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HUMAN MERCURY EXPOSURE IN RELATION TO FISH CONSUMPTION IN FIJI

By

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A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

Division of Chemistry

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CERTIFICATE OF AUTHENTICITY

I, Maureen Christina Kumar, hereby solemnly declare that this thesis is the result of my own research and that any information cited from literature or otherwise has been duly and appropriately acknowledged and referenced.

.....

Maureen Christina Kumar

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The research described in this thesis was performed under my supervision and to my knowledge is the sole work of Ms Maureen Christina Kumar.

.....

Professor William G.L. Aalbersberg

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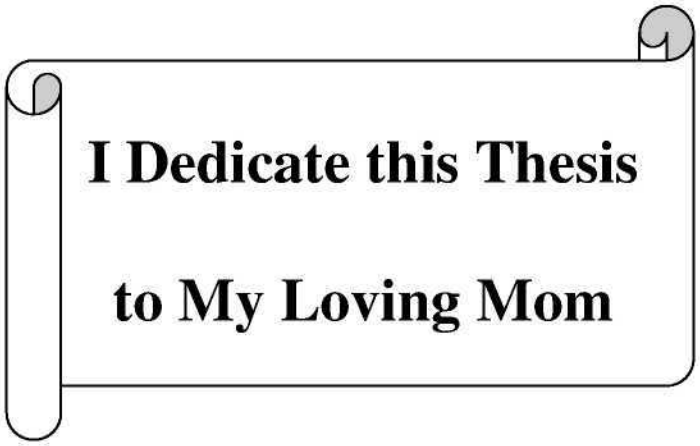
I Dedicate this Thesis
to My Loving Mom

ABBREVIATIONS

A number of abbreviations have been used throughout this thesis. The common ones include:

CRM	Certified Reference Material
FAO/WHO	Food and Agricultural Organization/World Health Organization
FFQ	Food Frequency Questionnaire
g	grams
[Hg]	mercury concentration
IVs	Independent Variables
L	liters
MDL	Method detection limit
mg	milligrams
mg/kg	milligrams per kilogram
ml	milliliters
mg/ml	milligram per milliliter
ppm	parts per million
ppb	parts per billion
PTWI	Potential Tolerable Weekly Intake
QC	Quality Control
µg/g	microgram per gram
µg/kg	microgram per kilogram
µg/L	microgram per liter
USEPA	United States Environmental Protection Agency
WHO	World Health Organization

ABSTRACT

Fish is the major source of mercury exposure in humans and data on mercury levels in fish and other seafoods from the Pacific Islands are scarce. Mercury (Hg) and its compounds pose a significant threat to human health, particularly to women who are pregnant or of childbearing age and young children.

The aim of the initial study was to measure total Hg content in several types of seafoods, which are commonly consumed in the Fiji Islands and calculate from the results whether there is a significant health risk arising from fish consumption.

Total Hg in the edible tissues of 200 seafood samples of different types (whole fish, fish steaks, shellfish, and canned fish) and species was analysed. Total Hg was determined by strong acid ($HNO_3/H_2SO_4/HCl$) digestion, addition of bromine chloride, reduction with sodium borohydride and analysis via flow injection cold vapour atomic absorption spectroscopy.

The total Hg levels in some of the large predatory fish species (marlin and swordfish) exceeded the Food and Agriculture Organization (FAO)/World Health Organization (WHO) Codex Alimentarius guideline level of 1 mg/kg. Other types of fish steaks, smaller reef fish, shellfish, canned tuna and mackerel had average levels below the guidelines. Although a limited amount of analyses were conducted on some fish species, it is clear that health risks, particularly to pregnant women, exist from consuming relatively small quantities (<1-2 portions per week) of a number of the larger fish species, such as shark, marlin, swordfish, sunfish, large

albacore tuna (canned and fresh), bigeye tuna, sailfish and large walu. For the marlin, swordfish, shark and sunfish the calculated safe level of human consumption is less than 1 portion size/week and for bigeye tuna it is about 1 portion per week. Frequent consumption of more than the recommended amount of these fish could lead to health problems. Issuing of fish consumption advisory is recommended since the total Hg levels in predatory fish are high and these fish are sold cheaply, more people tend to buy and consume these fish species. The mercury in hair study confirmed this need, however, careful risk communication is needed as fish consumption has many health benefits.

A follow-up study was initiated to determine human mercury exposure in the fish consuming population of Fiji. Hair was used as a biomarker of exposure. A total of 92 participants who volunteered their consent were part of this study of which 82 were fish consumers and 10 were a vegetarian control group. The fish consumers were from the Suburban Suva-Lami area included the participants from USP, Kalekana Settlement and Muaivuso village and Outer Island dwellers included participants from Dravuni and Daku villages of Kadavu. The method used for the determination of total Hg in human hair used was similar to that for fish tissue digestion mentioned earlier. The background total Hg in hair was 0.17 μg/g determined in the control group. In the total fish consuming population the men (n = 20) had total hair [Hg] of 5.06 μg/g and consumed an average of 7 serves of fish meals/week, women (n = 56) had hair [Hg] of 2.73 μg/g and consumed an average of 5 serves of fish meals/week and children (n = 6) had hair [Hg] of 3.09 μg/g and consumed average of 5 serves of fish meals/week. The mean total [Hg] in hair of

the overall fish consuming population (n = 82) was 3.33 μg/g consuming an average of 5.5 servings of fish meals/week.

The total hair [Hg] in all men exceeded the USEPA safety limit of approximately 1μg/g in hair and 85% of them exceeded the recommended FAO/WHO safety limit of 3μg/g in hair. Only 69% of the childbearing age women had total hair [Hg] below the FAO/WHO safety limit and 6% of the childbearing age women had hair [Hg] above WHO safety limit of 10 μg/g, an earlier safety limit derived from the Iraqi data which estimated level at which health effects occur but did not include some uncertainty factors included in later safety limit. In the total fish consuming population 44% of the participants have exceeded the FAO/WHO safety limit.

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CHAPTER 1.0

INTRODUCTION

Mercury (Hg) is a naturally occurring heavy metal that has provoked substantial human health concern worldwide (Lutter and Irwin, 2002). Mercury is part of the composition of the earth's crust and may be found in air, water, soil, aquatic sediments and living plants and animals. It occurs in several chemical forms, including elemental mercury (pure Hg) and both inorganic and organic Hg compounds. Most mercury in the atmosphere is elemental Hg vapour and inorganic Hg while most mercury in water, soil, plants and animals is inorganic and organic Hg, primarily methylmercury, MeHg (NRC, 2000; USEPA, 2001a). In the atmosphere the elemental Hg vapour (Hg^0) is converted to soluble inorganic Hg (Hg^{2+}), which returns to the surface of the earth in rainwater (WHO, 1990). In an aquatic ecosystem inorganic Hg is biomethylated by microorganisms into an organic form, methylmercury, and is the most toxic form of mercury and is bioaccumulated by aquatic organisms over their lifetime and biomagnifies at all trophic levels in the food chain (USEPA, 2001a; NRC, 2000).

Although occurring naturally, mercury is a global pollutant and concerns public health when it is elevated above natural background levels mainly through anthropogenic causes (UNEP, 2002). Mercury is very toxic to humans, and the recommended safe levels in drinking water are lower than for any other metal (1 µg/L) (WHO, 1996). The characteristic properties of mercury include extremely high toxicity, resistance to environmental degradation (which leads to its persistence in the environment), bioaccumulation within organisms and

biomagnification along the food chain, and its susceptibility to long distance transport (UNEP, 2002).

Because of the biomagnification process of MeHg, species at the top of the food chain, especially predatory fish and mammals, contain the highest levels of MeHg. Such species include whales, dolphins, swordfish, shark, marlin and these may bioaccumulate Hg to approximately 1 to 10 million times greater than dissolved Hg concentrations in surrounding waters (USEPA, 2001a). MeHg and elemental Hg are lipophilic, allowing them to pass through lipid membranes of the cells and facilitating their distribution to all tissues where the MeHg binds readily to the sulfurhydryl groups of the amino acid, cysteine, in the fish muscle tissue (USEPA, 2001a; Baird, 1999).

There are guidelines issued for levels of MeHg in fish intended for individual country governments to use to regulate fresh and processed fish and fish products moving in international trade. This includes the Food and Agricultural Organization/World Health Organization (FAO/WHO) Codex Alimentarius Commission guidelines and the European Union guidelines. The guidelines established by both international agencies are for maximum MeHg levels in predatory fish species of 1 mg/kg and 0.5 mg/kg for fish in general (FAO/WHO, 1991, 2005; European Commission Regulation, 2005).

Potential sources of human exposure to mercury include food (fish and other seafood products) contaminated with mercury, inhalation of mercury vapor in ambient air and exposure through dental amalgams and medical treatments (USEPA, 2001a; Hightower and Moore, 2003). Globally, consumption of fish and

sea mammals is by far the dominant source of MeHg among humans (Clarkson *et al.*, 2003; FAO/WHO, 2003; USEPA, 2001a; NRC, 2000). Individuals who may be exposed to higher than average levels of mercury include recreational and subsistence fishers who routinely consume large amounts of locally caught fish. At the same time pregnant women and women of childbearing age in certain ethnic groups (Asians, Pacific Islanders and Native Americans) eat much more fish than the general population. Because of the higher amounts of fish in their diet, women in these ethnic groups need to be aware of the level of mercury in the fish they eat (USEPA, 2001a; Hightower *et al.*, 2006).

It should be noted that fish and other seafood serve as a source of high quality protein and omega-3-fatty acids. Most of the Hg in fish tissue is in the form of MeHg (Bloom 1992, >95% MeHg; Kim 1995, >95% MeHg; USEPA 2001, 90-100% MeHg). About 95% of the MeHg ingested in fish is absorbed in the gastrointestinal tract (USEPA, 2001a; Clarkson, 2002). It is distributed to all tissues in a process completed in about 30 hours. About 5% is found in the blood compartment and 10% in the brain. The concentration in the red blood cells is about 20 times the concentration in plasma (Clarkson, 2002; USEPA, 2001a; NRC, 2000). In the elemental state (Hg^0), mercury is poorly absorbed in the intestine while ingested inorganic (Hg^{2+}) compounds are absorbed less than 10% on average (USEPA, 2001a; Barbosa *et al.*, 2001; Baird, 1999).

Elemental Hg and MeHg can readily cross the blood brain and human placental barrier presenting a two-fold hazard to the developing fetus resulting in higher levels of Hg in the fetal brain than the maternal brain (FAO/WHO, 2003). Once the MeHg crosses the maternal to fetal blood compartments, it binds to the red blood

cells and other fetal tissues. By the time of parturition cord blood MeHg is on average twice that of the maternal blood concentration (Hightower and Moore, 2003). MeHg binds to haemoglobin, and the high affinity to the fetal haemoglobin results in a higher Hg concentration in cord blood than in maternal blood (Stern and Smith, 2003). Elemental Mercury may penetrate the brain cell membrane where it forms inorganic mercury that has a long life (years) in the brain tissue (Clarkson, 2002).

The fetal brain is more susceptible than the adult brain to MeHg-induced damage as it inhibits the division and migration of neuronal cells and has the potential to cause irreversible damage to the growing central nervous system (Clarkson, 2003; USEPA, 1997a; NRC, 2000). The symptoms of acute mercury toxicity through high level exposures of MeHg may result in impaired central nervous system function, kidney damage and failure, gastrointestinal damage, cardiovascular collapse, shock and death (USEPA, 2001a). Chronic mercury toxicity results from both elemental and MeHg, which produce a variety of health effects at relatively high exposures. While recent studies indicate that lower dose exposure can have effects on the cardiovascular and immune system, neurotoxicity is the effect of greatest concern (USEPA, 2001a). In humans the indices of neurotoxicity include neuronal loss, ataxia, visual disturbances, impaired hearing, paralysis and death. Both central and peripheral nervous system exhibits signs of MeHg-induced damage (FAO/WHO, 2003).

Methylmercury first gained notoriety in Minamata, Japan after causing severe disabilities and death among people eating seafood contaminated through industrial discharge accumulating through the food chain (Booth and Zeller, 2005). In adults,

the earliest effects are non-specific symptoms such as paresthesia, malaise and blurred vision, with increasing exposure, signs appear such as concentric constriction of the visual field, deafness, dysarthria, ataxia, and ultimately coma and death. In infants exposed to high levels of MeHg during pregnancy, the clinical picture may be indistinguishable from cerebral palsy caused by other factors, the main pattern being microcephaly, hyperreflexia, and gross motor and mental impairment, sometimes associated with blindness or deafness (UNEP, 2002). In milder cases, the effects may only become apparent later during development as psychomotor and mental impairment and persistent pathological reflexes (WHO, 1990; NRC, 2000). There is emerging evidence that the cardiovascular and immune systems might be sites of MeHg toxicity from MeHg contaminated food (NRC, 2000).

A Swedish expert group (1971) estimated a threshold level for neurological effects in adults at about 50ppm MeHg in hair, an estimate confirmed by findings in Iraq. This level may be compared with an estimated threshold as low as 10ppm for prenatal effects (milestones of development and neurological change) in Iraq (Clarkson, 2002). Since then several epidemiological studies have been conducted in fish eating populations and large scale studies are continuing to this day focusing on neuropsychological development (Clarkson, 2002). At hair mercury concentrations of 30 ppm there is a strong possibility of subclinical effects of mercury poisoning (Kyle, 1981). In 2003 the Joint FAO/WHO Expert Committee on Food Additives (JECFA) used two epidemiological studies from the Republic of Seychelles and the Faroe Island and calculated MeHg concentration of 14 ppm in maternal hair as an estimate of the levels in maternal hair reflecting exposures that would be without appreciable adverse effects in the offspring in these two study

populations (FAO/WHO, 2003). At the same time the FAO/WHO JECFA revised the potential tolerable weekly intake (PTWI) of MeHg recommending it be reduced from 3.3 μg Hg/ kg body weight/week to 1.6 μg Hg/ kg body weight/week in order to sufficiently protect the most sensitive population, the developing fetus (FAO/WHO, 2003).

MeHg accumulates in animal and human tissues faster than it is excreted. Approximately 1% of the human body burden of MeHg is excreted daily (Clarkson, 2002). The average half-life in blood for MeHg in adults is 70 days, in children 90 days and lactating women 46 days (Swartout and Rice, 2000 as cited in Hightower and Moore, 2003).

Since the task of assessing dietary intake is relatively straightforward compared to assessment of multiple food types, retrospective dietary assessment methods such as the food consumption frequency method are simple and inexpensive and therefore used more often as the basis of dietary exposure assessments. Food frequency measures of usual frequency of consumption frequency of fish and shellfish are often used by researchers (NRC, 2000).

Scalp hair and blood are the most commonly used biological indicators to assess body burden following exposure to MeHg. In contrast to blood, hair sampling is noninvasive and can be done without medical supervision. More often hair is used as the sole biomarker of exposure (NRC, 2000). Body retention of mercury, like other metals, is dependent on dietary and physiological factors. In the exposed individuals mercury is taken up from the blood stream during hair formation and varies with the level of contamination with the fish source (Barbosa *et al.*, 2001).

Regardless of the levels of seafood consumption, mercury is preferentially taken up by the hair as the organic form (MeHg) than the inorganic form (Phelps *et al.*, 1980 in Barbosa *et al.*, 2001). Hair concentrations of MeHg are proportional to blood concentrations at the time of the formation of the hair strand. In general, the concentration of MeHg in hair is approximately 250 times the simultaneous concentration in the blood (FAO/WHO, 2003; USEPA, 2001a).

Several methods commonly used for the measurement of total mercury in hair are digestion with acids and cold-vapor atomic absorption spectrometry (CVAAS) or preconcentration on a gold amalgamator prior to using CVAAS (Feng *et al.*, 1998) or atomic fluorescence spectrometry (CVAFS) (Pellizzari *et al.*, 1999). Some samples are measured by ICP-MS after the sample is digested with concentrated nitric acid (Dickman *et al.*, 1999). Some samples are measured by graphite-furnace atomic absorption spectrometry (GFAAS) after preparing a slurry sample. Chen *et al.* (2002) analysed total mercury and MeHg in human hair by GFAAS using 2, 3-dimercaptopropane-1-sulfonate as a complexing agent. The hair samples for total mercury were completely dissolved in concentrated nitric acid by microwave digestion. In this study the total mercury in hair was determined using the flow injection Cold-Vapor AAS technique.

Mercury levels in fish and other seafoods and human mercury exposure through fish consumption have been extensively studied in various locations but such data are scarce in Fiji and other Pacific Island countries. In the Pacific Islands, there are very few studies of mercury in shellfish (Morrison *et al.*, 1997; 2001) from Fiji and some fish and canned tuna in Papua New Guinea (Kyle and Ghani, 1981; 1982a,b). Human mercury exposures through fish consumption studies are also limited in the

Pacific Islands with only the ones reported from Papua New Guinea in the early 1980s (Kyle, 1981; Kyle and Ghani, 1982b; Kyle and Ghani, 1983).

This is of great concern as fresh fish, shellfish and other seafood form one of the major sources of protein in the diet of most Pacific Island people. In addition data on mercury levels in tuna are also lacking, tuna are one of the largest income earnings for Fiji and other Pacific Island countries.

Preliminary work done by the researcher as part of a postgraduate chemistry course indicated that the total mercury in some of the large predatory fish species (marlin, swordfish, shark and sunfish) exceed the FAO/WHO guideline limit for predatory fish of 1 mg/kg and non-predatory fish of 0.5 mg/kg (FAO/WHO, 1991, 2005). There also was a clear indication of health risks particularly to the pregnant women and women of childbearing age from consuming relatively small quantities (<1-2 portions per week) of the above fish species. These findings are concerning as the larger sized fish are often the cheaper priced species (FJ$2-$4/ kg) which are sold as by-catch from the tuna trade. Although purchase and consumption data are lacking, it is believed that due to the low price, these fish are commonly and perhaps preferentially bought by local people and restaurants. It must be remembered that seafood makes up an important nutritional contribution to the diet in the Pacific Island countries and limited diet options are available to the outer island dwellers. The risk of consumption of these fish species by men, women (childbearing age and pregnant women) and children is not publicized in Fiji so a definite health risk exists.

1.1 AIMS AND OBJECTIVES

Phase I

To investigate total mercury levels in several types of seafood, which are commonly consumed in Fiji Islands and possible health implications

Objectives

- To measure total mercury concentrations in several seafood including predatory fish, fresh tuna, reef fish, shellfish, canned tuna and mackerel,

- to determine whether there is any correlation between the total mercury levels and fish weight and length for fresh tuna,

- to determine from the total mercury levels whether there is any health implications from fish consumption.

Phase II

To investigate the levels of total mercury in human hair as a measure of human mercury exposure of the fish eating population and to relate these exposure levels to their fish consumption patterns and to determine from the total hair mercury levels whether the fish eating population of Fiji would be at any health risks.

Objectives

- To validate the digestion method for determining total mercury (as Hg^{2+}) in human hair,

- to investigate the levels of total mercury in human hair as a measure of human mercury body burden and relate this to fish consumption data,

- to investigate whether there is any correlation between the frequency of fish consumption and the level of mercury body burden in the subjects under study,

- to determine from the mercury body burden results whether the fish consuming population of Fiji are at any health risk.

CHAPTER 2.0

LITERATURE REVIEW

2.1 Sources and Uses of Mercury and its Compounds

In the environment, Hg comes from natural and anthropogenic sources (Clarkson, 2002; NRC, 2000; WHO, 1990). Mercury occurs naturally in the metallic form and/or its sulfide ores such as cinnabar (HgS). Elemental Hg (Hg^0) is released from the earth's crust by volcanic activities, giving rise to natural background concentrations, which have been present in the environment for ages (Hansen and Gilman, 2005). The earth's crust contains 0.5 mg/kg, ambient air may contain 0.002-0.02 μg dm^{-3}, and the seawater contains about 0.03 mg dm^{-3} Hg (Morita *et al.*, 1998).

In addition to these background levels some Hg enters the environment as a result of anthropogenic activities. The primary anthropogenic source comes from combustion of fossil fuels, emission of coal fired electric power generation facilities, chloroalkali production, waste incineration and other industrial activities and now contribute to approximately 70% of the 5500 metric tons of mercury that are released into the earth's atmosphere each year (Hansen and Gilman, 2005; Trasande *et al.*, 2005; UNEP, 2002; Clarkson, 2002; Morita *et al.*, 1998). The world production was about 10000 tons in 1973 (WHO, 1989) and about 6500 tons in 1980. Recent estimates of anthropogenic emissions are in the order of 2000-3000 tons/year to 6000 tons/year (Morita *et al.*, 1998).

Mercury concentrates in the marine environment, especially in deep ocean waters, which contain approximately 74% of the global total, compared with approximately 24 and 2% in shallow oceans and the atmosphere respectively (Booth and Zeller, 2005).

Large quantities of liquid Hg are used to extract the sedimentary gold found in riverbeds. Pure gold is recovered when the mercury is evaporated from the amalgam by heating. It has been estimated that over 130 tons of mercury have been released each year into the Amazon basin alone (Clarkson, 2002). Elemental mercury finds extensive use industrially in lamps, batteries, and thermometers, as amalgam fillings and especially in the electrolytic manufacture of chlorine and sodium hydroxide. Mercury compounds have been used as catalysts, fungicides, herbicides, disinfectants, medicine, pigments and for other purposes (Clarkson, 2002; NRC, 2000; Morita *et al.*, 1998; WHO, 1990).

2.2 Environmental Transport, Distribution and Transformation of Mercury

Mercury exists in various chemical and physical forms and is persistent in the environment (UNEP, 2002). The three primary forms are elemental mercury (Hg^0), inorganic mercury (divalent mercury, Hg^{2+}) and methylmercury (an organic form of mercury). As mercury circulates through the environmental media such as air, water and sediments, it undergoes complex transformations. Most mercury in the atmosphere is mercury vapour (Hg^0), which circulates in the atmosphere and becomes

widely, dispersed and transported thousands of miles from its source of emission. Most mercury in water, soil, sediments or plants and animals is in the form of Hg^{2+} salts and organic forms of mercury (e.g. methylmercury, MeHg). Elemental mercury in gaseous form or bound to particles in the air is readily removed from the atmosphere by precipitation and also through dry deposition (USEPA, 1997a; Morita *et al.*, 1998; UNEP, 2002; WHO, 1990).

The air transport and deposition of mercury emissions depend on various factors including the chemical form of mercury emitted, stack height, characteristics of the area surrounding the site, topography and meteorology (UNEP, 2002). The mercury emitted in the air, usually in elemental or divalent forms, transport through the atmosphere and deposits onto land and waterbodies. The solubility of Hg^0 in water is not high enough to account for the mercury concentration found in seawater. In the atmosphere some of the Hg vapour undergoes photochemical reactions, whereby it is oxidised and transformed into relatively reactive and water-soluble species, Hg^{2+}, enabling its deposition on the earth's surface via precipitation. The residence time of Hg vapour is estimated to be between 0.4 to 3 years and, as a result, it accounts for its global distribution whereas the soluble form is assumed to have a residence time in the order of weeks (Hansen and Gilman, 2005; UNEP, 2002; USEPA, 1997a; WHO, 1990; Clarkson, 2002; NRC, 2000).

The deposited mercury on the earth's surface and open waterbodies is in part re-emitted to the atmosphere as Hg^0. The emission, deposition and re-

emission create difficulties in tracing the movement of Hg from its source to sink. The bottom sediment of the ocean is thought to be the ultimate sink where Hg is deposited in the form of the highly insoluble mercuric sulfide (Hansen and Gilman, 2005; Clarkson, 2002; WHO, 1990; Morita *et al.*, 1998).

The change in speciation from inorganic to methylated Hg is the first crucial step in the aquatic bioaccumulation process. Methylation occurs mostly in sediments in fresh and ocean waters but also in columns of fresh and seawaters (WHO, 1990). Fish intestinal contents and the outer slime of fish have also been found to methylate inorganic Hg (WHO, 1990). Methylation of inorganic Hg involves the non-enzymatic methylation of Hg^{2+} by methylcobalamine (analogous of vitamin B_{12}) that are produced as a result of bacterial synthesis. However, other pathways, both enzymic and non-enzymic, may play a role (WHO, 1990).

Microorganisms have also been isolated that carryout the reverse reaction:

$$CH_3Hg^+ \longrightarrow Hg^{2+} \longrightarrow Hg^0$$

The oxidation-reduction and methylation-demethylation reactions are assumed to be widespread in the environment, and each attains its own steady state with respect to the individual species of mercury. However, owing to the bioaccumulation of MeHg, methylation is more prevalent in the aquatic environment than demethylation (WHO, 1990).

MeHg is of particular concern because it is this form of Hg that biomagnifies in food webs especially the aquatic food webs and reaches concentration levels that are thousands of times greater than the surrounding water (USEPA, 2001a; WHO, 1990). Once MeHg is released from the microorganisms, it enters the food chain by rapid diffusion and tight binding to proteins in aquatic biota. Once absorbed, MeHg, which has high affinity for sulfur ligands, binds to the sulfur containing amino acid, L-cysteine. Through this reaction it loses some of its lipophilic character, but bound to amino acids it enters the protein pool. Due to its long biological half life, MeHg bioaccumulates in marine and fresh water organisms i.e. the older the animal, the higher the MeHg concentration, and also biomagnifies attaining its highest concentration in the tissues of fish and mammals placed at the highest trophic positions. As a result of food chain biomagnification, highest levels of MeHg are found in tissues of large long-lived predatory species such as ocean swordfish, king mackerel, tuna, shark, freshwater pike and bass (NRC, 2000; WHO, 1990; Morita *et al.*, 1998; Hansen and Gilman, 2005; COT, 2003). MeHg has a half-life of approximately two years in fish, thus large older fish particularly predatory species, will have accumulated considerably more MeHg than small younger fish (COT, 2003).

On land, some plants are known to concentrate Hg as less toxic chemical forms such as elemental Hg droplets or as HgS (Morita *et al.*, 1998).

2.3 Routes of Human Hg Exposure

Potential sources of human exposure to mercury include food (fish and other seafood products) contaminated with mercury, inhalation of mercury vapours in ambient air and exposure to mercury through dental amalgams and medical treatments (USEPA, 2001; Hightower and Moore, 2003).

Elemental Hg exposure occurs via dental fillings and to a lower extent from ambient air. Hg vapour is released from the dental amalgams into the mouth. The estimated average daily absorption of Hg vapour from dental fillings varies between 3 and 17 μg Hg and this depends on the number of dental fillings and other factors (UNEP, 2002; Clarkson *et al.*, 2003).

Occupational exposure may result where Hg and Hg compounds are produced, used in processes or incorporated into products. Reports of occupational exposure have been from chloro-alkali plants, Hg mines, Hg-based small scale gold and silver mines, refineries, thermometer factories, dental clinics with poor Hg handling practices and production of Hg based chemicals among others (ATSDR, 1999; UNEP, 2002).

Exposures to elemental and inorganic Hg can occur due to the use of skin lightening creams and soaps and the presence of Hg in some traditional medicines (such as certain traditional Asian and Chinese remedies).

Globally, dietary intake of fish, seafood and their derived products are by far the dominant source of exposure to methylmercury for the general

population (FAO/WHO, 2003; USEPA, 2001) although smaller amounts of inorganic Hg are present in other food sources but usually below the detection limit (below 20 μg/kg) (WHO, 1990).

Fish may concentrate MeHg either directly from the water or through consumption of other components of the food chain. Nearly all Hg that accumulates in fish is MeHg (NRC, 2000). Predatory fish such as king mackerel, pike, shark, swordfish, walleye, barracuda, large tuna, and marlin, as well as some marine mammals such as seals, toothed whales and dolphins may bioaccumulate mercury to approximately 1 to 10 million times greater than dissolved mercury concentrations found in surrounding waters (USEPA, 2001a). The substantial variation in human methylmercury exposure is based on the differences in frequency and amount of fish consumed and mercury concentration in the fish (NRC, 2000). Moderate consumption of fish with lower Hg levels is not likely to result in exposures of concern (UNEP, 2002).

Individuals who may be exposed to higher than average levels of mercury include recreational and subsistence fishers who routinely consume large amounts of locally caught fish. At the same time pregnant women and women of childbearing age in certain ethnic groups (Asian, Pacific Islanders and Native Americans) eat much more fish than the general population. Because of the higher amounts of fish in their diets, women in these ethnic groups need to be aware of the level of mercury in the fish they eat (USEPA, 2001a; Hightower et al. 2006).

Organic Hg exposure may also occur from the use of thimerosal (ethylmercury thiosalicylate) as a preservative in some vaccines and other pharmaceutical. The use of thimerosal vaccines is being phased out or significantly reduced in many countries, especially in vaccines intended for children (ATSDR, 1999; UNEP, 2002).

2.4 Benefits and Risks of Fish Consumption

Fish are important source of high quality protein for many people throughout the world and their importance in the diet has increased among the health conscious population. Fish provide omega-3 fatty acids that reduce cholesterol levels and the incidence of heart disease, stroke and preterm delivery (Burger *et al.*, 2005; Mahaffey *et al.*, 2004). Fish are also rich source of trace elements such as selenium, fluoride and iodine (Hansen and Gilman, 2005), taurine, calcium, copper and zinc (WHO, 2006).

Docosahexanoic acid (DHA) and eicopentanoic acid (EPA) are also essential for the development of the central nervous system. There is also some evidence that increased maternal intake of omega-3 fatty acids may prolong gestation in populations where shorter gestation periods and lower birth weights are observed, possibly due to lower background intake of omega-3 and fish intake (Hansen and Gilman, 2005).

The benefits from fish consumption are highlighted by the observation that children in the Seychelles showed enhanced visual motor coordination with

increasing MeHg exposure (Davidson *et al.*, 2000), and the children in the Faroes being studied for MeHg toxicity showed visual contrast sensitivity (Grandjean *et al.*, 1997). In both instances, the results were interpreted to mean that while fish consumption did increase exposure to MeHg, the consequently high intake of omega-3 polyunsaturated fatty acid gave superior nutrition (Custodio III, 2002).

The presence of pathogens, toxins and other chemical contaminants in fish can pose a risk to the population or subgroups. Contaminants such as PCBs, dioxin-like compounds, and heavy metals such a MeHg are present in variable levels in the fish tissue. The nutrients and contaminants may interact directly via the same mechanism or they may affect the same outcome via different mechanisms. Therefore the general approach adopted by most countries is to develop fish consumption guidelines for the general population and sensitive subgroup such as pregnant women and children. The consumption advisories and guidelines allow them to minimize exposure to contaminants while maximizing nutritional benefits (Burger *et al.*, 2005).

2.5 Toxicokinetics of Mercury

The toxicokinetics of Hg i.e. absorption, distribution, metabolism and excretion is dependent on the form of Hg a person is exposed to. The toxicokinetics of the three different forms of mercury are summarised below.

2.5.1 Elemental Mercury

Inhalation is the most important route of entry for elemental mercury. Following inhalation exposure, the absorption occurs efficiently and rapidly through the lungs. Approximately 80% of the inhaled Hg vapours are absorbed by the lung tissues (ATSDR, 1999; NRC, 2000; Clarkson, 2002; WHO, 1990; USEPA, 1997b). Elemental Hg is poorly absorbed by the gastrointestinal tract (less than 0.01% in rats), although increased blood levels of mercury have been measured in humans following accidental ingestion of several grams of metallic mercury (WHO, 1976, 1990). Airborne metallic mercury is also absorbed through the skin. The rate of dermal exposure increases with air concentration. The National Research Council stated that elemental Hg's average rate of absorption is 0.024 ng Hg/cm^3 for every 1 mg/m^3 in air (NRC, 2000).

Once absorbed, mercury vapour is readily distributed throughout the body in the bloodstream. Within a few minutes, elemental Hg is oxidised to Hg^{2+} in the erythrocytes, in a hydrogen peroxide pathway catalyzed by enzyme catalase. The maximum concentration of mercury in erythrocytes is seen after less than one hour whereas plasma levels peak after about 10 hours following short-term exposure to Hg vapour (WHO, 2000). Before oxidation, Hg^0 readily crosses cell membranes including the blood-brain and placental barriers. Following oxidation, Hg^{2+} is distributed in the body via blood. The main target organs for deposition of mercury following exposure to mercury vapour are the kidneys and the brain (WHO, 2000; NRC, 2000; Clarkson, 2002; USEPA, 1997b).

The elimination of Hg^0 occurs via urine, faeces, exhaled air, sweat and saliva. The pattern of exposure depends on the extent to which Hg^0 has been oxidised to Hg^{2+} (WHO, 2000; ATSDR, 1999; USEPA, 1997b). The half-life of elemental mercury in blood is 58 days in adults (NRC, 2000).

2.5.2 Inorganic Mercury

Inorganic mercury, Hg^{2+} can enter the body via inhalation, ingestion, or dermal exposure. Aerosols of Hg^{2+} can be absorbed through the lungs. The efficiency of absorption of ingested Hg^{2+} is between 7 and 15% and that of dermal absorption in guinea pigs is in 2-3% range (NRC, 2000). Absorption of Hg^{2+} through the gastrointestinal tract varies with the particular mercuric salt involved, absorption decreases with decreasing solubility. Absorption through skin may occur but there is no quantitative data available (ATSDR, 1999; USEPA, 1997b).

The ingested dose is rapidly distributed from the gastrointestinal tract to the blood and organs. Kidneys are the predominant sites for inorganic Hg accumulation. However accumulation also occurs in the cell of mucous membranes of the gastrointestinal tract, although a significant part of this accumulation is later eliminated owing to cell shedding and therefore never reaches the general circulation. Hg^{2+} is divided about equally between erythrocytes and plasma in the blood. In the erythrocytes Hg is probably bound to the sulfhydryl groups on the haemoglobin molecule and possibly also to glutathione. The distribution between different plasma-protein

fractions varies with dose and time following exposure. Inorganic mercury has limited capacity to penetrate the blood-brain or the placental barriers. However Hg^{2+} does accumulate in the placenta, fetal membranes and amniotic fluids. The distribution of Hg within the body and organs varies widely with dose and time following absorption. Hg^{2+} accumulates in the kidneys. Hg^{2+} can induce and bind to metallothioneine. A large proportion of Hg^{2+} in the kidneys is soluble and bound to metallothioneine (WHO, 2000; NRC, 2000; Clarkson, 2002, ATSDR, 1999; USEPA, 1997b).

Excretion of absorbed Hg^{2+} is mainly through faeces and urine but urinary route dominates when exposure is high (WHO, 1991; NRC, 2000). The half-life of inorganic mercury in blood is 1-2 months in adults (NRC, 2000).

2.5.3 Methylmercury

Oral exposure is the main route of exposure to MeHg. MeHg is also well absorbed in the gastrointestinal tract. Humans absorb 95% of the MeHg in fish they consume in the gastrointestinal tract to the bloodstream where it is distributed to all organs throughout the body (NRC, 2000; USEPA, 1997b). MeHg may also be absorbed by inhalation and through skin but to what extent has not been studied (ATSDR, 1999).

Distribution of MeHg to all tissues is complete in about 30 hours with approximately 5% found in blood compartment and about 10% in brain (Clarkson, 2002). The pattern of tissue distribution is much more uniform

than following inorganic Hg exposure, except in red blood cells, where the concentration is 10-20 times greater than the plasma concentration. MeHg is lipophilic and readily crosses the blood-brain and placental barriers. Levels in cord blood are proportional to but slightly higher than levels in maternal blood. MeHg is accumulated and concentrated in the fetus brain that is about 5-7 times higher than maternal blood (Clarkson, 2002). The mobility of MeHg in the body is not due to its lipid solubility. It is present in the body as water-soluble complexes mainly attached to the sulfur atom of thiol ligands. MeHg transport across the blood-brain barrier occurs via the neutral amino acid (MeHg-L-cysteine complex) carriers (Kajiwara *et al.*, 1996; NRC, 2000; Clarkson, 2002; USEPA, 1997b).

One fraction of the MeHg in the brain is slowly demethylated to inorganic Hg, which accumulates in certain brain structures. Although MeHg is the predominant form of mercury during exposure, Hg^{2+} slowly accumulates and resides for a longer period in the central nervous system. It is believed to be in inert form, probably insoluble mercury selenide (Clarkson, 2002; WHO, 1990). The primary route of excretion (90%) of the MeHg is via faeces. Demethylation of MeHg by microflora in the intestine is the key step in the excretion process. Both MeHg and inorganic Hg are excreted into bile conjugated with glutathione, followed by demethylation by gut microflora into inorganic form, and then eliminated from the body in faeces. Unless demethylated MeHg is reabsorbed in the intestine, it undergoes extensive enterohepatic cycling. The rate of excretion for MeHg in infants is not known, but is believed to be much lower than adults (NRC, 2000; Clarkson, 2002; WHO, 1990; USEPA, 1997b). The elimination of

MeHg from the body approximates first-order kinetics. The half-life of MeHg in adult blood is about 50 days and the whole-body half-life in adults is usually between 70-80 days (NRC, 2000). Individuals with long-term regular exposure to MeHg attain a steady-state body burden in about 1 year (NRC, 2000; Clarkson, 2002).

2.6 Human Health Effects Associated with Mercury Exposure

The most toxic effect of MeHg is on the central nervous system. The toxic action of MeHg on the developing brain differs in mechanism and outcomes from its action on the mature organs. Recent studies and reports have revealed that MeHg also affects other organs in addition to the central nervous system.

2.6.1 Mechanisms of Adverse Effects on Developing Central Nervous System

The first indications of susceptibility to the developing brain through prenatal exposure to MeHg arose from the outbreak of MeHg poisoning in Minamata, Japan in the 1950s, when it was found that mothers who were slightly poisoned gave birth to infants with severe cerebral palsy (WHO, 1990). The Iraqi outbreak in 1971-1972 confirmed the case of severe damage to the central nervous system in prenatally exposed infants.

The clinical picture is dose dependent. Cerebral palsy in infants with high maternal blood MeHg is indistinguishable from that caused by other factors. The main patterns are microcephaly, hyperreflexia, and gross motor and mental impairment and sometimes associated with blindness and deafness (NRC, 2000; WHO, 1990). In Japan the post-mortem observations indicated that there is damage throughout the brain in prenatal cases in contrast to adult exposure where there was predominance of focal lesions (WHO, 1990).

Prenatal cases in Japanese cases indicated disturbed development in the cytoarchitecture of the brain and the brain size was diminished in severe cases. Similar findings were reported in Iraqi infants. These observations suggested that the severe prenatal effects resulted from incomplete and abnormal migration of neuronal cells to the cerebellar and cerebral cortices, which is indicative of a general disturbance in the developmental growth of the brain (WHO, 1990; Clarkson, 2002; Goldman *et al.*, 2001).

MeHg is known to inhibit the cell division by metaphase arrest probably by disruption of microtubule proteins at lowest MeHg exposure. In vitro MeHg exposure resulted in primary neuroepithelial cell death and reduced cell production because of cell cycle inhibition during rapid phase of central nervous cell proliferation (Frustman *et al.*, 2002).

2.6.2 Mechanisms of Adverse Effects on Adult Central Nervous System

The central nervous system is the main target organ in adults. MeHg has a longer latent period of weeks and months between the time of exposure and onset of symptoms (Clarkson, 2002; Clarkson *et al.*, 2003; WHO, 1990). The sensory, visual and auditory functions, together with those of the brain areas especially the cerebellum concerned with coordination, are the most affected functions (WHO, 1990; NRC, 2000).

