeHg formation (Wood, 1972).
Figure 1.1. Major route of environment accumulation and transformation of Hg.

MeHg concentrations in fish tissues are of special concern because of the potential of MeHg to biomagnify through the food web in aquatic ecosystems (Hanisch, 1998). MeHg is generally accumulated more efficiently from food than inorganic forms (Pentreath, 1976). Fish preferentially excrete inorganic Hg and thus, accumulate MeHg (Jernelöv and Lann, 1971). The biological half-life of MeHg is longer than that of the inorganic form in fish (Stopford et al., 1975). As Hg accumulates in the edible portions of fish primarily as the MeHg (Kosatsky et al., 2000), MeHg is biomagnified in the lipid rich food of humans. Because of the importance of fishing, both commercial and subsistence, to Alaska’s economy, the Hg in fish has become a focus of research interest. A previous survey in 1976 showed that Hg levels in fish and marine mammal consuming Eskimos of the Yukon-Kuskokwim Delta were higher than in Eskimos of Anchorage, Alaska (Galster, 1976). Hg, as MeHg in fish, can represent a potential risk to wildlife consumers such as fish-eating birds and mammals and possibly to the fish themselves (Braune et al., 1999).
In the past several years, only a limited number of published studies examined the Hg levels in marine mammals in Alaska (Smith et al., 1975; Born et al., 1981; Becker et al., 1995), or Hg content of fish. In 1993, several species of freshwater fish from Kaiyuh Flats in West Central Alaska were analyzed. MeHg levels in Pike ranged from 91 to 832 ng/g (wet weight basis), with a mean value of 438 ng/g (Headlee, 1996). The MeHg levels in fish and shellfish samples in Norton Sound were low: 10ng/g in Saffron cod, 20ng/g in least cisco and 30ng/g in king crab (Rusanowski et al., 1987). In a limited study of fish in the Koyukuk Nowitna National Wildlife Refuge, Pike muscle MeHg levels varied from 70-2,900 ng/g (U.S. Dept. of the Interior, 1989). Low MeHg levels in salmon tissue were previously reported in Alaska waters by the FDA (1993). In a more recent study on subsistence fish in the Yukon-Kuskokwim Delta area of Alaska, the mean level of THg in freshwater fish was 368 ng/g (Duffy, 1999).

Because of the limited database, my objectives for this study were to determine both THg and MeHg levels in different tissues of selected subsistence fish from rivers in Western Alaska over a two-year period. From these data the Hg concentrations can be compared between fish species and between sample collection sites to determine whether or not the fish in the higher trophic level such as Pike contains higher body Hg burden than salmon which is in the lower trophic level. Also, I can judge whether or not significant species and sample collection sites differences for Hg concentrations exist. Because both THg and MeHg were measured in fish muscle and liver, I can determine the relative levels of MeHg versus THg in fish muscle and liver. This study provides a
sufficiently broad Hg database which will be useful to the State of Alaska and the federal U.S. FDA in decision making within the framework of environmental risk management. Additionally, I hope to estimate the potential for the biotransport of MeHg into Alaska. In the past, biotransport was not included in the biogeochemical cycling models of Hg in Alaska.
Chapter 2

Materials and Methods

2.1 Sample Collection:

Fish samples were collected in summer 1999 and 2000 from selected sites on 4 major rivers (Kuskokwim, Yukon, Nushagak, Kvichak) in Western Alaska (Figure 2.1 and 2.2). Descriptions of locations and species are given in Table 2.1 and 2.2. Fish samples included marine species (Chinook: *Oncorhynchus tsawyascha*; Chum: *O. keta*; Sockeye: *O. nerka*; Coho: *O. kisutch*) and freshwater species (Pike: *Esox lucius*; Grayling: *Thymallus arcticus*; Whitefish: *Caregonus nelsoni*). The fish length was measured in the field. The length for salmon is mid eye to fork of tail, for freshwater fish is snout to fork of tail. All fish samples were dissected for muscle and liver samples in the field and stored frozen until analysis.

In the laboratory, fish tissues including muscle and liver were lightly thawed. The surface muscle tissue was cut away to minimize potential contamination. A section of tissue of approximately 10g was dissected and then was homogenized by grinder (Kinematica GmbH PCU-2). Acid-washed Titanium knife, polyethylene gloves and polyethylene cutting board were used during dissection. About 1.0g of the homogenized sample was accurately weighed into a 40ml pre-cleaned vial with a Teflon cap and frozen until digestion.
Figure 2.1. Map of Western Alaska showing location of salmon sample collection sites.

X1 – Pilot Station on the Yukon River; X2 – Bethel on the Kuskokwim River;
X3 – Portage Creek on the Nushagak River; X4 – Levelock on the Kvichak River.
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Table 2.1. Description of 1999 sampling locations for salmon species.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of fish</th>
<th>River</th>
<th>Sampling location</th>
<th>Latitude</th>
<th>Longitude</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sockeye salmon</td>
<td>6</td>
<td>Kuskokwim</td>
<td>Bethel</td>
<td>60°47'</td>
<td>161°45'</td>
</tr>
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<td>Chum salmon</td>
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<td>Kuskokwim</td>
<td>Bethel</td>
<td>60°47'</td>
<td>161°45'</td>
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<td>Kuskokwim</td>
<td>Bethel</td>
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<tr>
<td>Coho salmon</td>
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<td>Kuskokwim</td>
<td>Bethel</td>
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<td>161°45'</td>
</tr>
<tr>
<td>Chum salmon</td>
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<td>Yukon</td>
<td>Pilot Station</td>
<td>61°56'</td>
<td>162°52'</td>
</tr>
<tr>
<td>Chinook salmon</td>
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<td>Yukon</td>
<td>Pilot Station</td>
<td>61°56'</td>
<td>162°52'</td>
</tr>
<tr>
<td>Coho salmon</td>
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<td>Yukon</td>
<td>Emmonak</td>
<td>62°46'</td>
<td>164°31'</td>
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<td>144°41'</td>
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<td>Nushagak</td>
<td>Portage Creek</td>
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<td>144°41'</td>
</tr>
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<td>Chinook salmon</td>
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<td>Portage Creek</td>
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<tr>
<td>Coho salmon</td>
<td>5</td>
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<td>Igiagig</td>
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Table 2.2. Description of 2000 sampling locations for salmon species.

<table>
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<th>Sampling location</th>
<th>Latitude</th>
<th>Longitude</th>
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<tr>
<td>Sockeye salmon</td>
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<td>Kuskokwim</td>
<td>Bethel</td>
<td>60°47'</td>
<td>161°45'</td>
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<td>Chum salmon</td>
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<td>161°45'</td>
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<td>George River</td>
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<td>Yukon</td>
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<td>Pilot Station</td>
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<td>Pilot Station</td>
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<td>Sockeye salmon</td>
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<td>Kvichak</td>
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Table 2.3. Description of 2000 sampling locations for freshwater fish species.

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<th>Longitude</th>
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<td>Grayling</td>
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<td>George River, Kuskokwim River</td>
<td>61°54'</td>
<td>157°42'</td>
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<tr>
<td>Pike</td>
<td>6</td>
<td>Aniak River, Kuskokwim River</td>
<td>61°34'</td>
<td>159°33'</td>
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<td>Aniak River, Kuskokwim River</td>
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<td>Pike</td>
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<td>Andraefsky River, Yukon River</td>
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<td>Grayling</td>
<td>6</td>
<td>Andraefsky River, Yukon River</td>
<td>62°00'</td>
<td>163°15'</td>
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</tbody>
</table>

2.2 Analytical Methods

2.2.1 Total Mercury (THg) in Tissues

Overview

Before analysis for THg, the homogenized sample is digested with HNO₃/H₂SO₄ (Bloom and Crecelius, 1983; Bloom and Fitzgerald, 1988). The digested sample is diluted with 0.2N BrCl. A known aliquot of the diluted sample is reduced in the bubbler with SnCl₂. THg is determined via the method of cold vapor atomic fluorescence technique (CVAFS), based on the emission of 253.7nm radiation by excited Hg⁰ atoms in an inert gas stream. Mercuric ions (Hg²⁺) in the oxidized sample are reduced to Hg⁰ with SnCl₂ and then purged onto gold-coated sand traps as a means of pre-concentration and interference removal. Mercury on the trap is thermally desorbed and the vapor is collected on a second gold trap. Then another desorption sends the Hg vapor to the
fluorescence cell. Fluorescence is measured as a function of THg collected, which is converted to concentration by the size of the aliquot purged. The typical detection limit is about 1ng/g as Hg. THg as defined by this method means all HNO₃/H₂SO₄ + BrCl oxidizable mercury forms and species. This includes but is not limited to Hg²⁺, Hg⁰, HgS, organocomplexed Hg(II) compounds, adsorbed particulate Hg, and several tested covalently bound organomercurials such as CH₃HgCl, (CH₃)₂Hg.

**Apparatus and Reagent**

**Apparatus:**

*Cold Vapor Atomic Fluorescence Spectrometry detector:* The CVAFS detector contains the following major components:

1. Four-watt low-pressure mercury vapor lamp (253.7 nm light source).
2. Far UV Quartz flow-through fluorescence cell.
3. UV-visible photo multiplier.
4. Flow meter.

*Acid fume pre-trap:* A 10cm x 0.9cm diameter Teflon tube containing 2-3 grams of reagent grade, non-indication 8-14 mesh soda-lime (Ca(OH)₂ + NaOH) aggregates, packet between portions of glass wool.
**Gold-coated sand columns:** Made from 10cm lengths of 6.5 mm O.D. X 4mm I.D. quartz tubing, with a quartz wool plug 2.0 cm from one end. The tube is filled with 3.4cm of gold-coated quartz sand (60/80 mesh), and the end then plugged with quartz wool.

**Teflon fittings:** Connections between components and columns are made using 6.4mm O.D. Teflon tubing, and Teflon friction-fit or threaded tubing connectors.

**Cold vapor generator:** A 125 ml Florence flask with standard taper 24/40 neck, fitted with a stopper having a coarse glass frit which extends to within 0.2 cm of the flask bottom.

**Reagents:**

**Nitric/Sulfuric acid:** Carefully add 300 ml of pre-analyzed low mercury concentrated sulfuric acid to 700 ml pre-analyzed low mercury concentrated nitric acid.

**20% Stannous Chloride:** A solution containing 200 g of SnCl₂·2H₂O and 100 ml concentrated HCl is brought to 1 L with high purity water. This solution is purged overnight with nitrogen to remove all traces of mercury. Store in the dark.
0.2N Bromine Monochloride: 27g of KBr are added to a 2.5 L bottle of concentrated HCl. A clean magnetic stir bar is placed in the bottle, and stirring for 1 hour in fume hood. Then 38g of low mercury KBrO₃ are slowly added to the acid.

Stock mercury standard: A NIST certified 10,000mg/L mercury atomic absorption standard is used as the basis of all lower concentration laboratory stock solutions.

Nitrogen: Grade 4.5 nitrogen which has been further purified by the removal of Hg using a gold-coated sand trap.

Argon: Grade 5.0 argon which has been further purified as above.

Sample Digestion

Approximately 1.0 gram of the homogenized sample is accurately weighted into a 40ml VWR pre-cleaned vial and 7.0ml of 7:3 v/v HNO₃+H₂SO₄ solution is pipetted in. The vial cap is tightly replaced and the samples are placed on a hot plate, heated to 125°C for 2 hours after the onset of refluxing or until all organic matter is dissolved. Upon cooling to room temperature, the digested sample is diluted to 37ml with a 10% (v/v) solution of 0.2N BrCl in milli-Q water.

Reduction Procedure

100 ml of water is placed in each bubbler, and 1.0 ml of SnCl₂ is added. The bubbler is purged with Hg-free N₂ for 20 minutes at 300ml/min. A gold sand trap is then connected to the output of the soda lime pre-trap, and the water purged another 20
minutes to obtain a bubble blank. Standards are analyzed by the addition of 0.1-0.5 ng aliquots of Hg standard, and 0.5 ml SnCl₂ to the bubblers, swirling to mix, and purging as above. To analyze samples, 0.3 ml of SnCl₂ and an aliquot of the digestate are pipetted into each bubbler. Gold-coated sand columns placed onto soda lime outlet, and the sample bubbled for 20 minutes. New samples may then be added to the bubblers, with additional aliquots of SnCl₂.

**Mercury Detection**

To analyze the mercury contained on a gold column, the Nichrome wire coil is placed around the column, and the column is inserted in the analyzer train between the incoming Hg-free helium and the second gold-coated sand column. Argon is passed through the columns into the analyzer at a rate of 30 ml/min for 2 minutes to dry off condensed water vapor. Electrical current (10 VAC) is then applied to the coil for 3 minutes, thermally desorbing the Hg as Hg⁰, which is carried by the Argon to the analytical gold column. After 4 minutes, the Nichrome coil is turned off, and a cooling fan directed toward the hot column is turned on. Next, the power to the Nichrome wire coil around the analytical column is turned on. This column is heated for 3 minutes. Following the recording of the peak, the analytical trap coil is turned off, and the cooling fan directed at it. The sample trap is then removed from the gas stream and the next sample column is placed in line, and the procedure is repeated.
Peaks generated using this technique should be very sharp and symmetrical. Broad or asymmetrical peaks are indicative of an analytical procedural problem, possibly including low gas flow, water vapor on the column, or an analytical column damaged by chemical fumes or overheating.

2.2.2 Methylmercury (MeHg) in Tissues

Overview

Before analysis for MeHg, the homogenized sample is digested with 25% KOH/methanol. The digested sample is diluted with methanol. A small aliquot of the digestate is added to milli-Q water, buffered at pH 5.0, and reacted with sodium tetraethyl borate. Aqueous phase ethylation is used to produce a volatile methyl, ethylmercury derivative of MeHg. Ethyl analogs are separated by isothermal GC and detected by using a cold vapor atomic fluorescence (CVAFS) detector. The typical detection limit is about 1ng/g as Hg. Methylmercury as defined by this method means all methylmercury forms and species found in the digestate. This includes but is not limited to CH$_3$Hg$^+$, CH$_3$HgCl, CH$_3$HgOH, and CH$_3$HgS-R.

Apparatus and Reagent

Apparatus:

In addition to the apparatus in section 2.2.1, the following were used:
Isothermal GC Unit: The column is made of \( \frac{1}{4} \) inch OD borosilicate glass column tubing with 4mm ID bore. The tube is formed into a 8 cm diameter coil of 1.0 m length with two 15cm arms extending in parallel up from the coil. The column is silanized, and packed with silanized glass wool plugs. The column is held in a small temperature-controlled isothermal oven made from a heating mantle interfaced with a Cole Parmer Digi-sense temperature controller. The column is held at a constant temperature of 100±2\(^\circ\)C using the temperature controller.

Pyrolytic organomercury breakdown column: This column consists of a 20 cm length of 7 mm O.D. by 4.5 mm I.D. quartz tubing with the central 10 cm packed with quartz wool. The column is wrapped with 1.5 m of 22 gage Nichrome wire which is electrically heated to about 700\(^\circ\)C with 30-34 volts from an auto transformer.

Ethylation reactor: A 125 ml Florence flask with standard taper 24/40 neck, fitted with a stopper having a coarse glass frit which extends to within 0.2 cm of the flask bottom.

Carbotrap column: Made from 10 cm lengths of silanized 6.5 mm O.D. X 4 mm I.D. quartz tubing, with a slight crimp or series of indentations 2.0 cm from one end. A small plug of silanized glass wool is placed into the tube, from the non-crimped end, compressing firmly against the crimped region for support. The tube is filled with 3.4 cm of Carbotrap (30/45 mesh), and the end then plugged with silanized glass wool.
Reagents:

Acetate buffer: 2 moles of reagent grade sodium acetate (272g) and 2 moles of glacial acetic acid (118ml) dissolved in D.I. water to give a final volume of 1.0 L.

25% Potassium Hydroxide/Methanol: 250g KOH are dissolved in methanol to make a final volume of 1.0 L.

Sodium tetraethyl borate solution: This reagent is purchased in 1.0 gram air-sealed bottles. 100ml of 1.2% KOH in deionized water is prepared in a Teflon bottle, and chilled to 0°C. The bottle of NaBEt₄ is then rapidly opened and about 5 ml of the KOH solution poured in. The reagent bottle is capped and shaken to dissolve the NaBEt₄. This is then all poured into the 100 ml bottle of KOH solution, and shaken to mix.

Methylmercury Stock Solution: Methylmercury solutions are prepared by serial dilution of an initial concentrated solution of methylmercuric chloride in deionized water containing 0.5% (v/v) glacial acetic acid and 0.2% (v/v) HCl.

Sample Digestion

Approximately 1.0 gram of the homogenized sample is accurately weighted into a 40ml VWR pre-cleaned vial. 10.0 ml of the 25% KOH/methanol reagent is added to each sample. The sample is then capped, shaken and placed on a hot plate at 90°C for 2-4
hours or until all soft tissue is visibly dissolved. The samples are then diluted up to 35 ml with methanol.

**Trapping Procedure**

100 ml of D.I. water and 500 μl of acetate buffer is added to a reaction vessel. Then an aliquot of the digestate is added to the bubbler. Addition of 35 μl sodium tetraethylborate activates aqueous phase ethylation. The bubbles are allowed to react for 17 minutes without bubbling. The Carbotrap column is then placed on the output of the bubbler using Teflon fitting. The bubbler is connected to the N₂ purge gas and the sample is purged for 17 minutes at a flow rate of 300 ml/min. At the end of this time, the trap is removed from the bubbler, and connected directly to the N₂ for 7 minutes.