Paresthesia, a numbness or "a pins and needle" sensation, malaise and blurred vision are the first symptoms to appear at low dose. Subsequently this progresses to dysarthria, cerebellar ataxia, deafness and constriction of visual fields (WHO, 1990; Clarkson, 2002). The sign and symptoms are caused by loss of neuronal cells in specific anatomical regions of the brain. For example, ataxia is a result of loss of granule cells in cerebellum, which lack the repair capacity and are the first cells in that area of the brain to succumb while the Purkinje cells are unaffected (Crinnion, 2000; WHO, 1990; Clarkson, 2002; Weiss *et al.*, 2002). Constriction of visual fields results from the loss of neurons from the visual cortex (Weiss *et al.*, 2002). Mercury inhibits enzyme activities critical for functioning of neuron systems and other tissues (Magour *et al.*, 1987 as cited in Crinnion, 2000).

2.6.3 High Dose MeHg Poisonings

The first MeHg poisoning episode took place in Minamata, Japan between 1953 and 1960. The source of MeHg was effluent from a chemical factory where Hg was used as a catalyst in the production of acetaldehyde. The bioaccumulation and biomagnification of MeHg in the food chain resulted in sufficiently high MeHg concentrations (40ppm) in fish and shellfish in these waters which formed the main staple diet for these people (Hansen and Gilman, 2005; WHO, 1990; Satoh, 2000). Over 2200 victims were certified with Minamata Disease. The overall prevalence of 59% of neurological and mental disorders was noted in Minamata region. Among all patients, 78 fatalities occurred and hair Hg concentrations ranged from 50-700 μg/g (Hansen and Gilman, 2005).

First discovery of fetal Minamata Disease was made in 1958. The mothers of these children had consumed contaminated fish and shellfish during pregnancy thus the infants were exposed in utero. Thirteen infants manifested a severe disease resembling cerebral palsy. The signs and symptoms in these children were mental retardation, cerebellar ataxia, primitive reflex and dysarthria, seizure and pyramidal signs (Watanabe and Satoh, 1996; WHO, 1990).

Studies 40 years after the Minimata incident and 30 years since fishing was banned still showed signs after this long latency period. In 1995 the male residents of fishing villages in the area reported complaints of stiffness, dysesthesia, hand tremor, dizziness, loss of pain sensation, cramping,

atrophy of the upper arm musculature, arthralgia, insomnia and lumbago. Female residents had higher incidence of leg tremor, loss of touch sensation, leg muscular atrophy and muscular weakness (Fukuda *et al.*, 1999 as cited in Crinnion, 2000).

Another outbreak occurred in Niigata, Japan in 1965 caused by acetaldehyde plant effluent. Approximately 700 patients resulted. In the Niigata episode only one infant displayed fetal Minamata Disease (Watanabe and Satoh, 1996).

Saito *et al.* (2004) studied adult subjects who had been exposed prenatally to MeHg from fish consumption during the Niigata poisoning to determine the long-term impact of exposure. The subjects were adult children born to mothers with elevated levels of exposure during the epidemic. The evaluation consisted of a questionnaire that focused on development, symptoms and current functions and standard neurological examination. A total of 40 families participated. These subjects were divided into four groups: Group A, the maternal hair levels was ≥50ppm; Group B, levels were 25-49ppm; Group C, levels were 10-24ppm and Group D included mothers diagnosed with Minimata Disease but hair Hg not measured. Subjects experienced symptoms such as muscle cramps (59%), gait disturbances (49%), disturbed hearing and vision (31%), disturbed speech (31%) and delayed walking (13%).

The largest mass outbreak occurred in rural Iraq in the winter of 1971-1972. The people were exposed to MeHg treated seed grain that was used for

baking of bread. The total number of victims was 6530, which included 459 deaths. The first effects were complaints of paresthesia or malaise followed by signs of ataxia, constriction of visual fields and loss of hearing. Some people also experienced muscular weakness, however most of the symptoms were attributed to damage to the central nervous system (Bakir *et al.*, 1973 as cited in WHO, 1990). The data from Iraq permitted the estimation of dose-response relationships and provided evidence of greater fetal brain sensitivity than adult brain. This resulted in a long-term daily intake of Hg concentration in blood of approximately 200 µg/L and hair concentration of about 50 µg/g (WHO, 1990).

2.6.4 Low Dose MeHg Exposure Studies

Three major epidemiological studies have been conducted in New Zealand, Republic of Seychelles Islands and Faroe Islands to assess the impact of MeHg dietary exposures on fetal brain.

A Study in New Zealand by Kjellstrom *et al.* (1986, 1989) investigated development of children who were prenatally exposed to MeHg by mothers' consumption of fish meals during pregnancy. At the age of four, the children were tested using the Denver Development Screen Test (DDST), which is a standardized test of a child's mental development. In children 52% prevalence in developmental delay was noted whose mothers had been exposed to high levels of MeHg. At the age of six, a follow-up study was conducted. Each child was tested with the Test of Language

Development (TOLD), the Wechsler Intelligence Scale for children and the McCarthy Scale of Children's Abilities. Regression analyses, which used the actual concentration of mercury in hair, did not find significant associations between mercury and children's test scores. However, this finding was highly influenced by the results for a single child whose mother had the highest mercury concentration in hair (86 mg/kg) in the cohort. When this child was excluded, the results were more indicative of an effect of mercury and scores of six tests were significantly associated with the concentration of mercury in mother's hair. The performance of all the six tests decreased with high prenatal MeHg exposure (as cited in Gilbert and Grant-Webster, 1995).

In the Seychelles Islands, the developmental effects of low level MeHg exposure in utero from consumption of marine fish have also been studied (Myers *et al.*, 1995; Grandjean *et al.*, 1997). Over 80% of the Seychellois women eat fish daily with a median of 12 meals per week during pregnancy (Shamlaye *et al.*, 1995). The MeHg concentration in ocean fish is 0.3 μg/g and is comparable to those consumed by the US population (Myers *et al.*, 2003). A cohort of 779 mother-infant pairs was enrolled in a longitudinal prospective study. The total Hg concentration in maternal hair ranged from 0.5-26.7 mg/kg with a median of 5.9 mg/kg. The children underwent neurological examination at 6.5 months of age. Some of the tests that were administered were the Fagan Test of Visual Recognition Memory and the Denver Development Screening Test-Revised (DDST-R). The authors found no association between maternal hair Hg level during pregnancy and an adverse neurodevelopmental outcome of the child at 6.5 months of age

(Myers *et al.*, 1995). At 19 and 29 months of age, no association between maternal hair levels and the Bayley Scales of Infant Development, Mental of Psychomotor Development Indices was found (Myers *et al.*, 1997).

A total of 711 out of the 779 cohort mother-child pairs initially recruited in the main Seychelles Child Development Study (SCDS) in 1989 were followed up at 66 months. The mean maternal hair Hg concentration was 6.8 mg/kg and mean Hg concentration in child hair at 66 months was 6.5 mg/kg. At 66 months no adverse outcomes were associated with either prenatal or postnatal MeHg exposure for the following tests: the McCarthy Scales of Children's Abilities, the Preschool Language Scale, the Woodcock-Johnson Applied Problems, and Letter and Word Recognition Tests at Achievement, the Bender Gestalt and Child Behaviour Checklist (Davidson *et al.*, 1998).

At 9 years of age the children were assessed for neurocognitive, language, memory, motor, perceptual motor and behavioral functions. Two out of 21 endpoints were associated with prenatal MeHg exposure and developmental outcomes. There was a significant decrease in performance on grooved pegboard time for the non-dominant hand in males. A significant improvement of the hyperactivity index of the Conners Teacher-Rating Scale was noted as prenatal exposure increased. The authors concluded that the SCDS longitudinal assessments at 9 years of age indicated no detectable adverse effects in a population consuming large quantities of wide variety of ocean fish (Myers *et al.*, 2003).

The Faroe Islands population is exposed to MeHg mainly from the consumption of pilot whale meat with very high MeHg concentration (around 2 μg/g) (USEPA, 2001). In 1986-1987 a cohort of 1022 consecutive singleton births were gathered in Faroe Islands. The high maternal exposure due to consumption of pilot whale meat was evident by the Hg concentration in cord blood and maternal hair. Of the 1033 children, 917 children at approximately 7 years of age underwent detailed neurobehavioural examination. Some of the neuropsychological tests administered were Finger Tapping, Hand-Eye Coordination, reaction time on a Continuous Performance Test, Wechsler Intelligence Scale for Children-Revised Digit Spans, Similarities and Block Designs, Bender Visual Motor Gestalt Test, Boston Naming Test and California Visual Learning Test for Children. Neuropsychological deficits were mostly in the domains of language, attention, and memory, while motor speed and visual spatial functions showed less robust decrements with increased MeHg exposure (Grandjean *et al.*, 1997). Developmental delays were found to be significantly associated with hair Hg concentrations below 10 μg/g. The calculations based on these data revealed that the lower 95% confidence limit for a doubling of a 5% abnormality response occurred at maternal hair levels of approximately 10 μg/g corresponding to cord blood concentration of 58 μg/L. Each doubling of prenatal MeHg exposure levels was associated with a developmental delay of 1-2 months (Budtz-Jorgensen *et al.*, 2000 as cited in Hansen and Gilman, 2005).

At the age of 7, the Faroe Islands birth cohort of 1000 children were examined for prenatal MeHg exposure, blood pressure, heart rate and heart rate variability. After adjustment for body weight, diastolic and systolic blood pressure increased by 13.9 mmHg and 14.6 mmHg when cord blood Hg concentrations increased from 1 to 10 μg/L. In boys heart rate variability decreased with increased MeHg exposures from 1 to 10 μg/L in cord blood at which variability was reduced by 47%. The findings suggested that prenatal MeHg exposure might affect the development of cardiovascular homeostasis (Sorensen *et al.*, 1999). These populations were re-examined at the age of 14 with electrophysiologic parameters of heart function, blood pressure and evoked potentials (Grandjean *et al.*, 2004; Murata *et al.*, 2004). A total of 878 of 1010 live cohort members were examined. Paired heart rate variability results from age 7 and 14 years showed a decrease of 25% in both low frequency and high frequency activities and these correlated well with blood pressures. The heart rate increased by 2.7% with each doubling of prenatal MeHg exposure and was associated with a decrease of 6.7% in low frequency and high frequency powers. A decrease in low frequency variability was associated with increased latency of brain auditory evoked potential peak III but adjustment for MeHg exposure attenuated this correlation. The study concluded that MeHg exposure was associated with decreased sympathetic and parasympathetic modulation of the heart rate variability.

Another smaller cohort was recruited in Faroe Islands, which consisted of 182 infants from a catchment area that included villages with greater access to fish and whales. The mean maternal hair Hg was 4.08 mg/kg (range 0.36-

16.3), mean cord blood Hg was 20.4 μg/L (range 1.90-102) and mean Hg in serum was 2.54 μg/L (range 0.7-8.74). In addition 18 pesticide metabolites and 28 PCB congeners were measured in maternal serum and breast milk, selenium was measured in cord blood, fatty acids (arachidonic acid, EPA, DHA, total omega-3 fatty acids) were measured in cord serum. The neurological optimality score (NOS), which assesses and infants functional abilities, reflexes and muscle tone was administered at the age of 2 weeks and several indices of thyroid function were measured in maternal and cord serum. A significant inverse relation was found between the mercury in cord blood and NOS score. A 10-fold increase in Hg concentration was associated with a deficit in NOS score that was equivalent to a 3-week reduction in gestational age. After adjustment for total PCBs and fatty acid concentrations did not have any effect on the results. Selenium did not appear to modify the effect of MeHg (Steuerwald *et al.*, 2000 in NRC, 2000).

2.6.5 Other MeHg Exposure Studies Related to Fish and Seafood Consumption

A study by Choy *et al.* (2002) compared blood Hg concentrations of couples with and without fertility problems in Hong Kong. The infertile group included 157 male and female partners undergoing in vitro fertilization. Another group comprised of 26 control couples attending antenatal care during second trimester of pregnancy. Blood Hg concentration of >50 μg/L was considered elevated. Infertile couples with

abnormal semen and infertile females with unexplained infertility had higher blood Hg concentration. Blood Hg concentration was positively correlated with the quantity of seafood consumption with infertile subjects with higher blood Hg levels consuming a large amount of seafood.

Another study found that hair Hg level correlated with subfertility in Hong Kong males. A typical male reaching 30 years was found to accumulate approximately 3.3 mg/kg of Hg in hair and by age of 60 the hair Hg level was about 7.5 mg/kg in a subfertile male. The age corrected risk indicated that compared with men with higher levels were twice as likely to be subfertile and some males with only 5 mg/kg hair Hg displayed signs of subfertility (Dickman *et al.*, 1998, 1999).

In participants (aged 15-81 years) from Brasilia Legal, Amazon were investigated for cytogenetic damage due to low level MeHg exposure. The total hair Hg levels ranged from 0.57-153.8 mg/kg (median = 13.5 mg/kg). The females had higher hair Hg levels than males. The investigation showed a clear relation between MeHg contamination and cytogenetic damage in lymphocytes at levels below 50 mg/kg in hair. The first apparent biological effect with increased hair MeHg was impairment of lymphocyte proliferation measured as mitotic index. The relation between hair Hg concentration and mitotic index suggested that this parameter, an indicator of changes in lymphocytes and their ability to respond to culture conditions may be an early marker of cytotoxicity and genotoxicity in humans (Amorim *et al.*, 2000).

Several studies have reported associations between cardiovascular disease and Hg mostly in the form of MeHg. Salonen *et al.* (1995) studied the relation of dietary intake of fish and Hg in a 7 year follow-up in 1833 Finnish men ages 42-60 years who were free of clinical coronary heart disease (CHD), stroke, claudication and cancer. The mean Hg concentration in hair was 1.92 mg/kg (range 0-15.67). There was and association between dietary intake of fish and Hg with significantly increased risk of acute myocardial infarction (AMI) and death from CHD and cardiovascular and any death. The men in the highest tertile (>2mg/kg) of hair Hg content had a 2-fold risk of AMI and a 2.9-fold risk of cardiovascular death compared to men with lower hair Hg levels. The daily intake of fish of more than 30g was associated with a 2.1-fold risk of AMI and a 2.4-fold risk of coronary mortality compared with men who consumed less than 30g of fish. During the average follow-up time of 13.9 years of these Finnish men (n = 1871) aged 42-60 years, 282 acute coronary events, 132 cardiovascular disease, 91 coronary heart disease and 525 all-cause death occurred (Virtanen *et al.*, 2005). It was found that high Hg content in hair was most strongly associated with fish and serum docosahexanoic acid and docosapentanoic acid concentration. The men in the highest third of hair Hg concentration (>2.03 mg/kg) had a 1.6-fold risk of acute coronary event, 1.68-fold risk of CVD, 1.56-fold of coronary heart disease death and 1.38-fold risk of any death compared with men in the lower two thirds. With each microgram of Hg in hair, the risk of acute coronary event increased by 11%, the risk of CVD death by 10%, the risk of CHD death by 13% and risk of any death by 5%. Furthermore the high Hg level in hair attenuated the beneficial effects

of fish oils on the risk of coronary events, CVD and CHD mortality (Virtanen *et al.*, 2005).

A case-controlled study was conducted in 684 men aged ≤70 years with first diagnosis of myocardial infarction living in the eight European countries or Israel and 724 controls representative of the same population. In this study an independent and graded association was found between toenail Hg levels and the risk of myocardial infarction. Furthermore Hg masked an inverse association between DHA and the risk of myocardial infarction that became evident only after adjustment for the Hg level. This study also concluded that the high Hg content may diminish the cardioprotective effect of fish intake (Guallar *et al.*, 2002).

The study by Yakoo *et al.* (2003) reported the results of neuropsychological testing and hair Hg concentrations in 129 adults aged 17-81 years from the six fishing villages downstream from an area of gold mining in which Hg used in the amalgamation process in Brazil. Tests of attention, memory, manual speed and dexterity, graphomotor speed, and mood were administered. The mean hair Hg concentration was 4.2 mg/kg (range 0.56-13.6). Hg levels were associated with fish consumption. An association was found between hair Hg levels and detectable alterations in performance on tests of fine motor speed and dexterity and concentration. Some aspects of verbal learning and memory disruption were also noted with Hg exposure. The magnitude of effects was found to increase with hair Hg concentrations. The authors concluded that these findings suggest that adult

cognitive function might be as sensitive as children's neurocognitive function.

2.6.6 Health Effects of Elemental and Inorganic Hg

Damage to the kidney is the key endpoint in exposure to inorganic Hg compounds. Autoimmune glomerulonnephritis formation is the most sensitive adverse effect observed following inorganic Hg exposure. The principal syndrome of acute Hg salt poisoning is stomatitis and digestive upset (Tchounwou *et al.*, 2003; ATSDR, 1999).

Effects on the nervous system appear to be the most sensitive outcome observed following elemental Hg exposure. Inhalation of Hg vapour causes intentional tremor, gingivitis, bizarre behaviour such as excessive shyness and even aggression, neuromuscular changes, headaches, polyneuropathy, memory loss and performance deficits in tests of cognitive function. At higher concentration observations such as kidney and thyroid effects, pulmonary dysfunctions, changes in vision and deafness are seen (USEPA, 1997; ATSDR, 1999; UNEP, 2002). Short-term exposure to high concentration of elemental Hg damages lining of mouth, lung irritation, causes tightness of chest, coughing, nausea, vomiting, diarrhea and increased blood pressure (Tchounwou *et al.*, 2003; ATSDR, 1999). A case of chronic Hg poisoning occurred in Papua New Guinea in a 24-year-old mine employee who had been stealing gold-amalgam and cooking it in his house which released Hg vapour during the process. He complained of

having weakness in his arms, trembling fingers, insomnia and inability to concentrate well at work. His symptoms improved when he was on field break but worsened when on duty. Investigations showed that he had very high Hg levels in blood and urine (Rety, 2002).

2.7 Reference Levels for Mercury and Mercury Compounds Set by International Agencies

A number of international agencies have estimated values for safe exposure levels for Hg and its compounds.

2.7.1 Methylmercury

2.7.1.1 The Joint FAO/WHO Expert Committee on Food Additives (JECFA) Evaluations

In 1972, JECFA established a Potential Tolerable Weekly Intake (PTWI) of 5 mg/kg bw/week for total Hg from which no more than two thirds (3.3 mg/kg bw/ week) should be from MeHg. The toxicity data from the poisoning incidents of Minamata and Niigata in Japan were used to derive this PTWI. The lowest Hg levels associated with the onset of clinical disease in adults in these incidents were reported to be 50 mg/g in hair and 200 mg/L in whole blood. Clinical effects such as peripheral neuropathy were displayed by individuals at these Hg levels and were considered to be more sensitive than the general population because there may have been

other individuals in Japan and other countries having higher hair Hg or blood levels with no experience of the above clinical effect (FAO/WHO, 1972).

Evidence of delayed achievement of developmental milestones, a history of seizures and abnormal reflexes were seen in the Iraqi population at maternal hair levels well below those associated with severe effects. A statistical analysis revealed that motor retardation arose above the background frequency of maternal hair mercury levels of 10-20 μg/g. The fetus was considered to be at greater risk. A prudent interpretation of the Iraqi data implied that a 5% risk might be associated with a peak mercury level of 10-20 μg/g in maternal hair (WHO, 1990).

In the 2000 JECFA evaluation of MeHg more attention was paid to possible prenatal and postnatal exposure by considering the large long-term prospective epidemiological studies conducted in the Seychelles Islands and the Faroes Islands. These studies aimed to identify the lowest dietary Hg exposure associated with subtle effects on the developing nervous system and this was done by following the neurodevelopment of children by testing their learning and spatial abilities at different ages. The JECFA found that though the mean Hg exposures during pregnancy that was assessed by maternal hair Hg were similar, the results of these two studies were conflicting. The regression analysis in the Faroes showed an association between MeHg exposure and impaired performance in neuropsychological tests, an association that remained even after excluding the results associated with greater than 10 μg/g maternal hair. In the Seychelles study,

the regression analysis showed no adverse effects but increased maternal hair concentration was associated with a small statistically significant improvement in the test scores on several different developmental outcomes. The investigators noted that this could be due to the beneficial nutritional effects of fish. An additional secondary analysis was performed where the results were split into subgroups based on maternal hair Hg level. The test scores in children with the highest Hg exposures (12-27 μg/g maternal hair) were not significantly different from those children with the lowest exposure (<3 μg/g maternal hair) (FAO/WHO, 2000).

Another study carried out in New Zealand on a smaller study group of six year old children used a similar batch of tests to the Seychelles Islands and had similar exposure to MeHg yet found MeHg related detrimental effects on behavioral test scores. The benchmark dose (BMD) for Hg in maternal hair, calculated from results of five tests, ranged from 32 to 74 mg/kg, and the corresponding BMDLs (lower 95% confidence limits on BMDs) ranged from 17 to 24 mg/kg. When the child with the highest maternal hair Hg was omitted BMDs ranged from 13 to 21 mg/kg and corresponding BMDLs ranged from 7.4 to 10 mg/kg (Crump *et al.*, 1998).

After the consideration of all the epidemiological results, JECFA concluded that it did not provide consistent evidence of neurodevelopmental effects in children whose mothers had hair levels of 20 μg/g or less. Since there was no evidence of consistent risk, JECFA decided not to revise the PTWI but with a recommendation that MeHg re-evaluation should be done as soon as latest data from the epidemiological studies become available (FAO/WHO,

2000). Therefore the PTWI of 3.3 μg/kg bw equivalent to 0.47 μg/kg bw/day was maintained.

In 2003, at its 61st meeting in June, the JECFA reviewed the new data from the Seychelles Child Development Study (SCDS), re-analysis of the Faroes and New Zealand studies and additional epidemiological data on reproductive toxicity, immunotoxicity, and cardiotoxicity and general medical status.

Neurodevelopmental assessment data of the SCDS at 8 years of age were consistent with the results obtained at younger ages and thus provided no evidence for inverse associations between maternal MeHg exposure and neurodevelopment in children. In addition, further re-analysis of the Seychellois data at 5.5 years of age did not alter the conclusion that in this population of frequent fish-consumers, no adverse effects of prenatal MeHg exposure have been detected.

No new data from the Faroes study were available. Additional new analysis of the existing data did not support the role of occasional exposure to higher levels of MeHg or polychlorinated biphenyls (PCBs) from consumption of whale meat in accounting for the positive associations of MeHg with neuropsychological deficits and visual function in this study.

Despite the additional evidence of immunotoxicity, cardiotoxicity and reproductive toxicity, JECFA concluded that neurotoxicity resulting from *in utero* exposure should be considered to be the most sensitive endpoint for

MeHg toxicity. The committee concluded that the PTWI should be based on the studies of this endpoint thus basing its evaluation on the Seychelles and Faroe Islands studies (FAO/WHO, 2003).

The maternal hair concentration of 15.3 μg/g corresponding to a no observed effect level (NOEL) for neurobehavioral effects was identified for the Seychelles Islands study (ATSDR, 1999). A benchmark dose lower confidence limit (BMDL) of 12 μg/g Hg in maternal hair was determined for the Faroe Islands (Rice *et al.*, 2003; NRC, 2000). The Committee used the average from the two studies and obtained a composite of 14 μg/g maternal hair Hg, as an estimate of the level in maternal hair reflecting exposures that would be without appreciable adverse effects in the offspring in these two study population. Dividing by the average hair: blood ratio of 250, allowed conversion of the 14 μg/g in hair to a maternal blood mercury level of 56 μg/L. From these values, JECFA calculated a MeHg steady-state daily ingestion rate of 1.5 μg/kg bw/day consistent with maternal hair concentration that would be without appreciable adverse effects in the offspring in these populations. Uncertainty factors were applied to the 1.5 μg/kg bw/day ingestion value. A factor of 2 was applied to allow for inter-individual variability in the hair: blood ratio and an uncertainty factor of 3.2 to account for the total human inter-individual variability for converting maternal blood concentration to a steady state dietary intake. Hence a total factor of 6.4 (2 x 3.2) was applied to the steady-state daily ingestion rate of 1.5 μg/kg bw/day to derive a PTWI of 1.6 μg/kg bw. This PTWI is considered sufficient to protect the developing fetus, the most sensitive subgroup of the population (FAO/WHO, 2003). This value would

approximately equal to 3 μg/g (14 μg/g divided by 6) in hair and 12 μg/L in blood (WHO, 2006).

2.7.1.2 United States Environmental Protection Agency (USEPA) Reference Dose (RfD)

USEPA defines an RfD as an estimate (with uncertainty spanning perhaps an order of magnitude) of a daily exposure to the human population (including the sensitive subgroups) that is likely to be without an appreciable risk of deleterious effects during a lifetime (USEPA, 1997a). The USEPA RfD is based on data from the Iraqi poisoning episode. The RfD is calculated from the Benchmark Dose Limit (BMDL). The BMDL is the 95% lower confidence limit on the maternal hair concentration corresponding to an extra 10% risk level. The USEPA used maternal hair Hg concentration of 11 μg/g as BMDL and used a hair: blood ratio of 250:1 to convert hair concentration to blood concentration. Thus 11 μg/g in hair corresponds to 44 μg/L blood. The following equation was used to obtain the daily dietary intake of MeHg of 1.1 μg/kg bw:

$$\mathbf{d = \frac{C \times b \times V}{A \times f \times bw}}$$

Where

d = daily dietary intake in μg/kg bw/day

C = concentration in blood (44 μg/L)

b = elimination constant (0.014 days $^{-1}$)

V = volume of blood in the body (5L)

A = absorption factor (expressed as the unit-less decimal fraction of 0.95)

f = fraction of daily intake taken up by blood (unit-less 0.05)

bw = body-weight default value of 60kg for an adult female.

A composite uncertainty factor of 10 was used to account for the human population variability. Hence the current RfD for MeHg developed by USEPA is 0.1 μg/kg bw/day (USEPA, 1997c). A daily intake of 0.1 μg Hg/kg bw/day by an adult woman is estimated to result in approximately 1 μg/g Hg concentration in hair, 4 to 5 μg/L in blood and 5 to 6 μg/L in cord blood (UNEP, 2005).

The US National Research Council (NRC) in 2000 reviewed this USEPA RfD. The Committee recommended that results from the Boston Naming Test in the Faroes study be used in the calculation of the RfD. NRC identified a benchmark dose lower confidence limit of 12 μg/g in maternal hair corresponding to 58 μg/L in cord blood. This was the lower 5% confidence limit of the lowest dose considered to produce a sufficiently reliable neurological endpoint in the Faroe Islands study. The NRC made a number of assumptions in deriving an estimate of MeHg intake and concluded a composite uncertainty factor of at least 10 to account for inter-individual variability. Hence on the basis of the available data, the Committee concluded that the value of USEPA's current RfD for MeHg of 0.1 μg/kg bw/day is scientifically justifiable for the protection of public health which is equivalent to approximately 1 μg/g in hair (NRC, 2000).

2.7.1.3 Agency of Toxic Substances and Disease Registry (ATSDR)

ATSDR developed a chronic oral minimal risk level (MRL) of 0.3 μg/kg bw/day for MeHg. The MRL was based on the no-observed adverse effect level (NOAEL) identified for neurodevelopmental effects in children in the Seychelles Child Development Study. An uncertainty factor of 4.5 was applied to account for the human pharmacokinetic and pharmacodynamic variability (ATSDR, 1999).

2.7.2 Elemental Hg

The USEPA established and RfD for elemental Hg of 0.3 $\mu g/m^3$, based on the lowest observed effect level (LOAEL) of 9 $\mu g/m^3$ and an uncertainty factor of 30 to account for sensitive human subpopulation and database deficiencies. A human occupational study use for the RfD investigated the neurological effects in humans exposed to elemental Hg in the workplace; hand tremors, increases in memory disturbances and evidence of autonomic dysfunction were observed and were basis for the LOAEL (USEPA, 1995a; UNEP, 2005).

ATSDR developed a chronic inhalation MRL of 0.2 $\mu g/m^3$ for metallic Hg vapour. The MRL was based on a LOAEL for increased frequency of tremors in occupationally exposed workers and an uncertainty factor of 30 to account for the use of a minimal LOAEL and human variability (ATSDR, 1999; WHO, 2003).

2.7.3 Inorganic Hg

A recent risk evaluation on inorganic Hg concluded that 2 μg/kg bw/day as tolerable intake for inorganic Hg based on data for occupational exposure (WHO, 2003). The USEPA established and RfD for mercuric chloride of 0.3 μg/kg bw/day based on the consensus decision of a panel of mercury experts who used several LOAELs ranging from 0.23 to 0.63 mg/kg bw/day and an uncertainty factor of 1000. The LOAELs were derived from several rat feeding, gavage, and subcutaneous injection studies in which autoimmune glomerulonephritis were observed (USEPA, 1995b).

2.7.4 International Guidelines for the Level of Mercury in Fish

Organization	**Guidelines**	
FAO/WHO Codex Alimentarius Guideline, 2005	1 mg/kg (large predatory fish)	0.5 mg/kg (other fish species)
European Commission Regulation, 2005	1 mg/kg (large listed predatory fish)	0.5 mg/kg (other fish and fishery products)
Food Standards Australia and New Zealand, 2004	1 mg/kg (for species known to contain high levels)	0.5 mg/kg (all other fish species and crustaceans and molluscs)
Health Canada, 2002		0.5 mg/kg (all fish sold at retail except shark, swordfish and fresh/frozen (not canned) tuna)

2.8 Role of Nutrition on Hg Metabolism

Diet can affect MeHg metabolism in the body. Selenium and vitamin E both help in reducing Hg toxicity, however in doing so, Hg decreases the availability of these nutrients to the tissues. In addition to these, intake of other antioxidants is highly recommended. Detoxification of Hg depletes glutathione thus supplements that increase glutathione levels should be employed including whey proteins, Vitamin C, milk thistle, and selenium all of which are highly necessary for any case of toxin overload (Chapman and Chan, 2000; Crinnion, 2000; Raymond and Ralston, 2004; Tchounwou *et al.*, 2003).

A recent 12 month long study involving 26 women from a fish eating village community in the Brazilian Amazon examined the influence of consumption of traditional foods on the relationship between fish consumption and Hg exposure. The study revealed strong relationship between fish consumption and Hg exposure which was modified by fruit consumption i.e. for the same number of fish meals, those who ate more tropical fruits had lower hair Hg levels and this relationship was observed for all periods of the year (Passos *et al.*, 2003). There are several mechanisms by which fruits may affect Hg levels. Phytochemicals may interact with toxic metals in several ways in the body: absorption and excretion, transport, binding to target proteins, metabolism and sequestration (Peraza *et al.*, 1998). A possible explanation is that the soluble dietary fiber of fruits could be interfering with absorption at the intestinal

level (Peraza *et al.*, 1998). A similar reaction was observed with wheat bran fiber (Rowland *et al.*, 1986).

2.8.1 Use of Chelation Therapy for Reduction of Hg Burden

The sulfur containing compounds such as dimercaptosuccinic acid (DMSA), dimercaptopropane sulfonate (DMPS) and N-acetylcysteine (NAC) have all been used effectively to reduce body burden of Hg. DMSA has been utilised in 1965 and has since then proved to be effective against mobilizing lead, cadmium, arsenic and Hg (Crinnion, 2000).

DMPS may benefit in reducing the nephrotoxicity of MeHg chloride. DMPS (oral dose at 10 mg/kg, intravenous dose at 3 mg/kg), DMSA (30 mg/kg), NAC (30mg/kg) along with potassium citrate (5g) were tested. Their effects on Hg excretion were comparable. When administered alone DMSA caused an increase in urinary Hg excretion of 163%, DMPS 135%, NAC 13% and potassium citrate 83%. The urinary Hg excretion increased to 163% for both DMPS and NAC when given with potassium citrate. It is recommended that these agents are administered in several day courses with rest periods in between (Crinnion, 2000).

Ballatori *et al.* (1998) studied the results of oral administration of NAC, an amino acid derivative that accelerates the urinary MeHg excretion in mice. The mice that received NAC (10 mg/ml) starting at 48 hours after MeHg administration excreted from 47-54% of ^{203}Hg in the urine in the next 48 hours. The ability of NAC to enhance MeHg excretion when given orally,

its relatively low toxicity and wide availability in the clinical setting indicates that it may serve as an ideal therapeutic agent for MeHg poisoning.

The children exposed to metallic Hg were treated with meso-2,3-dimercaptosuccinic acid orally which produced significant Hg diuresis in these children. No side effects were observed and DMSA appears to be an effective safe chelating agent for treatment of pediatric overexposures to metallic Hg (Forman *et al.*, 2000).

However an efficient and effective therapy that can be used for long-term chronic MeHg exposure in fish eating population is not yet available (Chapman and Chan, 2000).

2.9 Biomarker of Human Mercury Exposure

Biomarkers are useful tools for human exposure assessments. Biological samples that can be monitored as evidence of exposure to mercury include urine, hair and blood. Urine is most frequently monitored to follow chronic exposure to inorganic mercury vapour. Blood and hair are most commonly monitored to access body burden following exposure to methylmercury compounds. Fish consumption will markedly affect these levels. In contrast to blood sampling, hair sampling is simple, noninvasive and can be done without medical supervision. There is less risk of disease transmission in the sampling process of hair and fewer cultural issues are involved (NRC,

2000; UNEP, 2005). More often, hair is used as the sole biomarker of exposure (NRC, 2000). Hair concentrations of methylmercury are proportional to blood concentrations at the time of the formation of the hair strand. Once incorporated into the hair strand, its concentration remains unchanged for up to 11 years (WHO, 1990). In general, the MeHg concentration in hair is approximately 250 times the simultaneous concentration in the blood (FAO/WHO, 2003; USEPA, 2001). The normal level of Hg in hair is 1-2 ppm, but people who consume fish once or more times per day may have Hg levels in hair exceeding 10 ppm (UNEP, 2005).

Blood mercury concentration, unless supplemented by additional temporal exposure data, provides no clear information about the magnitude of timing of the exposures that yield the total mercury concentration observed in a given sample. In contrast, hair mercury concentration as a biomarker of methylmercury exposure has the advantages of being able to integrate exposure over a known and limited time and recapitulate the magnitude and the timing of exposure. The ability to obtain such information from hair is predicted on two assumptions: that growing hair shafts incorporate mercury from the circulating blood in proportion to the concentration of mercury in the blood, and that hair shafts grow at a constant rate that does not vary significantly among the individuals. The first of these assumptions is necessary to establish a quantitative relationship between hair mercury concentration and mercury intake, the blood mercury concentration being an intermediate kinetic compartment. The second assumption is necessary to establish a relationship between location along the hair strand and time of exposure (NRC, 2000). Thus the longitudinal analysis of mercury along a

strand of hair provides information about the timing of exposure of the individuals to methylmercury (WHO, 1990).

The growth rate of hair varies both within and among individuals. Among individuals, variation in hair growth rate occurs because individual hair follicles experience a cycle of growth, transition and terminal resting (Katz and Chatt, 1988 as cited in NRC, 2000). Direct incorporation of trace elements including methylmercury into the hair occurs only during the growth phase (NRC, 2000). An average growth rate of approximately 1 centimeter per month for scalp hair is commonly assumed. Thus it is possible to monitor exposure to methylmercury over a period of several months or years, depending on the length of a hair sample (NRC, 2000; Akagi and Naganuma, 2000; Boischio *et a*l., 2000).

Body retention of mercury, like other metals, is dependent on dietary and physiological factors. In the exposed individuals mercury is taken up from the blood stream during hair formation and varies with the level of contamination with the fish source (Barbosa *et al.*, 2001). Regardless of the levels of seafood consumption, mercury is preferentially taken up by hair as the organic form than the inorganic form (Phelps *et al.*, 1980 in Barbosa *et al.*, 2001).

2.9.1 Hair Sampling Technique and Analytical Methodologies

Hair has been used as a bioindicator in several environmental studies especially involving women, pregnant women and children. Two sampling

methods are available, single strand and bunch strands. The former requires more sensitive method and the determination of growth phase of each strand by microscopic examination of hair root (WHO, 1990). The best way to sample hair is to locate approximately 50-100 strands of hair at the back (occipital position) of the head. Using a hemostat clamp the hair to hold in place and cut them as close to the scalp as possible with a blunt surgical scissors. Before releasing the hemostat the hair strands are tied with a thread with proximal ends together to ensure that the hair strands remain in same alignment. The tied bunch of hair maybe stored in a resealable plastic bag or an envelope until analysed (WHO, 1990). Head hair sampling can pose some difficulties in cases where men are bald or have short hair. If the hair is too short to be cut and clipped together, then the hair can be cut directly into the storage bag using scissors or thinning shears (ICPS, 2000). The hair samples can be transferred at ambient temperature (ICPS, 2000; WHO, 1990).

Numerous analytical methods are available for the analysis of total Hg in hair. The original dithizone method was widely used up to the introduction of atomic absorption in the late 1960s. Basically it involved the formation of a coloured complex with dithizone after all the Hg in the sample had been converted to Hg^{2+} compounds by oxidation in strong acids. After neutralization of excess oxidant with a reducing agent, usually hydroxylamine, the coloured complex was extracted into a nonpolar solvent. After washing the extract, the colour intensity was measured on a spectrophotometer and the amount of Hg estimated from a standard curve.

The limit of detection was in the order 1-10 μg Hg so large quantities of sample were required for such media as blood and hair (WHO, 1990).

The cold vapour atomic absorption spectrometry (CVAAS) is one of the most widely used analytical methods for hair Hg analysis. CVAAS has adequate sensitivity to measure Hg at sub-ppm levels and has a low per sample cost compared to some newly developed methods. This method is also very specific and less prone to matrix interferences (UNEP, 2005).

Analysis of Hg in biological samples is complicated due to the presence of inorganic and organic forms of the metal that may be present. Therefore all the Hg in the sample is usually reduced to its elemental state prior to analysis but this is not appropriate when information about individual species is required. In reduction most methods require pre-digestion of the sample prior to reduction. Hg is relatively volatile and hence easily lost during sample preparation and analysis. In spite of these complications several methods have been developed for determining trace levels of Hg in biological samples (ASTDR, 1999).

Mercury is unique among the metals in that it has very high vapour pressure at relatively low temperature and can be introduced quantitatively to the spectrometer as a vapour without difficulty. Absorption at 253.7nm in the UV region has been measured with the use of Hg vapour lamps as well as hollow cathode lamps as the light source (Morita *et al.*, 1998). The way of liberating Hg from the aqueous or digested samples is reduction followed by volatilization and introduction of the Hg vapour with the aid of a gas

stream. Stannous chloride (Sn^{2+}) has been used as a reductant (Magos and Cernik, 1969; Lindstedt, 1970). Recently much work is done with sodium borohydride ($NaBH_4$) as the reductant. Table 1 shows some of the methods used to determine total mercury in hair. Many analytical chemists are publishing new analytical methodologies and techniques for environmental and biological samples. To validate these analytical methods, and to link to those methods to more established methods, accuracy examination is required by the use of appropriate certified reference material. The increased awareness and concern for public health has lead to much demand for routine methods that are fast and economic. These methods require validation and quality control (Morita *et al.*, 1998).

The hair samples need to be solubilized before analysis for total mercury. Some of the commonly used acids are concentrated ultrapure nitric acid, sulfuric acid, perchloric acid and hydrochloric acid. Some of the common oxidising agents used is hydrogen peroxide, hydroxylamine hydrochloride, and potassium permanganate and bromine monochloride. One or more of the above acids and oxidising agents can be used to digest the hair.

In this study a combination of concentrated analytical grade HCl, HNO_3 and H_2SO_4 were used to digest the hair samples (Louie *et al.*, 1985; Louie, 1983). An open hot water bath digestion was carried out at temperatures between 85-100°C and the samples were refluxed for 60-90 minutes. There is formation of stable $HgCl_4^{2-}$ ions in the HCl-HNO_3-H_2SO_4 mixture. This species is more stable to reduction to elemental Hg than is the Hg^{2+} predominating in mercury (II) salt solution prepared in acids in the absence

of Cl^- ions. To ensure good recovery of Hg, it is therefore preferable to add HCl to the sample before addition of HNO_3 and H_2SO_4. This ensures that Hg in samples is in the presence of an excess of Cl^- ions at all times (Louie *et al.*, 1985). Concentrated bromine monochloride was used in this study as an oxidising agent (Bloom, 1992; USEPA, 2001b,c).