**Mercury Detection**

The dried Carbotrap column is connected to the input side of the isothermal GC column. A Nichrome wire coil wound to supply about 400°C is placed around the Carbotrap, and argon carrier gas is connected to the Carbotrap. The output side of the GC column passes through the pyrolytic breakdown column, then the CVAFS cell, and finally through a flow meter. After allowing argon to flow through the column at least one minute, the Carbotrap column is heated with the Nichrome coil for a period of 30 seconds, transferring the mercury species to the GC column. Species are eluted according to molecular weights, with the following peaks:
1. A peak at about 1 minutes corresponding to Hg⁰, usually a decomposition product of diethyl mercury, as Hg⁰ is not trapped by Carbotrap. A small Hg⁰ peak is always present simply due to Hg released upon heating the Carbotrap.

2. A peak about 2 minutes corresponding to methyl ethyl mercury. This is the peak of interest, the ethylation product of methyl mercury.

3. A peak at about 3 minutes corresponding to diethyl mercury, resulting from the ethylation of Hg (II)-inorganic in the reagents and samples. However, this is not quantitative for Hg (II) as most Hg (II) is excluded by the distillation procedure.

2.3 Quality Control / Quality Assurance

To access the accuracy of THg and MeHg determinations, certified dogfish tissue (DORM-2) from the National Research Council of Canada was used. This material contains 4,470±370ng/g MeHg, 4,640±260ng/g THg. For THg, a solution is made by digesting 1.000 gram of the dogfish tissue in 25 ml of 7:3 (v/v) HNO₃ + H₂SO₄, and then diluting to 1000.0 ml with 0.0001 N BrCl solution (Bloom and Crecelius, 1987). For MeHg, a solution is prepared by digesting 1.000 gram of dogfish tissue in 20 ml of 25% KOH in methanol, and then diluting to 1000.0 ml with methanol (Bloom, 1989). My results show 100.3 ± 5.3 % (n=16) of THg recovery and 93.2 ± 10.5 % (n=16) of MeHg recovery. A check standard and a blank were run after every 10 samples. A duplicate and a spike of samples were performed once for each run of 20 samples. The relative percent
difference of the duplicate pair is $2.85 \pm 3.4 \%$ (n=21) for THg and $11.4 \pm 10.0 \%$ (n=15) for MeHg. The Percent Recovery for duplicate matrix spike samples is $100.1 \pm 8.6 \%$ (n=21) for THg and $101.2 \pm 13.5 \%$ (n=16) for MeHg.

In addition to the analysis of certified standard tissue, selected samples were sent to Frontier Geosciences (Seattle, WA.) for blind analysis. The mean relative percent difference was 9.58%.

2.4 Statistical procedures

Data were statistically analyzed using STATISTCA ‘99 Edition and Microsoft Excel. The analysis of variance (ANOVA) was used for testing species and sampling sites differences. Tukey’s honest significant difference test was used to compare multiple parameters if ANOVA showed significant differences. Shapiro-Wilk W test was used to test normality of Hg distribution (95% confidence level) in the tissue samples. Because Hg concentrations were not normally distributed both in muscle and liver samples in salmon, Hg data were Log-transformed to normalize their variance. The levels of significance for all comparisons was $p \leq 0.05$. 

3.1 Hg in Salmon

The mean concentrations of THg and MeHg in salmon tissues at four rivers are given in Table 3.1. THg in salmon muscle had mean concentrations for the different species ranging between 34 and 96 ng/g (wet weight). The mean concentrations of MeHg in salmon muscle ranged between 23 and 78 ng/g. In salmon liver, the mean concentrations of THg varied from 54 to 112 ng/g, the mean levels of MeHg ranged from 29 to 76 ng/g.

Over a two-year period (1999-2000), the THg in salmon muscle had a mean value of 62 ng/g with a range from 25 to 137 ng/g and the MeHg had a mean value of 48ng/g with a range from 9 to 121 ng/g. Muscle Hg concentrations were not normally distributed (p<0.001) (Figure 3.1). The THg in salmon liver had a mean value of 84 ng/g with a range from 32 to 172 ng/g and was significantly higher than the THg in salmon muscle (p< 0.001). Liver Hg concentrations were not normally distributed (p<0.002) (Figure 3.2). The MeHg concentration in salmon liver had a mean value of 52 ng/g with a range from 16 to 118 ng/g. There was no significant difference of MeHg concentration between salmon muscle and liver (p=0.1).
Table 3.1. Arithmetic mean concentrations (ng/g ww) with standard deviation (in parenthesis) of total mercury (THg) and methylmercury (MeHg) in muscles and livers of salmon species from rivers draining into the East Bering Sea, Alaska.

### Muscle

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<th>MeHg</th>
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<th>THg</th>
<th>MeHg</th>
<th># Fish</th>
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<th>MeHg</th>
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Figure 3.1. Shapiro-Wilk W test for normality of Hg distribution in salmon muscle over a two-year period, 1999-2000.
Figure 3.2. Shapiro-Wilk W test for normality of Hg distribution in salmon liver over a two-year period, 1999-2000.
3.1.1. Salmon Species and Sampling Sites as Confounding Variables

In 1999, I found highly significant species differences for both THg and MeHg in salmon muscle (p<0.001) (Table 3.2 and 3.3). The THg concentration in Chinook muscle from the Yukon river was significantly lower than Chinook from the Kuskokwim river (p=0.018) and Nushagak river (p=0.022) and the MeHg concentration in Chinook muscle from the Yukon river was significantly lower than from the Kuskokwim river (p=0.017) and Nushagak river (p=0.022) (Figure 3.3). There were no significant variations between rivers for Chum, Coho and Sockeye salmon for THg and MeHg in muscle. Tukey's HSD test was used to compare the Hg levels in salmon species (Table 3.4 and 3.5). The THg concentrations in Chinook and Chum salmon muscle were higher than in Sockeye and Coho salmon muscle. The MeHg concentration in Chinook salmon muscle was higher than in Sockeye and Coho salmon muscle. There was no significant difference for the MeHg concentration between Chum and Coho muscle. In 1999 salmon liver samples, I found significant differences between species only for THg (p=0.011) (Table 3.6 and 3.7). The THg concentration in Chinook liver from the Yukon river was significantly lower than from the Kuskokwim river (p=0.032) and the MeHg concentration in Chinook liver from the Yukon river was significantly lower than from the Kuskokwim river (p=0.018). The MeHg concentration in Coho salmon liver from the Kvichak river was lower than from the Kuskokwim river (p=0.022) in 1999 (Figure 3.4).
Table 3.2. Univariate tests of significance for Log-transformed THg in salmon muscle, 1999.

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(1) SS = Sums of Squares  
(2) MS = Mean Square  
(3) F = F distribution  
(4) P = Probability

Table 3.3. Univariate tests of significance for Log-transformed MeHg in salmon muscle, 1999.

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Table 3.4. Tukey HSD test for Log-transformed THg in 1999 salmon muscle samples.

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<td>3.5725</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>2</td>
<td>Chum</td>
<td></td>
<td>.000168*</td>
<td></td>
<td>.075260</td>
</tr>
<tr>
<td>3</td>
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<td>.000162*</td>
<td>.695999</td>
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<td>.000635*</td>
</tr>
<tr>
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<td>.075260</td>
<td>.013473*</td>
<td>.000635*</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.5. Turkey HSD test for Log-transformed MeHg in 1999 salmon muscle samples.

<table>
<thead>
<tr>
<th>Cell No.</th>
<th>Species</th>
<th>Sockeye</th>
<th>Chum</th>
<th>Chinook</th>
<th>Coho</th>
</tr>
</thead>
<tbody>
<tr>
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</tr>
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<td>Chum</td>
<td>.000165*</td>
<td></td>
<td>.742355</td>
<td>.156014</td>
</tr>
<tr>
<td>3</td>
<td>Chinook</td>
<td>.000161*</td>
<td>.742355</td>
<td></td>
<td>.014576*</td>
</tr>
<tr>
<td>4</td>
<td>Coho</td>
<td>.004205*</td>
<td>.156014</td>
<td>.014576*</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.3. Mean THg and MeHg concentrations (ng/g wet weight) in 1999 muscle samples of four salmon species in four rivers.
Table 3.6. Univariate tests of significance for Log-transformed THg in salmon liver, 1999.

<table>
<thead>
<tr>
<th>Effect</th>
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<th>Degr. of Freedom</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
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<td>.111</td>
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<tr>
<td>Species</td>
<td>1.324*</td>
<td>3*</td>
<td>.441*</td>
<td>4.06*</td>
<td>.011349*</td>
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</tbody>
</table>

Table 3.7. Univariate tests of significance for Log-transformed MeHg in salmon liver, 1999.

<table>
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<tr>
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<th>Degr. of Freedom</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
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<tr>
<td>Site</td>
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<td>.4142</td>
<td>2.329</td>
<td>.084867</td>
</tr>
<tr>
<td>Species</td>
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<td>.4785</td>
<td>2.690</td>
<td>.055290</td>
</tr>
</tbody>
</table>
Figure 3.4. Mean THg and MeHg concentrations (ng/g wet weight) in 1999 liver samples of four salmon species in four rivers.
In 2000, I also found significant species differences for both THg ($p=0.003$) and MeHg ($p=0.0014$) in salmon muscle while there were no significant river systems differences for THg ($p=0.446$) and MeHg ($p=0.306$) in salmon muscle (Table 3.8 and 3.9). Tukey’s HSD test was used to compare the Hg levels in salmon species (Table 3.10 and 3.11). The THg and MeHg concentrations in Chum salmon muscle were higher than in Sockeye and Coho salmon muscle. The THg and MeHg concentrations in Chinook salmon muscle were not significantly higher than in Sockeye and Coho salmon muscle in 2000. There was no significant difference of the THg and MeHg concentrations between Sockeye and Coho muscle (Figure 3.5). In 2000 salmon liver samples, I did not find significant species differences for THg and MeHg (Table 3.12 and 3.13). However, the THg concentration in Coho salmon liver from the Yukon river was lower than from the Nushagak river ($p=0.003$) and the Kuskokwim river ($p=0.004$), and the MeHg concentration in Coho salmon liver from the Yukon river was also significantly lower than from the Nushagak river ($p<0.001$) and the Kuskokwim river ($p=0.014$) (Figure 3.6).

**Table 3.8.** Univariate tests of significance for Log-transformed THg in salmon muscle, 2000.

<table>
<thead>
<tr>
<th>Effect</th>
<th>SS</th>
<th>Degr. of Freedom</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>.170</td>
<td>3</td>
<td>.057</td>
<td>.90</td>
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</tr>
<tr>
<td>Species</td>
<td>.979*</td>
<td>3*</td>
<td>.326*</td>
<td>5.18*</td>
<td>.003000*</td>
</tr>
</tbody>
</table>
### Table 3.9. Univariate tests of significance for Log-transformed MeHg in salmon muscle, 2000.

<table>
<thead>
<tr>
<th>Effect</th>
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<th>Degr. of Freedom</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
<td>.369</td>
<td>3</td>
<td>.123</td>
<td>1.23</td>
<td>.306253</td>
</tr>
<tr>
<td>Species</td>
<td>1.749*</td>
<td>3*</td>
<td>.583*</td>
<td>5.84*</td>
<td>.001445*</td>
</tr>
</tbody>
</table>

### Table 3.10. Tukey HSD test for Log-transformed THg in 2000 salmon muscle samples.

<table>
<thead>
<tr>
<th>Cell No.</th>
<th>Species</th>
<th>Sockeye</th>
<th>Chum</th>
<th>Chinook</th>
<th>Coho</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sockeye</td>
<td>4.0227</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Chum</td>
<td>.002996*</td>
<td></td>
<td>.224001</td>
<td>.997827</td>
</tr>
<tr>
<td>3</td>
<td>Chinook</td>
<td>.224001</td>
<td>.318754</td>
<td>.306241</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Coho</td>
<td>.997827</td>
<td>.005167*</td>
<td>.306241</td>
<td></td>
</tr>
</tbody>
</table>

### Table 3.11. Tukey HSD test for Log-transformed MeHg in 2000 salmon muscle samples.

<table>
<thead>
<tr>
<th>Cell No.</th>
<th>Species</th>
<th>Sockeye</th>
<th>Chum</th>
<th>Chinook</th>
<th>Coho</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sockeye</td>
<td>3.6872</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Chum</td>
<td>.005213*</td>
<td></td>
<td>.215502</td>
<td>.997439</td>
</tr>
<tr>
<td>3</td>
<td>Chinook</td>
<td>.215502</td>
<td>.432637</td>
<td>.148689</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Coho</td>
<td>.997439</td>
<td>.002931*</td>
<td>.148689</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.5. Mean THg and MeHg concentrations (ng/g wet weight) in 2000 muscle samples of four salmon species in four rivers.
**Table 3.12.** Univariate tests of significance for Log-transformed THg in salmon liver, 2000.

<table>
<thead>
<tr>
<th>Effect</th>
<th>SS</th>
<th>Degr. of Freedom</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
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<td>.050</td>
<td>.65</td>
<td>.586851</td>
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<td>Species</td>
<td>.367</td>
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<td>.122</td>
<td>1.58</td>
<td>.203439</td>
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</table>

**Table 3.13.** Univariate tests of significance for Log-transformed MeHg in salmon liver, 2000.

<table>
<thead>
<tr>
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<th>SS</th>
<th>Degr. of Freedom</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
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<td>3</td>
<td>.097</td>
<td>.693</td>
<td>.560108</td>
</tr>
<tr>
<td>Species</td>
<td>.596</td>
<td>3</td>
<td>.199</td>
<td>1.413</td>
<td>.248103</td>
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</tbody>
</table>
Figure 3.6. Mean THg and MeHg concentrations (ng/g wet weight) in 2000 liver samples of four salmon species in four rivers.
Over a two-year period (1999-2000), I found highly significant species differences for both THg and MeHg in salmon muscle (p<0.001) (Table 3.14 and 3.15). The THg concentration in Chinook muscle from the Yukon river was significantly lower than from the Kuskokwim river (P=0.035) and the MeHg concentration in Sockeye muscle from the Kuskokwim river was significantly lower than from the Kvichak river (P=0.025) (Figure 3.7). Tukey’s HSD test was used to compare the Hg levels in salmon species (Table 3.16 and 3.17). The THg and MeHg concentrations in Chinook and Chum salmon muscle were higher than in Sockeye and Coho salmon muscle. There were no significant differences of the MeHg and THg concentrations between Sockeye and Coho muscle (Figure 3.7). In salmon liver samples, there were no significant species differences for THg and MeHg (Table 3.18 and 3.19). The THg concentration in Coho salmon liver from the Yukon river was lower than from the Nushagak river (p=0.021). The MeHg concentration in Coho salmon liver from the Yukon river was lower than from the Nushagak river (p=0.019) and the Kuskokwim river (p=0.021). The MeHg level in Coho salmon liver from the Kvichak river was significantly lower than from the Nushagak river (p=0.034) (Figure 3.8).

<table>
<thead>
<tr>
<th>Effect</th>
<th>SS</th>
<th>Degr. of Freedom</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
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<td>.080</td>
<td>.88</td>
<td>.452613</td>
</tr>
<tr>
<td>Species</td>
<td>4.086*</td>
<td>3*</td>
<td>1.362*</td>
<td>15.02*</td>
<td>.000000*</td>
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</tbody>
</table>

Table 3.15. Univariate tests of significance for Log-transformed MeHg in salmon muscle, 1999 – 2000.

<table>
<thead>
<tr>
<th>Effect</th>
<th>SS</th>
<th>Degr. of Freedom</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
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<td>.042</td>
<td>.30</td>
<td>.825156</td>
</tr>
<tr>
<td>Species</td>
<td>6.665*</td>
<td>3*</td>
<td>2.222*</td>
<td>15.86*</td>
<td>.000000*</td>
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</tbody>
</table>

Table 3.16. Tukey HSD test for Log-transformed THg in salmon muscle samples over a two-year period (1999-2000).

<table>
<thead>
<tr>
<th>Cell No.</th>
<th>Species</th>
<th>Sockeye</th>
<th>Chum</th>
<th>Chinook</th>
<th>Coho</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Sockeye</td>
<td>3.8426</td>
<td>.000008*</td>
<td>.000010*</td>
<td>.538215</td>
</tr>
<tr>
<td>2</td>
<td>Chum</td>
<td>.000008*</td>
<td></td>
<td>.992527</td>
<td>.000194*</td>
</tr>
<tr>
<td>3</td>
<td>Chinook</td>
<td>.000010*</td>
<td>.992527</td>
<td></td>
<td>.000624*</td>
</tr>
<tr>
<td>4</td>
<td>Coho</td>
<td>.538215</td>
<td>.000194*</td>
<td></td>
<td>.000624*</td>
</tr>
</tbody>
</table>
Table 3.17. Tukey HSD test for Log-transformed MeHg in salmon muscle samples over a two-year period (1999-2000).

<table>
<thead>
<tr>
<th>Cell No.</th>
<th>Species</th>
<th>Sockeye</th>
<th>Chum</th>
<th>Chinook</th>
<th>Coho</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>3.4712</td>
<td>3.9804</td>
<td>3.9624</td>
<td>3.6547</td>
</tr>
<tr>
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<td></td>
<td>.000008*</td>
<td>.000008*</td>
<td>.198952</td>
</tr>
<tr>
<td>2</td>
<td>Chum</td>
<td>.000008*</td>
<td></td>
<td>.997024</td>
<td>.001427*</td>
</tr>
<tr>
<td>3</td>
<td>Chinook</td>
<td>.000008*</td>
<td>.997024</td>
<td></td>
<td>.003011*</td>
</tr>
<tr>
<td>4</td>
<td>Coho</td>
<td>.198952</td>
<td>.001427*</td>
<td>.003011*</td>
<td></td>
</tr>
</tbody>
</table>
Figure 3.7. Mean THg and MeHg concentrations (ng/g wet weight) in muscle samples of four salmon species in four rivers over a two-year period (1999-2000).
Table 3.18. Univariate tests of significance for Log-transformed THg in salmon liver, 1999 – 2000.