Table 1: Determination of total mercury in hair methodology used by other researchers (continued on next page)

Hair digestion method for total Hg	Analytical Method	Method Detection Limit	Percentage recovery (%)	Reference
Addition of 1ml of 30% H_2SO_4 in HNO_3 to 5mg hair in vial, sealed and oven digested at 90°C for 6-8 hrs, cooled and 2ml of deionised water added. To 2ml of aliquot 1ml HCl and 1ml of bromide/bromate solution added in a 25ml volumetric flask. Mixture allowed to stand overnight at room temperature to convert all ionic forms of Hg to Hg^{2+}. Hydroxylamine hydrochloride added to decolourise the solution and made to volume with deionised water.	Cold vapour Atomic Fluorescence Spectrophotometer	12 ± 5 ppb	100 ± 3	Pellizari *et al.*, 1999
No sample digestion. Thermal decomposition and amalgamation and measured at 253.7 nm.	Direct Hg Analyzer 80	No data	90 - 110	Oken *et al.*, 2005
Alkaline digestion. ~20mg hair treated with 1% L-cysteine, 45% NaOH and 1% NaCl and heated at 90-95 °C for 20 minutes for complete solubilisation.	Cold vapour Atomic Fluorescence Spectrophotometer	LOD in Hair 0.004-0.05 μg/g	No Data	Bjornberg *et al.*, 2003

Hair digestion method for total Hg	Analytical Method	Method Detection Limit	Percentage recovery (%)	Reference
No sample digestion. Oxygen combustion-gold amalgamation method.	Atomic Absorption Spectroscopy using Hg detector	No data	No data	Yasutake *et al.*, 2004
Hair sample dissolved in high purity HNO_3 at 80°C till the solution was clear using standard microwave digestion procedure.	ICP-MS	No data	No data	Dickman *et al.*, 1999
Hair dissolved in ultrapure trace-metal grade HNO_3 and digested at 100°C for 15 minutes in temperature controlled heating block in acid digestion fumehood.	Flow Injection Hg system Atomic Absorption Spectroscopy	No data	No data	Ip *et al.*, 2004
No sample digestion.	Gold amalgamation Cold Vapour AAS	No data	97.7 – 101.4	Feng *et al.*, 1998
Hair digested in ultrapure analytical grade HNO_3, (2ml) H_2SO_4 (2.5ml) and 15ml $KMNO_4$ and 8ml potassium persulfate in a microwave at 95°C for 1 hour.	Cold Vapour HG-4 instrument	0.02 µg/g	No data	Stern *et al.*, 2001

Hair digestion method for total Hg	Analytical Method	Method Detection Limit	Percentage recovery (%)	Reference
~0.05-0.1 g hair samples decomposed with 2.5ml HNO_3 under pressure (300kPa) in teflon vessels at 130°C. Determination by direct reduction to Hg^0 with an aqueous solution of 10% $SnCl_2$, 6% NH_2OH, 6% NaCl and 1N H_2SO_4.	Atomic Absorption Spectrophotometry (Perkin Elmer 2280)	0.01 µg/g	No data	Gaggi *et al.*, 1996
Hair digested using 30:70 mixture of H_2SO_4 and HNO_3.	Cold vapour Atomic Fluorescence Spectroscopy	Range from 0.0006 – 0.06 µg/g	96.2	McDowell *et al.*, 2004
Hair digested with 1ml 8.3mM cysteine, 2ml of 11.3M NaOH, heated at 90°C for 15 minutes. Digests cooled in ice bath and diluted with 7ml 171mM NaCl and weight of digest noted. 1ml of digest reacted with 1ml 8.3 mM cysteine, 20ml 171mM NaCl, 10ml 8M H_2SO_4, 1ml $SnCl_2.2H_2O/CdCl_2$ and 20ml 13.3 M NaOH to produce vapour that was passed through a water solution of tri-n-butyl-phosphate in ice.	Cold Vapour Atomic Absorption Spectrometry	No data	No data	Barbosa *et al.*, 1998

Hair digestion method for total Hg	Analytical Method	Method Detection Limit	Percentage recovery (%)	Reference
200mg hair digested for 48 hours in HNO_3 at room temperature. An aliquot was introduced into an alkaline reduction solution ($SnCl_2$ + $CdCl_2$) for determination	Cold Vapour Atomic Absorption Spectrometry	No data	No data	Frery *et al.*, 2001
20mg hair acid digested with 0.5ml of HNO_3, 0.5ml of $HClO_4$ and 2ml H_2SO_4 at 200°C for 30 minutes. Reduction to Hg vapour by adding 10% of $SnCl_2$.	Cold Vapour Atomic Absorption Spectrometry	No data	No data	Nakai *et al.*, 2004
25mg of hair sample digested with HNO_3 (0.4ml) at 90°C for 10 minutes in a 7ml teflon microreaction vessel. The pH of the acidic hair mixture adjusted to 5.0-6.0 with NaOH and then passed through a clean-up Sep-Pak C18 cartridge. DMPS and sodium acetate buffer (pH=6) were added to the eluate to form a Hg-DMPS complex. This complex was preconcentrated on two Sea-Pak C18 cartridges and each was eluted with methanol and adjusted to 2ml. 50µL was introduced into graphite cuvette and then atomized according to a temperature program.	Graphite Furnace Atomic Absorption Spectrophotometry	0.064 µg/g	95.8 – 98.2%	Chen *et al.*, 2002

Hair digestion method for total Hg	Analytical Method	Method Detection Limit	Percentage recovery (%)	Reference
Approximately 100-200 mg dry sample was irradiated simultaneously in a reactor with a thermal flux density of 1.10^{13} $n.cm^{-2}$ for 44 hours. After a cooling time of 1-3 days the samples irradiated together with the polyethylene bags were digested in the presence of 100 µg Hg carrier with 1 ml concentrated HNO_3 and 2.2ml of H_2SO_4 in sealed teflon bombs in an oven at 150°C for 3.5 hours. The digested samples diluted to 15ml and extracted with $CHCl_3$. The aqueous phase was extracted twice with 10 ml of Zinc-diethyldithiocarbamate, Zn-DDC.	Neutron Activation Analysis	0.0036 µg	No data	Zhuang *et al.*, 1989.

CHAPTER 3.0

RESEARCH METHODOLOGY

3.1 Sample Collection and Preservation (Fish and other Seafoods)

The glassware was reserved for mercury analysis. All the plastic and glassware were soaked in 10% HNO_3 bath for at least 24 hours. After acid cleaning the plastic and glassware were rinsed five times with distilled water and dried in the laminar flow clean cabinet. Using acid-cleaned stainless steel knife, portions of fish muscle tissue were taken from each fish (the inner portion of the flesh) and minced to about 1-2mm pieces (this was done using an acid-cleaned stainless steel knife and clean-gloved hands to avoid contamination). The minced tissues were stored in clean plastic bags, labeled, sealed and frozen for analysis.

Two hundred samples of different seafoods (fish, canned fish, and shellfish) were collected and analyzed for total mercury content. Table 2 shows both the scientific (*genus-species*) and local names of fish species analysed in this study. The samples were collected from various sources as outlined below:

- Albacore and Yellowfin Tuna: Samples of albacore and yellowfin tuna were supplied by a commercial fishing company, the Fijifish Marketing Group. They also supplied general location data for where the fish were caught, and length and weight data for the whole fish. Samples were analysed separately.

- Freshwater mussels (local name: *Kai*) and estuarine/seawater shellfish (local name: *Kaikoso*): Three heaps of each type was bought from the Suva market with location of catch for each noted. Seawater was collected from the USP jetty to soak the shellfish samples overnight, while the freshwater mussels were soaked in normal tap water, to allow the samples to expel most of the sand and other materials ingested during feeding. This is a normal practice carried out before consuming these seafoods. Ten average sized samples were randomly selected from each heap, forced open, contents removed and homogenized in a wet mill. The homogenized samples from different locations were transferred into clean plastic bags and frozen for analysis. Each homogenate was subsequently analysed for mercury.

- Fish steaks: Steaks of various fish species (marlin, swordfish, sailfish, sunfish, walu, shark, wahoo, mahi mahi, skipjack and kalia-blacksnapper) were bought from the local fish shops. The steak diameter was measured across the vertebral column and the samples were packed in clean plastic bags and frozen. Each steak was analysed separately.

- Reef fish (kaikai): A large bundle of reef fish was purchased from the Suva market. Five fish were randomly selected from the bundle, length and weight recorded, packed in clean plastic bags and frozen. Each fish was analysed separately.

- Canned Tuna and Mackerel: Three brands of canned albacore tuna, two brands of canned skipjack tuna and four brands of canned mackerel were bought from local supermarkets. Each can was opened, the contents emptied in a clean plastic bag, mashed and frozen until analysis. The contents of each can were analysed separately.

Table 2: Scientific and local names (where available) of fish and shellfish species analysed in this study

	Scientific Name	Local Name
Albacore Tuna	*Thunnus alalunga*	
Yellowfin Tuna	*Thunnus albacares*	
Skipjack Tuna	*Katsuwanas pelamis*	
Bigeye tuna	*Thunnus obesus*	
Spanish Mackerel	*Scomberomorus commerson*	Walu
Striped marlin [1]	*Tetrapturus audax*	
Blue marlin [1]	*Makaira mazara*	
Barracuda.	*Sphyraena sp*	Oqo
Swordfish	*Xiphias gladius*	
Sailfish	*Istiophorus platypterus*	
Opah	*Lamprius regius*	
Sunfish	*Mola mola*	
Mahi Mahi	*Coryphaena sp.*	Maimai
Black snapper	*Macolor niger*	
Reef fish	-	Kaikai
Goatfish	*Parupeneus barberinus*	Mataroko
Parrot Fish	*Scarus sp.*	Ulavi
Rabbit fish	*Siganus puntatus*	Nuqa
Peacock cod	*Cephalopholis argus*	Kawakawa
Unicornfish	*Naso unicornis*	Ta
Shellfish	*Anadara antiquata*	Kaikoso
Freshwater mussels	*Batissa violacea*	Kai

[1] The exact species of marlin supplied by the fish company was unknown so the two species they catch are listed in this table

3.2 Reagent Preparation

3.2.1 Only analytical grade reagents were used, unless otherwise stated.

3.2.2 Hydrochloric acid (3% v/v) carrier solution was prepared by adding 30 ml of concentrated HCl (10M) to approximately 500 ml distilled water in a 1L volumetric flask and made up to the mark with distilled water.

3.2.3 Sodium borohydride ($NaBH_4$) reducing agent solution (0.02% $NaBH_4$ (w/v) in 0.005% NaOH) was prepared by dissolving $NaBH_4$ (1g) and NaOH (0.25g) in a 500 ml volumetric flask and made to the mark with distilled water. This reducing solution was subjected to a 10-fold dilution. Foaming may occur when certain samples high in protein are mixed with the borohydride reagent. Adding a silicone antifoaming agent (1 drop in the 10-fold diluted reductant solution) when necessary can control the foaming. This solution was prepared freshly.

3.2.4 Bromine monochloride (BrCl) was prepared by dissolving reagent grade potassium bromide (KBr) (5.4g) in 500 ml of concentrated ultra trace HCl or less depending on the amount required for the analysis. The solution was stirred for an hour in the fumehood after which reagent grade potassium bromate ($KBrO_3$) (7.6g) was added to the solution while stirring.

(Caution: This needed to be done slowly and in the fume hood because large quantities of free halogens were produced). As $KBrO_3$ was added to the

solution, the colour changed from yellow to red to orange. The bottle was capped loosely and allowed to stir for an additional hour. The Bromine monochloride solution was prepared on a daily basis.

To reduce the Hg content of the reagents, both the KBr and the $KBrO_3$ was muffled overnight at 250°C. The reagents were out of the muffle furnace while still hot and KBr was dissolved in HCl as soon as possible.

3.2.5 Mercury Stock (1,000 mg/L): Merck commercial "SPECTROSOL" Hg stock solution (1,000 mg/L) was used.

3.2.5.1 Mercury Working Stock Solutions (10 mg/L and 200 µg/L)

10 mg/L Hg: Using a calibrated automatic pipette 1 ml of the mercury stock solution was added to about 50 ml distilled water in a 100 ml flask. Approximately 10 ml of HCl was added and the solution was made to the mark with distilled. This solution was stable for 1 week when kept tightly stoppered in the refrigerator.

200 µg/L Hg: Using a calibrated automatic pipette 2 ml of the 10 mg/L working stock solution was added to about 50 ml distilled water in a 100 ml flask. Added 3 ml of HCl and made to the mark with distilled water. This solution was prepared daily.

Mercury Standards (0, 2, 4, 8, 20 µg/L): To separate 100 ml volumetric flasks 0, 1, 2, 4 and 10 ml respectively of the 200 µg/L Hg working stock solution was added using a calibrated auto-pipette. Then 3 ml of concentrated HCl was added to each flask and made up to the mark with distilled water. These standards contained 0, 2, 4, 8 and 20 µg/L Hg respectively. The 0 µg/L was used as the standard blank. These solutions were kept tightly stoppered at all times and prepared fresh daily.

3.3 Safety Procedures

In this study the researcher was exposed to very low concentrations of mercury in the laboratory during sample analysis. The Institute of Applied Science laboratory is OHS complied and strict safety procedures were adhered to in the laboratory during the analysis to minimise mercury exposure to the analyst. A long sleeve lab coat was worn on top of normal clothing. Safety specs, shoes, handgloves and facemasks were worn during sample preparation and analysis. The laboratory has well equipped fumehoods and Laminar flow cabinets where all the sample digestion and preparation was carried out. The instrument used for analysis (flow injection CVAAS) had well equipped vent system above the burner to prevent any gaseous mercury vapour from escaping.

3.4 Instrumentation

The digested hair samples were analysed for total mercury using the flow injection cold vapour atomic absorption spectrometry technique. All the total Hg measurements were performed with Perkin Elmer Model 3100 Atomic Absorption Spectrophotometer and Perkin Elmer Flow Injection Analysis System (FIAS) 100 unit equipped with Perkin Elmer AS90 Autosampler (see picture attached in Appendix 3.0a). The cold vapour technique first involves the reaction of the Hg^{2+} ions in the digest solution with a reducing agent, $NaBH_4$. This reduction reaction generates a volatile Hg vapour, which is collected in the gas-liquid separator and is transported to the quartz cell by means of an argon carrier gas. In the quartz cell the volatile Hg vapour is converted to gaseous Hg atoms (Hg^0). A light beam is directed through the quartz cell into a monochromator and onto a detector that measures the amount of light absorbed. Peak height measurement is normally the preferred quantification procedure for flow injection Hg analysis. Peak height measurements can be performed in less time than peak area that provides no analytical advantages (Perkin Elmer, 1992).

Table 3: Instrument parameters

Integration time	20seconds
Data Processing	Peak Height
Lamp	Hallow cathode Hg Lamp
Slit	0.7 nm
Wavelength	253.7 nm
Cell Temperature	100 °C
Volume of sample	500 µL

3.4.1 Sample digestion Procedure– FISH

Fish samples were digested by the following hydrochloric-nitric-sulphuric acid digestion procedure. Digestion blanks consisting of just the digestion acids were run alongside the samples. Approximately 2g of the fish tissue were weighed into 50-mL acid-cleaned boiling tubes (Note: for certain large predatory fish species such as swordfish contained high levels of mercury so less tissue was used in these instances to avoid having to dilute the sample during analysis). For the CRM (i.e. BCR CRM 464) 0.2g of CRM was all weighed into separate boiling tubes.

To each boiling tube 1ml of hydrochloric acid, 5ml of nitric acid and 2.5mL of sulphuric acid, were added and capped with an acid-cleaned glass marble and left for an hour at ambient temperature. The samples were heated in a boiling water bath (temperature between 85-<100°C) in the fume hood. Refluxing was continued for 2 hours until the digestion was complete. This was generally indicated when the digest was light in colour or did not change in appearance with continued refluxing. The samples were cooled and filtered (using filter paper # 54, width 9cm) into an acid-cleaned 100 ml volumetric flask. The boiling tubes were rinsed a few times with distilled water and the rinsings were added into the volumetric flask. BrCl (5ml) was added per 100ml sample and made up to the mark with distilled water.

3.5 Sampling Design for Hair Sampling and Analysis

3.5.1 Study Location

Due to the nature of the research, convenience sampling was carried out. Figure 1 shows the map of Fiji Islands indicating the study sites.

3.5.1.1 Kalekana Settlement, Lami

This is a coastal Fijian settlement near Fiji Fish and Marketing Group outlet in Lami. This is a low income generating area and there are greater chances of these people buying the fish sold by the Fiji Fish outlet as by-catches in cheap prices. Most men in this settlement work for the company and get free or discounts for the fish. An assumption is also that they may also be practicing traditional fishing for the household consumption.

3.5.1.2 Muaivuso Village, Lami

This is a coastal Fijian Village in Lami and the villagers have a common fishing ground. This village was chosen because fish and other seafood have been a major source of protein in their diet in the past and it may still be now. People in this village heavily depend on reef fish and canned tuna and mackerel.

3.5.1.3 Dravuni Village and Daku Village, Kadavu

These are two island coastal Fijian Villages in Kadavu. These outer island villages were chosen because fish and other seafood have been a major source of protein in their diet in the past and it may still be now. The USP research station is located on Dravuni Island so access was regular. This also means that Dravuni will have greater access to town bought goods compared to Daku.

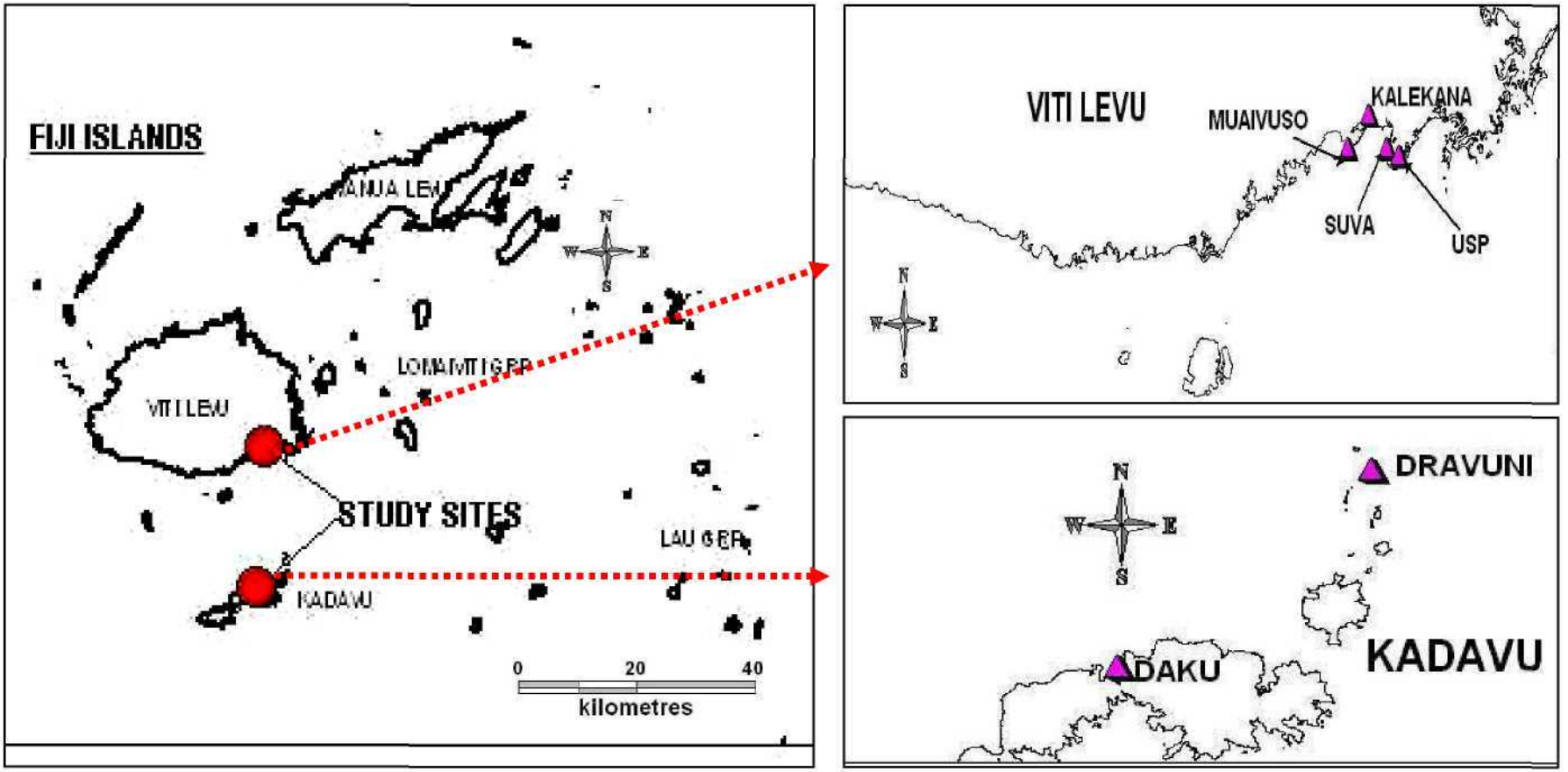
3.5.1.4 Control Group

It was very difficult to identify vegetarians in a Fijian community as a group. Therefore Indian vegetarians were included as control group in order to determine the background levels of mercury in human hair. Indian vegetarian Students and staff around the USP Campus were included in this project.

3.5.2 Population Size

In all of the villages adult males (15 years and above), females (15 years and above) and children (2-<15 years of age) were considered during the sampling. From each village the individuals who self volunteered to be part of this project were included. Therefore the total number of participants in this entire project was 92.

Figure 1: Map of Fiji Islands showing the study sites



3.5.2.1 Kalekana Settlement, Lami

A total of 22 hair samples were collected from this settlement, adult males (n=5, ages 19-71yrs), adult females (n=12, ages 15-57yrs) and children (n=5, ages 5-12 yrs).

3.5.2.2 Druvuni Village, Kadavu

A total of 21 hair samples were collected from this village, adult males (n=10, ages 24-69yrs), adult females (n=10, ages 19-45yrs) and children (n=1, age 8yrs).

3.5.2.3 Daku Village, Kadavu

A total of 7 hair samples were collected from females aged between 19-52yrs.

3.5.2.4 Muaivuso Village, Lami

A total of 22 hair samples were collected from Muaivuso village, adult males (n=3, ages 39-71yrs), adult females (n=19, ages 19-88yrs).

3.5.2.4 USP Staff and students

A total of 10 hair samples were collected from individuals irrespective of their villages. Individuals (adult males and females) with both Indian and

Fijian backgrounds were part of this group. This included 2 adult males (ages 32-46yrs) and 8 adult females (ages 24-46yrs).

3.5.2.5 Control Group

A total of 10 vegetarian Indian adult males and females from around the USP campus volunteered to be part of this group. Of this there were 3 males (ages 21-30yrs) and 7 females (ages 23-51yrs).

3.5.3 Inclusion/Exclusion Criteria

Participants who were able to give full consent were the only ones included in this study. Children less than two years of age were excluded. Individuals with very short hair (<2 cm in length) were excluded (except for where the legal guardian gave consent and insisted of their child's participation) since it made sampling difficult and chances that not enough hair could collected for analytical requirement.

3.5.4 Sampling Design

This study was approved by the National Health Review Committee (NHRC, Fiji Ministry of Health), Fiji National Research Ethics Review Committee

(FNRERC, Fiji School of Medicine) and the University of the South Pacific (USP), School of Pure and Applied Science (SPAS) Postgraduate Committee.

3.5.4.1 Participant Consent

The village elders (the village chief - Turaga-ni-koro) at the respective study sites were approached in a traditional manner (with the help of an interpreter) to obtain their approval to carry out this study in their village/settlement. On this initial visit the details of this study was explained to the respective Turaga-ni-koro. Copies of the consent form and food frequency questionnaire that was translated into Fijian language were handed to each village chief. The participants were given enough time (at least a week) to decide on their participation. After this visit the village chief approached their villagers at a village gathering and described the nature of this study to them. The villagers were given the choice of volunteering for their participation due to the nature of the study. Once the villagers had agreed, the chief of each village gave their approval for this study to be carried out in their villages.

On the second visit the volunteering participants gathered at their common village halls. The details of the study were again described to the volunteering participants with the help of an interpreter. Those participants who agreed to participate in this study were asked to fill and sign a written Consent Form (attached in Appendix 1).

The Consent Form, which had been translated into Fijian were filled by each participant to be fully informed of his/her participation in this study and provide full consent to the undertakings of this study. For those participants who were under 21 years of age the legal guardians were chosen to give full consent. Each participant was assured of confidentiality of the information that was gathered from him or her and the results of their hair analysis for total mercury.

3.5.4.2 Dietary Assessments

Also on the second visit to the respective villagers, the fish consumption patterns of the participants were gathered through a Food Frequency Questionnaire that had been translated into Fijian language (see Appendix 2). The interview was conducted by the researcher with the help of an interpreter on face to face basis with each adult participant and with an adult family member present with children. One food Frequency Questionnaire was administered per each participant.

3.5.4.2.1 Food Frequency Questionnaire (FFQ)

Frequency of fish consumption pattern was gathered from each participant. This questionnaire included details of the frequency of fish meals (one meal/day, one meal/week, ≥2 meals/day, ≥2 meals/week, 1-3 meals/month or rarely). The different seafood

categories included were large deep-sea predatory fish (swordfish, shark, marlin, sunfish, sailfish, walu), fresh tuna (albacore, yellowfin, skipjack, bigeye), reef fish, canned tuna and mackerel, shellfish. The origin of the seafood (the source of their fish and seafood supply e.g. from fish shops, Fiji Fish outlet, bought from fishermen, personally fished by a household member) was also being noted. The questionnaire also included information such as the age at which the children started consuming fish meals and whether fish and other seafood has been one of the major food sources for them. Following the interview, each participant's body weight was been noted.

Average total number of fish meals per week was evaluated from the FFQs. For categories "≥2 meals/day and ≥2 meals/week" were taken as minimum of two and a maximum of 3 meals per day and per week. Therefore the average frequency ranged from a minimum to maximum number of meals consumed per week (e.g. 3-6 fish meals/week).

3.5.5 Hair Collection

Participant ID, name, age and sex of donor, location and date of sampling were recorded on the label pasted on a transparent acid cleaned polyethylene plastic sampling bag. Hair samples were taken from the occipital area (from the back of the head and as close as possible (3-5mm) from the scalp) using

gloved hands and blunt-tip stainless steel scissors to avoid accidents. It was ensured that the acquiring of the hair samples would not have any significant cosmetic effect on the participants. After each hair sample was obtained, the scissors was wiped with tissue paper. The hair was cut in an amount equivalent to about 0.5-1.0 gram (~150-200 strands if the hair is ~10cm long). At the same time it was noted that the length and the hair texture would affect the weight of the hair so it was ensured that the amount of hair collected would be sufficient enough for the analytical method employed. The hair was tied with a thread with proximal ends on the same side and transferred into the bag and stapled (see pictures attached in Appendix 3.0 b-e). Samples collected from each location was packaged together and brought to the Institute of Applied Science analytical chemistry laboratory, USP.

3.5.6 Hair Pretreatment and Preparation

Adhering dust and grease can be removed from the hair by one of the following solvents: hexane, alcohol, water, acetone, diethylether or detergents. However, the use of only water and acetone recommended by both the International Atomic Energy Agency (IAEA) and WHO was followed (Horvat, 1995).

The hair samples of each participant were placed in an acid cleaned beaker and washed successively with acetone-water-water-acetone. Each time the sample was completely covered with the solvent and stirred at frequent intervals for 10 minutes. The hair was left to dry in a clean laminar flow

cabinet after final acetone wash. Once dry, the length of the hair sample to be used was measured using a ruler and the colour of hair noted. The measured length of the hair samples were cut using an acid cleaned scissors into segments as fine and short as possible (~1mm) on a clean white sheet of paper. By folding the paper the hair samples were transferred into acid cleaned glass vials having appropriate identification labels and stored in dry dark cabinet at room temperature. Long term storage of hair has shown that mercury is stable for a period of few years if stored dry and in darkness at room temperature (Horvat, 1995).

3.5.7 Digestion of Hair Samples

Reagent blanks consisting of only the digestion acids were carried through the sample preparation procedure along with each batch of samples analysed. The hair samples (approximately 100mg) were weighed into a 75ml boiling tubes in duplicates. To each sample were added 1ml of 10M HCl, 5ml of 15M HNO_3 followed by 2.5ml of 18M H_2SO_4 and covered with an acid-cleaned glass marble. The samples were left overnight to acid digest at ambient temperature. The samples were heated in a boiling hot water bath (between 85-<100°C) and the refluxing was continued for 60 to 90 minutes (see pictures in Appendix 3.0 f-g). The samples were cooled and filtered into acid-cleaned 50ml volumetric flasks. Concentrated BrCl (2.5ml) was added per 50ml sample and the solution made up to the final volume using distilled water. The samples were allowed to sit overnight at room temperature to convert all forms of ionic Hg to Hg^{2+}.

3.6 Quality Control (QC)

3.6.1 The samples were analysed in duplicates, i.e. two separate portions of the samples were weighed in separate boiling tubes and carried through the entire digestion and preparation steps and analysed for total Hg separately. The duplicate determinations agreeing within 10% of their average was accepted as satisfactory.

3.6.2 Duplicate blanks consisting of only the digestion acids were included with each batch of determinations. Analysis of blanks provided information on the concentration contributed by the reagents that are used in the sample digestion and preparation steps.

3.6.3 Certified Reference Material (CRM) was run alongside the samples in duplicates to verify the accuracy of the analysis.

NOTE

With every batch of CRM analysis the correction to dry weight of the Hg CRM was made from a moisture determination on a separate portion of the CRM. Accurately weighed approximately 0.1g of CRM (*initial mass*) into a metal dish and dried in an oven at ~105°C for 3-4 hours, cooled in a desiccator and reweighed. The drying procedure was repeated until a constant dried CRM weight was obtained (*final mass*). The Hg content measured in the CRM was then corrected to a dry weight by multiplying the concentration by the mass correction factor.

A BCR CRM No. 464 was used for total Hg determination in the fish tissues (certified at 5.24 ± 0.10 μg/g) and a BCR CRM No. 397 (certified at 12.3 ± 0.5 μg/g) was used for total Hg determination in human hair.

3.6.4 Spike recovery measurements were performed on selected samples in every batch of analysis.

The fish tissue samples were spiked with a known amount of Hg using a known concentration of Hg solution and the level of Hg spikes were between 0.2-0.8 μg.

The human hair samples were spiked with the BCR CRM No. 397. A known mass (~25-30 mg) were added to the samples and carried through the entire digestion and preparation procedures. The level of spikes for human hair ranged from 0.308-0.369 μg Hg.

3.6.5 Method Detection Limit (MDL): A sample with the lowest level of total Hg was chosen to determine the MDL of the method employed. In this case a composite was prepared (n=10) from control samples obtained from the vegetarian participants. Seven replicates of this composite sample were spiked with BCR CRM No. 397 and seven replicates were unspiked. These samples were carried through the employed procedure and analysed for total Hg. The mean and standard deviation (SD) were calculated for the seven

unspiked samples. Method detection limit was determined by multiplying the SD of the composite control sample by a factor of 3.14 in µg/g.

3.6.6 Analytical Method Performance

The sample digestion methods were validated prior to any sample analysis. The accuracy and precision of the method were checked with the BCR CRM No. 464 for the fish tissues and BCR CRM No. 397 for the human hair matrix. The samples were carried through the entire digestion procedure and analysed to demonstrate method performance on separate days. The spike recovery measurements were performed by spiking a known amount of Hg with known Hg concentration at levels in the calibration range and treated through the whole procedure. The samples were analysed with CVAAS technique. The instrument was calibrated with aqueous Hg standards. Instrument performance was monitored by running QC checks. This included running a calibration standard blank and an 8-ppb calibration standard after analysis of every 10 samples (refer to the Quality Control Chart in Appendix 3.1). Also the instrument performed re-calibration of the calibration curve using the standard blank and the highest calibration standard (20ppb) after every 10 samples.

3.6.7 Instrument Calibration

The quantification of total Hg using CVAAS was carried out using batch specific standard calibration curves. Five aqueous calibration standards

(0,2,4,8,20µg/L) were used for calibration. Calibration blank and calibration standards ere analysed for Hg from the lowest concentration to the highest. The linearity of the calibration curve was confirmed to be greater than 0.99 for acceptability.

3.6.8 Analysis of Samples

Prior to any sample analysis the above set of calibration standards were used to construct a calibration curve. The calibration blank and the 8ppb calibration standard served as QC check standards. Samples were digested and analysed in batches consisting of 5-7 samples. The order of analysis was sample blanks, certified CRM, and samples. At every 10^{th} sample the QC check standards were analysed with re-calibration of the standard curve after each 10 runs. For each duplicate sample prepared two replicate readings was taken and averaged for each duplicate to obtain the Hg concentration in that particular duplicate. All the duplicates had to meet the ±10% for acceptance. The BCR CRM No. 464 (5.24 ± 0.10 µg/g, range 5.04-5.44µg/g) and BCR CRM 397 (12.3 ± 0.5 µg/g, range 11.3-13.3 µg/g) had to agree within 2 SD of their certified mean for acceptance. The percentage recovery had to fall between 90-110% for acceptability.

3.6.9 Calculations

The following formulae were used for the calculation of total Hg and % recovery in the fish tissues and the hair samples.

Hg concentrations are read directly off the instrument printout in µg/L and fish tissue concentrations are calculated by the following equation:

$$[Hg]\ (mg/kg) = \frac{\text{Concentration Measured (µg/L) x Extract Volume (0.1 L) x DF}}{\text{Sample weight (g)}}$$

Note: DF is the Dilution Factor (usually 1). Samples concentrations were corrected for the average digestion blank concentration if this was above the method detection limit.

SRM mercury concentration

For the SRM the wet weight concentration (mg/kg) was obtained using the equation above and corrected to dry weight (mg/kg) by the following equation:

$$\text{Corrected [SRM Hg] (mg/kg)} = \text{Mass Correction Factor x [SRM Hg from wet weight]}$$

where the Mass Correction Factor $= \frac{\text{Initial Mass of SRM}}{\text{Final Mass of SRM}}$

Spike recovery calculation

Spike recoveries were calculated by the following procedures:

$$\text{Calculated amount of Hg in sample before spiking (µg)} = \text{Average [Hg] measured in unspiked portions of the same tissue as that which was spiked (mg/kg)} \times \text{Mass of sample in the spiked sample (g)}$$

Measured Amount of Hg in spiked sample (µg)	=	Concentration Measured (µg/L) X Extract Volume (0.1 L) x DF

Hg Recovered from spiked sample (µg)	=	Measured amount of Hg in spiked sample (µg)	-	Calculated amount of Hg in sample before spiking (µg)

$$\% \text{ Recovery} = \frac{\text{Hg recovered from spiked sample (µg)} \times 100\%}{\text{Actual amount of Hg spiked (0.8µg)}}$$

3.6.10 Result Evaluation

The results for total mercury concentration in hair were statistically evaluated using the SPSS statistical software version 10.

The food frequency data and the total Hg levels in hair was used to calculate the PTWI of the participants and compared with WHO recommended PTWI. A portion of fish meal or serving size was taken as 150g for adults and 75g for children.

The results of total mercury in hair concentration would provide the human mercury body burden from the consumption of fish. The levels of total Hg concentration in hair would also reveal whether the participant is at any health risk.

3.6.11 Participant Benefits and Follow Up

The participants were given the feedback of their results using the Result Feedback Form (Appendix 4.0). A few Participants from Kalekana Settlement had high total hair Hg level above 10 μg/g. This level was recommended by WHO in 1990 and seems a reasonable compromise between the 3 μg/g level in hair recommended by the JECFA using safety factors for the 14 μg/g level assumed to affect the fetus. If 3 μg/g were used most participants would be considered "at risk", which did not seem a good risk communication strategy. The issue of balancing risk with benefits in mercury consumption is an ongoing debate and was the subject of a WHO workshop in January, 2006 which recommended that the upcoming JECFA meeting in mid-2006 reconsider the 3 μg/g hair Hg level.

A visit was made to this Settlement and these participants were explained their results and the possible cause for the high mercury level.

CHAPTER 4.0

RESULTS

4.1 Phase I: Mercury Levels in Fijian Seafoods

4.1.1 Method Performance for the Determination of Total Mercury in Fish Tissue

No significant mercury level was detected in the sample blanks indicating that the contamination from the laboratory was minimal. The coefficient of variance for a fish sample analysed ten times for total mercury was 4%. Several samples were analysed in duplicates and the results typically agreed within 10% of their average. A certified reference material, BCR CRM 464 (total and methylmercury in Tuna SRM) was analysed with every batch of samples. The concentration of total Hg determined was in the range of 5.07-5.38 μg/g. The certified total Hg concentration in the BCR CRM 464 was 5.24 ± 0.10 μg/g. Spiked recovery measurements were in the range of 91-103%. The method detection limit for determining total mercury in fish tissue was 0.02 μg/g based on 2g of the fish sample. In addition to this an 8μg/L Hg standard was used as a QC check standard which was run in between samples and gave [Hg] readings in the range of 7.57-8.73 μg/L with an average of 8.15 ± 0.29 μg/L.

4.1.2 Mercury Levels in Fijian Seafoods in Comparison to FAO/WHO Codex Alimentarius Guidelines

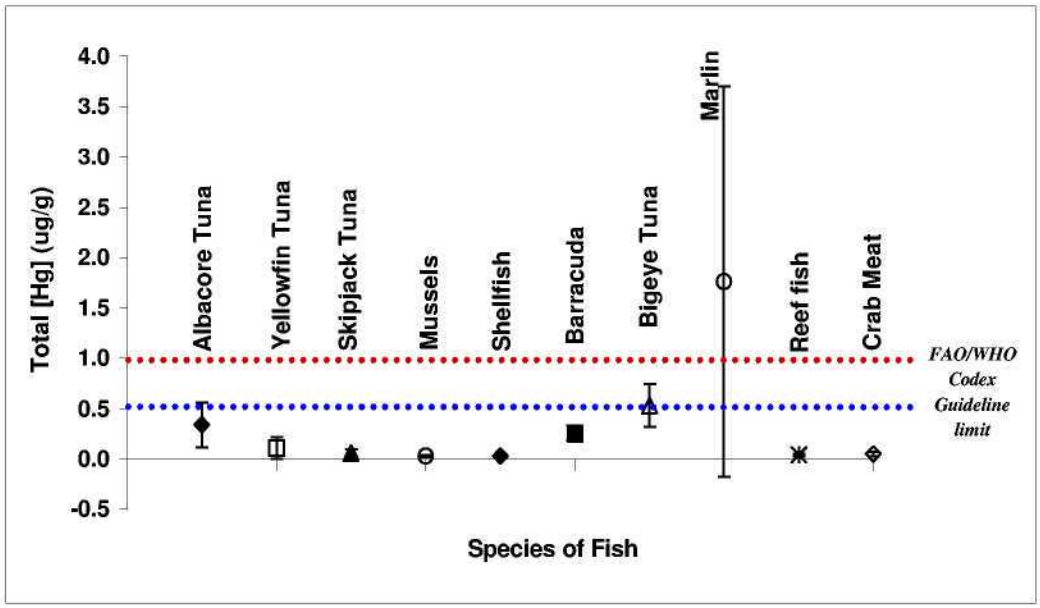
The summary of the results for the various seafood species analysed between 2003 to 2004 are shown in Tables 4-6 for fresh fish/shellfish species, canned fish and fish steaks respectively. Complete data set summary is attached in Appendix 5.0. All concentrations are reported on a wet weight basis. It should be noted that this study was an initial survey of mercury levels in some Fijian seafood samples, as little previous data were available. Due to the large number of seafood types analysed, the number of samples that were able to be analysed on certain seafood species was limited.

4.1.2.1 Mercury in Fresh Fish and Shellfish

The summary results for the fresh fish and shellfish species along with average length and weight data are shown in Table 4. The data are also shown graphically in Figure 2 in comparison to the FAO/WHO codex guideline limits (FAO/WHO, 2005). Marlin, bigeye tuna, and albacore tuna all recorded mercury levels on certain samples that exceeded the guideline limits. The marlin had a very high standard deviation in mercury concentrations. However, statistical tests showed that none of the *average* levels were statistically significantly above the 0.5 μg/g guideline limit (FAO/WHO, 2005). However, in the case of bigeye tuna and marlin more samples need to be analysed to obtain more representative data on mercury levels.

Table 4: Mercury levels (range and average) in different fresh fish and shellfish from the Fiji Islands, with length and weight data where available. (Note: n = number of samples, SD = standard deviation)

Seafood Sample	n	Average Length (cm)	Average Weight (kg)	Range [Hg] (µg/g)	Average [Hg] (µg/g) ± SD
Albacore Tuna	31	72.7	21.3	0.03 – 1.01	0.34 ± 0.22
Yellowfin Tuna	24	71.3	15.2	<0.02 – 0.40	0.11 ± 0.11
Skipjack Tuna	12	45.7	2.4	<0.02 – 0.16	0.06 ± 0.04
Bigeye Tuna	3	103.3	28.3	0.28 – 0.80	0.53 ± 0.21
Marlin	5	167.6	67.4	0.45 – 5.60	1.76 ± 1.94
Reef fish	5	17.2	0.09	<0.02 – 0.04	0.04 ± 0.01
Barracuda	4	61.3	1.32	0.18 – 0.38	0.26 ± 0.07
Mussels	3	-	-	<0.02 – 0.04	0.03 ± 0.01
Shellfish	3	-	-	<0.02 – 0.05	0.03 ± 0.01
Crab Meat	3	13.3	-	0.03 – 0.07	0.05 ± 0.02
Parrot fish (ulavi)	2	31-35	0.75	<0.02	<0.02
Wahoo	1	92	6	0.17	0.17
Goatfish (mataroko)	1	28	0.31	0.03	0.03
Rabbit fish (nuqa)	1	32	0.5	0.15	0.15
Peacock cod (kawakawa)	1	33	0.62	<0.02	<0.02
Unicorn fish (ta)	1	39	1.07	<0.02	<0.02
Opah	1	111	65	0.27	0.27



FAO/WHO Codex limit of 1µg/g for predatory fish

FAO/WHO Codex limit of 0.5 µg/g for non predatory fish

Figure 2: Average total mercury concentrations and SD (error bars) for different fresh fish and shellfish in comparison to FAO/WHO codex guidelines

The tuna data were also analysed to see if a significant correlation existed between total mercury levels and length and weight. Figure 3 shows the concentration of mercury in these two tuna species plotted against length and weight. There was a significant positive correlation between mercury levels with the length of yellowfin tuna ($p<0.05$ level of significance) but not for albacore. For total mercury versus fish weight there was no significant correlation noted for either yellowfin or albacore tuna. There was also a statistically significant difference between average mercury levels in the albacore and yellowfin tuna, with albacore having higher levels.

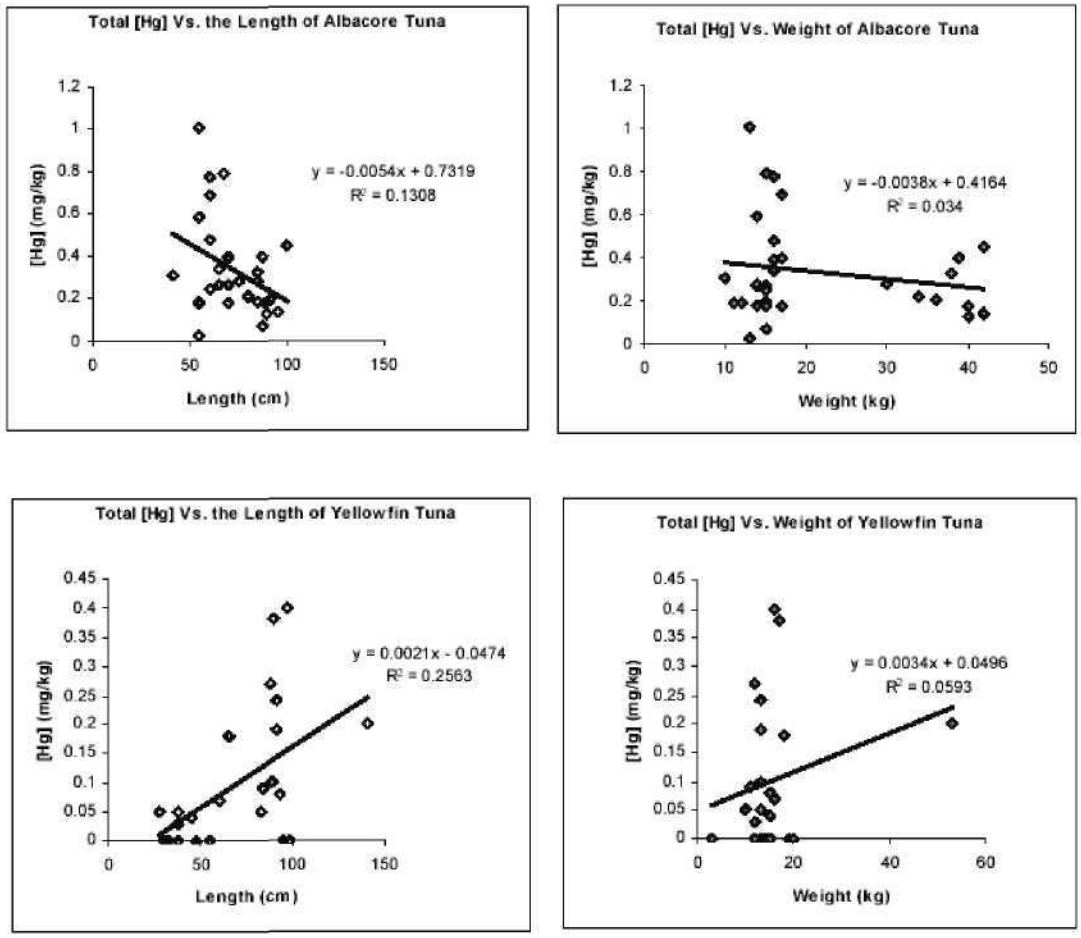


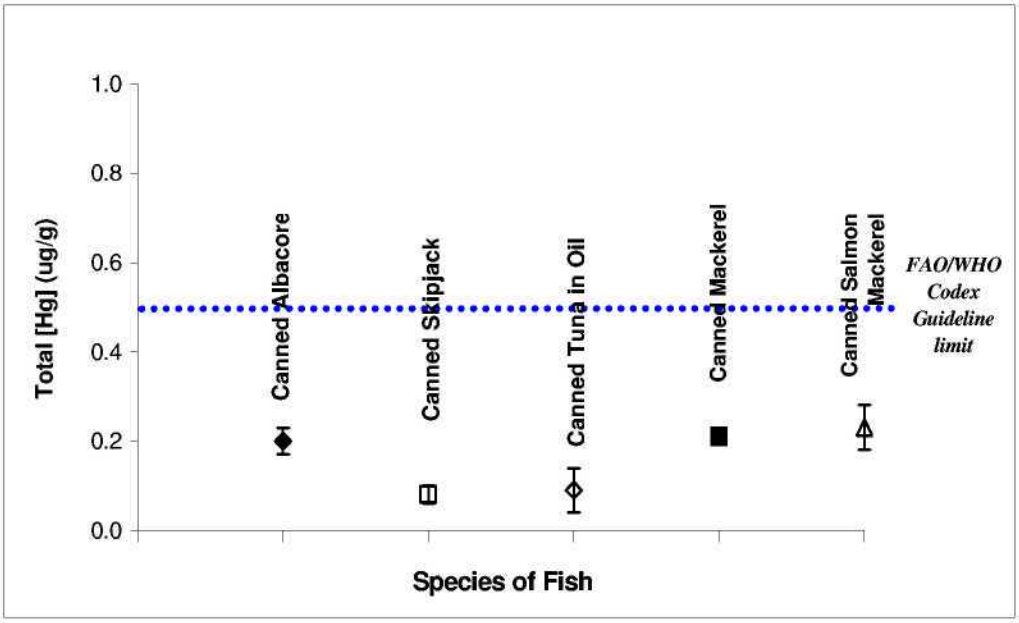
Figure 3: Mercury levels in albacore and yellowfin tuna versus fish weight and Length

4.1.2.2 Mercury Levels in Canned fish

The summary of the results for the various canned fish analysed is shown in Table 5 and Figure 4. Most of the canned fish had a reasonably similar mercury content of approximately 0.1-0.2 µg/g. None of the concentrations measured exceeded WHO/FAO guidelines.

Table 5: Mercury levels (range and average) in canned fish sold in the Fiji Islands. (Note: n = number of samples, SD = standard deviation)

Canned Fish Type	n	[Hg] range (µg/g)	[Hg] average (µg/g) ± SD
Canned Albacore	6	0.16 – 0.27	0.20 ± 0.03
Canned Skipjack	9	0.06 – 0.11	0.08 ± 0.02
Canned Tuna in oil	3	0.05 – 0.16	0.09 ± 0.05
Canned Mackerel	6	0.18 – 0.22	0.21 ± 0.01
Canned Salmon Style Mackerel	6	0.17 – 0.29	0.23 ± 0.05



FAO/WHO Codex limit of 0.5 µg/g for non predatory fish

Figure 4: Average total mercury concentrations and SD (error bars) for different canned fish in comparison to FAO/WHO codex guidelines

4.1.2.3 Mercury Levels in Fish Steaks

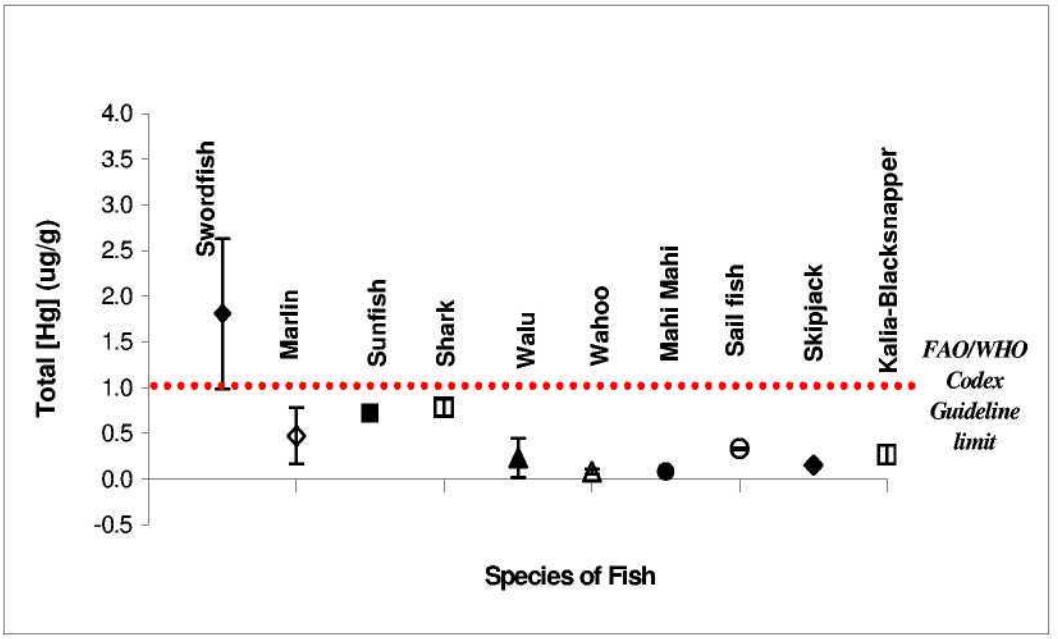
The summary of the results for the various fish steaks analysed is shown in Table 6 and Figure 5. Several of the fish species (swordfish, sunfish, shark and marlin) had average mercury concentrations, which exceeded FAO/WHO guidelines (0.5 μg/g). Statistical testing showed that the average levels of mercury in sunfish, swordfish and shark were significantly greater ($p<0.05$, denoted by '*' in table) than the recommended health guidelines. The highest average mercury concentration was noted in swordfish followed by shark, sunfish and marlin steaks.

Table 6: Mercury levels (range and average) in fish steaks from the Fiji Islands.

Steak type	n	Diameter of Steak (cm)	Range [Hg] (μg/g)	Average [Hg] (μg/g) ± SD
Marlin	19	19.1	<0.02 – 1.01	0.47 ± 0.31
Sunfish	5	23.6	0.67 – 0.78	0.72 ± 0.07 *
Walu	17	12.5	<0.02 – 0.87	0.23 ± 0.21
Swordfish	5	23.4	0.99 – 2.81	1.81 ± 0.82 *
Shark	7	14.1	0.57 – 0.85	0.78 ± 0.09 *
Wahoo	4	13.5	0.05 – 0.12	0.08 ± 0.03
Mahi Mahi	3	21.3	0.05 – 0.11	0.08 ± 0.02
Sail Fish	2	23.5	0.32 – 0.34	0.33 ± 0.01
Kalia-Blacksnapper	2	23.0	0.17 – 0.34	0.26 ± 0.09
Skipjack	5	14.6	0.11 – 0.19	0.15 ± 0.03

The relationship between the total mercury levels with the size of the fish steaks was also analysed. Although limited analyses were conducted, a significant positive

correlation in total mercury with steak size was found for swordfish and marlin ($p < 0.01$ level of significance) and to a lesser extent for walu ($p < 0.05$ significance level). No correlation was found with other steaks but in many cases there were not enough samples analysed to provide representative data.



FAO/WHO Codex limit of 1µg/g for predatory fish

Figure 5: Average total mercury concentrations and SD (error bars) for different fish steaks in comparison to FAO/WHO codex guidelines

4.2 Phase II: Mercury Levels in Human Hair and Fish Consumption Data

4.2.1 Analytical Method Performance

The method performance for total mercury measurement in hair was evaluated. The linear calibration curves were plotted using the mercury concentration versus the response as a measure of peak height for the mercury standards. An example of the linear calibration curve obtained during the analysis of hair sample is shown in Figure 6. The correlation coefficient, R^2, obtained was 0.9997 confirming the linearity of the calibration curves. During the sample analyses the calibration curve with a correlation coefficient of 0.99 or more was accepted for linearity.

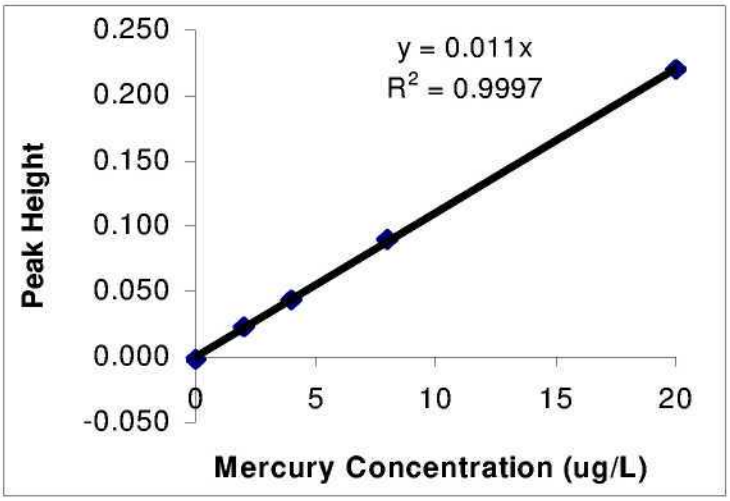


Figure 6: Instrument calibration curve for the mercury standards

The hair sample digestion procedure outlined in the methodology was validated prior to any sample analysis. A certified reference material, BCR CRM 397 was used for this purpose. Validation was performed in three separate trials on three separate days. Each trial included sample blanks, CRM and percentage recovery measures in triplicates. For spike recovery measurements a separate hair sample was used. A triplicate unspiked and a triplicate spike of this hair sample was prepared. To the spiked replicates a known mass of the CRM 397 was added resulting in a level of spike of 0.3075 μg Hg. The results of the three separate trails are summarised in Tables 7 and 8.

Table 7: The total [Hg] (μg/g) in the BCR CRM No. 397 obtained during the three trials of method validation

Trial	No of replicate	[Hg] in BCR CRM 397 (μg/g)
1	1	11.70
	2	11.80
	3	11.82
2	4	12.00
	5	12.26
	6	11.92
3	7	12.16
	8	12.15
	9	12.32
	10	12.03
	Mean ± SD	12.02 ± 0.21

Table 8: Percentage spike recovery data obtained during the trials of method validation

Trials	No. of replicates	% Recovery
1	1	91.0
	2	95.4
	3	105.6
	4	97.7
2	5	95.4
	6	98.9
	7	101.4
3	8	95.8
	9	96.6
	10	98.3
	Mean ± SD	97.6 ± 3.9

The average Hg concentration obtained in the BCR CRM 397 (n = 10) from the three separate trial was 12.02 ± 0.21 µg/g (range 11.70-12.32) which is 97.9% of the certified value of 12.30 ± 0.50 µg/g. The coefficient of variance between the ten replicates of the CRM was 1.75%. The % difference between the duplicates was less than 10%. The average spike recoveries obtained was 97.6 ± 3.9 % (range 91.0-105.6%). The coefficient of variance between the percentage recoveries (n = 10) was 4%. The % recoveries were within the acceptable range of 90-110%. Based on these results, the performance of the method was acceptable, thus the hair samples were analysed using the method described in the methodology.

4.2.2 *Quality Control (QC) Results for Sample Analysis*

The hair sample analyses for total mercury determination were performed over an 8-month period. The QC procedures outlined in the methodology were used to assess the method performance for each batch of analysis.

The method detection limit (MDL) was determined using a composite hair sample obtained from the Control Group participants (n = 10) who had never consumed meat or seafoods. Their hair mercury levels were considered to be the lowest and served as background Hg concentration in hair. Seven replicates of this composited hair sample were analysed separately together with spiked recoveries, sample blanks and hair CRM. The mean Hg concentration and SD in these seven replicates were 0.243 ± 0.005 μg/g. The MDL was determined to be 3.14 times the SD. Thus the MDL for determining total Hg in hair was 0.02 μg/g and sample weight of 100mg was sufficient to measure total Hg in hair using the method employed.

The CRM was included in duplicate in all the sample batch analysis. The mean total Hg concentration obtained for the BCR CRM No. 397 (n = 37) was 11.89 ± 0.29 μg/g (range 11.33-12.43). This value is within 96.7% of the certified mean of 12.30 ± 0.50 μg/g. All the values obtained for the CRM were within the two SD of the certified mean. The precision of the analysis was assessed from the analysis of the CRM on separate days. The coefficient of variance for the BCR CRM 397 (n = 37) was calculated to be 2.44%. The percentage differences between the duplicates were within 10% of their

mean. The % recovery measurements were performed in duplicates with each batch of analysis. The % recovery obtained ranged from 90.7-105.1% with an average of 95.2 ± 3.7% (n = 34).

A QC calibration standard, 8 µg/L, was used as a QC check sample analysed at every 10^{th} sample and at the end of each analysis. The Hg concentration obtained for this QC check sample was within ± 10% acceptance criteria. The Hg concentration of this 8 µg/L QC check sample obtained ranged from 7.62-8.59 µg/L with an average of 8.16 ± 0.29 µg/L.

The sample blank readings were very low indicating that there was no contamination contributed from the reagents and the laboratory environment.

4.2.3 Total Mercury Levels in Human Hair

A total of 92 participants took part in this study of which 89.1% were fish consumers and 10.9% were vegetarians (did not consume any seafoods, meat and eggs) and served as a control group. From this total number of participants, 85.9% (n = 79) were Fijians and 14.1% were Indians. A majority of the participants (n = 72) were from one of these four coastal Fijian villages (Kalekana Settlement, Muaivuso village, Dravuni and Daku villages). Dravuni and Daku villages belong to an island group of Kadavu while Kalekana is located in suburban Lami area and Muaivuso in rural Lami area.

Nearly all participants from Daku and Muaivuso reported having a high reef fish diet while a few participants from Dravuni reported eating predatory fish and fresh tuna in addition to their normal reef fish meals, whereas the participants from Kalekana consumed more of the predatory fish and fresh tuna meals compared to reef fish. Apart from fresh fish, canned mackerel and tuna form a second major source of seafood diet for the residents of Muaivuso, Dravuni and Daku while canned mackerel is consumed rarely compared to canned tuna by Kalekana residents. Nearly all the fish consuming participants reported eating fish since childhood. A majority of the participants confirmed that they start feeding fish meals to infants from 4-6 months onwards. The young children consume the same fish meals as adults but in smaller portions than adults.

The results of total hair mercury levels and number of fish meals consumed per week (average and range) are summarized in Table 9 and shown graphically in Figure 7, for adult females, males and children from different study locations in Fiji with age and body weight data. The background total [Hg] in hair was 0.17 ± 0.03 μg/g determined in the control group. The male participants (n = 5) from Kalekana had the highest total hair Hg level of 7.93 ± 2.27 μg/g (range 5.84-10.84). Likewise females (n = 12) from Kalekana had the highest total [Hg] in hair of 6.50 ± 4.07 μg/g (range 2.65-15.28). The consumption of predatory fish and fresh tuna dominates over reef fish and canned tuna in Kalekana. The males consume an average total of 3.4 servings of fish per week and females 1.7 servings of fish per week and this reflects males having higher total hair Hg level than females.

Table 9: Mercury levels in hair and number of fish servings per week (range and average) in participants from different study sites in Fiji with age, gender and body weight data. (Note n = number of participants)

Study Location	n	Average age (yrs)	Average body weight (kg)	Gender	Average total no. of fish meals/week	Average total hair [Hg] ± SD (µg/g)
Kalekana Settlement Lami	12	42.4 ± 14.9 (range 15-57)	65.8 ± 13.6 (range 40-92)	Female	1.7 ± 1.4 (range 0-5)	6.50 ± 4.07 (range 2.65-15.28)
	5	48 ± 21.5 (range 19-71)	70.2 ± 14.5 (range 50-87)	Male	3.4 ± 1.6 (range 1-5)	7.93 ± 2.27 (range 5.84-10.84)
	5	8.4 ± 3.3 (range 5-12)	17.8 ± 5.9 (range 12-27)	Children	3.6 ± 3.4 (range 0-7.5)	3.05 ± 1.30 (range 1.11-5.17)
Dravuni Village Kadavu	10	40.3 ± 13.2 (range 19-45)	83 ± 10.7 (range 68-100)	Female	7.8 ± 3.9 (range 2-14.5)	3.01 ± 1.66 (range 1.19-7.05)
	10	31.9 ± 8.5 (range 24-69)	73.6 ± 15.6 (range 45-102)	Male	8.6 ± 2.8 (range 3.5-12.5)	4.42 ± 1.26 (range 2.80-7.08)
	1	8	25	Children	11.5	3.32
Daku Village Kadavu	7	34.9 ± 13.2 (range 19-52)	96.6 ± 18.7 (range 65-116)	Female	8.7 ± 1.8 (range 7.5-11.5)	1.51 ± 0.24 (range 1.24-1.84)
Muaivuso Village Lami	19	47.5 ± 21.7 (range 19-88)	76.3 ± 12.8 (range 55-95)	Female	5.6 ± 3.2 (range 0.5-12)	1.34 ± 0.57 (range 0.47-2.74)
	3	52 ± 16.8 (range 39-71)	87.3 ± 6.8 (range 82-95)	Male	9.3 ± 4.6 (range 4-12)	3.49 ± 1.98 (range 1.75-5.64)
Staff of USP Suva	8	36.6 ± 10 (range 24-52)	66.5 ± 16.9 (range 50-100)	Female	3.1 ± 1.6 (range 0.5-5.5)	1.10 ± 0.61 (range 0.34-2.23)
	2	39.9 ± 9.9 (range 32-46)	91 ± 1.3 (range 90-92)	Male	3.3 ± 1.1 (range 2.5-4)	3.44 ± 2.53 (range 1.65-5.23)
Control Group Suva	10	29.6 ± 8.7 (range 21-51)	61.7 ± 15.2 (range 44-85)	Female & Male	0 (Vegetarians)	0.17 ± 0.03 (range 0.10-0.20)

The result summary for the fish consumption frequency and the total [Hg] in hair for each individual is attached in Appendix 6.0.

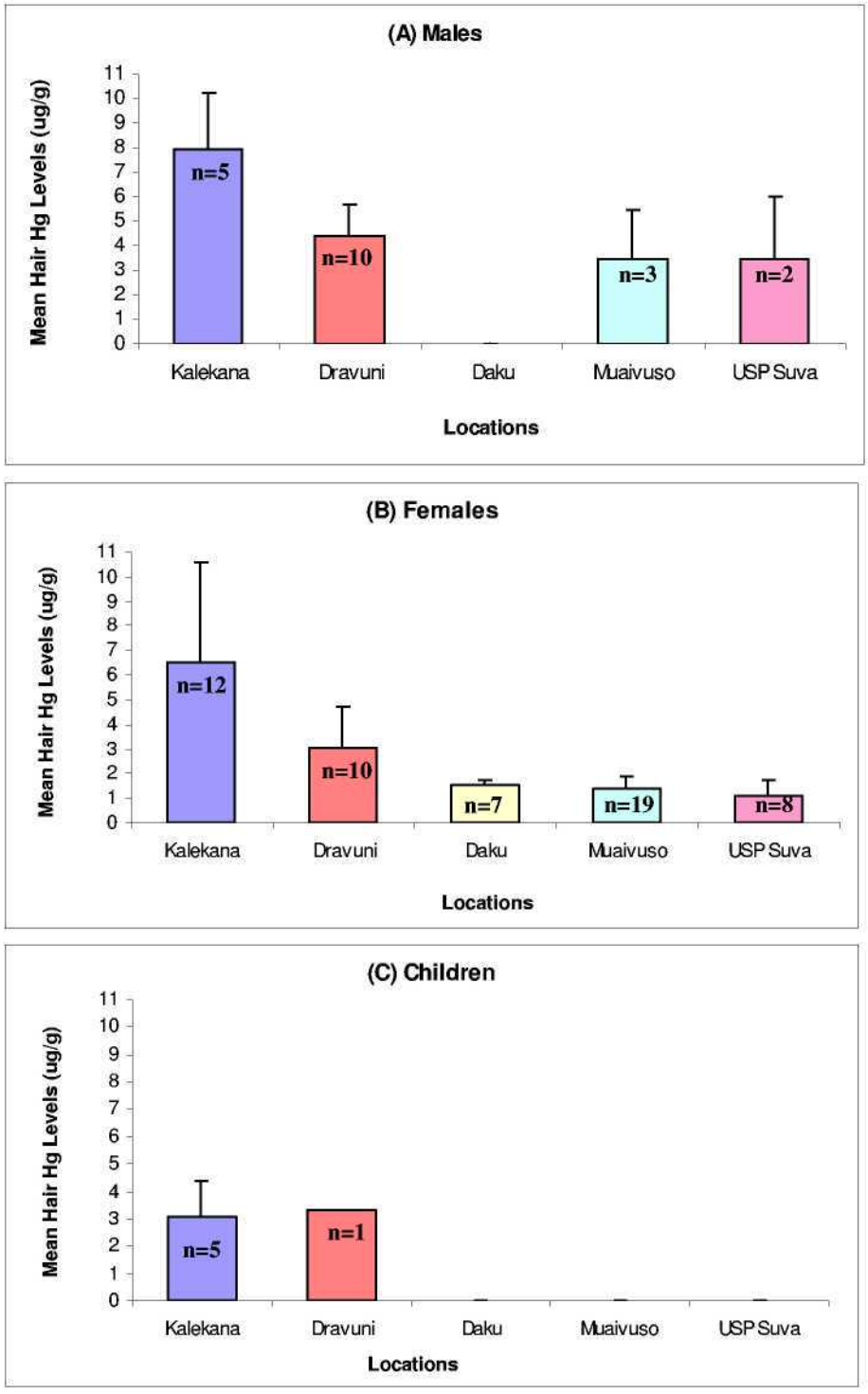


Figure 7: Graph showing mean [Hg] in hair of (A) males, (B) females and (C) children consuming fish and other seafoods from different study sites of Fiji

From the other three coastal villages that depend largely on reef fish, the males and females from Dravuni have higher total hair Hg of 4.42 and 3.01 μg/g. The males consume an average total of 8.6 fish servings per week and the females 7.8 fish servings per week. The female residents of Daku consume nearly the same number of fish meals (8.7 fish servings/week) as females from Dravuni but their total hair Hg level is lower (1.51 μg/g,) than females from Dravuni.

The males from Muaivuso eat an average total of 9.3 fish servings per week compared to males from Dravuni (8.6 fish servings/week) but have a slightly lower total [Hg] in hair of 3.49 μg/g because the males from Dravuni consume predatory fish and fresh tuna as well. Female participants from Muaivuso and USP consume less number of fish meals than Dravuni and Daku. Their total [Hg] in hair is much lower than Dravuni females but similar to Daku though these females consume more fish meals.

A total of six children were part of this study. The children from Kalekana (n=5) ate an average total of 3.6 fish servings per week having total [Hg] in hair of 3.05 ± 1.30 μg/g (range 1.11-5.17) while another child from Dravuni who reported eating a total of 11.5 fish servings per week has a total [Hg] in hair of 3.32 μg/g.

All the data for the fish consuming population were pooled to obtain an average total [Hg] in hair of men, women and children that is presented in Table 10 and shown graphically in Figure 8. In general the men (n = 20)

consume an average total of 7 fish servings per week and having total [Hg] in hair of 5.06 ± 2.38 µg/g which is higher compared to women. The women (n = 56) who consume an average of 5 fish servings per week have total [Hg] in hair of 2.73 ± 2.92 µg/g. The children have higher total [Hg] in hair (3.09 ± 1.30 µg/g) than women though they consume same number of fish servings per week. Overall the total [Hg] in hair of the fish consuming population (n = 82) of Fiji is 3.33 ± 2.88 µg/g consuming an average total of 5.5 fish servings per week (Table 10, Figure 8).

Table 10: Mercury levels in hair and number of fish servings per week (range and average) consumed by the fish consuming population of Fiji with age, gender and body weight data. (Note n = no. of participants)

Gender	n	Average age (yrs)	Average body weight (kg)	Average total number of fish meals/week	Average total hair [Hg] ± SD (µg/g)
Women	56	40.5 ± 16.6 (range 15-88)	74.7 ± 17 (range 40-116)	5.0 ± 3.6 (range 0.5-14.5)	2.73 ± 2.92 (range 0.34-15.28)
Men	20	43.8 ± 15 (range 19-71)	81.3 ± 12.1 (range 50-100)	7.0 ± 3.6 (range 1-12.5)	5.06 ± 2.38 (range 1.65-10.84)
Children	6	8.3 ± 2.9 (range 5-12)	19 ± 6 (range 12-27)	5.0 ± 4.4 (range 0 – 11.5)	3.09 ± 1.30 (range 1.11-5.17)
Total fish consuming population	82	38.9 ± 17.9 (range 5-88)	72.2 ± 21.6 (range 12-116)	5.5 ± 3.7 (range 0-14.5)	3.33 ± 2.88 (range 0.34-15.28)

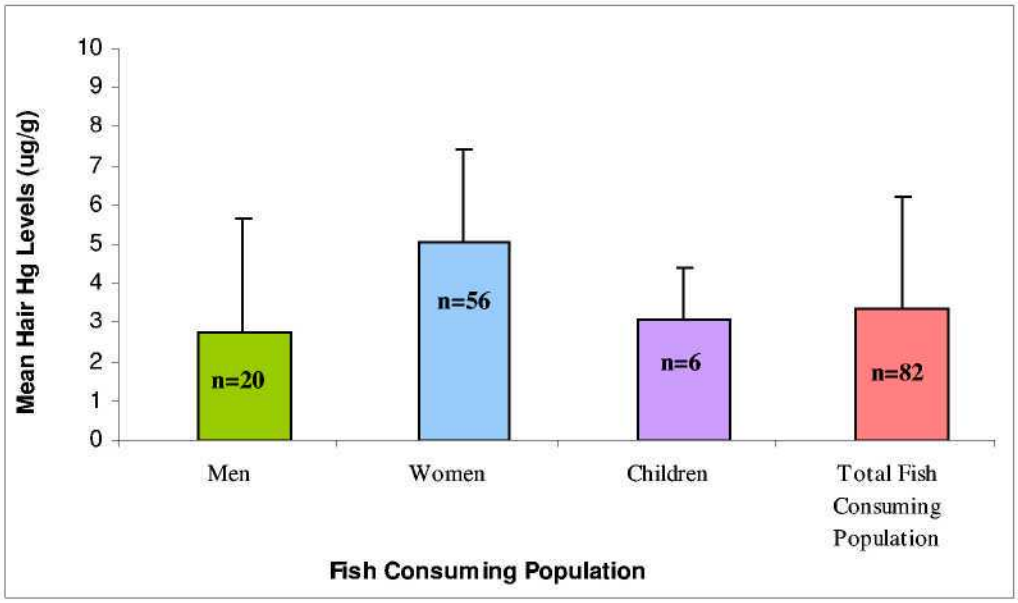


Figure 8: Graph showing mean [Hg] in hair of the fish consuming men, women, children and total fish consuming population of Fiji

4.2.4 Correlation between Total [Hg] in Hair and Fish Consumption Patterns

The Statistical Package for Social Sciences (SPSS version 10) software was used to perform all the statistical analysis in this study. Statistical significant level was set to $p<0.05$. There were a total of 82 fish consuming individuals, which included men (above 15 years, n = 20), women (above 15 years, n = 56) and six children (2-<15 years) categorized as total fish consuming population. This total fish consuming population included individuals from Kalekana Settlement (n=22) situated in the suburbs of Lami near the Fiji Fish outlet, Muaivuso village (n=22) not far from Kalekana but situated in rural coastal area in Lami, Dravuni village (n=21) in Kadavu hosting the USP research station and have easier access to facilities and Daku village (n=7) in Kadavu which is an inland village. The ten individuals from Suva (USP) had more western diet. Since the number of individuals falling in this diet

category was only ten no statistical analyses were performed separately on this group of individuals.

The two villages from Kadavu have a different type of fish diet though they both are from the same island. Dravuni has easier access to town bought goods and services. Ships and other vessels have access to this village. Though these people are from an outer island, their diet is in transition between traditional Fijian and western. These people eat predatory fish and fresh tuna frequently together with reef fish, shellfish and canned fish. These people also depend on reef fish caught by the village fishermen, whereas people from Daku village have more traditional fishing habits and fish diet. These people depend more on reef fish, canned tuna and mackerel. Most of these people eat reef fish at least twice a day.

Kalekana and Muaivuso villages are situated in Lami not far away from each other but still the pattern of fish diet is very different. Kalekana is situated near the Fiji Fish and Marketing group processing plant while Muaivuso is an inland rural village near the coast. Most men from Kalekana work on Fiji Fish fishing vessels and the processing plant. These people get fish at a discount directly from the fishing vessels and they also get most of their fish from the Fiji fish outlet where most of the predatory fish and tuna are sold as by-catches. Therefore these people have easier access to fish hence not many people do personal fishing for the family. These people have frequent consumption of predatory fish and fresh tuna together with other fish. The fish consumption pattern of these people is also in transition between western

and traditional Fijian diet. Muaivuso village however, is situated not far away from Fiji Fish but these people rarely buy fish from this outlet and rarely consume predatory fish and fresh tuna. The village men practice traditional fishing in their common fish ground. These people consume more reef fish and canned tuna and mackerel thus have more traditional fish diet.

Hence statistical analyses were performed on the total fish consuming population and according to the type of fish diet of the people from these four villages. Thus in total twenty-nine individuals from Daku village in Kadavu and Muaivuso village in Lami had very similar type of fish diet i.e. more traditional Fijian diet. A total of forty-three individuals from Dravuni village in Kadavu and Kalekana Settlement in Lami have similar fish diet pattern that is in transition between western and traditional Fijian. Therefore the data from Daku and Muaivuso village were pooled and analysed together under traditional Fijian diet and the data from Kalekana and Dravuni village were pooled and analysed together under fish diet in transition between western and traditional Fijian.

The Spearman's correlation was used to see how the consumption of different fish categories (i.e. predatory fish, fresh tuna, reef fish, canned mackerel, canned tuna and shellfish) correlated individually with the total [Hg] in hair of the fish consuming population of Fiji. This analysis was performed in regards to the number of servings of each of these fish consumed per week, total number of fish servings per week and the calculated amount of Hg

intake from each of these fish per week and total calculated amount of Hg intake from all fish meals per week with total hair Hg levels.

In addition multiple linear regressions were used to determine the influence of the consumption of each of the six categories of fish on total [Hg] in hair. This is because the individuals ate a combination of fish meals per week and not just only one of it. The number of independent variables (IVs) that can be included in a regression model is determined by the number of samples (i.e. roughly = number of samples divided by ten), not all the six fish categories could be selected in some of the analyses. For example, when performing the analyses on individuals having traditional fish diet (n = 29), only three IVs should be included in these analyses (29/10 = 2.9). The IVs selected for the analyses were based on the absence of any significant correlations between them and the preferred fish (i.e. a larger number of individuals consuming a particular fish) that was consumed by the individuals from the six fish categories.

4.2.4.1 Spearman's Correlations between the Total [Hg] in Hair and Consumption of the Number of Servings of Fish from Six Different Fish Categories for the Total Fish Consuming Population, Individuals Having Traditional Fijian Diet and Individuals Having Diet in Transition between Western and Traditional Fijian

The Spearman's correlation data are presented in Tables 11 and all the significant correlations are shown graphically in Figure 9. There was a low

positive correlation between total [Hg] in hair and the number of servings of predatory fish ($r = 0.378$, $p = 0.000$) and fresh tuna ($r = 0.347$, $p = 0.001$) per week for the total fish consuming population (Figure 9a-b). These correlations were significant at the 0.01 level of significance. Other fish categories showed negative correlations that were insignificant.

A moderate significant positive correlation was noted between total [Hg] in hair and number of servings per week of predatory fish ($r = 0.553$, $p = 0.002$) while low significant positive correlations were observed for fresh tuna ($r = 0.370$, $p = 0.048$) and total number of fish servings per week ($r = 0.386$, $p = 0.039$) in the participants from Muaivuso and Daku having traditional fish diet with rare consumption of predatory fish and fresh tuna (Table 11, Figure 9c-e). In addition number of servings of reef fish, canned mackerel, canned tuna and shellfish showed slight positive correlation, which were insignificant (Table 11).

A low significant positive correlation was noted between total [Hg] in hair and the number of servings per week of predatory fish ($r = 0.345$, $p = 0.024$) and low significant negative correlation was noted for number of servings of canned mackerel ($r = -0.329$, $p = 0.031$) in the participants from Kalekana and Dravuni having fish diet in transition between western and traditional Fijian (Table 11, Figure 9f-g). A low insignificant positive correlations were observed for the number of servings of fresh tuna and shellfish while reef fish, canned tuna and total number of fish servings showed low insignificant negative correlations with total [Hg] in hair of these participants.