<table>
<thead>
<tr>
<th>Effect</th>
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<th>Degr. of Freedom</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
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<td>.147</td>
<td>1.27</td>
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<td>.194</td>
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<td>.177187</td>
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</tbody>
</table>

Table 3.19. Univariate tests of significance for Log-transformed MeHg in salmon liver, 1999 – 2000.

<table>
<thead>
<tr>
<th>Effect</th>
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<th>Degr. of Freedom</th>
<th>MS</th>
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<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Site</td>
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<td>.450</td>
<td>2.32</td>
<td>.078657</td>
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<tr>
<td>Species</td>
<td>.268</td>
<td>3</td>
<td>.089</td>
<td>.46</td>
<td>.710311</td>
</tr>
</tbody>
</table>
Figure 3.8. Mean THg and MeHg concentrations (ng/g wet weight) in liver samples of four salmon species in four rivers over a two-year period (1999-2000).
3.1.2 Relationship Between MeHg and THg in Salmon

In salmon muscles and livers, MeHg was proportional to THg. Overall, the percentage of the MeHg to THg in muscle was 78% while salmon liver showed a lower percentage of MeHg at 63% (Figure 3.9 and 3.10).

3.1.3 Relationship Between Hg Concentration and Salmon Length

I observed that both THg and MeHg in Chinook muscle increased with fish length (Figure 3.11 and 3.12). However, significant positive correlations were not found between Hg concentrations and fish lengths in Chum, Sockeye, and Coho salmon (Figure 3.13-3.18). Only my sample of Chinook salmon contained a wide enough range of sizes, with fish length ranging between 400 to 950mm, to show a good correlation. Other salmon species in my sample had lengths in a relatively smaller range (500-650 mm). The slight decrease line in Figure 3.13-3.18 is related to a few individual fish at the extremes of the size range analyzed.
Figure 3.9. Relationship between MeHg and THg in salmon muscle, 1999-2000

\[ y = 0.7778x \]

\[ R^2 = 0.8489 \]
Figure 3.10. Relationship between MeHg and THg in salmon liver, 1999-2000
Figure 3.11. Relationship between THg and length in Chinook salmon muscle, 1999-2000

\[ y = 10.039e^{0.0025x} \]

\[ R^2 = 0.5336 \]
Figure 3.12. Relationship between MeHg and length in Chinook salmon muscle, 1999-2000

\[ y = 6.1837e^{0.0028x} \]

\[ R^2 = 0.5122 \]
Figure 3.13. Relationship between THg and length in Sockeye salmon muscle, 1999-2000

\[ y = 64.439e^{-0.0006x} \]

\[ R^2 = 0.0108 \]
Figure 3.14. Relationship between MeHg and length in Sockeye salmon muscle, 1999-2000

\[ y = 99.394e^{-0.002x} \]

\[ R^2 = 0.0617 \]
Figure 3.15. Relationship between THg and length in Chum salmon muscle, 1999-2000

\[ y = 343.66e^{-0.0027x} \]

\[ R^2 = 0.1098 \]
Figure 3.16. Relationship between MeHg and length in Chum salmon muscle, 1999-2000

\[ y = 629.54e^{0.0041x} \]

\[ R^2 = 0.1743 \]
Figure 3.17. Relationship between THg and length in Coho salmon muscle, 1999-2000
Figure 3.18. Relationship between MeHg and length in Coho salmon muscle, 1999-2000

\[ y = 48.468e^{-0.0004x} \]
\[ R^2 = 0.0032 \]
3.2 Hg in Freshwater Fish

The mean concentrations of THg and MeHg in muscles and livers of freshwater fish species in the Yukon and Kuskokwim rivers are summarized in Table 3.20 and 3.21.

I found the significant river systems difference for THg and MeHg in Pike and Grayling (Table 3.22). The mean concentrations of THg and MeHg in muscles of Pike and Grayling from the Yukon were about 3 times higher than in the same species from the Kuskokwim (Figure 3.19). There were no significant differences between THg and MeHg concentrations in Pike muscle and Grayling muscle. The THg concentrations in Pike muscle were not significantly different from the THg concentrations in Pike liver (p=0.83). However, the MeHg concentrations in Pike liver were significantly lower than in Pike muscle (p<0.05). The mean THg and MeHg levels (Table 3.20) in Whitefish were significantly lower than those in Pike and Grayling (i.e. for MeHg in muscle, Whitefish: 26 ng/g; Pike: 578 ng/g).
**Table 3.20.** Concentrations of THg (ng/g wet weight) (Mean ± SD) in Alaska freshwater fish species in two rivers.

<table>
<thead>
<tr>
<th>Year</th>
<th>Species</th>
<th>Tissue</th>
<th>Yukon</th>
<th>Kuskokwim</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>Pike</td>
<td>Muscle</td>
<td>1506±298 (n=6)</td>
<td>628±359 (n=15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver</td>
<td>1731±861 (n=6)</td>
<td>471±594 (n=15)</td>
</tr>
<tr>
<td>2000</td>
<td>Grayling</td>
<td>Muscle</td>
<td>264±30 (n=4)</td>
<td>78±14.6 (n=6)</td>
</tr>
<tr>
<td>2000</td>
<td>Whitefish</td>
<td>Muscle</td>
<td>32±13 (n=6)</td>
<td>57±20 (n=6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3.21.** Concentration of MeHg (ng/g wet weight) (Mean ± SD) in Alaska freshwater fish species in two rivers.

<table>
<thead>
<tr>
<th>Year</th>
<th>Species</th>
<th>Tissue</th>
<th>Yukon</th>
<th>Kuskokwim</th>
</tr>
</thead>
<tbody>
<tr>
<td>2000</td>
<td>Pike</td>
<td>Muscle</td>
<td>1531±345 (n=6)</td>
<td>578±371 (n=15)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver</td>
<td>1199±596 (n=6)</td>
<td>258±232 (n=15)</td>
</tr>
<tr>
<td>2000</td>
<td>Grayling</td>
<td>Muscle</td>
<td>249±35 (n=4)</td>
<td>75±10 (n=6)</td>
</tr>
<tr>
<td>2000</td>
<td>White fish</td>
<td>Muscle</td>
<td>26±11 (n=6)</td>
<td>31±16 (n=6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liver</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.22. Outcome of comparison of the mercury concentrations in freshwater fish species between the Yukon and Kuskokwim river.

<table>
<thead>
<tr>
<th>Species</th>
<th>Liver</th>
<th>Muscle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MeHg</td>
<td>THg</td>
</tr>
<tr>
<td>Pike</td>
<td>p&lt;0.001</td>
<td>p=0.001</td>
</tr>
<tr>
<td>Grayling</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

n/a, not available.
Figure 3.19. Mean THg and MeHg concentrations (ng/g wet weight) in muscle samples of freshwater fish species in two rivers (2000).
3.2.1. Relationship Between MeHg and THg in Freshwater Fish

In Pike muscles and livers, MeHg was also proportional to THg. The percentage of the MeHg to THg in Pike muscle was 100% (Figure 3.20) while Pike liver showed a lower percentage of MeHg at 62% (Figure 3.21). The MeHg in Grayling muscle constituted 94% of THg (Figure 3.22).

3.2.2. Relationship Between Hg Concentration and Freshwater Fish Length

The Hg concentrations increased with fish lengths in all three freshwater species. The typical relationship between Hg and length is slightly curvilinear with higher concentrations found in larger fish (Figure 3.23-3.27).
Figure 3.20. Relationship between MeHg and THg in Pike muscle, 2000
Figure 3.21. Relationship between MeHg and THg in Pike liver, 2000

\[ y = 0.6238x \]

\[ R^2 = 0.9206 \]
Figure 3.22. Relationship between MeHg and THg in Grayling muscle, 2000
Figure 3.23. Relationship between THg and length in Pike muscle, 2000
Figure 3.24. Relationship between MeHg and length in Pike muscle, 2000

$y = 42.113e^{0.005x}$

$R^2 = 0.5093$
Figure 3.25. Relationship between THg and length in Grayling muscle, 2000

\[ y = 3.8513e^{0.011x} \]

\[ R^2 = 0.8931 \]
Figure 3.26. Relationship between MeHg and length in Grayling muscle, 2000

\[ y = 4.5113e^{0.0103x} \]

\[ R^2 = 0.8236 \]
Figure 3.27. Relationship between THg and length in Whitefish muscle, 2000
Chapter 4
Discussion

4.1 Mercury in Fish

4.1.1 Salmon

Hg content in salmon was the lowest measured in the different types of fish collected in this study. The low Hg concentrations in the salmon may result from their migratory nature in that salmon barely feed during the time they return to fresh water (Gray et al., 2000). Concentrations of Hg in salmon presented in this study were similar to those presented in other studies in Alaska. Gray et al., (2000) reported the Hg contents were also low in muscle samples (<100 ng/g) of Coho, Chinook, and Chum salmon collected throughout southwestern Alaska. The Chinook salmon collected from Alaska water contained on average 39 ng/g THg (Bloom 1992). A 1993 salmon research project found low tissue levels of MeHg, with the highest level reported as 60 ng/g among 16 fish tested from Alaska waters (Adams, 1993).