Table 11: Summary of Spearman's correlation for the number of servings of different fish categories per week in relation to the total [Hg] in hair of the total fish consuming population, individuals from Muaivuso and Daku having traditional Fijian fish diet and individuals from Kalekana and Dravuni having fish diet in transition between western and traditional Fijian

Total Hg concentration in hair Vs total number of servings/week of:	Total Population Consuming Fish		Individuals from Muaivuso and Daku having Traditional Fijian Fish Diet		Individuals from Kalekana and Dravuni having Fish Diet in Transition between Western and Traditional Fijian	
	Spearman's Correlation, r	Significance (2-tailed)	Spearman's Correlation, r	Significance (2-tailed)	Spearman's Correlation, r	Significance (2-tailed)
Predatory fish	0.378 (low positive correlation)	p= 0.000 **	0.553 (moderate positive correlation)	p= 0.002**	0.345 (low positive correlation)	p= 0.024 *
Fresh tuna	0.347 (low positive correlation)	p= 0.001 **	0.370 (low positive correlation)	p= 0.048 *	0.168 (slight positive correlation)	p= 0.281 ***
Reef fish	-0.146 (low negative correlation)	p= 0.190 ***	0.106 (slight positive correlation)	p= 0.584***	0.295 (low positive correlation)	p= 0.055 ***
Canned mackerel	-0.206 (slight negative correlation)	p= 0.063 ***	0.163 (slight positive correlation)	p= 0.397 ***	-0.329 (low negative correlation)	p= 0.031 *
Canned tuna	-0.147 (low negative correlation)	p= 0.189 ***	-0.049 (slight negative correlation)	p= 0.801 ***	-0.237 (low positive correlation)	p= 0.126 ***
Shellfish	-0.002 (slight negative correlation)	p= 0.985 ***	0.242 (low positive correlation)	p= 0.205 ***	-0.192 (slight negative correlation)	p= 0.219 ***
All fish meals	-0.002 (slight negative correlation)	p= 0.984 ***	0.386 (low positive correlation)	p= 0.039 *	0.262 (low positive correlation)	p= 0.178 ***

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

*** Correlation is insignificant (p > 0.05)

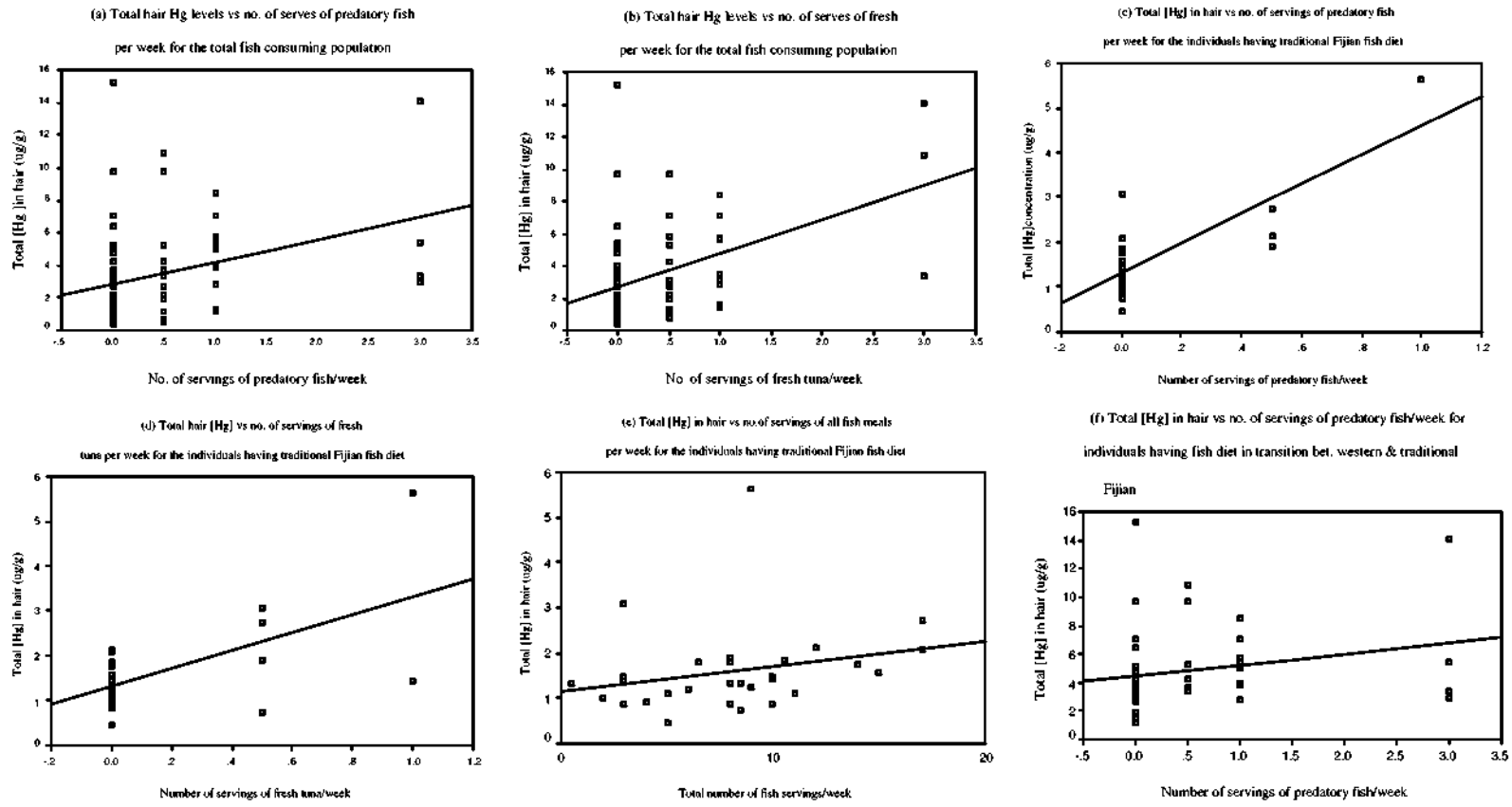


Figure 9: All significantly Correlated Relationship between the total hair Hg levels and number of servings of fish from different categories per week for the total fish consuming population, individuals from Muaivuso and Daku having traditional Fijian fish diet and individuals from Kalekana and Dravuni having fish diet in transition between western and traditional Fijian

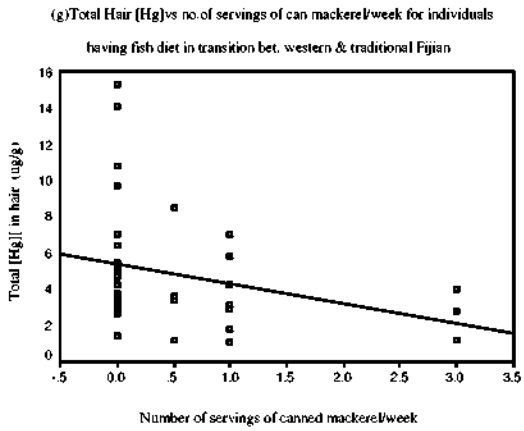


Figure 9 cont.: All significantly Correlated Relationship between the total hair Hg levels and number of servings of fish from different categories per week for the total fish consuming population, individuals from Muaivuso and Daku having traditional Fijian fish diet and individuals from Kalekana and Dravuni having fish diet in transition between western and traditional Fijian

4.2.4.2 Multiple Linear Regression Analyses between the Total [Hg] in hair and the Consumption of the Number of Servings of Fish from Six Different Categories for the Total Fish Consuming Population, Individuals Having Traditional Fijian Fish Diet and Individuals Having Fish Diet in Transition between Western and Traditional Fijian

The multiple regression analyses were performed on the total fish consuming population, individuals from Muaivuso and Daku having traditional Fijian diet and individuals from Kalekana and Dravuni having fish diet in transition between western and traditional Fijian. The total population included 82 fish consuming participants allowing for all six categories to be used in the multiple regression analysis. However the number of servings of shellfish per week was the only IV that did not correlate with the other five IVs and was included in the analysis together with number of servings per week of fresh tuna, reef fish and

canned tuna, which did not correlate with each other and were consumed by most of the individuals. The combination of the number of servings of fresh tuna, reef fish, canned tuna and shellfish per week explained 24.4% of the variation in the total [Hg] in hair (Table 12, Appendix 8.0a-b), a relationship that was significant ($F_{4,77} = 6.226$, $p_{model} = 0.000$, Appendix 8.0c). Of the four IVs put into the regression model only the number of servings of fresh tuna per week had the significant influence on the total Hg concentration in hair ($p_{fresh\ tuna} = 0.000$, Table 12, Appendix 8.0d). The total Hg level in hair increased with the number of servings of fresh tuna meals per week (figure 10).

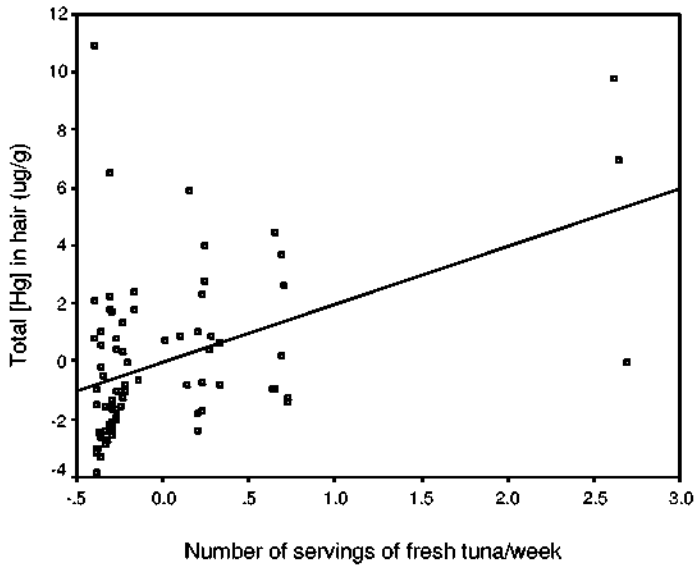


Figure 10: Relationship between the total [Hg] in hair and number of servings of fresh tuna meals per week within total fish consuming population

The total number of individuals from Muaivuso and Daku having traditional Fijian fish diet equals 29 therefore three IVs can be included in the multiple regression analysis. The number of servings of reef fish, canned mackerel,

canned tuna and shellfish were the uncorrelated IVs. Three IVs, number of servings of reef fish, canned mackerel and canned tuna per week were used in the multiple regression analysis because these three variables showed no correlations with each other and a majority of the participants preferred consuming these fish. The combination of reef fish, canned tuna and canned mackerel insignificantly explained 7.2% of the variation in the total [Hg] in the hair of these individuals ($p_{model} = 0.595$, Table 12, Appendix 8.1b-c). None of the three IVs had any significant influence on the total Hg in hair of these individuals (Table 12, Appendix 8.1d).

A total of 43 individuals were from Kalekana and Dravuni, who have a fish diet in transition between western and traditional Fijian. Only four IVs should be selected for the multiple regression analysis. The only non-correlated IV included the number of servings per week of fresh tuna. However only three IVs could be selected for multiple regression analysis, which were the number of servings of predatory fish, fresh tuna and reef fish that did not correlate with each other and most of these individuals consumed frequently. The combination of predatory fish, fresh and reef fish significantly explained 18.8% of the total [Hg] in hair of these individuals ($p_{model} = 0.041$, Table 12, Appendix 8.2 b-c). Of the three IVs only the number of servings of fresh tuna per week significantly influenced the total [Hg] in hair ($p_{fresh\ tuna} = 0.048$; Table 12, Appendix 8.2d). The total [Hg] in hair increased with the number of servings of fresh tuna meals per week (Figure 11).

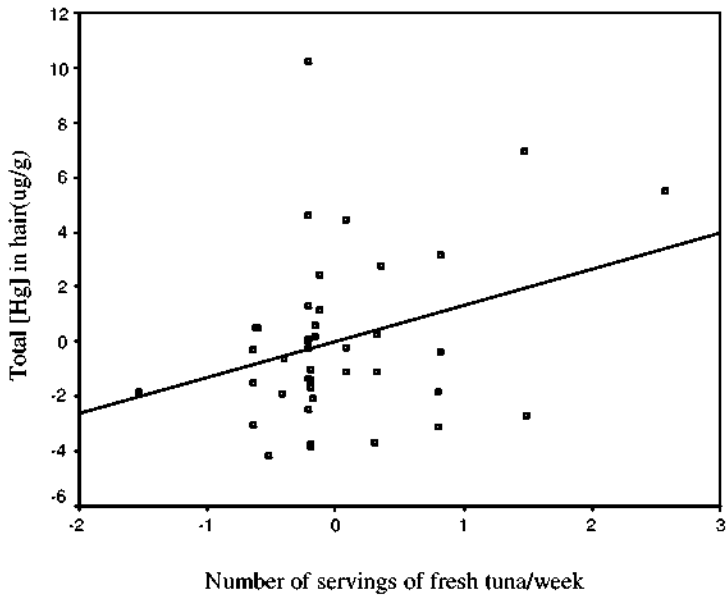


Figure 11: Relationship between the total [Hg] in hair and the number of servings of fresh tuna meals consumed per week by the individuals having fish diet in transition between western and traditional Fijian

Table 12: Summary of the Multiple Regression Results for the number of servings from different fish categories per week in relation to total [Hg] in hair of the Total fish consuming population, individuals from Muaivuso and Daku having traditional Fijian fish diet and individuals from Kalekana and Dravuni having fish diet in transition between western and traditional Fijian

	No. of servings of fish meals per week
Total Fish Consuming Population (n = 82)	**Non Correlated IVs:** Shellfish **Selected IVs:** Fresh tuna, reef fish, canned tuna & shellfish $R^2 = 0.244$ $F_{4,77} = 6.226$, $p_{model} = 0.000$ **Correlated IV Coefficients**: Fresh tuna ($p_{Fresh\ tuna} = 0.000$)
Individuals from Muaivuso and Daku having Traditional Fijian Fish Diet	**Non Correlated IVs:** Reef fish, canned mackerel, canned tuna & shellfish **Selected IVs:** Reef fish, canned mackerel & canned tuna $R^2 = 0.072$ $F_{3,25} = 0.642$, $p_{model} = 0.595$ **Correlated IV Coefficients:** None
Individuals from Kalekana and Dravuni having Fish Diet in Transition between Western and Traditional Fijian	**Non Correlated IVs:** Fresh tuna **Selected IVs:** Predatory fish, fresh tun & reef fish $R^2 = 0.188$ $F_{3.39} = 3.019$, $p_{model} = 0.041$ **Correlated IV Coefficients:** Fresh tuna ($p_{fresh\ tuna} = 0.048$)

4.2.4.3 Spearman's Correlations between the Total [Hg] in Hair and the Calculated Amount of Hg Intake from Six Different Categories of Fish for the Total Fish Consuming Population, Individuals Having Traditional Fijian Diet and Individuals Having Diet in Transition between Western and Traditional Fijian

The [Hg] in fish from the initial phase of this study were used in calculation for the amount of Hg intake from each fish category. Table 13 shows the [Hg] in the different categories of fish eaten by these fish consuming individuals.

Table 13: Summary of the average total [Hg] in the different categories of fish

Fish Categories	Types of Fish	Average [Hg] in µg/kg
Predatory fish	Marlin, shark, swordfish, sunfish, sailfish, walu, black snapper, barracuda, Wahoo, mahi-mahi	530
Fresh tuna	Bigeye, albacore, yellowfin, skipjack tuna	270
Reef fish	Kai kai, sabutu, kawakawa, parrotfish, nuqa,	40
Canned mackerel	Salmon style, canned mackerel	220
Canned tuna	Canned albacore and skipjack tuna	140
Shellfish	Fresh water & salt water mussels	30

A standard portion size of 150 grams for adults and 75 grams for children were used in the calculations. The number of servings of each fish meal was multiplied with the portion size (in kg) and this was multiplied with the [Hg]

in fish tissue (μg/kg) and then divided by the body weight of each individual to obtain the calculated amount of Hg intake from each fish category (data summary attached in Appendix 7.0). The Spearman's correlation data are presented in Table 14 and all the significant correlations are shown graphically in Figure 12.

For the total fish consuming population there is a significant low positive correlation between the total [Hg] in hair and the calculated amount of Hg intake per week from predatory fish ($r = 0.362$, $p = 0.001$) and fresh tuna ($r = 0.345$, $p = 0.001$). These correlations are significant at the 0.01 level (Figure 12a-b). The calculated total amount of Hg intake from all fish meals per week showed slight positive correlation which is insignificant ($r = 0.102$, $p = 0.364$). Other IVs showed insignificant negative correlations except for canned mackerel, which gave a significant correlation at the 0.05 level ($r = -0.230$, $p = 0.037$; Figure 12 c).

The individuals from Muaivuso and Daku having a traditional Fijian fish diet showed moderate significant positive correlation between the amount of Hg intake per week from predatory fish ($r = 0.552$, $p= 0.002$) and total [Hg] in their hair (Figure 12d). Low positive correlations were also noted for the calculated amount of Hg intake from fresh tuna, shellfish and total calculated Hg intake from all fish meals per week, but these correlations were insignificant. The amount of Hg intake from reef fish and canned mackerel showed slight insignificant positive correlation and canned tuna showed slight insignificant negative correlation.

The individuals from Kalekana and Dravuni having fish diet in transition between western and traditional Fijian showed low significant correlation between the calculated amount of Hg intake from predatory fish ($r = 0.319$, $p = 0.037$) and the total [Hg] in hair (Figure 12e). While low significant negative correlations were observed between the calculated amount of Hg intake from reef fish ($r = -0.367$, $p = 0.015$), canned mackerel ($r = -0.352$, $p = 0.021$) and canned tuna ($r = -0.315$, $p = 0.040$; Figure 12f-h). Slight insignificant positive correlations were noted for the calculated amount of Hg intake from fresh tuna and shellfish whereas slight insignificant negative correlation existed between total calculated amount of Hg intake from all fish meals per week and total [Hg] in hair.

Table 14: Summary of Spearman's Correlation for the calculated amount of Hg intake from different fish categories per week in relation to total [Hg] in hair of the total fish consuming population, individuals from Muaivuso and Daku having traditional Fijian fish diet and individuals from Kalekana and Dravuni having fish diet in transition between western and traditional Fijian

Total Hg concentration in hair Vs the calculated amount of Hg intake/week from:	Total Population Consuming Fish		Individuals from Muaivuso and Daku having Traditional Fijian Fish Diet		Individuals from Kalekana and Dravuni having Fish Diet in Transition between Western and Traditional Fijian	
	Spearman's Correlation, r	Significance (2-tailed)	Spearman's Correlation, r	Significance (2-tailed)	Spearman's Correlation, r	Significance (2-tailed)
Predatory fish	0.362 (low positive correlation)	p= 0.001 **	0.552 (moderate positive correlation)	p= 0.002 **	0.319 (low positive correlation)	p= 0.037 **
Fresh tuna	0.345 (low positive correlation)	p= 0.001 **	0.359 (low positive correlation)	p= 0.055 ***	0.148 (slight positive correlation)	p= 0.344 ***
Reef fish	-0.164 (slight negative correlation)	p= 0.141***	0.060 (slight positive correlation)	p= 0.757 ***	-0.367 (low negative correlation)	p= 0.015 *
Canned mackerel	-0.230 (low negative correlation)	p= 0.037 *	0.087 (slight positive correlation)	p= 0.654 ***	-0.352 (low negative correlation)	p= 0.021 *
Canned tuna	-0.179 (slight negative correlation)	p= 0.109 ***	-0.072 (slight negative correlation)	p= 0.709 ***	-0.315 (low negative correlation)	p= 0.040 *
Shellfish	-0.019 (slight negative correlation)	p= 0.864 ***	0.280 (low positive correlation)	p= 0.141***	0.180 (slight positive correlation)	p= 0.249 ***
All fish meals	0.102 (slight positive correlation)	p= 0.364 ***	0.344 (low positive correlation)	p= 0.067 ***	-0.150 (low negative correlation)	p= 0.336 ***

* Correlation is significant at the 0.05 level (2-tailed)

** Correlation is significant at the 0.01 level (2-tailed)

*** Correlation is insignificant ($p > 0.05$)

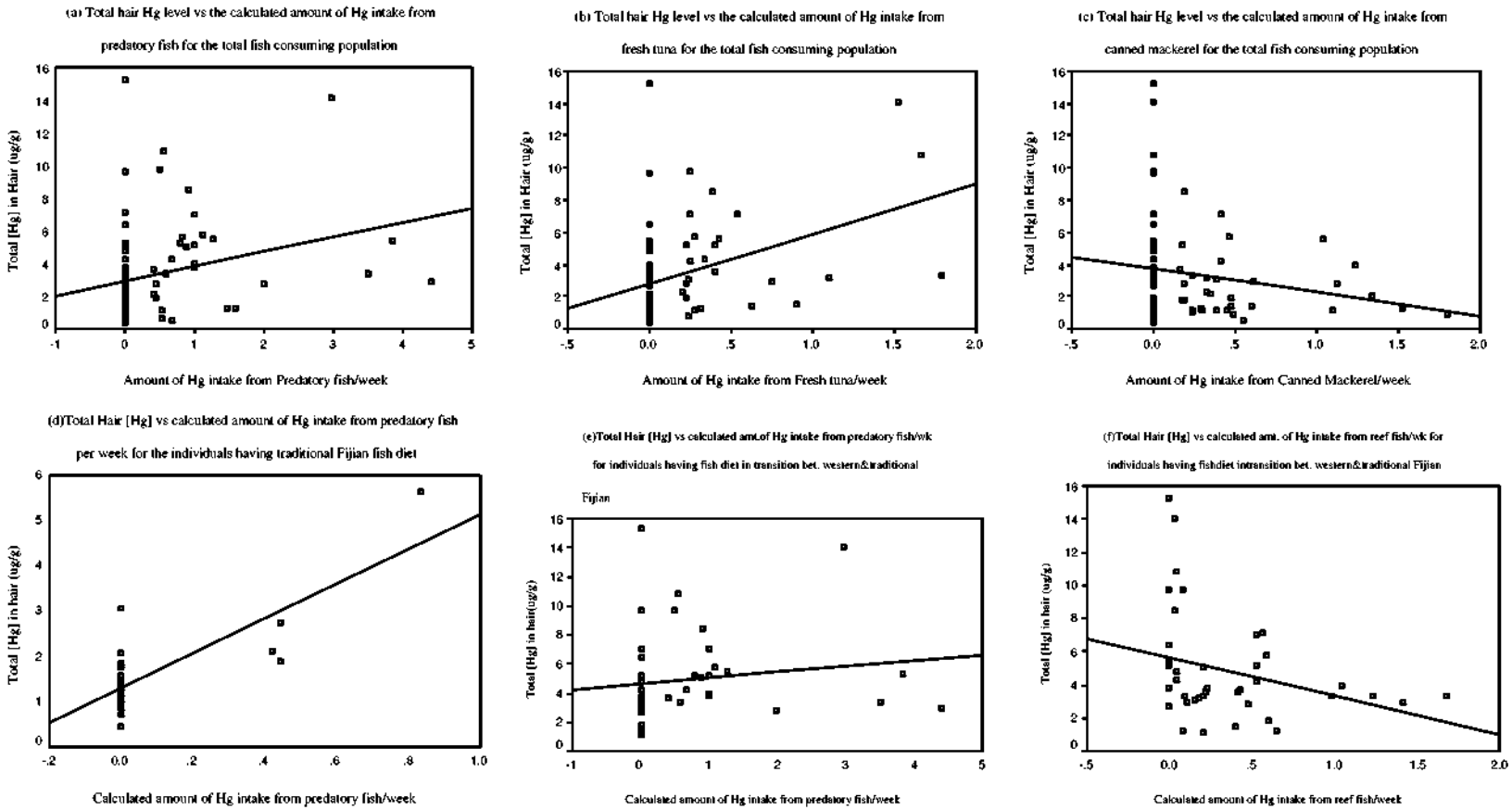
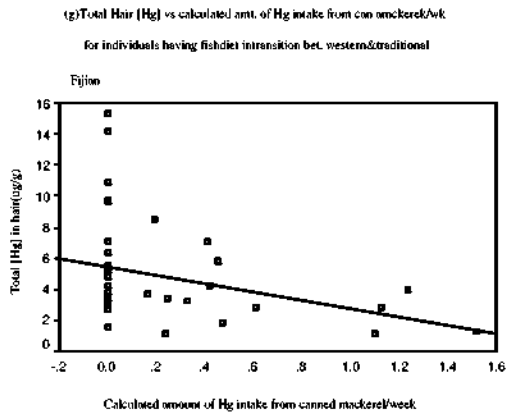


Figure 12: All significantly Correlated Relationship between the total [Hg] in hair and the calculated amount of Hg intake from different fish categories per week for the total fish consuming population, individuals from Muaivuso and Daku having traditional Fijian fish diet and individuals from Kalekana and Dravuni having fish diet in transition between western and traditional Fijian



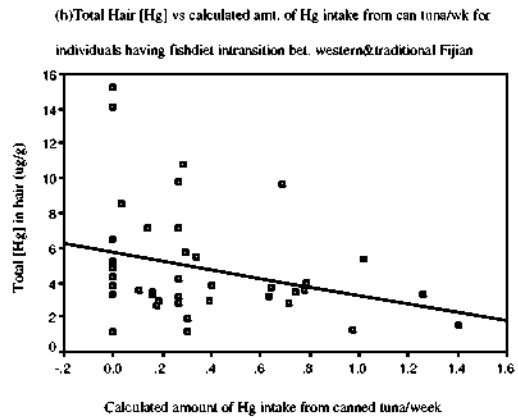


Figure 12 Cont.: All significantly Correlated Relationship between the total [Hg] in hair and the calculated amount of Hg intake from different fish categories per week for the total fish consuming population, individuals from Muaivuso and Daku having traditional Fijian fish diet and individuals from Kalekana and Dravuni having fish diet in transition between western and traditional Fijian

4.2.4.4 Multiple Linear Regression Analyses between the Total [Hg] in Hair and Calculated Amount of Hg Intake from Six Different Categories of fish for the Total Fish Consuming Population, Individuals Having Traditional Fijian Fish Diet and Individuals Having Fish Diet in Transition between Western and Traditional Fijian

The multiple linear regression analyses were performed on the total fish consuming population, individuals from Muaivuso and Daku having traditional Fijian fish diet and individuals from Kalekana and Dravuni having fish diet in transition between western and traditional Fijian. There were 82 fish consuming individuals in the total population therefore all six IVs should be included in the multiple regression analyses. However the calculated amount of Hg intake from reef fish and shellfish were the only non correlated IVs that were included in the regression together with fresh tuna and canned mackerel that did not correlate with each other and most of these individuals preferred consuming these fish. A combination of fresh tuna, reef fish, canned mackerel and shellfish significantly explained 22.8% of the variations in the total [Hg] in hair ($p_{model} = 0.000$; Table 15, Appendix 8.3b-c). Of these IVs fresh tuna ($p_{fresh\ tuna} = 0.000$) and canned mackerel ($p_{canned\ mackerel} = 0.044$) significantly influenced the total hair Hg levels in these individuals (Table 15, Appendix 8.3d). The total hair Hg level increased with the calculated amount of Hg intake from fresh tuna per week (Figure 13) and decreased with the calculated amount of Hg intake from canned mackerel (Figure 14).

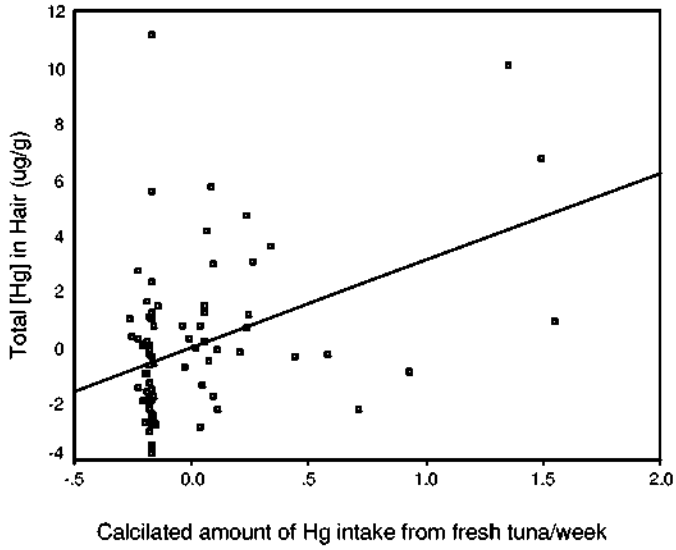


Figure 13: Relationship between the total [Hg] in hair and the calculated amount of Hg intake from fresh tuna per week for the total fish consuming population

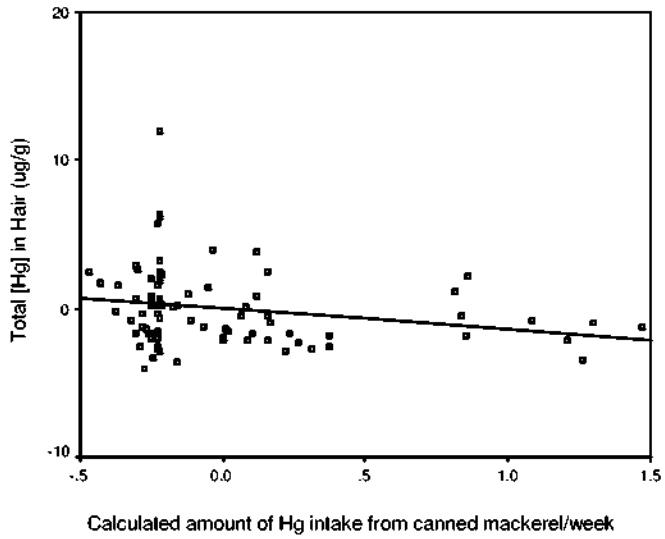


Figure 14: Relationship between the total [Hg] in hair and the calculated amount of Hg intake from canned mackerel for the total fish consuming population

The multiple regression analysis was performed on the individuals from Muaivuso and Daku having fish diet in transition between western and traditional Fijian. The only uncorrelated IV was calculated amount of Hg intake from shellfish. The IVs included in the multiple regression analysis were the calculated amount of Hg intake from reef fish & canned mackerel since these IVs were not correlated with each other and most of these individuals preferred consuming these fish. The combination of these IVs insignificantly explained 3% of the variation in the total Hg levels in hair of these individuals ($p_{model} = 0.676$; Table 15, Appendix 8.4 b-c). None of the IVs had any significant influence on the total [Hg] in hair of these individuals.

There were a total of 43 individuals from Kalekana and Dravuni having fish diet in transition between western and traditional Fijian. Only four IVs should be included in the linear regression analysis. The calculated amount of Hg intake per week from fresh tuna was the only uncorrelated IV. Only three IVs could be included in the multiple linear regression analysis, which were predatory fish, fresh tuna and reef fish that did not correlate with each other and most of these individuals preferred consuming these fish. The combination of these three IVs insignificantly explained 17.9% of the total [Hg] in the hair of these individuals ($p_{model} = 0.051$; Table 15, Appendix 8.5 b-c). Of these IVs only the calculated amount of Hg intake from reef fish had significant influence on the total [Hg] in hair ($p_{reef\ fish} = 0.033$; Table 15, Appendix 8.5d). The total [Hg] decreased with the calculated amount of Hg intake from reef fish (Figure 15).

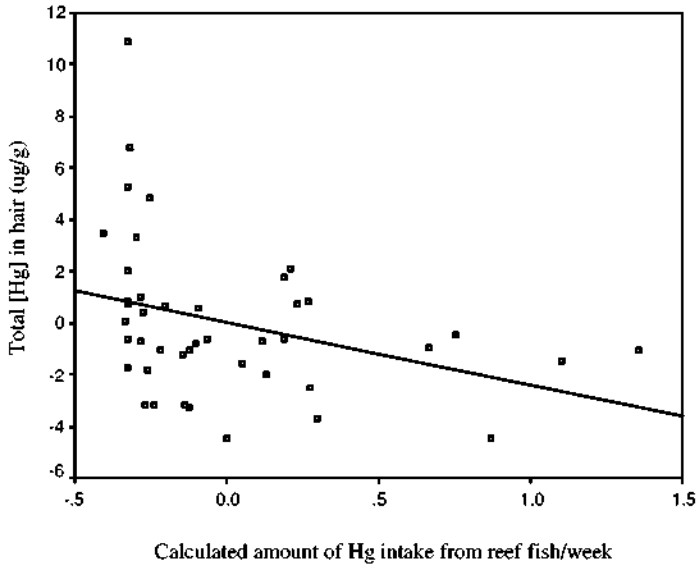


Figure 15: Relationship between total [Hg] in hair and the calculated amount of Hg intake from reef fish in the individuals having fish diet in transition between western and traditional Fijian

Table 15: Summary of Multiple Regression Results for the calculated amount of Hg intake from different fish categories per week in relation to total [Hg] in hair of the total fish consuming population, individuals from Muaivuso and Daku having traditional Fijian fish diet and individuals from Kalekana and Dravuni having fish diet in transition between western and traditional Fijian

	Calculated amount of Hg intake from fish per week
Total Fish Consuming Population (n = 82)	**Non Correlated IVs**: Reef fish, shellfish **Selected IVs:** Fresh tuna, reef fish, canned mackerel, shellfish $R^2 = 0.228$ $F_{4,77} = 5.691$, $p_{model} = 0.000$ **Correlated IV Coefficients:** Fresh tuna ($p_{fresh\ tuna} = 0.000$) & canned mackerel ($p_{canned\ mackerel} = 0.044$)
Individuals from Muaivuso and Daku having Traditional Fijian Fish Diet	**Non Correlated IVs:** Shellfish **Selected IVs:** Reef fish & canned mackerel $R^2 = 0.030$ $F_{3,25} = 0.398$, $p_{model} = 0.676$ **Correlated IV Coefficients:** None
Individuals from Kalekana and Dravuni having Fish Diet in Transition between Western and Traditional Fijian	**Non Correlated IVs:** Fresh tuna **Selected IVs:** Predatory fish, fresh tuna, reef fish $R^2 = 0.179$ $F_{3,39} = 2.827$, $p_{model} = 0.051$ **Correlated IV Coefficients:** Reef fish ($p_{reef\ fish} = 0.033$)

CHAPTER 5.0

DISCUSSION

5.1 Phase I: Mercury Levels in Fijian Seafoods

In most adult fish, 90-100% of the mercury is methyl-mercury, which is primarily found in the fish muscle tissue, bound to protein molecules (Bloom, 1992; USEPA, 2001a; Kim, 1995). Therefore the total mercury results can also be assumed to largely represent methylmercury concentrations. In the present study, large fish species such as marlin, swordfish, and sunfish had levels of mercury that exceeded guideline limits (Tables 4 and 6, Figures 2 and 5, section 4.1.2). These results were similar to that found in previous studies (see Table 16). These large predatory fish have a high trophic position and therefore they bioaccumulate a lot of mercury from their food and elimination of methylmercury from fish is slow.

A positive correlation with total mercury and fish length was found only for yellowfin tuna. Positive correlations between mercury concentration and length have been previously found in sharks (De Pinho *et al.*, 2002; Lacerda *et al.*, 2000), cartilaginous fish such as ghost shark (Storelli *et al.*, 2002) and the long finned eels (Redmayne *et al.*, 2000) but these fish all have a high fat content. Similarly, a positive correlation between mercury concentration and fish weight was found in the giant perch from Lake Murray in PNG (Kyle and Ghani, 1982b). The increase in mercury with length of yellowfin tuna observed in the present study is most likely due to increasing

bioaccumulation of mercury as the fish grow. However, it is noted that this correlation was relatively weak so it is difficult to predict mercury levels with any accuracy given the weight and length of a fish. The tuna samples were taken from several different locations around the Fiji Islands so concentrations of mercury in the water and prey species are likely to be different for similar sized fish taken from different locations. Closer examination of the albacore tuna data showed that several samples taken from near Koro Island showed high mercury levels for smaller length fish, which contributed to making the regression non-significant. This finding requires further examination but it could be due higher levels of mercury in the water, as Koro is a more recently formed island in comparison to others in Fiji. The area also contains undersea hydrothermal vents so more mercury methylating bacteria might be present. An alternative is that the growth rate of the albacore tuna is greater than for yellowfin and that this leads to less mercury present (on a weight for weight basis) for the large fish.

There was a statistically significant difference between average mercury levels in the albacore and yellowfin tuna, with the albacore having higher mercury levels. However, this may be due to the albacore tuna analysed having a slightly longer length. Methylmercury is fat-soluble, thus different species of tuna may also vary in their fat content depending on their diet. Fish such as shark and eels have quite high fat content and thus have high capacity for methylmercury bioaccumulation relative to their weight (Lacerda *et al.*, 2000; Storelli and Marcotrigiano, 2001). Other differences between similarly sized species could be due to the difference in the mercury metabolism ability

of different species, differences in feeding habits, ecology, fish growth rate and mercury levels in the ambient water. Tuna are known to be migratory species thus the geographical variability is another factor that maybe contributing to their difference in mercury levels. This is because of differences in the Hg levels in the seawater and the food sources at different localities. Mercury levels may be due to geothermal or volcanic activity in the sea and the land areas. Geographical variability has also been reported as a major source of differences among and between species. Distinct variations in mercury levels were observed in rainbow trout in lakes influenced by different levels of geothermal input in New Zealand (Kim, 1995).

A strong positive correlation also existed between mercury levels and the size of swordfish, marlin and walu steaks. It is recognized that for steaks cut from an individual fish, steaks of varying sizes can result depending on whether the steak is cut nearer the tail or head. On average, however, a steak with larger diameter is more likely to come from a larger fish and hence more bioaccumulation of mercury is likely to have occurred as discussed above. The total mercury concentration in marlin (Table 4, section 4.1.2.1) is high compared to the marlin steaks (Table 6, section 4.1.2.3). This is because these samples analysed (n=5, Table 4) came from much larger sized marlin (as per length and weight data in Table 4).

In the present study, small reef fish and the mussels and the shellfish had very low mercury levels. These organisms are very small and at the lower trophic levels and thus there is low degree of mercury bioaccumulation in them. The

major source of mercury in mussels and shellfish would be from particles and plankton in the water. It should be noted that several of these fish and shellfish samples were caught near to the major city of Suva, which has significant sewage and industrial discharges to the ocean. Land-based pollution could lead to elevated Hg levels but there does not appear to be a problem for the samples we examined.

Mercury levels in canned tuna and mackerel in the current study were relatively low and below the recommended FAO/WHO guideline limit. The level of mercury in canned mackerel in the present study was lower than that found for Japanese mackerel but was similar to that in New Zealand mackerel (Kyle and Ghani, 1981). In comparison with the canned albacore tuna, the canned skipjack tuna have quite low levels of mercury, which may be due to it being a smaller sized fish on average. The canned tuna values found in the present study was at the lower range of what has been reported previously. Values ranging from 0.1-1.0 μg/g have been reported for canned tuna from various countries (Holden, 1973) and a US study in 1992 found nearly 20% of canned tuna contained 0.3-0.5 mg/kg mercury and 10 % exceeded the 0.5 mg/kg Hg limit, and in 1995 15% contained 0.3-0.5 mg/kg mercury. Canned tuna is the most commonly consumed fish in US, averaging 10 cans per person per year (Johnson, 1999). Similar data from the Pacific are lacking but canned fish does form a significant part of the diet and consumption may also be increasing due to increasing urbanization and less reliance on subsistence living. The levels of mercury in comparison to other studies are shown in Table 16.