4.1.2 Freshwater Fish

Mean THg concentrations in Pike and Grayling were lower than the FDA action level of 1.0 mg Hg / kg body weight. However, seven Pike collected from Andrefski River exceeded the action level. Pike, which is a fish-eating fish, on average contained the higher concentration. The Hg values I observed for Whitefish were lower than those Duffy et al., (1999) reported for Whitefish while the Hg contents in Grayling were
similar to those Duffy et al., (1999) reported. Gray et al., (2000) observed high Hg levels (up to 420 ng/g in muscle) in Grayling collected downstream from the old cinnabar mines in Western Alaska. The range of Hg levels in the Tanana Chiefs study for Pike in Western Alaska ranged from 91 ng/g to 832 ng/g. These values were lower than my results reported here and the THg concentrations in Pike muscle (from 300ng/g to 1444 ng/g) reported in Bloom’s study (Bloom 1992). Duffy et al., (1999) reported the THg concentrations in Pike muscle had a mean value of 1128 ng/g with a range from 225 ng/g to 1824 ng/g and the Andrefski River showed the highest levels. Gray et al., (2000) reported the Hg concentrations in Pike muscle were as high as 310 ng/g. Other studies reported that the average concentrations in various fish were approximately 200 ng/g (Egeland et al., 1998).

4.2 Relationship Between the Hg Content and Fish Length

It has been shown that the bioaccumulation of mercury in fish is generally size dependent (Jackson, 1990). Because of the accumulation of MeHg with increasing time of exposure, Hg concentrations in fish tend to rise with an increase in age, and therefore, the fish size (Johnels et al., 1967; Scott and Armstrong 1972; Huckabee et al., 1979). Basically, the age is the more preferred parameter (Derksen and Green 1987), but since the direct measurements of age were not available, the fish length or body weight can be used for the approximation of age. Norstrom et al., (1976) showed that the body weight was also a suitable parameter because MeHg concentrations and accumulation kinetics varied as a function of weight. In a study of several species from many lakes in
Manitoba and northwestern Ontario, Scott and Armstrong (1972) considered length more reliable because it is less prone to major short-term fluctuations (weight being strongly affected by feeding). In a study of fish in northern Manitoba, Derksen and Green (1987) showed that the Hg concentrations in Walleye and Pike correlated with length more significantly than with the body weight. In my study, fish length was used since it was judged to be both suitable and preferable.

Both Chinook salmon and freshwater fish species from Western Alaska gave a significant correlation between the Hg concentrations and fish lengths while the Chum, Coho, and Sockeye salmon unexpectedly showed slight negative correlations. Other workers have reported poor correlations between Hg concentration and fish length too (McGregor 1980; Bodaly et al., 1984). Jackson (1990) found that Pike and Whitefish in the lakes and reservoirs of Northern Manitoba, Canada, gave positive correlations between Hg concentrations and fish lengths whereas Shiner and Perch gave either negative correlations or much lower correlation coefficients. On the one hand, the older, larger individuals in the Pike and Whitefish populations tended to have larger accumulations of Hg because they had longer exposure to MeHg, which is absorbed more rapidly than it is excreted (Huckabee et al., 1979), also their low growth rates and large body sizes, and clearance of MeHg from their tissues were not rapid enough to dilute the MeHg (Norstrom et al., 1976; Huckabee et al., 1979). On the other hand, the high growth rates of Shiner and Perch resulted in growth dilution of their body burdens of MeHg, offsetting the tendency of their high metabolic rates to accelerate MeHg uptake.
and thus compensate the effect of bioaccumulation. Another factor may be relatively rapid excretion of MeHg owing to small body size. The growth dilution could not be used to explain the slight negative correlation between Hg content and length in Chum, Coho and Sockeye salmon since they had the similar body size and growth rate to Chinook salmon. The size ranges of Chum, Coho and Sockeye salmon in my study, however, may be relatively small (500-650 mm) to show the correlation between Hg concentration and fish length. The slight decrease in correlation is most likely related to a few individual fish at the extremes of the size range analyzed. The high mean concentrations of both forms of mercury in Chinook salmon muscle are probably related to a number of possible reasons: 1) their larger size and, thus longer ocean period, and 2) their predominantly piscivorous behavior. Both Sockeye and Coho are considered planktivorous and have lower mean levels of mercury in their muscles.

4.3 The Proportion of MeHg to THg in Muscle or Liver Tissues of Fish Collected from Western Alaska

Ratios calculated from THg and MeHg concentrations in fish muscles and livers are in Table 4.1. Grayling could not be included because of a lack of Hg information for liver. The ratio of MeHg to THg in Chinook muscle had a mean value of 0.78, which was relatively lower than 1.05 reported by Bloom (1992). The proportion of MeHg to THg in Pike muscle had a mean value of 0.94, which was similar to 0.86 reported by Jackson (1990), 1.03 by Bloom (1992), and 1.02 by Duffy et al., (1999). The MeHg comprised 81% of THg in Whitefish muscle, which is similar to 84% (Jackson 1990) but
lower than 100% (Duffy et al., 1999). The ratios of MeHg to THg in livers of all fish species were lower than in muscles (Figure 4.1) which indicated that demethylation may occur in the liver continuously.

The mean $[\text{MeHg}]_{\text{liver}}/[\text{MeHg}]_{\text{muscle}}$ ratio was much higher in Whitefish (1.2) and salmon species (1.12-1.58) than in Pike (0.5), and THg data for liver and muscle gave essentially the same result (Table 4.1 and Figure 4.2). Interestingly, this result was very similar to what Jackson reported in his studies. Jackson (1990) found that the ratios of MeHg in liver to MeHg in muscle in Whitefish (1.45-1.88) were higher than in Pike (0.54-0.70) and he concluded that the higher the mean $[\text{MeHg}]_{\text{liver}}/[\text{MeHg}]_{\text{muscle}}$ ratio of a fish species, the weaker the tendency of that species to accumulate MeHg from its environment. Therefore, Whitefish and salmon collected from Western Alaska have a weaker ability to accumulate environmental MeHg than Pike. Being lipophilic, MeHg is readily taken up and retained by fish tissues and is not excreted readily. However, in salmon and Whitefish, MeHg is probably bonded to water-soluble molecules to form hydrophilic complexes, and then excreted readily (Norseth and Clarkson 1971; Ruohtula and Miettinen 1975). The biochemical compounds to which MeHg can be bonded include the amino acid cysteine and sulfur-bearing peptides and proteins since those compounds can bind MeHg by means of dissociated sulfhydryl groups ($-\text{S}^\cdot$). Another possibility is that MeHg is demethylated in liver before it reaches the muscle or is demethylated in the muscle itself (Burrows and Krenkel 1973). The salmon and Whitefish show high $[\text{MeHg}]_{\text{liver}}/[\text{MeHg}]_{\text{muscle}}$ ratio. This appears to be consistent with
the possibility of demethylation in the muscle with the assistance of those biochemical compounds.

**Table 4.1.** Ratios computed from THg and MeHg concentrations in muscle and liver tissue of salmon, Pike and Whitefish collected from Western Alaska in a two-year period, 1999-2000. N = number of samples.

<table>
<thead>
<tr>
<th>Species</th>
<th>N</th>
<th>MeHg/THg</th>
<th>Liver THg/Muscle THg</th>
<th>Liver MeHg/Muscle MeHg</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Muscle</td>
<td>Liver</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>N</td>
<td>Mean</td>
<td>Range</td>
</tr>
<tr>
<td>Chinook</td>
<td>36</td>
<td>0.78</td>
<td>0.5-1.0</td>
<td>36</td>
</tr>
<tr>
<td>Chum</td>
<td>36</td>
<td>0.78</td>
<td>0.5-1.1</td>
<td>36</td>
</tr>
<tr>
<td>Coho</td>
<td>35</td>
<td>0.76</td>
<td>0.5-1.2</td>
<td>35</td>
</tr>
<tr>
<td>Sockeye</td>
<td>30</td>
<td>0.70</td>
<td>0.3-1.0</td>
<td>30</td>
</tr>
<tr>
<td>Pike</td>
<td>21</td>
<td>0.94</td>
<td>0.8-1.2</td>
<td>21</td>
</tr>
<tr>
<td>Whitefish</td>
<td>6</td>
<td>0.81</td>
<td>0.7-0.9</td>
<td>6</td>
</tr>
</tbody>
</table>
Figure 4.1. Mean ratios of Liver MeHg to Liver THg and Muscle MeHg to Muscle THg in Pike, salmon and Whitefish collected from Western Alaska.
**Figure 4.2.** Mean ratios of Liver MeHg to Muscle MeHg and Liver THg to Muscle THg in Pike, salmon and Whitefish collected from Western Alaska.

### 4.4 Exposure Assessment and Consumption Limits

The exposure to mercury through ingestion of fish was assessed according to methods outlined by USEPA (1989). We used the following equation to calculate the mercury exposure:

\[
\text{Ingestion (mg/kg/d)} = \frac{\text{CF} \times \text{IR} \times \text{EF} \times \text{ED}}{\text{BW} \times \text{AT}}
\]
Where CF is the mercury concentration in fish (mg/kg), IR is the ingestion rate (kg/meal), EF is the exposure frequency (meals/yr), ED is the exposure duration (yr), BW is the body weight (kg), and AT is the averaging time (ED × 365 d/yr). In this study, the ingestion rate used for Alaska subsistence adults is about 0.2 kg/meal (Nobman, et al., 1992), for general population is 0.227 kg/meal (Huggett, et al., 2001). Average exposure frequency for the general population in United States is 48 meals/yr (Huggett, et al., 2001), for Alaska subsistence adults is 96 meals/yr. The typical exposure duration for assessing noncancerogenic effects is 30 year. The average body weight for general adults and Alaska subsistence adults is 70 kg, for children is 14.5 kg (USEPA, 1989). In this analysis, we focus on the MeHg in fish and assume that 100% of MeHg ingested is absorbed into the blood stream.

A hazard index (HI) for each fish species was calculated by dividing the ingestion by the EPA suggested reference dose (RfD) for MeHg. This RfD is the daily dose of MeHg that can be safely consumed over a lifetime. The RfD for MeHg is $1.0 \times 10^{-4}$ mg/kg/d (USEPA, 1997b), that is, 100 nanograms per kilogram body weight per day. An HI less than one implies that toxic effects probably will not occur to people who consume the fish, if the HI is greater than one, toxic effects are predicted to occur (USEPA, 1989).

Monthly consumption limits were calculated using an alternative and more recent method from USEPA (1997b) based on the following equation:
\[
\text{Consumption limits (kg/mo)} = \frac{\text{RfD} \times \text{BW} \times 30.44 \text{ d/mo}}{C}
\]

Where RfD is the reference dose \( (1.0 \times 10^{-4} \text{ mg/kg/d}) \), BW is the body weight (70 or 14.5 kg as applicable to age), and C is the MeHg concentration in fish (mg/kg).

In this study, the mean MeHg concentration in muscle considered for Chinook salmon and Chum salmon is 0.06 mg/kg, for Coho salmon is 0.04 mg/kg, for Sockeye salmon is 0.03 mg/kg, and for Pike and Grayling are 0.86 mg/kg and 0.144 mg/kg respectively. Although the mean MeHg concentration in Pike muscle did not exceed the FDA threshold level of 1.0 mg/kg, seven of the Pike analyzed had MeHg concentrations in muscles above 1.0 mg/kg. Using the EPA guidelines, Alaska subsistence adults would be exposed to as much as \( 6 \times 10^{-4} \text{ mg/kg/d} \) MeHg if consuming 100% Pike while general adults and children would be exposed, if eating 100% Pike, to as much as \( 3.7 \times 10^{-4} \) and \( 1.8 \times 10^{-3} \text{ mg/kg/d} \) MeHg respectively (Figure 4.3).

The hazard indices for consuming salmon indicate that it is safe for all groups to consume 100% Coho or Sockeye salmon while it is probably hazardous for general children to consume 100% Chinook or Chum salmon (Figure 4.4). Consuming 100% Grayling is only hazardous for general children while it is hazardous for all groups to consume large amount of Alaska Pike. It is more hazardous for all children to consume all these fish than Alaska subsistence adults and general adults (Figure 4.4).
Consumption limits for general adults show that 3.6 kg Chinook salmon or 7.1 kg Sockeye salmon may be eaten per month while 0.24 kg Pike or 1.5 kg Grayling may be eaten per month. Children should limit consumption to 0.7 kg Chinook salmon or 1.5 kg Sockeye salmon a month, 0.05 kg Pike a month or 0.3 kg Grayling a month (Figure 4.5).
Figure 4.3. MeHg ingestion associated with human consumption of 100% individual species of fish from Western Alaska. (Ingestion (mg/kg/d) = CF × IR × EF × ED / (BW × AT); Ingestion rate for general adults and children is 0.227 kg/meal, for Alaska subsistence adults is 0.2 kg/meal; Exposure frequency for general adults and children is 48 meals/yr, for subsistence adults is 96 meals/yr; The average body weight for adults is 70 kg, for children is 14.5 kg; The mean MeHg concentration in Chinook and Chum muscle is 0.06 mg/kg, in Coho muscle is 0.04 mg/kg, in Sockeye muscle is 0.03 mg/kg. The mean MeHg concentrations in Pike and Grayling muscles are 0.86 mg/kg and 0.144 mg/kg respectively. Assume 100% of MeHg ingested is absorbed into the blood stream.)
Figure 4.4. MeHg hazard associated with human consumption of 100% individual species of fish from Western Alaska. (MeHg Hazard index = MeHg Ingestion / RfD; RfD = $1.0 \times 10^{-4}$ mg/kg/d; See Figure 4.3 for details about MeHg Ingestion.)
Figure 4.5. Consumption limits associated with human consumption of 100% individual species of fish from Western Alaska. (Consumption limits (kg/month) = (RfD × BW × 30.44 d/month) / MeHg concentration in fish (mg/kg); See Figure 4.3 and 4.4 for details about RfD and body weight.)

4.5 Salmon as Biotransporters for MeHg to Alaska

As industrial development proceeds and global climate change occurs as predicted, increased transport of Hg into river systems from exposed cinnabar deposits and military activities might occur (Gray et al., 2000). Also, it is believed that the removal of mercury from the atmosphere is driven by temperature-dependent photochemical processes, which create water-soluble reactive species (Lindberg, 2000).
Pacific salmon (*Oncorhynchus spp.*) complete their life cycle by returning to the spawning sites where they were reared to spawn a new generation and die. The quantity of biomass introduced inland by the yearly escapement is significant. In 1980, based on escapement data (i.e., number of salmon allowed to escape commercial and subsistence fisheries to spawn) (ADF&G 1999), Sockeye salmon contributed approximately $5 \times 10^6$ kg of organic matter to the Kvichak River, in the Bristol Bay region of Alaska. Over 99% of this salmon biomass is derived from the marine growth period and represents a substantial new source of nutrients to the regions, aquatic and terrestrial food webs (Watkinson 2000; Bilby et al., 1996; Kline et al., 1993).

Returning salmon also transport anthropogenic contaminants, such as PCBs, from their ocean feeding grounds to the spawning grounds (Ewald et al., 1998). My studies show that salmon transport MeHg, one of the most neurotoxic forms of mercury, directly to the spawning ground and, thus, are potentially exposed to the associated food webs. In recent years the evidence, even with uncertainties, indicates that on global scale anthropogenic emissions of mercury have increased (Hanisch 1998; Fitzgerald et al., 1998). Atmospheric deposition is believed to be the principal source for oceanic Hg and much of this Hg is of anthropogenic origin (Mason and Fitzgerald, 1996; Lindberg, 2000). The ability of organisms in the aquatic and terrestrial food web to adapt to increasing levels of MeHg or other contaminants over evolutionary short periods is unknown.
Is biotransport of Hg by salmon a significant input relative to other sources of mercury in Western Alaska? Accurate regional data for Alaska is lacking to quantitatively complete a mass balance for the range of potential natural and anthropogenic Hg inputs at this time (Duffy, 2000). In Western Alaska there is no active mercury anthropogenic sources. Historically, there has been some use of elemental mercury in placer gold mines in this region. Natural mercury ore deposits, principally cinnabar (HgS), represent the largest local source of mercury (Nelson et al., 1977; Gray et al., 2000). Erosion from undisturbed ore deposits and old cinnabar mining sites provide a mercury source to the watersheds in this region.

Nelson et al (1977) estimated that the Kuskokwim River transports 16,700 kg/yr of Hg, principally as cinnabar, to the marine environment. The majority of the transported mercury is in the suspended material in the water column. The biologically mediated conversion of the cinnabar to organic mercury, such as MeHg, is low (Gray et al., 2000). Cinnabar is quite resistant to weathering and has a low solubility in waters at neutral to slightly alkaline pH, typical of waters in Western Alaska (Nelson et al., 1977; Gray et al., 2000; Biester et al., 2000).