Table 16: Levels of mercury for seafoods found in previous studies compared to those found in present study (table continued on next page) Note: nd = not detected

Species	Location	[Hg] mg/kg	Reference
Tuna species			
Yellowfin	Fiji Islands	<0.02-0.40	This Study
Albacore	Fiji Islands	0.03-1.01	This Study
Skipjack	Fiji Islands	<0.02-0.16	This Study
Tuna	unknown	0.35	Louie, 1983
Yellowfin Tuna	Bismark Sea, PNG	0.09	Sorentino, 1979
Skipjack Tuna	Bismark Sea, PNG	0.03	Sorentino, 1979
Bluefin Tuna	North Atlantic Ocean	3.41	USEPA, 2001a
Bluefin Tuna	Mediterranean Sea	1.02	Storelli and Marcotrigiano, 2001
Marlin			
Marlin	Fiji Islands	<0.02-5.6	This study
Blue Marlin	unknown	0.72	Bloom, 1992
Striped Marlin	unknown	1.0-2.0	USEPA, 1997b
White Marlin	unknown	0.7-0.8	USEPA, 1997b
Marlin	Australia	0.57	NSW Health Department, 2001
Swordfish			
Swordfish Steaks	Fiji Islands	0.99-2.81	This Study
Swordfish	unknown	0.43	Bloom, 1992
Swordfish	Australia	0.98	NSW Health Department, 2001
Shark			
Shark Steaks	Fiji Islands	0.57-0.85	This Study
Sharks	South East Brazil	9.40-17.9	Larcerda *et al.*, 2000
Ghost shark	Mediterranean Sea	1.30-5.16	Storelli *et al.*, 2002
Saw shark	Lake Murray,PNG	0.37	Kyle and Ghani, 1982a
Whaler shark	West Bougainvillea, PNG	0.33	Sorentino, 1979
Atlantic Shark	Atlantic Ocean	0.6-2.87	USEPA, 1997b
Hammerhead Shark	unknown	2.0-3.0	USEPA, 1997b
Shark	Australia	0.48	NSW Health Department, 2001
Shellfish			
Shellfish	Fiji Islands	<0.02-0.05	This Study
Shellfish	Laucala Bay, Fiji	0.06	Morrison *et al.*, 2000
Shellfish	Astrolabe Lagoon, Fiji	0.34(dry wt)	Morrison *et al.*, 1997
Shellfish	Tonga	nd	Brown and Morrison, 2000

Species	Location	[Hg] mg/kg	Reference
Canned fish			
Canned Albacore	Fiji	0.16-0.27	This Study
Canned Skipjack	Fiji	0.06-0.11	This Study
Canned Tuna in oil	Fiji	0.05-0.16	This Study
Canned Mackerel	Fiji	0.18-0.22	This Study
Canned salmon Mackerel	Fiji	0.17-0.29	This Study
Canned Tuna	Various Countries	0.1-1.0	Holden, 1973
Canned Tuna	Australia	0.78	Kyle and Ghani, 1981
Canned Salmon	Australia	0.32	Kyle and Ghani, 1981
Canned Pink Salmon	United States	0.08	Kyle and Ghani, 1981
Canned Red Salmon	Canada	0.13	Kyle and Ghani, 1981
Canned Mackerel	Japan	0.48	Kyle and Ghani, 1981
Canned Mackerel	New Zealand	0.18	Kyle and Ghani, 1981
Canned Tuna	Papua New Guinea	0.13-1.01	Kyle and Ghani, 1983
Canned Salmon	Papua New Guinea	0.13-0.31	Kyle and Ghani, 1983
Canned Mackerel	Papua New Guinea	0.05-0.51	Kyle and Ghani, 1983
Canned Sardine	Papua New Guinea	0.05-0.14	Kyle and Ghani, 1983
Canned Pilchard	Papua New Guinea	0.02-0.07	Kyle and Ghani, 1983
Lobster and crabs			
Crab	Fiji	0.03-0.07	This Study
American lobster	Unknown	0.5-2.0	USEPA, 1997b
Miscellaneous Fish			
Rainbow Trout	New Zealand	0.18-1.84	Kim, 1995
Freshwater anchovy	Lake Murray,PNG	0.64	Kyle and Ghani, 1982a
Dolphins	Japan	4.70-15.0	Endo *et al.*, 2003
Predatory Whales	Japan	1.64-46.9	Endo *et al.*, 2003
Filter-feeding Whales	Japan	0.02-0.10	Endo *et al.*, 2003
Electric Ray	Mediterranean Sea	1.65-3.59	Storelli *et al.*, 2002
Eagle Ray (M . aquila)	Mediterranean Sea	0.67-1.01	Storelli *et al.*, 2002
Freshwater Eels	New Zealand	0.12-0.65	Redmayne *et al.*, 2000
Barramundi	Papua New Guinea	0.32-0.57	Kyle and Ghani,1982a
Broad snouted catfish	Papua New Guinea	0.12-0.31	Kyle and Ghani, 1982a,b
Sapik gar pike	Lake Murray, PNG	0.44	Kyle and Ghani, 1982a
Mackerel	Port Moresby, PNG	0.13-0.15	Sorentino, 1979
Atlantic Barracuda	Unknown	2.0-3.0	USEPA, 1997b
Barracuda	Fiji	0.18-0.38	This Study
Sailfish	Unknown	0.5-0.6	USEPA, 1997b
Sailfish	Fiji	0.32-0.34	This Study

5.1.1 Health Implications of Mercury Levels in Fijian Seafoods

An indication of the amount of fish or shellfish that a person could safely consume in one week without exceeding the Joint FAO/WHO Expert Committee on Food Additives (FAO/WHO, 2003) Provisional Tolerable Weekly Intake (PTWI) of 1.6 μg methylmercury/kg of body weight/week is shown graphically in Figures 16a-d. The safe human consumption level (g/week) was calculated by multiplying the FAO/WHO JECFA (2003) PTWI with body weight and then dividing by the average level of mercury in a particular fish (see Tables 4-6, section 4.1.2). The calculations were performed for a range (10-120 kg) of individual body weights with heavier individuals theoretically able to consume more mercury without adverse health effects than lighter individuals. The default average human body weight used by JECFA is 60 kg but it is unlikely that this would be the same in the Pacific Islands.

The calculated PTWIs are based on total mercury levels as methylmercury was not measured, but as described earlier methylmercury forms nearly 100% of the total mercury levels in fish although there is some question whether this is true for the shellfish.

It should be noted that some samples had limited samples taken so values can be considered only as a general guideline until further analyses are performed. However, it is clear that health risks, particularly to children and pregnant women, exist from consuming relatively small quantities (<1-2 portions per week) of a number of the larger predatory fish species, such as

swordfish, marlin, shark, sunfish and bigeye tuna (Figures 16a and b). One portion of fish is considered equal to 150g, which is about the weight of one small fillet of fish. Frequent consumption of more than the recommended amount of these fish by pregnant women and women of childbearing age could be harmful to a developing fetus.

These findings are concerning as the larger-sized fish are often the cheaper priced species (FJ $2-$4/kg) which are sold as by-catch from the tuna trade (see Figure 17). Although purchasing and consumption data are lacking, it is believed that due to the low price, these fish are commonly and perhaps preferentially bought by local people and restaurants. The risk of consumption of these larger-sized species of fish by pregnant women is not publicized in Fiji so a definite health risk exists. Canned fish can be consumed in moderate quantities (4-7 cans per week depending on body weight) without a risk of health effects (Figure 16c) and shellfish and reef could be consumed in very large amounts (2-3 kg/week for an average sized person, Figure 16d).

The analyses in the current study were performed on raw (uncooked) fish tissue but mercury is not significantly removed by normal cooking processes (USEPA, 2001a). Skinning or trimming of fats cannot reduce the mercury concentration in fish, the reason being that methylmercury is bound to protein of the muscle tissue that cannot be removed. Even the concentration of Hg remains unchanged by the use of different cooking methods. Instead the cooking process reduces the moisture in the fish, rendering Hg levels in

cooked fish more concentrated than in uncooked fish but this small increase is insignificant (USEPA, 2001a; UNEP, 2005).

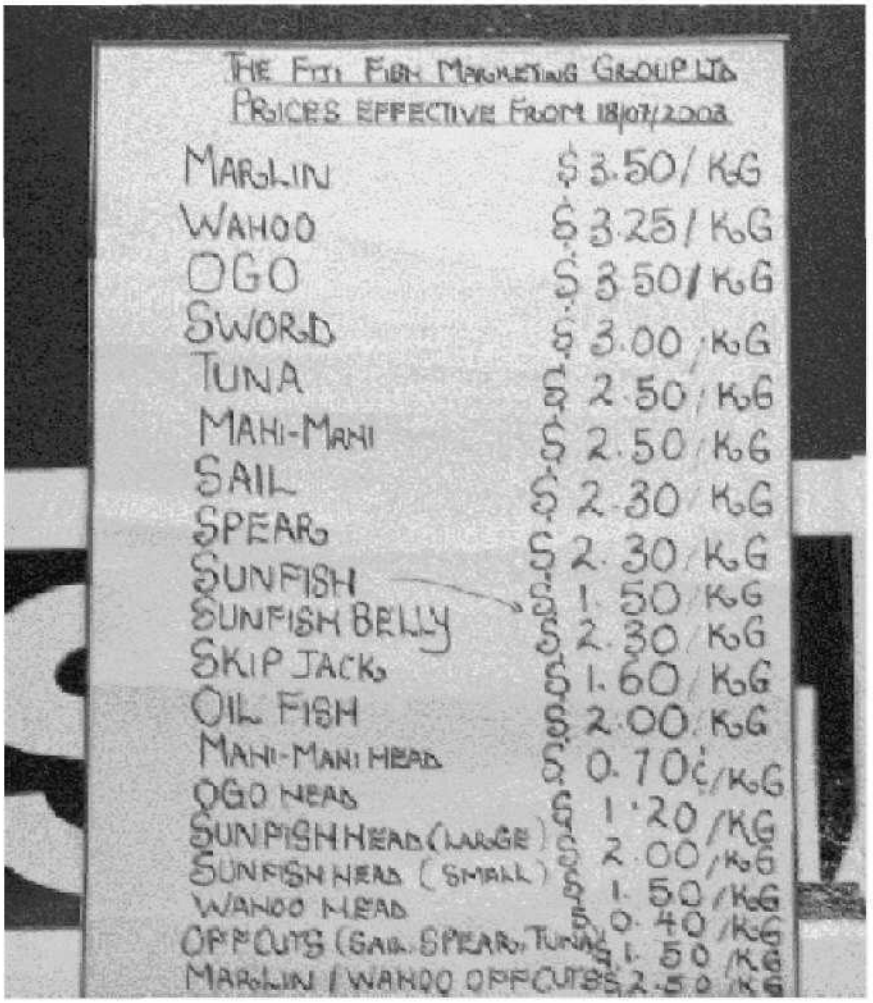
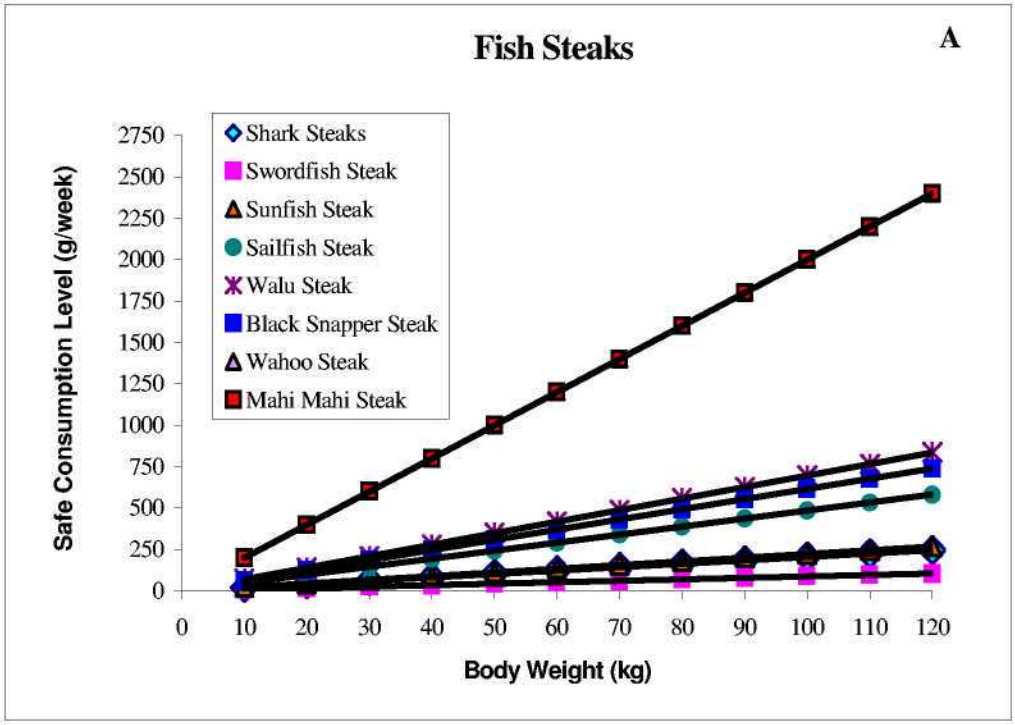


Figure 17: Fish price list in Suva, Fiji showing the low price for a number of species, which contain high mercury, levels.



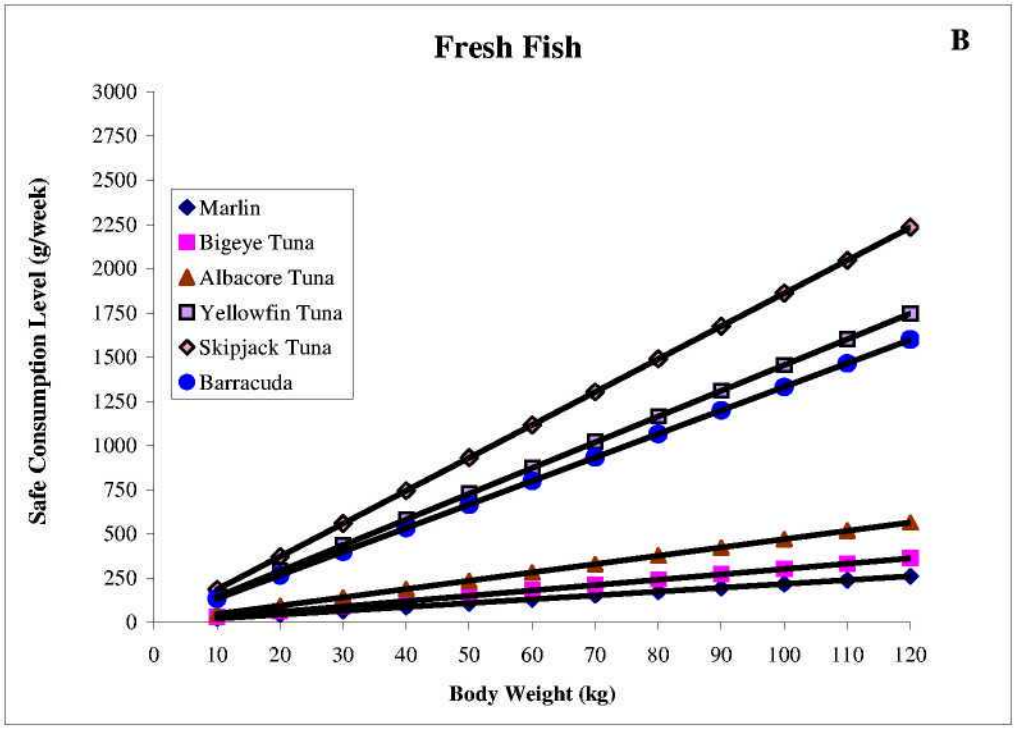
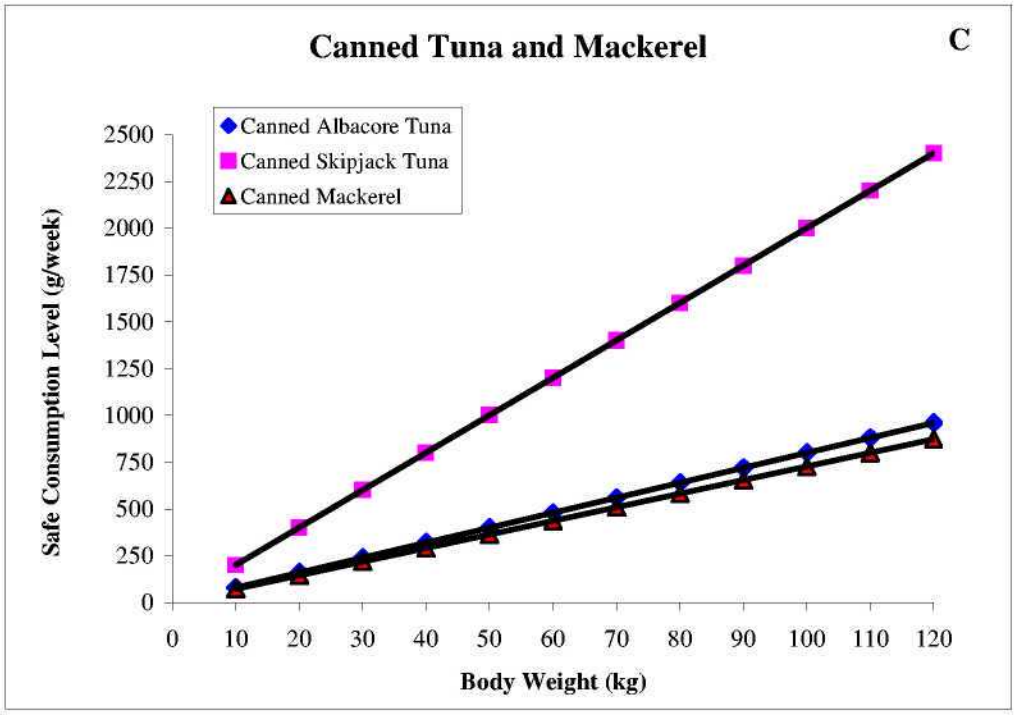


Figure 16: Provisional Tolerable Weekly Intake (PTWI) of: A. Fish Steaks and B. Fresh Fish based on different body weights (kg).



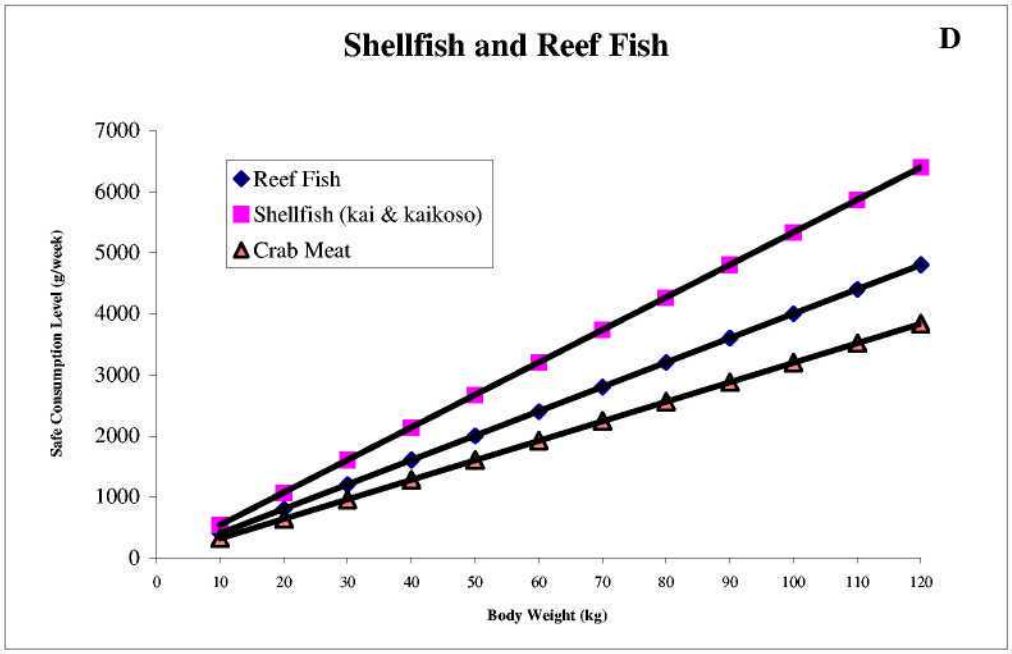


Figure 16 Cont.: Provisional Tolerable Weekly Intake (PTWI) of: C. Canned Tuna and mackerel and D. Shellfish, Crabs and Reef fish based on different body weights (kg).

5.2 Phase II: Total Mercury in Human Hair in Relation to Fish Consumption

Fish form one of the major source of protein in the diet of island dwellers and the coastal villages in Fiji. A majority of the island dwellers (Dravuni and Daku) and the suburban coastal villages (Muaivuso) practice traditional fishing in their common fishing grounds or obtain their fish from other village fishermen or sometimes from fish markets when there is no other source of supply. Canned mackerel and canned tuna are usually bought from local supermarkets or village shops. Kalekana is located in a suburban coastal Lami area and is just near the Fiji Fish and Marketing Group factory. Most of the men work on Fiji Fish fishing vessels and at the processing plant. These villagers buy most of their fish from the Fiji Fish outlet. These by-catches are very cheap, easily obtained and much affordable for most of these people. Also men working on fishing boats get free fish or buy them at a discount.

The men and women from Kalekana Settlement have the highest total [Hg] in hair compared to other study participants. This is due to the consumption of large predatory fish and fresh tuna that forms the major fish diet rather than reef fish in this population. The predatory fish occupy the highest trophic positions and accumulate more mercury in their tissue over their lifetime (USEPA, 1997b). These predatory fish (average 0.53 μg/g of total Hg) and fresh tuna (0.27 μg/g) thus have much higher concentration of mercury in the tissue than reef fish (0.04 μg/g) and shellfish (0.03 μg/g). Even though the total average fish meal per week in these participants is lower than those from Dravuni, Daku and Muaivuso, the predatory fish and fresh tuna consumption

leads to much higher total mercury intake per week due to high Hg levels in these fish. Also the amount of fish consumed per meal and the frequency of consumption affects the total Hg intake. The males from Kalekana were consuming 3.4 fish servings per week compared to 1.7 fish servings per week by females. Also the portion sizes for males could be bigger than those of females that contributed to higher Hg level in hair of males.

The other coastal villagers eat more fish meals per week but have lower total [Hg] in hair than the residents of Kalekana because their major source of Hg intake is from reef fish, canned tuna and mackerel. Canned mackerel has higher total mercury (0.22 μg/g) than canned tuna (0.14 μg/g). The total hair Hg level was found to be higher in the females and males from Dravuni compared to those from Daku and Muaivuso. Some of the participants from Dravuni reported eating either predatory fish or fresh tuna once per week. These participants were found to have higher hair Hg levels than those who only consumed reef fish, canned tuna and mackerel. Meanwhile one out of the three males from Muaivuso reported eating one meal of predatory and one meal of fresh tuna per week, which contributed to higher total hair Hg level in this participant compared to the other males and the females from this village who rarely consumed predatory and fresh tuna.

The children from Kalekana were aged between 5-12 years consuming an average of 3.6 fish servings per week had total [Hg] in hair of 3.05 μg/g. Whereas another child from Dravuni reported eating 11.5 fish servings per week had higher total [Hg] in hair of 3.32 μg/g. This child ate reef fish meals

at least twice a day. The mothers reported that children eat the same fish meals as adults and at the same frequency but the only difference is the smaller portion size than adults. The infants from 4-6 months onwards are served with fish meals. These total Hg levels in children are higher than some of the females consuming similar amount of fish meals. This could be due to their small age and lower body weights compared to adults thus have higher Hg level per kg of body weight.

5.2.1 Correlations between Total [Hg] in Hair and Fish Consumption Patterns

In general the consumption of fish from different fish categories in this study has correlated differently with total [Hg] in hair but does show positive correlations between total [Hg] in hair and fish consumption.

For the total fish consuming population this study revealed low positive Spearman correlations between the total [Hg] in hair and number of servings and the calculated amount of Hg intake from predatory fish and fresh tuna per week. These correlations are significant at 0.01 level (see Table 11 & 14, section 4.2.4.1 & 4.2.4.3). The multiple regression analyses showed that only the number of servings of fresh tuna per week significantly influences the total [Hg] in hair, which increases with the number of servings of predatory fish meals per week (see Table 12, section 4.2.4.2). While multiple regression analysis on the calculated amount of Hg intake showed that only the

consumption of fresh tuna and canned mackerel had significant influence on the total [Hg] in hair (Table 15, section 4.2.4.4).

In the fish consuming individuals from Muaivuso and Daku who have traditional Fijian fish diet it is observed that significant positive correlation exists between the number of servings and the calculated amount of Hg intake from predatory fish and fresh tuna and total [Hg] in hair while total number of fish servings per week also correlated positively with total [Hg] in hair of these people (Table 11 & 14; section 4.2.4.2 & 4.2.4.4). Linear regression analyses revealed that only the calculated amount of Hg intake from fresh tuna and canned mackerel significantly influenced the total [Hg] in hair of these people (Table 15, section 4.2.4.3).

In the fish consuming individuals from the Kalekana and Dravuni who have fish diet in transition between the western and traditional Fijian, it was seen that significant positive correlations existed between the number of servings and the calculated amount of Hg intake from predatory fish and total [Hg] in hair. While significant negative correlations were observed between the number of servings and calculated amount of Hg intake from canned mackerel and number of servings of reef fish and the total [Hg] in hair of these individuals (Table 11 & 14, section 4.2.4.1 & 4.2.4.3). Linear regression analysis showed that the number of servings of fresh tuna and calculated amount of Hg intake from reef fish had significant influence on the total [Hg] in hair (Table 12 & 15, section 4.2.4.2 & 4.2.4.4).

Other authors have reported similar correlations in previous studies done elsewhere. Oken *et al.* (2005) reported a moderate association between maternal fish consumption and hair Hg content (Spearman r = 0.47) in the US cohort. Consumption of each group of fish was correlated with hair Hg, with Spearman correlation coefficients with hair Hg ranging from r = 0.43 for canned tuna to r = 0.23 for white meat fish. Hightower and Moore (2003) found that swordfish had the highest correlation with blood Hg level (Pearson's r = 0.71, p = 0.001). This was the only fish with significant positive correlation with Hg level. Red snapper and sole consumption were negatively correlated with Hg level but only red snapper was statistically significant (Pearson's r = -0.39, p = 0.03). The authors from the New Zealand study found that consumption of snapper was most closely correlated with hair Hg levels among other possible choices of fish type (NRC, 2000). Another research reported that consumption of several fish species was significantly associated with total hair Hg. For example, intake of cod, saithe, pickled herring, other canned fish, salmon, fresh marine fish, spawn and shellfish gave correlations on the order of 0.2-0.3 ($p<0.05$) with total Hg in hair. In the multiple regression analysis consumption of deep frozen fish, pickled herring and marine fish remained significant ($p < 0.001$) (Bjornberg *et al.*, 2003). While Ask *et al.* (2002) found that placental MeHg concentration increased with freshwater fish intake (multiple linear regression adjusted $r^2 = 0.14$, $p \leq 0.003$).

Furthermore positive significant correlations between fish consumption and hair Hg levels have also been reported by Ip *et al.* (2004) in Chinese children

(r = 0.51) and Holsbeck *et al.* (1996) in Bangladesh males (r = 0.88, p < 0.001). A significant weak correlation between hair Hg and estimated total fish intake per year was found by Stern *et al.* (2001) in the New Jersey pregnant women (r = 0.18, p = 0.02). In the Wayana Amerindian population in the South of French Guiana Frery *et al.* (2001) found that the Hg intake data was significantly correlated with the corresponding hair data of the individuals (r = 0.36, p < 0.01). Kyle and Ghani (1983) did not find any significant correlation between fish consumption and hair Hg levels for a coastal group of participants from Papua New Guinea.

In general individuals in this study showed large variability in hair Hg levels. This could be due to the lack or overestimation of the information given in the food frequency questionnaire. The other factors responsible for this could be interindividual variation in toxicokinetics and physiology among different individuals, a wide range of Hg levels in fish consumed and the timing of the test in relation to the fish consumed (Hightower and Moore, 2003; NRC, 2000). Also in this study the participants consumed a mixture of different fish from different fish categories in a week and most participants reported consumption of different fish at different frequencies. For the calculation of the amount of Hg intake from each fish category an average Hg concentration in fish tissue analysed in the initial phase of this study was used. There could be a possibility that the actual Hg levels in fish consumed were less than or higher than the Hg concentration used for calculation. The length of hair used for analyses in this study varied with participants due to the amount of hair required for analysis. In this study the Food Frequency Questionnaire was not

pretested with community members to evaluate content validity and information on the frequency of consumption other food groups such as carbohydrates, meat and poultry, tropical fruits and vegetable and milk and dairy products was not collected.

5.2.2 *Comparison of Hair Mercury Levels to Previous Studies*

The relation between MeHg oral dose and body burden expressed as human Hg exposure through fish consumption versus Hg levels in hair may vary among certain ethnic groups (Canuel *et al.*, 2006). Hightower *et al.* (2006) reported that Asians, Pacific Islanders and native Americans had higher blood Hg levels than other groups. The total Hg levels in hair of men and women in this study was found to be lower than those reported by Gaggi *et al.* (1996), Boischio *et al.* (2000), Barbosa *et al.* (2001), Frery *et al.* (2001), Dolbec *et al.* (2000) and Kyle and Ghani (1982a) (see Table 17). The Hg levels in hair of children were higher than those reported by Ip *et al.* 2004 and McDowell *et al.* 2004 but lower than Barbosa *et al.* 1998 and 2001(see Table 17).

The overall population mean of this study is slightly lower but comparable to that reported by Yakoo *et al.* (2003).

Table 17: Levels of total Hg concentrations in hair in relation to fish consumption patterns in previous studies compared to those found in the present study

Study Location	Total [Hg] in hair (μg/g)	Fish Consumption Patterns	References
Fiji Islands			
Women (n=56)	2.73 ± 2.92	• average of 5.2 fish servings /week	
Men (n=20)	5.06 ± 2.38	• average of 6.9 fish servings/week	This Study
Children (n=6)	3.09 ± 1.30	• average of 4.9 fish servings/week	
Overall Population mean (n = 82)	3.33 ± 2.88	• average of 5.6 fish servings/week	
United States US Pregnancy and child cohort (n = 135 mother-child pairs)	0.55 (0.02-2.38) maternal hair	Average maternal consumption of 1.2 fish servings/week	Oken *et al.*, 2005
China Chinese children (n = 137) aged 4-11 yrs	2.20	Children who consumed > 3 fish meals/week had twice the Hg level than those consuming 1-3 meals/week & 3-fold than those who never ate fish	Ip *et al.*, 2004
United States (NHANES)		The mean Hg in hair increased with increasing frequency of fish consumption	McDowell *et al.*, 2004
Children =	0.22		
Non-pregnant women =	0.47		
Pregnant women =	0.43		
Brazil Fishing Communities of Baixada Cuiabana in Pantanal Region Adults (n=129) aged 17-81 yrs	4.20	Hg exposure was associated with fish consumption	Yakoo *et al.*, 2003

Study Location	Total [Hg] in hair (µg/g)	Fish Consumption Patterns	References
Sweden Swedish Pregnant women (n = 123)	0.35 (median) (0.5-1.5)	Consumption of fish on an average of 6.5 times per month i.e. 25 grams/day during the year they became pregnant.	Bjornberg *et al.*, 2003
Philippines School Children	0.28-20.39	Fish is a major diet	Narveez, 2002
New Jersey Pregnant women (n=189)	0.53	Consumption of 83 fish meals per year which included 30 meals of canned tuna per year	Stern *et al.*, 2001
Brazil Communities along Negro River Basin Amazon • Children (n=73) aged <15 Yrs • Adults (n=76) aged >15 yrs	18.52 21.40	Most of the population consume fish at least twice a day	Barbosa *et al.*, 2001
French Guiana Wayana Amerindian Population	11.40	Most are high fish consumers. Men between 15 & 45 years consume around 340g of fish/day	Frery *et al.*, 2001
Canada Inuit Pregnant women, Northern Quebec	4.5	Most ate fish at least once a week, 32.6% ate 3-6 fish meals/week and 9.2% ate fish daily	Muckle *et al.*, 2001
Tapajos River N = 98 (12 – 79 years) Men = Women =	 12.2 9.90	Fish was included in an average of 61.8% of total meals eaten in a 7-day period	Dolbec *et al.*, 2000

Study Location	Total [Hg] in hair (μg/g)	Fish Consumption Patterns	References
Brazilian Amazon Ribeirinhos along upper Madeira River. Individuals (n=10) living in same household & sharing same fish meals	8-339	Up to 7 different fish species were included in the same meal. Heavy fish eaters.	Boischio *et al.*, 2000
Brazil Inhabitants of fishing villages along Tapajos River Basin	10.20-35.90	Fish Consumers	Akagi and Naganuma, 2000
Hong Kong Fertile males & females (n = 67) & Fertile vegetarian males & females (n = 45)	4.07 2.56 1.21	individuals who consume ≥ 4 fish meal/week individuals consuming less fish meals individuals who consumed no fish	Dickman *et al.*, 1999
EPA Region V Great Lakes Area (Minnesota, Wisconsin, Illinois, Indiana, Ohio & Michigan)	0.29	Fish consumers	Pellizzari *et al.*, 1999
French Guiana Pregnant women n = 109 Other adults n = 225 Children n = 136	1.6 3.4 2.5	Fish consumption contributed most to Hg exposure	Cordier *et al.*, 1998
China (n=64 males aged between 40 & 49 yrs) **Indonesia** (n=55 males aged between 40&49 yrs) **Japan** (n=243 males aged between 40 & 49 yrs)	1.69 3.13 4.62	Fish consumers	Feng *et al.*, 1998

Study Location	Total [Hg] in hair (μg/g)	Fish Consumption Patterns	References
Amazonian Region Child Bearing age women n = 28 Indians n = 98 Non-Indians Children n = 54 Indians n = 71 Non-Indians	 8.11 14.08 7.30 10.82	Non-Indians consumed more fish estimated at 200g/ day than Indians	Barbosa *et al.*, 1998
Amazon Tapajos River Region	11.40	Consumption of contaminated fish	Castilhos *et al.*, 1998
Seychelles Islands N= 779 mother-child pairs 6.5 months 66 months - Maternal - Children	Maternal hair 0.5-26.7 6.8 6.5	80% of the women eat ocean fish daily with a median of 12 fish meals/week	Davidson *et al.*, 1998 Shamlaye *et al.*, 1995 Myers *et al.*, 1995
Madeira Portugal Fishermen & their families in Camara de Lobos Women (n =22) Men (n = 58)	 16.22 39.76	Men consumed more fish than women. The men mostly eat fish during their fishing expeditions	Gaggi *et al.*, 1996
Bangladesh Males (n = 219)	 0.44 (range 0.02-0.95)	Moderate fish consumption averaging to 2.1 kg per month. The Hg levels in fish from Bangladesh are low	Holsbeck *et al.*, 1996
Papua New Guinea Populations in Wau-Bulou area Background [Hg] Fish eating population	 0.55 1.2 (0.39-3.00)	The fish eating population consume fish from contaminated stream.	Saeki *et al.*, 1996

Study Location	Total [Hg] in hair (μg/g)	Fish Consumption Patterns	References
Atlantic Coast of Southern Spain • Huelva City Professional fishermen (n=37) Full term pregnant women (n=34) • Algeciras City Professional Fisher men (n=34) Full term pregnant women(n=17) • Control from an inland Seville City Male fish consumers (n=16) Pregnant women (n=17)	10.41 2.40 8.36 5.94 5.53 0.99	For the residents of Huelva and Algeciras city fish is a major food. The fish consumers eat tuna or swordfish at least three times per week	Lopez-Artiguez *et al.*, 1994
Papua New Guinea Residents of Lake Murray Western Province Volunteers from Lake Murray (n = 114) Volunteers from Suki (n = 51) Volunteers from Runginae (n = 45)	15.40 (3.2 -50.0) 6.40 (0.62 -25.7) 2.40 (0.33 -9.0)	Main source of Hg from consumption of giant perch 2-3 times/day Low fish consumers Controls have very low fish diet	Kyle and Ghani, 1982a
Papua New Guinea Port Moresby Residents Coastal region (n = 121) Noncoastal region(n=122)	2.60 (0.4-14.4) 1.70 (0.2-5.2)	Residents eat more of fresh fish with a mean of 12.6 meals/week Residents eat more canned fish with mean of 10 fish meals/week	Kyle and Ghani, 1983

Study Location	Total [Hg] in hair (μg/g)	Fish Consumption Patterns	References
Papua New Guinea			
Dorogori village on the coast	Males: 4.10 Females: 4.40	Males and females from the Dorogori coastal village consumed more fish than other villagers	Suzuki *et al.*, 1991 as cited in Feng *et al.*, 1998
Ume village ~6 km from coast	Males: 3.80 Females: 3.40		
Wonie Village 25 km from coast	Males: 1.50 Females: 1.00		

5.2.3 Public Health Implications of Mercury Levels in Human Hair in Relation to Fish Consumption

Nearly all the participants in the study had hair Hg levels below 10 μg/g WHO safety limit (WHO, 1990) except for two adult females and one adult male from Kalekana Settlement in the suburban Suva-Lami area. The male was 70 years old with hair [Hg] of 10.84 μg/g and reported eating 5 fish servings per week that included 3 servings of fresh tuna and 0.5 servings of predatory fish per week. The women were of ages 43 years with total [Hg] in hair of 15.28 μg/g and 39 years old with total [Hg] in hair of 14.09 μg/g. The former reported rarely eating fish while the latter consumed an average of 5 fish servings per week of which she reported eating two meals each of predatory fish and fresh tuna per week. The reason for the highest hair Hg concentration of 15.28 μg/g with rare fish consumption could not be

explained as upon revisiting, the participant informed of giving the correct information in the food frequency questionnaire. A second hair sample could not be taken as the participant had applied hair colour.

The total fish eating population was divided into subgroups: total population, men (15 years and above, n = 20), women (15 years and above, n = 56), childbearing age women (ages 15-45 years, n =36) and children (2-<15 years, n = 6). Furthermore the Hg levels in these individuals are pooled in five categories of hair Hg levels □1ppm, >1 - □3ppm, >3 - □6ppm, >6 - □10ppm and >10ppm. These are shown graphically in Figure 18 a-e.