In comparison, spawning salmon, which have accumulated 99% of their biomass, including most of their body burden of mercury from the ocean, return Hg in readily bioavailable form. The salmon biomass is delivered not as a dispersed atmospheric source of Hg, but as a concentrated MeHg source within the aquatic system. A return of
2.25 \times 10^6$ Sockeye salmon in 1980 (about $5 \times 10^6$ kg) to the Kvichak River represents an estimated input of 0.1 kg of MeHg into surface water. Methylation of the remaining inorganic mercury in the salmon carcasses under anoxic conditions in the streambed may contribute to additional loading.

Twenty year Sockeye mean escapement data for 8 Alaska Bristol Bay region rivers (ADF&G 1999) was combined with my MeHg mean value for Sockeye to evaluate the magnitude of biotransport over time. Table 4.2 lists the estimated 20-year total mass loading for MeHg to Bristol Bay river ecosystems, showing about 22 kg MeHg transported from the ocean. My data support the hypothesis of Ewald et al., (1998), that salmon biomass is an additional transport pathway for MeHg, in addition to atmospheric and local geological sources of Hg, to Alaska’s interior fluvial waters.
Table 4.2. Estimated methylmercury biotransport to 8 Bristol Bay, AK rivers over a 20-year period (1979-1998).

<table>
<thead>
<tr>
<th>Bristol Bay, AK River Drainage</th>
<th>Mean Sockeye Escapement(1)</th>
<th>20 year total input grams(2) MeHg Average</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kvichak River</td>
<td>6,054,000</td>
<td>10,171</td>
</tr>
<tr>
<td>Naknek River</td>
<td>1,521,000</td>
<td>2,555</td>
</tr>
<tr>
<td>Egegik River</td>
<td>1,371,000</td>
<td>2,303</td>
</tr>
<tr>
<td>Ugashik River</td>
<td>1,303,000</td>
<td>2,189</td>
</tr>
<tr>
<td>Wood River</td>
<td>1,326,000</td>
<td>2,228</td>
</tr>
<tr>
<td>Igushik River</td>
<td>465,000</td>
<td>781</td>
</tr>
<tr>
<td>Nushagak River</td>
<td>626,000</td>
<td>1,052</td>
</tr>
<tr>
<td>Togiak River</td>
<td>192,000</td>
<td>323</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>12,858,000</strong></td>
<td><strong>21,601</strong></td>
</tr>
</tbody>
</table>

(1) Alaska Department of Fish & Game, Annual Management Report, Bristol Bay, Area, 1999 Appendix Table 1.

(2) MeHg level based on muscle average of 35 ng/g ww.

The mass of Hg biotransported is small compared with the regional natural input, but one must also consider speciation and bioavailability of Hg. Biotransported mercury is in the organic form readily bioavailable, coexists with other biotransported contaminants, such as PCBs and easily incorporates into the food web, thus, not requiring complex processing in regional biogeochemical cycles. Biotransport has been determined to be a critical pathway for the input of nutrients into the food web, especially as a nitrogen source for Sockeye salmon nursery lakes (Kline et al., 1993).
Increased input of anthropogenic mercury to the ocean surface over the last 100 years has been suggested by geochemical modeling efforts (Mason and Fitzgerald 1996). The evidence suggests that contaminants incorporated into salmon while feeding in the pelagic environment provide a direct pathway to the food web organisms of the spawning ground, including the maturing salmon fry and smolt. If this additional input has been occurring, it is important to understand how biotransport contributes to the mercury cycling in the salmon spawning areas. Does the mercury introduced in one season completely cycle through and out of the system or does some amount remain to accumulate? What are the critical loadings at which damage to the organisms within this food web occur? Based on piscivorous behavior of Pike, the loading for MeHg may already be approached or exceeded. It is crucial to continue work in this area to determine how the biotransport of mercury and other contaminants move through the ecosystem and the ecotoxicological implications.

We have just begun to address the questions about mercury in Alaska. In a few cases such as river otters, biomagnification of mercury was observed (Ben-David et al., 2001; Duffy, 2000). It is to be expected that mercury will have increased residence time in cold water and that aquatic systems will be affected by atmospheric deposition of mercury. We know that the organic rich soils of Western Alaska are favorable for the methylation of mercury. However, there are few measures of atmospheric levels of mercury in Western Alaska or the Aleutians. As more data is gathered, additional research in Alaska will contribute to important scientific and health questions such as “What is the relative
importance of physical or biological transport mechanisms in the redistribution of mercury?” This is a key question, since global climate change scenarios suggest an increase in severe storms which could resuspend mercury – as was seen recently in the mercury contamination of the U.S. Carolinas by hurricane-generated river flooding of industrial storage sites.
Chapter 5

References


Alaska Department of Fish and Game. ADF and Game, Division of Commercial Fisheries Annual Management Report-Bristol Bay Area 1999. Anchorage, AK.


**Appendix. Hg Data.**

**Table 1. Hg concentrations in 1999 salmon muscles.**

<table>
<thead>
<tr>
<th>Sample #</th>
<th>Sampling site</th>
<th>Species</th>
<th>Lng (mm)</th>
<th>Tissue</th>
<th>Date of collection</th>
<th>THg(ng/g)</th>
<th>MeHg(ng/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1A</td>
<td>Kuskokwim</td>
<td>Sockeye</td>
<td>550</td>
<td>Muscle</td>
<td>7/10/99</td>
<td>25.21</td>
<td>17.49</td>
</tr>
<tr>
<td>2A</td>
<td>Kuskokwim</td>
<td>Sockeye</td>
<td>543</td>
<td>Muscle</td>
<td>7/10/99</td>
<td>34.27</td>
<td>20.5</td>
</tr>
<tr>
<td>3A</td>
<td>Kuskokwim</td>
<td>Sockeye</td>
<td>610</td>
<td>Muscle</td>
<td>7/10/99</td>
<td>40.46</td>
<td>22.25</td>
</tr>
<tr>
<td>4</td>
<td>Kuskokwim</td>
<td>Sockeye</td>
<td>545</td>
<td>Muscle</td>
<td>7/10/99</td>
<td>36.1</td>
<td>26.1</td>
</tr>
<tr>
<td>5</td>
<td>Kuskokwim</td>
<td>Sockeye</td>
<td>500</td>
<td>Muscle</td>
<td>7/10/99</td>
<td>29.16</td>
<td>25.45</td>
</tr>
<tr>
<td>6</td>
<td>Kuskokwim</td>
<td>Sockeye</td>
<td>594</td>
<td>Muscle</td>
<td>7/10/99</td>
<td>38.7</td>
<td>23.45</td>
</tr>
<tr>
<td>7A</td>
<td>Kuskokwim</td>
<td>Chum</td>
<td>575</td>
<td>Muscle</td>
<td>7/10/99</td>
<td>54.4</td>
<td>50.25</td>
</tr>
<tr>
<td>8A</td>
<td>Kuskokwim</td>
<td>Chum</td>
<td>605</td>
<td>Muscle</td>
<td>7/10/99</td>
<td>38.28</td>
<td>27.36</td>
</tr>
<tr>
<td>9A</td>
<td>Kuskokwim</td>
<td>Chum</td>
<td>668</td>
<td>Muscle</td>
<td>7/10/99</td>
<td>49.93</td>
<td>44.16</td>
</tr>
<tr>
<td>10</td>
<td>Kuskokwim</td>
<td>Chum</td>
<td>555</td>
<td>Muscle</td>
<td>7/10/99</td>
<td>76.73</td>
<td>63.06</td>
</tr>
<tr>
<td>11</td>
<td>Kuskokwim</td>
<td>Chum</td>
<td>625</td>
<td>Muscle</td>
<td>7/10/99</td>
<td>57.75</td>
<td>37.46</td>
</tr>
<tr>
<td>12</td>
<td>Kuskokwim</td>
<td>Chum</td>
<td>566</td>
<td>Muscle</td>
<td>7/10/99</td>
<td>71.91</td>
<td>42.47</td>
</tr>
<tr>
<td>13A</td>
<td>Kuskokwim</td>
<td>Chinook</td>
<td>905</td>
<td>Muscle</td>
<td>7/10/99</td>
<td>115.58</td>
<td>78.72</td>
</tr>
<tr>
<td>14A</td>
<td>Kuskokwim</td>
<td>Chinook</td>
<td>895</td>
<td>Muscle</td>
<td>7/10/99</td>
<td>129.89</td>
<td>120.6</td>
</tr>
<tr>
<td>15A</td>
<td>Kuskokwim</td>
<td>Chinook</td>
<td>820</td>
<td>Muscle</td>
<td>7/10/99</td>
<td>94.97</td>
<td>86.64</td>
</tr>
<tr>
<td>16</td>
<td>Kuskokwim</td>
<td>Chinook</td>
<td>855</td>
<td>Muscle</td>
<td>7/10/99</td>
<td>59.79</td>
<td>48.67</td>
</tr>
<tr>
<td>17</td>
<td>Kuskokwim</td>
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* Pre-cleaned vial broke when sample was digested.
Table 5. Hg concentrations in 2000 freshwater fish muscles.

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Table 6. Hg concentrations in 2000 freshwater fish livers.

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