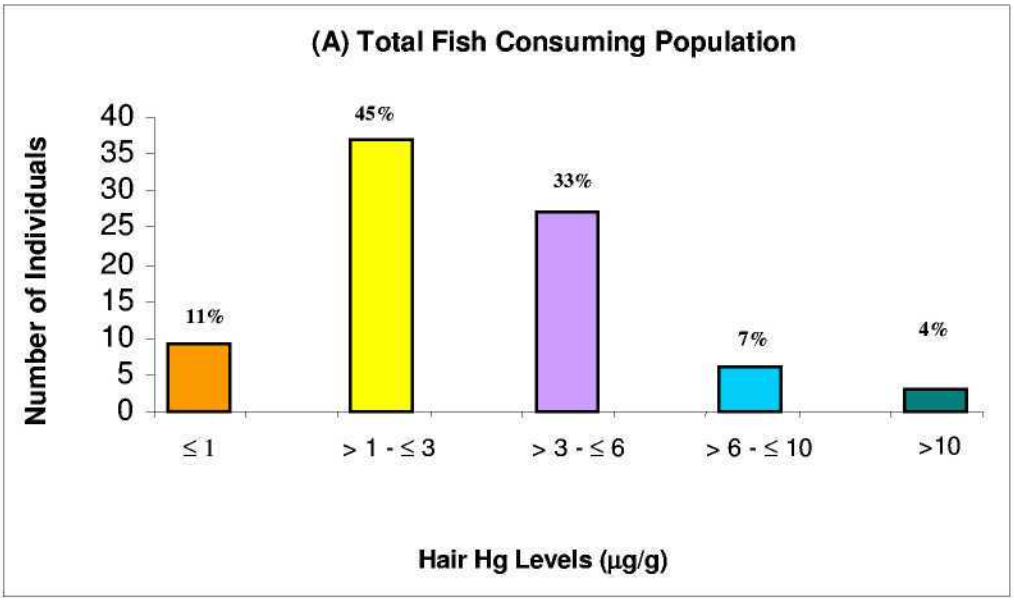
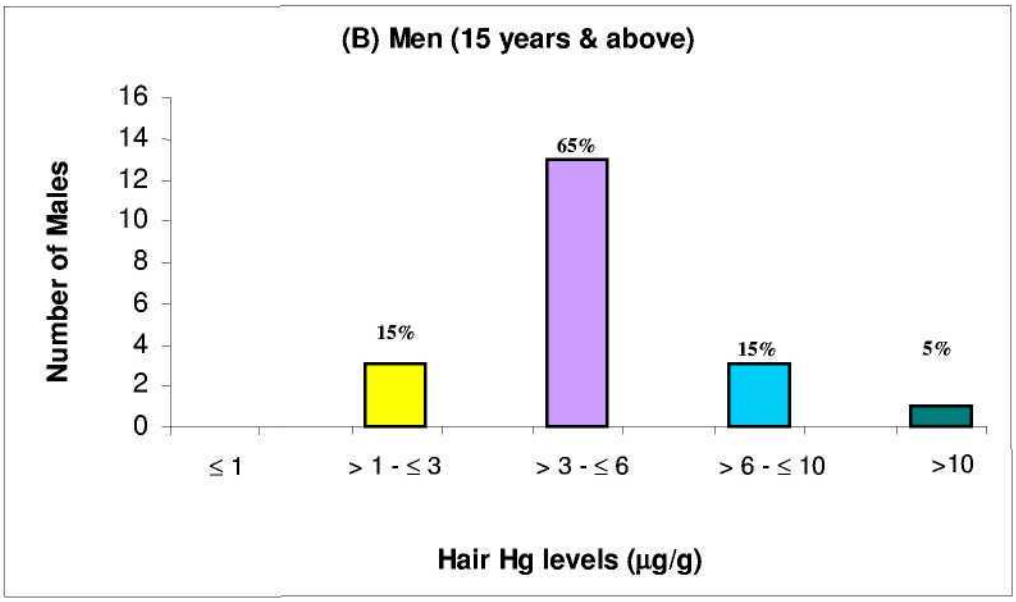


Figure 18: Distribution of total hair Hg levels in hair of (A) the total fish consuming population



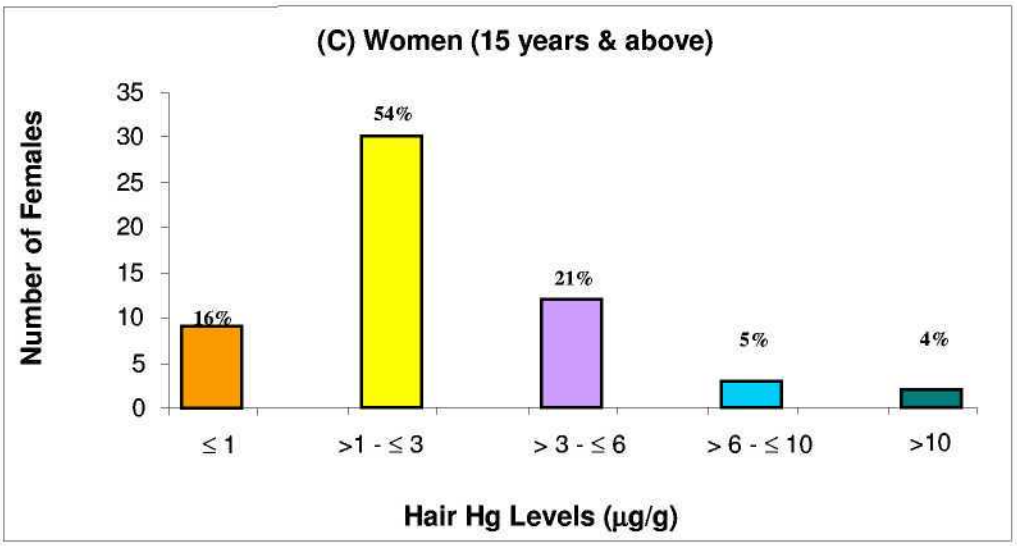
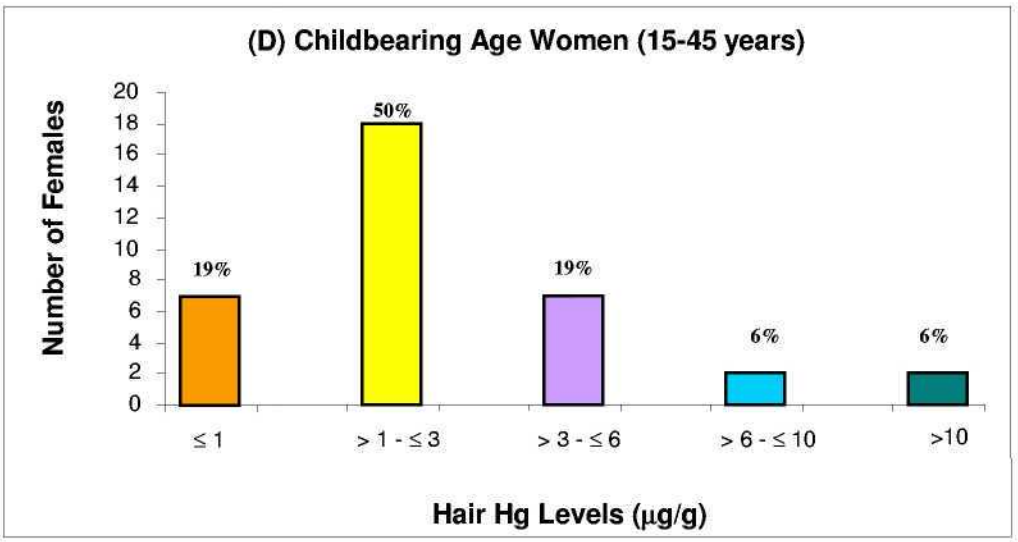


Figure 18 cont.: Distribution of total hair Hg levels in hair of (B) men (C) women



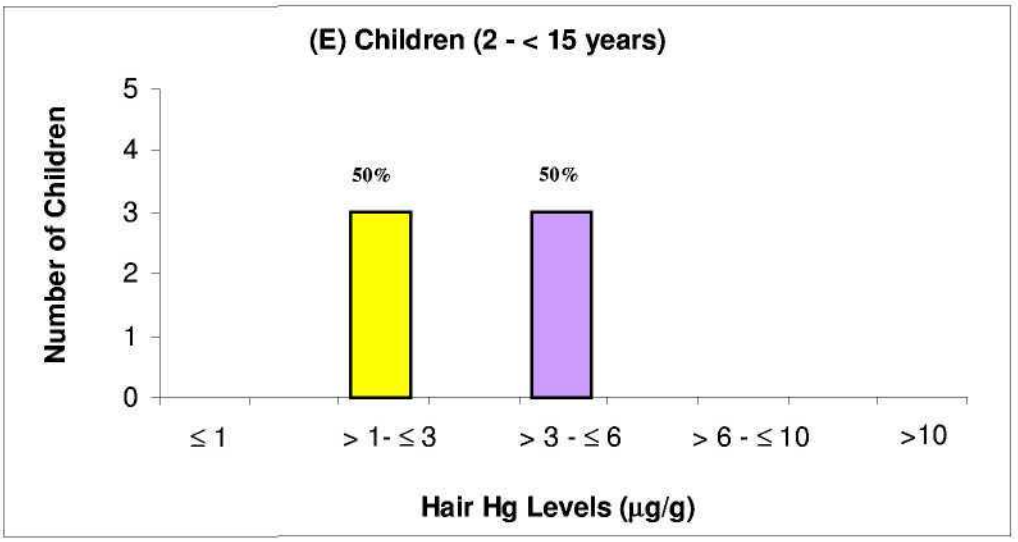


Figure 18 cont.: Distribution of total hair Hg levels in hair of (D) childbearing age women; (E) children

Figure 18a shows the distribution of total Hg in hair of the total fish consuming individuals. Only 11% of these individuals have total [Hg] in hair below the USEPA safety limit of 1µg/g in hair. While 44% of these

individuals exceeded the FAO/WHO recommended safety limit of 3 μg/g in hair and 4% exceeded the WHO safety limit of 10 μg/g in hair.

A total of twenty men were part of the total fish consuming population. Figure 18b shows that all men exceeded the USEPA safety limit of 1μg/g and 85% exceeded the FAO/WHO recommended safety limit of 3 μg/g and 5% are over the WHO safety limit of 10 μg/g.

A total of 56 women were part of the total fish consuming population and of these 36 were of childbearing age group (15-45 years of age). From Figure 18c it is noted that only 16% of the total women have total hair [Hg] below the USEPA safety limit while only 30% exceeded the FAO/WHO safety limit of 3 μg/g and 4% exceeded the 10μg/g WHO safety limit.

Figure 18d shows that 81% of childbearing age women have total hair [Hg] exceeding the USEPA limit. The FAO/WHO limit of 3 μg/g has been exceeded by 31% of these women while 6% of them have exceeded the 10μg/g WHO safety limit.

Only six children were part of this study and consumed fish. All of these children have total hair [Hg] above the USEPA safety limit. Fifty percent of them had hair Hg levels between >1- □3ppm and 50% had Hg levels between >3 – □6ppm (Figure 18e).

In a US pregnancy and child cohort study in 135 mother-child pairs Oken *et al.* (2005) studied maternal hair Hg levels and infant cognition. The mean maternal hair Hg concentration was 0.55 μg/g and 10% of them had Hg levels exceeding the USEPA limit. The mean visual recognition memory (VRM) score was 59.8 at mean age of 6.5 months. Maternal fish intake was positively correlated with the VRM score but Hg was negatively associated. After the adjustment for the maternal and infant characteristics, with each additional weekly fish serving was associated with VRM score that was 4.0 points higher. However an increase of 1ppm Hg in maternal hair was associated with a decrement in VRM score of 7.5 points. Hence 7% of the participants who consumed >2 weekly fish servings had infants with VRM scores that were 12.0 points higher than those who consumed ≤2 fish servings per week. On the other hand the 10% offsprings with maternal hair Hg >1.2ppm had VRM scores 9.3 points lower than those with hair Hg ≤1.2ppm. In comparison, this study reveals that only 19% of the childbearing age women have hair Hg levels below the USEPA limit. Therefore those women who are above this limit would be at greater risk if they are planning to become pregnant since the developing fetus would be at higher risk.

A study in the Philippine school children revealed hair Hg concentrations in these children ranging from 0.28-20.39 μg/g with fish being the major diet. Summary of examinations showed that predominant findings include underheight, gingival discolouration, adenopathy, underweight, and dermatological abnormalities among children. A significant neurological finding included 17.1% with cranial nerve abnormalities. Seventeen (5%)

children had sensory deficits and showed reflex abnormalities while 13 (3.9%) had cerebellar deficits and 5 (1.5%) suffered from motor nerve abnormalities (Narveez, 2002). Frery *et al.* (2001) reported that all Amerindian children under 1 year had hair Hg levels of 5 μg/g since they ingest approximately 3 μg Hg/day taking into account nursing.

Epidemiological studies are underway in the Seychelles Islands and the Faroe Island to assess prenatal exposure in relation to fish consumption. In the Seychelles no adverse outcomes were found in children with prenatal and postnatal Hg exposure at 6.5, 19, 29, or 66 months of age (Davidson *et al.*, 1998; Myers *et al.*, 1997). A follow up assessment at 9 years of age detected not adverse effects (Myers *et al.*, 2003).

Studies in Faroe Islands reported developmental delays at 7 years of age significantly associated with hair Hg concentrations below 10 μg/g (Grandjean *et al.*, 1997). Grandjean *et al.* (2003) administered clinical examination to 917 Faroese children at 7 years of age with focus on nervous system function. Eight of the neuropsychological tests administered showed deficits significantly associated with cord blood Hg concentration after confounder adjustment. This study supported previous findings that maternal hair Hg exposure during pregnancy is associated with neuropsychological deficits detectable at 7 years of age from this cohort and that this association is evident in women with stable exposures throughout pregnancy. A follow up to 14 years found an association between MeHg exposures and decreased

sympathetic and parasympathetic heart rate variability in these children (Grandjean *et al.*, 2004).

Children from two coastal villages in Italian coastal area were enrolled between 1999-2001. Out of 243 children 53 children underwent a follow-up at mean age of 25.1 months. Thirty-eight percent of the mothers reported eating <1 serving (150g) per week of fresh fish during pregnancy. The children whose mothers MeHg level was □1ppm were 47% more likely to perform as expected or poorly in DDST fine motor area as compared to children whose mothers hair MeHg level was <1ppm. The results remained unchanged after adjustment to other potential confounders. From the linear regression of the month of delay or advance development of fine motor-adaptive area on maternal hair MeHg, a delay of 0.52 months in development was estimated for each ppm increase in methylmercury concentration. The results did not change after adjustment for other confounders. These pilot findings were suggestive of an association between children's fine motor skills and their prenatal MeHg exposure from maternal fish consumption. However the authors concluded that a small number of cohorts have been tested and more extensive testing with more sensitive and specific tests are needed to determine if these findings persist (Barbone *et al.*, 2004).

In the New Zealand study the prenatally exposed children at age 4 showed 52% prevalence in developmental delay. A follow-up study at age 6 found out that the performance with the Test of Language Development (TOLD), the Wechsler Intelligence Scale for Children and the McCarthy Scale of

Children's Abilities decreased with high prenatal MeHg exposure (Kjellstrom *et al.*, 1986; Kjellstrom *et al.*, 1989 as cited in Gilbert and Grant-Webster, 1995 and NRC, 2000). The maternal hair Hg levels were > 6ppm with a mean of 8.3ppm. In this study 12% of the childbearing age women have hair Hg levels exceeding 6ppm.

In this study the number of children volunteers was small in number hence a representative data was not achieved but it was noted that infant fron 4-6 months onwards are fed with fish meals. Younger children eat the same fish meals at the same frequency as adults hence the children following this lead may be at health risks in Fiji. The six children included in this study have hair Hg levels ranging from 1.11-5.17 μg/g with a mean of 3.09 μg/g.

New Jersey pregnant women (n = 189) had mean hair Hg levels of 0.53 μg/g with a range from 0.02-9.1 μg/g. These women consumed an average of 83 fish meals per year which consisted commonly of 30 meals per year of canned tuna. Most women had hair Hg levels below the USEPA limit. Three cases exceeded 4 μg/g and 2 cases exceeded 6 μg/g. The authors concluded that based on scientific literature the above exposures may present an elevated risk of toxicity (Stern *et al.*, 2001).

The indigenous Indian women and children of the Amazonian region showed that most of them had hair Hg levels below 10 μg/g while 60% of the non-Indian women and children were above it. Overall 1% of the non-Indian women had hair Hg levels exceeding 50 μg/g. Considering that body half-life

of Hg is estimated to be 70 days and that fish are widely consumed, these women are carrying body loads of Hg approaching the threshold for fetal intoxication. This study found a decrease in the maternal hair Hg concentration during pregnancy, with decreases of 20% from first trimester to third trimester of pregnancy. The results of this study indicated that placental transfer of Hg to the fetus is more important than transfer during lactation (Barbosa *et al.*, 1998).

A correlation between elevated Hg levels and male subfertility has been reported by Dickman *et al.* (1999). The individuals who have been eating Hg contaminated fish for many years suggests that a daily intake of 0.3-0.7 mg/kg body weight may be sufficient to inhibit spermatogenesis in some Hong Kong males. In some of these males only 5 μg/g hair Hg levels displayed signs of subfertility.

A Study by Yakoo *et al.* (2003) assessed the neuropsychological deficits and hair Hg levels in adults in Brazil. The mean total hair Hg levels in these adults were 4.2 μg/g. The study found an association of hair Hg levels with detectable alterations in performance on tests of fine motor speed and dexterity and concentration. Some aspects of learning and memory disruption were also noted with Hg exposure.

The Finnish men in the highest third of hair Hg concentration (>2.03 mg/kg) had a 1.6-fold risk of acute coronary event, 1.68-fold risk of CVD, 1.56-fold of coronary heart disease death and 1.38-fold risk of any death compared with

men in the lower two thirds. With each microgram of Hg in hair, the risk of acute coronary event increased by 11%, the risk of CVD death by 10%, the risk of CHD death by 13% and risk of any death by 5%. Furthermore the high Hg level in hair attenuated the beneficial effects of fish oils on the risk of coronary events, CVD and CHD mortality (Virtanen *et al.*, 2005). A case-controlled study was conducted in 684 men aged ≤ 70 years with first diagnosis of myocardial infarction living in the eight European countries or Israel and 724 controls representative of the same population. In this study an independent and graded association was found between toenail Hg levels and the risk of myocardial infarction. Furthermore Hg masked an inverse association between DHA and the risk of myocardial infarction that became evident only after adjustment for the Hg level. This study also concluded that the high Hg content may diminish the cardioprotective effect of fish intake (Guallar *et al.*, 2002). Rissanen *et al.* (2000) also confirmed that fish-oil-derived fatty acids reduce the risk of acute coronary events however, high mercury content in fish could attenuate this protective effect.

A closer look at the results of this study reveals that majority of these fish consuming participants have exceeded both the USEPA and FAO/WHO safety limit and may be at potential health risks. If the above mentioned study data on developmental delays, male subfertilities, neurodevelopmental deficits and cardiovascular risks holds for children and adults, the hair Hg levels achieved by most of this study population indicate a general adult population and children at risk.

In this study men had hair Hg levels of 5.06 μg/g which is higher than the levels at which male subfertility, myocardial infarction and cardiovascular disease were reported.

The childbearing age women have hair Hg levels, which have shown developmental, cardiovascular risk and neurological deficits in the offsprings. The hair Hg levels in children has exceeded the USEPA and FAO/WHO safety limits and taking into account their age there is a possibility of health risks. With a mean hair Hg level of 4.2 mg/kg (range 0.56-13.60) in the fish consuming adults from Brazil Yakoo *et al.* (2003) found an association between hair Hg levels and detectable alterations in performance on tests of fine motor speed and dexterity and concentration. Verbal learning and memory disruptions were noted with Hg exposure and the magnitude of these effects increased with hair Hg levels. This study suggests that the adult cognitive function might be as sensitive as children and there is could be a possibility that the fish consuming adult population of Fiji could be at neuropsychological risk.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

The objectives of this study have been achieved. From the initial phase of this study 200 samples of Fijian seafoods were been analysed and it was concluded that mercury levels in the high trophic position fish that are predatory in nature such as swordfish and marlin exceeded the FAO/WHO Codex Alimentarius Limit set at 1 μg/g for predatory fish. Bigeye tuna, sunfish and shark species exceeded the FAO/WHO the codex limit of 0.5 μg/g set for other fish. A positive correlation between mercury concentrations and length of yellowfin tuna was observed but not for albacore tuna. No correlations were found between the fish weight and mercury concentration in tuna. The frequent consumption of more than the recommended portion of these fish by children, pregnant and childbearing age women could be harmful. These fish are available at a cheaper price and no data are available in Fiji on the risk of consumption of these large species of fish thus it was concluded that a definite health risk existed.

In a follow-up study the method for the determination of total mercury levels in hair was successfully validated and used for the determination of mercury in hair in a second phase of the study. It was concluded that the people

consuming larger predatory fish and fresh tuna were found to have higher total [Hg] in hair compared to those consuming only reef fish, canned tuna, canned mackerel and shellfish. From the total hair mercury levels in relation to fish consumption by the general population it was concluded that most of the participants exceeded the FAO/WHO recommended safety limit of 3 μg/g in hair and USEPA recommended safety limit of 1 μg/g in hair. Significant positive correlations were observed for the total hair mercury levels in relation to the fish consumption pattern especially with consumption of fresh tuna and predatory fish. Though the number of individuals targeted was small the study revealed that in comparing the results of this study to previous studies, the mercury levels at which the health effects such as cardiovascular risks, male subfertility, neurodevelopmental delays and neurological deficits occur are similar and comparable to those levels obtained in this study. Therefore the fish consuming population of Fiji could be at potential health risks. Because of the neurotoxic potency of MeHg, it is essential that the general public be made aware of which fish species tend to have higher Hg levels and which fish are safe to consume. The level of Hg in predatory fish justifies for a general fish consumption advisory for the people of Fiji.

6.2 Recommendations and Future Research

1) There is a need to measure mercury levels in seafoods from the various other Pacific Island countries as mercury levels may differ from location to location due to different levels of natural inputs and localized sources of

contamination. A good idea would be the establishment of a long-term South Pacific mercury monitoring program.

2) Further mercury analyses should be performed because the number of samples that could be taken from individual species was limited due to the large number of fish types analysed in this study. More emphasis should be placed on the large predatory species in subsequent research. The levels of methylmercury in water and sediments should be investigated for various fresh and marine waterways to obtain an idea of ambient levels.

3) The sample population in the hair Hg study was small. The number of children was only six. Since Fijian coastal villages were targeted in most cases the young children did not get their first hair cut which is a sacred Fijian custom and thus restricted their participation in the study. People from more coastal villages and the rest of the sites in Fiji should be targeted as most people from all ethnic groups consume fish and could be at health risks.

4) This was the first preliminary study of this nature conducted in Fiji so all the people were unaware of the risks involved from consuming large predatory fish and tuna more often. There is a need for a proper advisory that can conduct public education so that people are aware of such issues. The high mercury levels in predatory fish calls for a general fish advisory to be issued for the general population. This advisory needs to inform the public of the fish species that contain high levels of mercury and those species that are safe

for consumption and also inform about the risks involved in consuming more than the recommended amount of these fish.

5) A particular group should be targeted. In this case we included the general population who volunteered their consents. Proper communication strategies are needed so that specific groups such as children, pregnant and childbearing age women, men and sport and subsistence fishermen could be part of future studies.

6) The Food Frequency Questionnaire administered was not done in detail. The questionnaire needs to be checked and validated with the community members of the study sites to better assess their diet. In this case for example, the large deep-sea fish were categorized as predatory fish in general thus we were not able to know specifically which of the predatory fish were consumed. Also our questionnaire did not include other food and beverage groups which may influence hair Hg levels.

7) The length of hair sample used in this study was not uniform because in most cases the mass of hair would not make up the mass needed for efficient analyses. A representative amount of hair should be collected so that the length of hair used could be uniform giving the same length of exposure in all participants. Also more efficient hair digestion technique such as the use of microwave digestion should be validated for future research which may allow less amount of sample to be used and prevent any losses during digestion.

8) The most common bioindicators now used are cord blood, cord tissue and whole blood in addition to hair. In future studies if prenatal exposure is assessed then the above biomarkers will serve as accurate exposure biomarkers to determine exposure outcomes.

9) The general practitioners, pediatricians, child health professionals and other health officers and nurses should be made aware of this problem in Fiji because they could be better educators and their contribution to patients health could be very receptive. This is lacking in Fiji may be due to lack of knowledge in this area of research. There should be a separate advisory body formed to discuss the importance of environmental contaminants affecting human health so that this group can educate the public. This group of people should play more active roles in the environmental health matters.

CHAPTER 7.0

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Appendix 1.0

University of the South Pacific

Master of Science Research in Chemistry

Human Mercury Exposure in Relation to Fish Consumption in Fiji

Researcher: Maureen Christina Kumar

Consent Form (Hair Sampling Component)

Introduction

Mercury is a heavy metal contaminant and a toxin to the central nervous system. It is found in high levels in fish. It causes a variety of health problems to humans. Some of which are:

- Damage to the developing nervous system in the foetus and young children, IQ deficits and leaning disability.
- Causes cardiovascular problems
- Found to inhibit reproductive capabilities in males.

Aim of this research:

- To investigate the levels of total mercury in human hair as a measure of human mercury exposure and relate this to fish consumption.
- To determine from the above results whether the fish consuming population are at any health risk.

Who will be part of this component?

Adult males (15 years and above), adult females (15 years and above) and children between 2 and <15 years of age.

What will your participation in the study involve?

- You will be given time to consider your participation.
- This study will involve interviews that will include answering of questions by the head of the household or the person who prepares food. During this you will inform the investigator about the fish consumption pattern of your family. This will in take about half an hour.
- Height and weight measurements will take place after the interview.
- Following this hair samples will be collected from you if you have given consent to participate in the study. Hair will be taken from the back of your head using a scissors and this will not have any significant cosmetic effects on you. Hair sampling will take place at your household and this will take another half an hour.
- Your hair samples will be analysed for total mercury.

How will you benefit from this study?

- You will be given feedback on your height and weight measurements and total mercury level in your hair soon after the completion of the analysis.
- The interview data on fish consumption pattern and the analysis of your hair for total mercury level will determine whether your body has been exposed to high mercury levels as a result of consuming fish and other seafoods.
- If you are found to have adverse level of mercury in your hair and are at a potential health risk, you will be referred to Ministry of Health. Upon referral the General Practitioner or the nearest Health Center or Hospital will assist with the diet intervention programme for two months. After two months the researcher will revisit these participants to resample their hair. It will therefore be requested that these participants refrain from having a hair cut within these two months.

Your rights and confidentiality:

- You can decline to take part in this study.
- You may withdraw your consent at any time and be assured that no adverse consequences will result from withdrawal in part or whole study.
- You will not have to answer any questions in the interview that you do not wish to answer.
- You are assured that your participation and data provided will be completely confidential. You will provide your name and contact information so that you can be contacted if any problems arise in your hair mercury level. Your name will not be used in analysis or in any report of the study.

Ethical Approval

- This study has received approval from the Rewa/Kadavu provincial Council.
- This study has also received ethical approval from the Fiji National Research Ethics Review Committee.

Please go over the statements carefully and circle either "YES" or "NO" for each of them. Ask your interviewer any question necessary for you to understand these statements.

1	I confirm that the researcher has explained to me the details of the study	Yes / No
2	My questions have been answered to my satisfaction, and I understand that I may ask further questions at any time.	Yes / No
3	I confirm that the researcher has explained to me why my hair is being taken.	Yes / No

4	That the hair samples taken will not be for any commercial purposes.	Yes / No
5	That I have a right to refuse giving my hair sample for mercury analysis if I do not want to do so.	Yes / No
6	I understand and agree that I will be given the results of my hair mercury level, and that should I be found to have a problem with my hair mercury level, I will be referred to the Ministry of Health and my General Practitioner will be informed.	Yes / No
7	I understand that my participation in this study is confidential and that no material that could identify me will be used in the analysis of the study findings and that I will not be identified in any of the reports on the study.	Yes / No

I hereby provide INFORMED CONSENT to take part in the research on Human Mercury Exposure in Relation to Fish Consumption undertaken by the researcher.

Name: _____ Sign/Thumbprint: _____

In the Presence of: _____ Sign/Thumbprint: _____

Name of the Researcher: _____

For participants under 21 years old, a parent or guardian must sign this form on their behalf.

Parent/Guardian: _____ Sign/Thumbprint: _____

In the Presence of: _____ Sign/Thumbprint: _____

Name of the Researcher: _____

Appendix 2.0

University of the South Pacific

Master of Science Research in Chemistry

Human Mercury Exposure in Relation to Fish Consumption in Fiji

Researcher: Maureen Christina Kumar

Food Frequency Questionnaire

Participant ID: _____ Date: _____
Participant Name: _____ Occupation: _____
Date of Birth: _____
Ethnicity: _____ Sex: Male / Female (Circle one)

Age Group (tick one): Children 2-<15 years ☐ Adult 15 years and above ☐

Body Weight (kg): _____ Height (cm): _____

Location/Village: _____

Q. Is fish one of the major food sources for you and your family?

_____

Fish and other Seafood Consumption Patterns

How often do you and your family consume the following foods? (Please tick (√) where appropriate).

Fish and other seafood	Once per day	Once per week	2 or more times per day	2 or more times per week	1-3 times per month	Rarely
Large Deep Sea fish e.g., marlin, walu, swordfish, shark, sun fish and any others						
Tuna (albacore, yellowfin, skipjack, bigeye tuna)						
Reef Fish						
Canned Tuna						
Canned Mackerel						
Kai & kaikoso						
Any other seafood						

Q. If possible could you mention the common names of some of the type of fish that you and your family eat that is not in the table above?

_____
_____
_____
_____
_____

Q. How long have you been eating fish on a regular basis?

_____
_____
_____
_____

Q. Are fish meals a regular component of the diet of small children?

_____
_____
_____

Q. The fish and seafoods you consume at home is (tick (√) which ever is appropriate for you):

- Personally fished or fished by the family men
  _____
- Bought from the village fishermen
  _____
- Bought from the fish shops
  _____
- Bought from other shops e.g. supermarkets
  _____
- Bought from any other sources (specify)
  _____

Is there any thing else regarding your fish and other seafood diet that you want to mention?

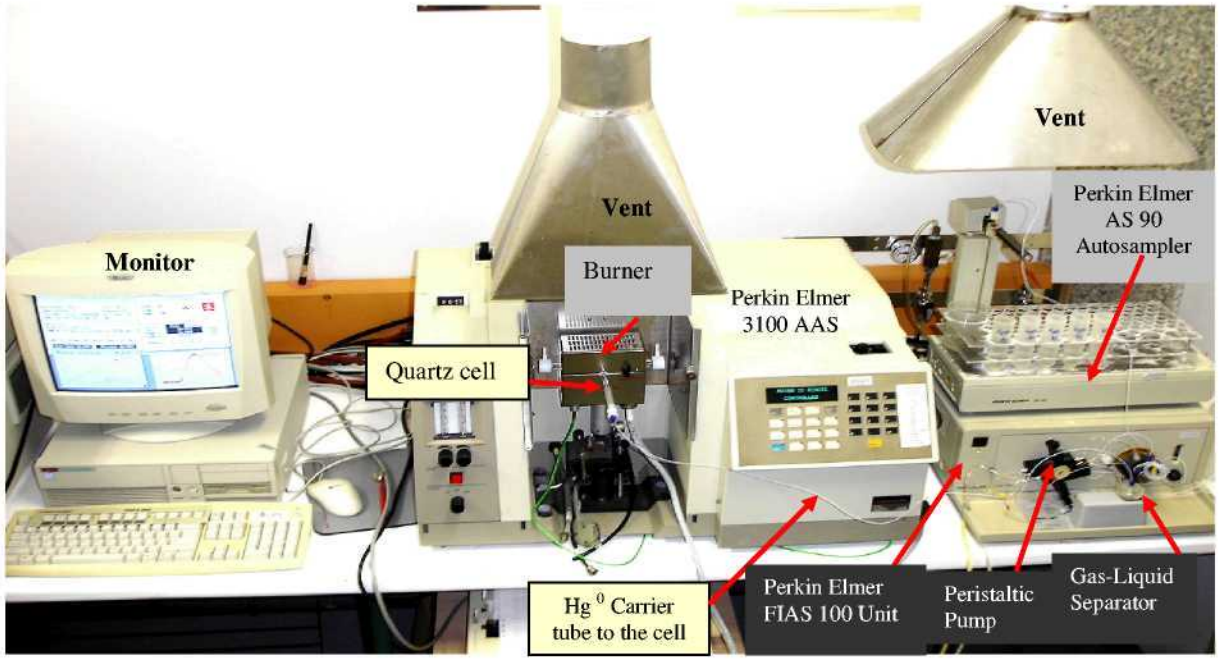
_____
_____
_____
_____
_____
_____
_____

Thank You
Researcher: Maureen Christina Kumar

Appendix 3.0

Photo Gallery

Instrumentation



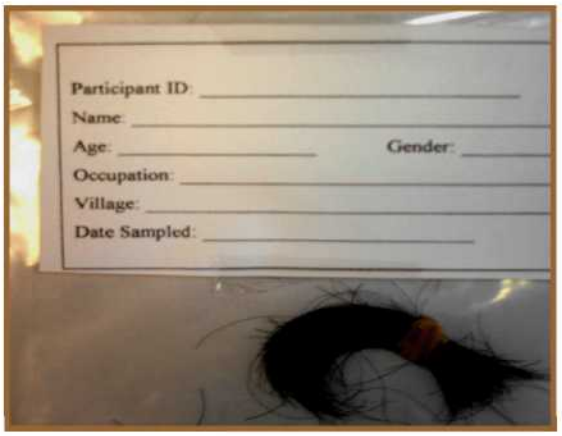
Picture a: Perkin Elmer Model 3100 Atomic Absorption Spectrophotometer and Perkin Elmer Flow Injection Analysis System (FIAS) 100 unit equipped with Perkin Elmer AS90 Autosampler.

Hair Sampling Technique

b. Identification of sample location on the scalp and amount of hair to be cut. Surrounding hair secured with hair clips.

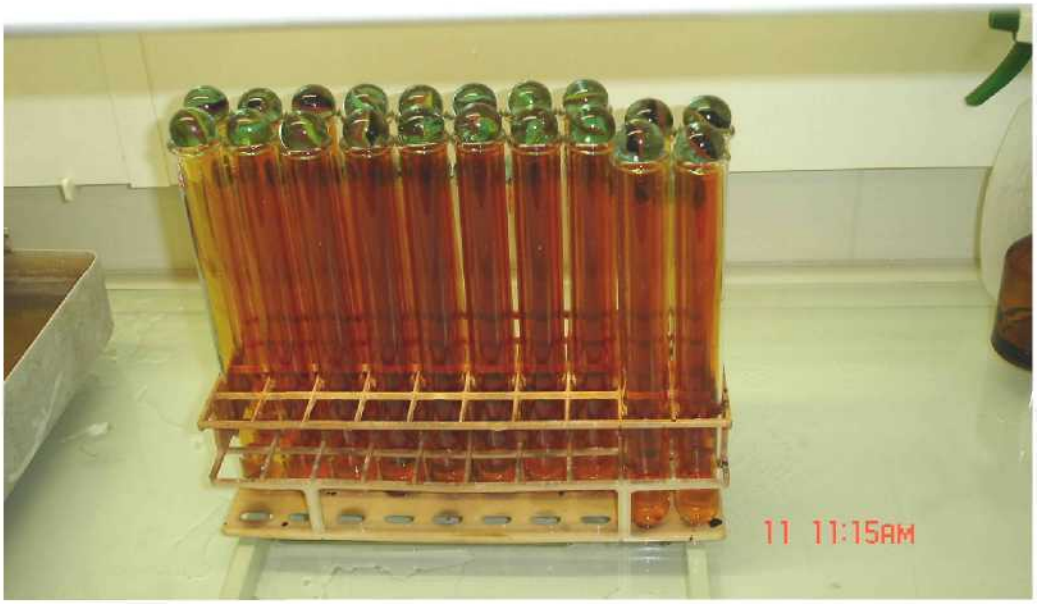
c. Hair sample clamped using a hemostat leaving enough space to cut between the clamp and scalp.

d. The hair sample tied with a thread with proximal ends on one side.



e. Entire hair sample placed into a plastic bag with identification label.

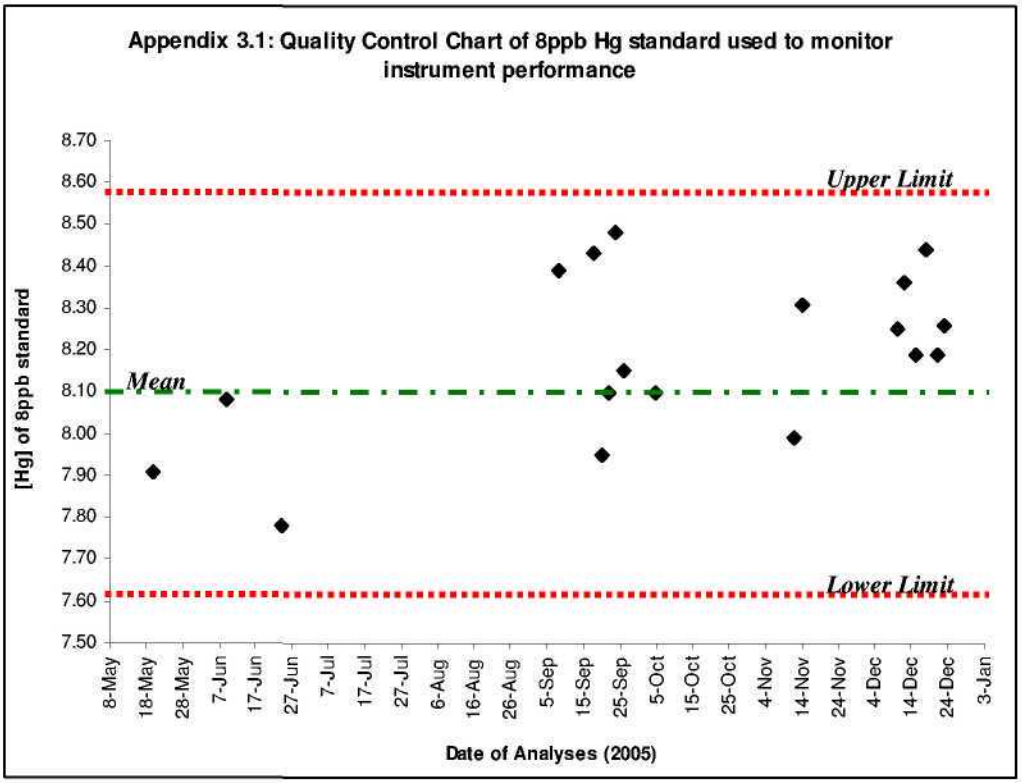
Sample Digestion Pictures



Picture f: Hair samples left to digest overnight in concentrated hydrochloric: nitric: sulfuric acid at ambient temperature

Picture g: Samples digested in a hot water bath at 85-<100 °C under reflux for 60 – 90 minutes

Appendix 3.1: *Quality Control Chart of 8ppb Hg Standard*



Appendix 4.0

University of the South Pacific
Master of Science Research in Chemistry

Human Mercury Exposure in Relation to Fish Consumption in Fiji

Researcher: Maureen Christina Kumar

Result Feedback Form

1. Sample Code: _____

   Date Received: _____ Date Sampled: _____

   Date of Analysis: _____

   Sample Location: _____
2. Laboratory: _____

   Analyst: _____
3. Results and estimated % Uncertainty

   Total Mercury in Hair: _____±_____
4. *Data on Subject*

   Participant Name: _____

   Age: _____ Sex: _____

   Height: _____ Body Weight: _____

   Occupation: _____

   Village/Location: _____
5. Remarks and observations relevant to Result Interpretation.

   _____
   _____
   _____
   _____
   _____

Director (Institute of Applied Science, University of the South Pacific, Suva)

_____

Appendix 5.0

Result Summary for Total Mercury in Fish Tissues

#	Type of Fish	Length (cm)	Weight (kg)	Location	[Hg] µg/g
1	Albacore Tuna	55	13	Lau/Cikobia, Fiji	0.03
2	Albacore Tuna	55	14	Lau/Cikobia, Fiji	0.18
3	Albacore Tuna	65	14	Lau/Cikobia, Fiji	0.27
4	Albacore Tuna	55	11	Lau/Cikobia, Fiji	0.19
5	Albacore Tuna	70	17	Lau/Cikobia, Fiji	0.40
6	Albacore Tuna	70	15	Lau/Cikobia, Fiji	0.27
7	Albacore Tuna	41	10	Lau/Cikobia, Fiji	0.31
8	Albacore Tuna	90	40	Lau Group, Fiji	0.18
9	Albacore Tuna	90	40	Lau Group, Fiji	0.13
10	Albacore Tuna	95	42	Lau Group, Fiji	0.14
11	Albacore Tuna	100	42	Lau Group, Fiji	0.45
12	Albacore Tuna	80	36	Lau Group, Fiji	0.21
13	Albacore Tuna	85	38	Lau Group, Fiji	0.33
14	Albacore Tuna	87.5	39	Lau Group, Fiji	0.40
15	Albacore Tuna	75	30	Lau Group, Fiji	0.28
16	Albacore Tuna	80	34	Lau Group, Fiji	0.22
17	Albacore Tuna	70	17	Koro Island, Fiji	0.18
18	Albacore Tuna	70	16	Koro Island, Fiji	0.39
19	Albacore Tuna	60	17	Koro Island, Fiji	0.69
20	Albacore Tuna	67.5	15	Koro Island, Fiji	0.79
21	Albacore Tuna	60	16	Koro Island, Fiji	0.48
22	Albacore Tuna	55	14	Koro Island, Fiji	0.59
23	Albacore Tuna	60	16	Koro Island, Fiji	0.78
24	Albacore Tuna	65	16	Koro Island, Fiji	0.34
25	Albacore Tuna	55	13	Koro Island, Fiji	1.01
26	Albacore Tuna	60	15	Koro Island, Fiji	0.25
27	Albacore Tuna	87	15	North Lau Group, Fiji	0.07
28	Albacore Tuna	85	12	North Lau Group, Fiji	0.19
29	Albacore Tuna	91	15	North Lau Group, Fiji	0.20
30	Albacore Tuna	85	14	North Lau Group, Fiji	0.28
31	Albacore Tuna	89	15	North Lau Group, Fiji	0.18
32	Yellowfin Tuna	37.5	13	Yasawa/Lau Grp, Fiji	0.05
33	Yellowfin Tuna	37.5	12	Yasawa/Lau Grp, Fiji	0.03
34	Yellowfin Tuna	45	15	Yasawa/Lau Grp, Fiji	0.04
35	Yellowfin Tuna	37.5	14	Yasawa/Lau Grp, Fiji	<0.02
36	Yellowfin Tuna	27.5	10	Yasawa/Lau Grp, Fiji	0.05
37	Yellowfin Tuna	30	13	Yasawa/Lau Grp, Fiji	<0.02
38	Yellowfin Tuna	32.5	12	Yasawa/Lau Grp, Fiji	<0.02
39	Yellowfin Tuna	65	18	Yasawa/Lau Grp, Fiji	0.18
40	Yellowfin Tuna	60	16	Yasawa/Lau Grp, Fiji	0.07
41	Yellowfin Tuna	55	12	Yasawa/Lau Grp, Fiji	<0.02
42	Yellowfin Tuna	95	19	Kadavu&Lau Grp, Fiji	<0.02
43	Yellowfin Tuna	95	15	Vanuatu	<0.02
44	Yellowfin Tuna	98	20	Vanuatu	<0.02
45	Yellowfin Tuna	141	53	Taveuni, Fiji	0.20
46	Yellowfin Tuna	97	16	Lau Group, Fiji	0.40

47	Yellowfin Tuna	48	2.84	Suva Market	<0.02
48	Yellowfin Tuna	89	13	Off Yasawa Grp, Fiji	0.10
49	Yellowfin Tuna	93	15	Off Yasawa Grp, Fiji	0.08
50	Yellowfin Tuna	83	10	Off Yasawa Grp, Fiji	0.05
51	Yellowfin Tuna	90	17	Off Yasawa Grp, Fiji	0.38
52	Yellowfin Tuna	88	12	Off Yasawa Grp, Fiji	0.27
53	Yellowfin Tuna	91	13	Off Yasawa Grp, Fiji	0.19
54	Yellowfin Tuna	84	11	Off Yasawa Grp, Fiji	0.09
55	Yellowfin Tuna	91	13	Off Yasawa Grp, Fiji	0.24
56	Skipjack Tuna	45	2.75	Kiribati (Tarawa Island)	0.03
57	Skipjack Tuna	45	2.75	Kiribati (Tarawa Island)	0.08
58	Skipjack Tuna	45	2.75	Kiribati (Tarawa Island)	0.03
59	Skipjack Tuna	45	2.75	Kiribati (Tarawa Island)	0.05
60	Skipjack Tuna	45	2.75	Kiribati (Tarawa Island)	<0.02
61	Skipjack Tuna	45	2.75	Kiribati (Tarawa Island)	0.04
62	Skipjack Tuna	45	2.75	Kiribati (Tarawa Island)	0.03
63	Skipjack Tuna	45	2.75	Kiribati (Tarawa Island)	0.05
64	Skipjack Tuna	45	2.75	Kiribati (Tarawa Island)	0.08
65	Skipjack Tuna	45	2.75	Kiribati (Tarawa Island)	0.03
66	Skipjack Tuna	47	1.85	Suva Market	0.12
67	Skipjack Tuna	45	1.83	Suva Market	0.16
68	Bigeye Tuna	86	13	Taveuni, Fiji	0.28
69	Bigeye Tuna	120	40	Lau Group, Fiji	0.80
70	Bigeye Tuna	104	32	Lau Group, Fiji	0.52
71	Wahoo	92	6	Kadavu/Lau Group, Fiji	0.17
72	Marlin	216	118	Yasawa Group, Fiji	5.60
73	Marlin	184	78	Unknown	1.22
74	Marlin	145	47	Unknown	1.00
75	Marlin	143	47	Unknown	0.45
76	Marlin	150	47	Unknown	0.54
77	Reef Fish (Kaikai)	16	0.08	Suva Market	0.04
78	Reef Fish (Kaikai)	17	0.09	Suva Market	0.03
79	Reef Fish (Kaikai)	18	0.11	Suva Market	<0.02
80	Reef Fish (Kaikai)	17	0.08	Suva Market	0.05
81	Reef Fish (Kaikai)	18	0.08	Suva Market	0.04
82	Parrot Fish (ulavi)	35	0.98	Suva Market	<0.02
83	Parrot Fish (ulavi)	31	0.51	Suva Market	<0.02
84	Goatfish (mataroko)	28	0.31	Suva Market	0.03
85	Rabbitfish (nuqa)	32	0.50	Suva Market	0.15
86	Peacock cod (Kawakawa)	33	0.62	Suva Market	<0.02
87	Unicornfish (ta)	39	1.07	Suva Market	<0.02
88	Opah	111	65	Kadavu/Lau Grp, Fiji	0.27
89	Barracuda	80	2.64	Suva Market	0.26
90	Barracuda	60	1.23	Suva Market	0.38
91	Barracuda	60	0.98	Suva Market	0.23
92	Barracuda	45	0.41	Suva Market	0.18
93	Mussels (Kai)			Nasinu River	<0.02
94	Mussels (Kai)			Nasinu River	<0.02
95	Mussels (Kai)			Rewa River	0.04
96	Shellfish (kaikoso)			Rewa River	0.05
97	Shellfish (kaikoso)			Rewa River	<0.02
98	Shellfish (kaikoso)			Navua River	0.03

Results Summary for Total Mercury in Canned Tuna and Mackerel

#	Type of Canned Fish	Brand	Packed By	[Hg] µg/g
99	Albacore Tuna Flakes in Oil	Old Capital Special	PAFCO, Fiji	0.19
100	Albacore Tuna Flakes in Oil	Old Capital Special	PAFCO, Fiji	0.18
101	Albacore Tuna Flakes in Oil	Old Capital Special	PAFCO, Fiji	0.21
102	Albacore Tuna Flakes in Oil	Old Capital Special	PAFCO, Fiji	0.19
103	Albacore Tuna Flakes in Oil	Old Capital Special	PAFCO, Fiji	0.16
104	Tuna Flakes (light Meat) in Canola Oil	Ocean Master	Fish Canners (Fiji) Ltd	0.07
105	Tuna Flakes in Canola Oil salt added	Ocean Master	Fish Canners (Fiji) Ltd	0.16
106	Skipjack Tuna Flakes in Oil	Pacific Choice	Product of Thailand	0.07
107	Skipjack Tuna Flakes in Oil	Pacific Choice	Product of Thailand	0.08
108	Skipjack Tuna Flakes in Oil	Pacific Choice	Product of Thailand	0.09
109	Tuna (Light Meat)	Just	Packed for MH's	0.05
110	Albacore Tuna Flakes in Oil	Sun Bell	PAFCO, Fiji	0.27
111	Skipjack Tuna Flakes	Koro Sea	PAFCO, Fiji	0.10
112	Skipjack Tuna Flakes	Koro Sea	PAFCO, Fiji	0.11
113	Skipjack Tuna Flakes	Koro Sea	PAFCO, Fiji	0.10
114	Skipjack Tuna Chunks in Brine	Sun Bell	PAFCO, Fiji	0.06
115	Skipjack Tuna Chunks in Brine	Sun Bell	PAFCO, Fiji	0.07
116	Skipjack Tuna Chunks in Brine	Sun Bell	PAFCO, Fiji	0.07
117	Mackerel in Natural Oil	Just	Packed for MH's	0.22
118	Mackerel in Natural Oil	Just	Packed for MH's	0.22
119	Mackerel in Natural Oil	Just	Packed for MH's	0.22
120	Salmon Style Mackerel in Natural Oil	Sunrise Gold	Fish Canners (Fiji) Ltd	0.27
121	Salmon Style Mackerel in Natural Oil	Sunrise Gold	Fish Canners (Fiji) Ltd	0.27
122	Salmon Style Mackerel in Natural Oil	Sunrise Gold	Fish Canners (Fiji) Ltd	0.29
123	Salmon Style Mackerel in Natural Oil	Ocean	Vo-Ko Industries Ltd, Fiji	0.18
124	Salmon Style Mackerel in Natural Oil	Ocean	Vo-Ko Industries Ltd, Fiji	0.17
125	Salmon Style Mackerel in Natural Oil	Ocean	Vo-Ko Industries Ltd, Fiji	0.17
126	Mackerel in Natural Oil Salt Added	Seaking	Vo-Ko Industries Ltd, Fiji	0.18
127	Mackerel in Natural Oil Salt Added	Seaking	Vo-Ko Industries Ltd, Fiji	0.20
128	Mackerel in Natural Oil Salt Added	Seaking	Vo-Ko Industries Ltd, Fiji	0.21

Results Summary for Total Mercury in Different Fish Steaks

#	Type of Fish Steaks	Diameter of Steak (cm)	[Hg] µg/g
129	Marlin Steaks	10	<0.02
130	Marlin Steaks	23	0.77
131	Marlin Steaks	25	0.79
132	Marlin Steaks	14	0.08
133	Marlin Steaks	15	0.44
134	Marlin Steaks	14	0.39
135	Marlin Steaks	12	0.25
136	Marlin Steaks	23	0.37
137	Marlin Steaks	21	0.39
138	Marlin Steaks	15	0.43
139	Marlin Steaks	11	0.05
140	Marlin Steaks	10	0.13
141	Marlin Steaks	12	0.14
142	Marlin Steaks	26	0.92
143	Marlin Steaks	25	0.79
144	Marlin Steaks	26	0.57
145	Marlin Steaks	23	0.55
146	Marlin Steaks	28	0.87
147	Marlin Steaks	29	1.01
148	Walu Steaks	11	0.07
149	Walu Steaks	10	<0.02
150	Walu Steaks	13	0.67
151	Walu Steaks	10	<0.02
152	Walu Steaks	16	0.87
153	Walu Steaks	11	0.14
154	Walu Steaks	11	0.17
155	Walu Steaks	10	0.11
156	Walu Steaks	9	0.14
157	Walu Steaks	16	0.22
158	Walu Steaks	14	0.17
159	Walu Steaks	13	0.20
160	Walu Steaks	12	0.21
161	Walu Steaks	12	0.21
162	Walu Steaks	13	0.21
163	Walu Steaks	15	0.26
164	Walu Steaks	17	0.30
165	Wahoo Steaks	11	0.05
166	Wahoo Steaks	15	0.12
167	Wahoo Steaks	14	0.07
168	Wahoo Steaks	14	0.06
169	Swordfish Steaks	21	0.99
170	Swordfish Steaks	23	1.37
171	Swordfish Steaks	21	1.07
172	Swordfish Steaks	27	2.81
173	Swordfish Steaks	25	2.79
174	Shark Steaks	17	0.84
175	Shark Steaks	7	0.85
176	Shark Steaks	13	0.85
177	Shark Steaks	15	0.84

178	Shark Steaks	16	0.74
179	Shark Steaks	12	0.76
180	Shark Steaks	19	0.57
181	Mahi Mahi Steaks	20	0.05
182	Mahi Mahi Steaks	22	0.11
183	Mahi Mahi Steaks	22	0.07
184	Sail Fish Steaks	25	0.34
185	Sail Fish Steaks	22	0.32
186	Sun Fish Steaks	22	0.78
187	Sun Fish Steaks	21	0.76
188	Sun Fish Steaks	22	0.78
189	Sun Fish Steaks	22	0.61
190	Sun Fish Steaks	31	0.67
191	Kalia-Blacksnapper Steak	27	0.34
192	Kalia-Blacksnapper Steak	19	0.17
193	Skipjack Tuna Steaks	15	0.16
194	Skipjack Tuna Steaks	15	0.11
195	Skipjack Tuna Steaks	12	0.17
196	Skipjack Tuna Steaks	16	0.12
197	Skipjack Tuna Steaks	15	0.19
198	Crab Meat	15	0.06
199	Crab Meat	14	0.03
200	Crab Meat	11	0.07

Appendix 6.0 Summary of the Total [Hg] in hair and Fish Consumption Frequency Data for the Total Population

Participant ID	Age (yrs)	Body Wt (kg)	gender	Approximate Length of Hair used in analyses (cm)	Frequency of the types of Seafoods Consumed						Total number of fish meals/week	Average total [Hg] in Hair (µg/g)
					Predatory fish	Fresh Tuna	Reef Fish	Canned Mackerel	Canned Tuna	Shellfish (Kai/Kaikoso)		
Kalekana – 001	71	73	M	5	0.5	3	0.5	0	1	0	5	10.84
Kalekana – 002	19	62	M	2	3	0	0	0	3	0	6	5.36
Kalekana – 003	63	87	M	3.5	1	1	0.5	0.5	0.5	0.5	4	8.45
Kalekana – 004	48	60	F	7	0.5	0.5	0.5	0	0	3	4.5	4.28
Kalekana – 005	22	52	F	6	0	0	0	0	1	0	1	3.77
Kalekana – 006	24	92	F	15	0	0	0	0	3	0	3	9.67
Kalekana – 007	47	63	F	12	1	0	0	0	1	1	3	5.47
Kalekana – 008	49	60	F	7	0	0	0	0	0.5	0	0.5	2.65
Kalekana – 009	5	15	M	1	0	0	1	1	0	0	2	1.11
Kalekana – 010	11	20	F	23	1	0	0	0	0.5	7	8.5	2.76
Kalekana – 011	12	27	F	4.5	3	1	1	1	1	0	7	2.90
Kalekana – 012	33	79	M	4	0.5	0.5	1	0	1	0	3	9.74
Kalekana – 013	57	65	F	7	0	0	0	0	0	0	0	5.14
Kalekana – 014	43	75	F	6	0	0	0	0	0	0	0	15.28
Kalekana – 015	5	12	F	7	0	0	0	0	0	0	0	5.17
Kalekana – 016	15	40	F	15	0	1	1	0	0.5	0	2.5	3.12

Participant ID	Age (yrs)	Body Wt (kg)	gender	Approximate Length of Hair used in analyses (cm)	Frequency of the types of Seafoods Consumed						Total number of fish meals/week	Average total [Hg] in Hair (µg/g)
					Predatory fish	Fresh Tuna	Reef Fish	Canned Mackerel	Canned Tuna	Shellfish (Kai/Kaikoso)		
Kalekana – 017	47	60	F	9	0	0	0.5	0	0	0.5	1	4.77
Kalekana – 018	39	80	F	7	3	3	0.5	0	0	0	6.5	14.09
Kalekana – 019	64	75	F	7	0	0	0	0	0	0	0	6.43
Kalekana – 020	52	50	M	2	0.5	0.5	0	0	0	0	1	5.25
Kalekana – 021	9	15	F	5	0	0	1	0	0	0	1	3.29
Kalekana – 022	54	67	F	8	0.5	0	1	0	0.5	0.5	2.5	3.37
Dravuni - 001	69	98	M	2	0.5	0	7	0.5	3	0	10	3.67
Dravuni - 002	48	75	M	2.5	0	1	7	0	0.5	0	8.5	7.08
Dravuni - 003	46	90	M	2.5	1	0	3	0	0	0	4	5.03
Dravuni – 004	32	79	F	5	1	0	3	0	0	0	4	3.80
Dravuni – 005	45	102	F	8	0	1	7	0	0.5	0	8.5	3.53
Dravuni – 006	45	80	M	1	1	0	7	0	0	0.5	8.5	5.16
Dravuni – 007	33	100	M	1.5	0	0	3	1	3	0	7	3.19
Dravuni – 008	39	85	F	6	0	0	14	0	3	0	17	3.37
Dravuni – 009	20	70	F	5	0	0	1	0.5	1	0	2.5	1.19
Dravuni – 010	8	25	F	2.5	0	0	14	0	3	0	17	3.32
Dravuni – 011	30	88	M	2.5	0	0.5	7	3	3	0	13.5	2.80
Dravuni – 012	19	45	F	6	0	1	3	0	3	0	7	1.51
Dravuni – 013	35	72	M	5	1	0.5	7	1	1	0.5	11	5.76

Participant ID	Age (yrs)	Body Wt (kg)	gender	Approximate Length of Hair used in analyses (cm)	Frequency of the types of Seafoods Consumed						Total number of fish meals/week	Average total [Hg] in Hair (µg/g)
					Predatory fish	Fresh Tuna	Reef Fish	Canned Mackerel	Canned Tuna	Shellfish (Kai/Kaikoso)		
Dravuni – 014	45	68	M	6	3	3	7	0.5	0.5	0	14	3.36
Dravuni – 015	31	65	F	2	0	0.5	1	3	3	0	7.5	1.24
Dravuni - 016	39	81	F	8	0	0	3	0	3	0	6	3.60
Dravuni - 017	28	80	M	2.5	1	0	14	3	3	0	17	3.98
Dravuni – 018	24	79	M	4	0	0.5	7	1	1	0	9.5	4.21
Dravuni - 019	32	80	F	6	1	0.5	7	1	1	0.5	11	7.05
Dravuni - 020	25	70	F	5	0	0	7	1	1	0	9	1.87
Dravuni - 021	37	59	F	6	0	0	14	0	0.5	0	14.5	2.94
Daku - 001	52	110	F	7	0	0	7	0	0.5	3	10.5	1.84
Daku – 002	49	96	F	9	0	0	7	0.5	0	0.5	8	1.80
Daku – 003	42	114	F	8	0	0	7	1	0	1	9	1.24
Daku – 004	26	116	F	8	0	0	7	1	0	0.5	8.5	1.33
Daku – 005	19	94	F	6	0	0	7	0	0	1	8	1.34
Daku – 006	22	65	F	8	0	0	3	3	3	1	10	1.46
Daku - 007	34	82	F	7	0	0	14	0	1	0	15	1.56
Muaivuso - 001	49	90	F	7	0.5	0.5	14	0.5	1	1	17	2.74
Muaivuso – 002	20	55	F	8	0	0	7	3	0	0	10	0.89
Muaivuso - 003	79	69	F	8	0	0	1	0.5	0.5	0	2	1.03

Participant ID	Age (yrs)	Body Wt (kg)	gender	Approximate Length of Hair used in analyses (cm)	Frequency of the types of Seafoods Consumed						Total number of fish meals/week	Average total [Hg] in Hair (µg/g)
					Predatory fish	Fresh Tuna	Reef Fish	Canned Mackerel	Canned Tuna	Shellfish (Kai/Kaikoso)		
Muaivuso – 004	43	90	F	4	0.5	0.5	3	0	1	3	8	1.89
Muaivuso – 005	25	95	F	8	0.5	0	7	1	3	0.5	12	2.14
Muaivuso – 006	28	67	F	5	0	0	1	0	3	1	5	1.13
Muaivuso – 007	59	90	F	7	0	0	3	0	0	0	3	1.46
Muaivuso – 008	26	90	F	6	0	0	3	0.5	3	0	6.5	1.80
Muaivuso – 009	55	85	F	8	0	0	3	0	1	0	4	0.93
Muaivuso - 010	72	80	F	8	0	0	0.5	0	0	0	0.5	1.32
Muaivuso – 011	21	74	F	6	0	0	3	14	1	0	17	2.07
Muaivuso – 012	76	67	F	5	0	0	0.5	1	1	0.5	3	0.87
Muaivuso – 013	88	55	F	7	0	0	0.5	1	1	0.5	3	1.37
Muaivuso – 014	64	70	F	8	0	0	3	0	3	0	6	1.19
Muaivuso – 015	46	65	F	6	0	1	3	3	3	0	10	1.42
Muaivuso – 016	35	60	F	8	0	0	1	1	3	0	5	0.47
Muaivuso – 017	71	82	M	2	0	0	14	0	0	0	14	1.75
Muaivuso – 018	19	87	F	7	0	0.5	7	0	1	0	8.5	0.73
Muaivuso – 019	58	85	F	7	0	0	7	1	3	0	11	1.09
Muaivuso - 020	40	76	F	7	0	0	7	0	1	0	8	0.86
Muaivuso - 021	39	85	M	3	0	0.5	0.5	1	1	0	3	3.09

Participant ID	Age (yrs)	Body Wt (kg)	gender	Approximate Length of Hair used in analyses (cm)	Frequency of the types of Seafoods Consumed							
					Predatory fish	Fresh Tuna	Reef Fish	Canned Mackerel	Canned Tuna	Shellfish (Kai/Kaikoso)	Total number of fish meals/week	Average total [Hg] in Hair (µg/g)
Muaivuso – 022	46	95	M	1.3	1	1	1	3	3	0	9	5.64
Suva (USP) - 001	52	74	F	6	0.5	0.5	1	1	3	0.5	6.5	1.15
Suva (USP) – 002	29	50	F	6	0	0	0	0	0.5	0	0.5	0.34
Suva (USP) – 003	43	70	F	6	0	0	1	1	3	0	5	1.46
Suva (USP) – 004	35	74	F	7	0.5	0	0.5	0	1	0	2	0.62
Suva (USP) – 005	42	100	F	5	0	0.5	0.5	1	3	0.5	5.5	2.23
Suva (USP) – 006	25	54	F	8	1	0	0	0.5	0.5	0	2	1.19
Suva (USP) – 007	24	50	F	8	1	0	3	0	1	0.5	5.5	1.27
Suva (USP) – 008	46	92	M	2.5	0	0.5	1	0.5	3	0.5	5.5	5.23
Suva (USP) – 009	43	60	F	7	0.5	0	0.5	0	3	0	4	0.50
Suva (USP) - 010	32	90	M	2	0	0	0.5	0	0.5	0	1	1.65

Control Group

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Appendix 7.0 Summary of the Total [Hg] in hair and the Calculated Amount of Hg Intake from Each Category of Fish for the Total Fish Consuming Population

Participant ID	Age (yrs)	Body Wt (kg)	gender	Approximate Length of Hair used in analyses (cm)	Amount of Hg intake from each fish category per kg body weight per week						Calculated PTWI (µgHg/kg bw/week	Average total [Hg] in Hair (µg/g)
					Predatory fish (530 µg/kg)	Fresh Tuna (270 µg/kg)	Reef Fish (40 µg/kg)	Canned Mackerel (220 µg/kg)	Canned Tuna (140µg/kg)	Shellfish (Kai/Kaikoso) (30 µg/kg)		
Kalekana – 001	71	73	M	5	0.545	1.664	0.041	0	0.288	0	2.54	10.84
Kalekana – 002	19	62	M	2	3.847	0	0	0	1.016	0	4.86	5.36
Kalekana – 003	63	87	M	3.5	0.914	0.391	0.034	0.190	0.034	0.026	1.59	8.45
Kalekana – 004	48	60	F	7	0.663	0.338	0.05	0	0	0.225	1.28	4.28
Kalekana – 005	22	52	F	6	0	0	0	0	0.404	0	0.40	3.77
Kalekana – 006	24	92	F	15	0	0	0	0	0.69	0	0.69	9.67
Kalekana – 007	47	63	F	12	1.262	0	0	0	0.333	0.071	1.67	5.47
Kalekana – 008	49	60	F	7	0	0	0	0	0.175	0	0.18	2.65
Kalekana – 009	5	15	M	1	0	0	0.20	1.10	0	0	1.30	1.11
Kalekana – 010	11	20	F	23	1.988	0	0	0	0.263	0.788	3.04	2.76
Kalekana – 011	12	27	F	4.5	4.417	0.75	0.111	0.611	0.389	0	6.28	2.90
Kalekana – 012	33	79	M	4	0.503	0.256	0.076	0	0.266	0	1.10	9.74
Kalekana – 013	57	65	F	7	0	0	0	0	0	0	0	5.14
Kalekana – 014	43	75	F	6	0	0	0	0	0	0	0	15.28
Kalekana – 015	5	12	F	7	0	0	0	0	0	0	0	5.17
Kalekana – 016	15	40	F	15	0	1.103	0.15	0	0.263	0	1.52	3.12

Participant ID	Age (yrs)	Body Wt (kg)	gender	Approximate Length of Hair used in analyses (cm)	Amount of Hg intake from each fish category per kg body weight per week						Calculated PTWI (µgHg/kg bw/week	Average total [Hg] in Hair (µg/g)
					Predatory fish (530 µg/kg)	Fresh Tuna (270 µg/kg)	Reef Fish (40 µg/kg)	Canned Mackerel (220 µg/kg)	Canned Tuna (140µg/kg)	Shellfish (Kai/Kaikoso) (30 µg/kg)		
Kalekana – 017	47	60	F	9	0	0	0.05	0	0	0.038	0.09	4.77
Kalekana – 018	39	80	F	7	2.981	1.519	0.038	0	0	0	4.54	14.09
Kalekana – 019	64	75	F	7	0	0	0	0	0	0	0	6.43
Kalekana – 020	52	50	M	2	0.795	0.405	0	0	0	0	1.20	5.25
Kalekana – 021	9	15	F	5	0	0	0.20	0	0	0	0.20	3.29
Kalekana – 022	54	67	F	8	0.593	0	0.09	0	0.157	0.034	0.874	3.37
Dravuni - 001	69	98	M	2	0.406	0	0.429	0.168	0.643	0	1.65	3.67
Dravuni - 002	48	75	M	2.5	0	0.54	0.56	0	0.14	0	1.10	7.08
Dravuni - 003	46	90	M	2.5	0.883	0	0.20	0	0	0	1.08	5.03
Dravuni – 004	32	79	F	5	1.006	0	0.228	0	0	0	1.23	3.80
Dravuni – 005	45	102	F	8	0	0.397	0.412	0	0.103	0	0.91	3.53
Dravuni – 006	45	80	M	1	0.994	0	0.525	0	0	0.028	1.55	5.16
Dravuni – 007	33	100	M	1.5	0	0	0.18	0.33	0.63	0	1.14	3.19
Dravuni – 008	39	85	F	6	0	0	0.988	0	0.741	0	1.73	3.37
Dravuni – 009	20	70	F	5	0	0	0.086	0.236	0.30	0	0.62	1.19
Dravuni – 010	8	25	F	2.5	0	0	1.68	0	1.26	0	2.94	3.32
Dravuni – 011	30	88	M	2.5	0	0.230	0.477	1.125	0.716	0	2.55	2.80
Dravuni – 012	19	45	F	6	0	0.90	0.40	0	1.40	0	2.7	1.51
Dravuni – 013	35	72	M	5	1.104	0.281	0.583	0.458	0.292	0.031	2.75	5.76 ± 0.08

Participant ID	Age (yrs)	Body Wt (kg)	gender	Approximate Length of Hair used in analyses (cm)	Amount of Hg intake from each fish category per kg body weight per week						Calculated PTWI (µgHg/kg bw/week	Average total [Hg] in Hair (µg/g)
					Predatory fish (530 µg/kg)	Fresh Tuna (270 µg/kg)	Reef Fish (40 µg/kg)	Canned Mackerel (220 µg/kg)	Canned Tuna (140µg/kg)	Shellfish (Kai/Kaikoso) (30 µg/kg)		
Dravuni – 014	45	68	M	6	□w□□i	□w□□□i	□w□□□i	□w□□□i	□w□□□i	□i	□w□□i	□w□□ii
Dravuni – 015	31	65	F	2	□i	□w□□□ii	□w□□□i	□w□□i	□w□□□i	□i	□w□□	□w□□ii
Dravuni - 016	39	81	F	8	□i	□i	□w□□□i	□i	□w□□□i	□i	□w□□i	□w□□iii
Dravuni - 017	28	80	M	2.5	□w□□□i	□i	□w□□i	□w□□□i	□w□□□i	□i	□w□□i	□w□□ii
Dravuni – 018	24	79	M	4	□i	□w□□□i	□w□□□i	□w□□□i	□w□□□i	□i	□w□□i	□w□□ii
Dravuni - 019	32	80	F	6	□w□□□i	□w□□□i	□w□□□i	□w□□□i	□w□□□i	□w□□□i	□w□□i	□w□□ii
Dravuni - 020	25	70	F	5	□i	□i	□w□□i	□w□□□i	□w□□i	□i	□w□□i	□w□□ii
Dravuni - 021	37	59	F	6	□i	□i	□w□□□i	□i	□w□□□i	□i	□w□□i	□w□□ii
Daku - 001	52	110	F	7	□i	□i	□w□□□i	□i	□w□□□i	□w□□□i	□w□□i	□w□□ii
Daku – 002	49	96	F	9	□i	□i	□w□□□i	□w□□□i	□i	□w□□□i	□w□□i	□w□□ii
Daku – 003	42	114	F	8	□i	□i	□w□□□i	□w□□□i	□i	□w□□□i	□w□□i	□w□□ii
Daku – 004	26	116	F	8	□i	□i	□w□□□i	□w□□□i	□i	□w□□□i	□w□□i	□w□□ii
Daku – 005	19	94	F	6	□i	□i	□w□□□i	□i	□i	□w□□□i	□w□□i	□w□□ii
Daku – 006	22	65	F	8	□i	□i	□w□□□i	□w□□□i	□w□□□i	□w□□□i	□w□□i	□w□□i
Daku - 007	34	82	F	7	□i	□i	□w□□□i	□i	□w□□□i	□i	□w□□i	□w□□i
Muaivuso - 001	49	90	F	7	□w□□□i	□w□□□i	□w□□□i	□w□□□i	□w□□□i	□w□□i	□w□□i	□w□□ii
Muaivuso-002	20	55	F	8	□i	□i	□w□□i	□w□i	□i	□i	□w□□i	□w□□i
Muaivuso - 003	79	69	F	8	□i	□i	□w□□□i	□w□□□i	□w□□□i	□i	□w□□i	□w□□ii

Participant ID	Age (yrs)	Body Wt (kg)	gender	Approximate Length of Hair used in analyses (cm)	Predatory fish (530 µg/kg)	Fresh Tuna (270 µg/kg)	Reef Fish (40 µg/kg)	Canned Mackerel (220 µg/kg)	Canned Tuna (140µg/kg)	Shellfish (Kai/Kaikoso) (30 µg/kg)	Calculated PTWI (µgHg/kg bw/week	Average total [Hg] in Hair (µg/g)
Muaivuso-004	43	90	F	4								
Muaivuso-005	25	95	F	8								
Muaivuso-006	28	67	F	5								
Muaivuso-007	59	90	F	7								
Muaivuso-008	26	90	F	6								
Muaivuso-009	55	85	F	8								
Muaivuso-010	72	80	F	8								
Muaivuso-011	21	74	F	6								
Muaivuso-012	76	67	F	5								
Muaivuso-013	88	55	F	7								
Muaivuso-014	64	70	F	8								
Muaivuso-015	46	65	F	6								
Muaivuso-016	35	60	F	8								
Muaivuso-017	71	82	M	2								
Muaivuso-018	19	87	F	7								
Muaivuso-019	58	85	F	7								
Muaivuso-020	40	76	F	7								
Muaivuso-021	39	85	M	3								

Participant ID	Age (yrs)	Body Wt (kg)	gender	Approximate Length of Hair used in analyses (cm)	□□□□□□i□□i□□i□□□□□□i□□□□i□□□□i□□□□i□□□□□□□□i□□□i□□i □□□□i□□□□□□i□□□i□□□□							
					Predatory fish (530 µg/kg)	Fresh Tuna (270 µg/kg)	Reef Fish (40 µg/kg)	Canned Mackerel (220 µg/kg)	Canned Tuna (140µg/kg)	Shellfish (Kai/Kaikoso) (30 µg/kg)	Calculated PTWI (µgHg/kg bw/week	Average total [Hg] in Hair (µg/g)
Muaivuso-022	46	95	M	1.3	□w□□□i	□w□□□i	□w□□□i	□w□□□i	□w□□□i	□i	□w□□i	□w□□ii
Suva (USP)-001	52	74	F	6	□w□□□i	□w□□□i	□w□□□i	□w□□□i	□w□□□i	□w□□i	□w□□i	□w□□iii
Suva (USP)-002	29	50	F	6	□i	□i	□i	□i	□w□□i	□i	□w□□i	□w□□iii
Suva (USP)-003	43	70	F	6	□i	□i	□w□□□i	□w□□□i	□w□i	□i	□w□□i	□w□□ii
Suva (USP)-004	35	74	F	7	□w□□□i	□i	□w□□□i	□i	□w□□□i	□i	□w□□i	□w□□ii
Suva (USP)-005	42	100	F	5	□i	□w□□□i	□w□□i	□w□□i	□w□□i	□w□□□i	□w□□i	□w□□ii
Suva (USP)-006	25	54	F	8	□w□□i	□i	□i	□w□□□i	□w□□□i	□i	□w□□i	□w□□ii
Suva (USP)-007	24	50	F	8	□w□□i	□i	□w□□i	□i	□w□□i	□w□□□i	□w□□i	□w□□ii
Suva (USP)-008	46	92	M	2.5	□i	□w□□i	□w□□□i	□w□□□i	□w□□□i	□w□□□i	□w□□i	□w□□ii
Suva (USP)-009	43	60	F	7	□w□□□i	□i	□w□□i	□i	□w□□i	□i	□w□□i	□w□□i
Suva (USP)-010	32	90	M	2	□i	□i	□w□□□i	□i	□w□□□i	□i	□w□□i	□w□□iii

APPENDIX 8.0: Linear Regression Analysis Data Summary

Appendix 8.0

8.0 (a) Descriptive Statistics

	Mean	Std. Deviation	N
Total Hg in hair	3.3251	2.8943	82
No. of servings of fresh tuna/week	.2988	.6229	82
No.of servings of reef fish/week	3.6768	4.1391	82
No. of servings of canned tuna/week	1.2683	1.2100	82
No. of servings of shellfish/week	.3598	.9631	82

8.0 (b) Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.494[a]	.244	.205	2.5804	.244	6.226	4	77	.000

a. Predictors: (Constant), No. of servings of shellfish/week, No.of servings of reef fish/week, No. of servings of fresh tuna/week, No. of servings of canned tuna/week

b. Dependent Variable: Total Hg in hair

8.0 (c) ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	165.833	4	41.458	6.226	.000[a]
	Residual	512.701	77	6.658		
	Total	678.533	81			

a. Predictors: (Constant), No. of servings of shellfish/week, No.of servings of reef fish/week, No. of servings of fresh tuna/week, No. of servings of canned tuna/week

b. Dependent Variable: Total Hg in hair

8.0 (d) Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	3.584	.535		6.695	.000
	No. of servings of fresh tuna/week	1.995	.463	.429	4.305	.000
	No.of servings of reef fish/week	-9.33E-02	.070	-.133	-1.340	.184
	No. of servings of canned tuna/week	-.349	.240	-.146	-1.456	.149
	No. of servings of shellfish/week	-.192	.302	-.064	-.637	.526

a. Dependent Variable: Total Hg in hair

Appendix 8.1

8.1 (a) Descriptive Statistics

	Mean	Std. Deviation	N
total hair Hg concentration (ug/g)	1.6017	.9657	29
Number of servings of reef fish/week	4.8966	4.0518	29
Number of servings of canned tuna/week	1.3103	1.2205	29
Number of servings of canned mackerel/week	1.2414	2.6478	29

8.1 (b) Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.267[a]	.072	-.040	.9848	.072	.642	3	25	.595

a. Predictors: (Constant), Number of servings of canned mackerel/week, Number of servings of canned tuna/week, Number of servings of reef fish/week

b. Dependent Variable: total hair Hg concentration (ug/g)

8.1 (c) ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	1.868	3	.623	.642	.595[a]
	Residual	24.245	25	.970		
	Total	26.114	28			

a. Predictors: (Constant), Number of servings of canned mackerel/week, Number of servings of canned tuna/week, Number of servings of reef fish/week

b. Dependent Variable: total hair Hg concentration (ug/g)

8.1 (d) Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.242	.421		2.952	.007
	Number of servings of reef fish/week	1.727E-02	.049	.072	.354	.726
	Number of servings of canned tuna/week	.140	.160	.177	.872	.391
	Number of servings of canned mackerel/week	7.414E-02	.071	.203	1.040	.308

a. Dependent Variable: total hair Hg concentration (ug/g)

Appendix 8.2

8.2 (a) Descriptive Statistics

	Mean	Std. Deviation	N
Total hair Hg concentration (ug/g)	4.8970	3.1290	43
Number of servings of predatory fish/week	.5581	.8878	43
Number of of servings of fresh tuna/week	.4419	.7959	43
Number of servings of reef fish/week	3.5233	4.3493	43

8.2 (b) Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.434[a]	.188	.126	2.9252	.188	3.019	3	39	.041

a. Predictors: (Constant), Number of servings of reef fish/week, Number of of servings of fresh tuna/week , Number of servings of predatory fish/week

b. Dependent Variable: Total hair Hg concentration (ug/g)

8.2 (c) ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	77.502	3	25.834	3.019	.041[a]
	Residual	333.708	39	8.557		
	Total	411.210	42			

a. Predictors: (Constant), Number of servings of reef fish/week, Number of of servings of fresh tuna/week , Number of servings of predatory fish/week

b. Dependent Variable: Total hair Hg concentration (ug/g)

8.2 (d) Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	4.811	.670		7.180	.000
	Number of servings of predatory fish/week	.124	.585	.035	.211	.834
	Number of of servings of fresh tuna/week	1.332	.652	.339	2.043	.048
	Number of servings of reef fish/week	-.162	.104	-.226	-1.558	.127

a. Dependent Variable: Total hair Hg concentration (ug/g)

Appendix 8.3

8.3 (a) Descriptive Statistics

	Mean	Std. Deviation	N
Total [Hg] in Hair (ug/g)	3.3251	2.8943	82
Amount of Hg intake from Fresh tuna/week	.1713	.3635	82
Amount of Hg intake from Reef fish/week	.3041	.3578	82
Amount of Hg intake from Canned Mackerel/week	.2634	.4321	82
Amount of Hg intake from Shellfish/week	4.527E-02	.1453	82

8.3 (b) Model Summary[b]

					Change Statistics				
Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	R Square Change	F Change	df1	df2	Sig. F Change
1	.478[a]	.228	.188	2.6079	.228	5.691	4	77	.000

a. Predictors: (Constant), Amount of Hg intake from Shellfish/week, Amount of Hg intake from Canned Mackerel/week, Amount of Hg intake from Fresh tuna/week, Amount of Hg intake from Reef fish/week

b. Dependent Variable: Total [Hg] in Hair (ug/g)

8.3 (c) ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	154.830	4	38.708	5.691	.000[a]
	Residual	523.703	77	6.801		
	Total	678.533	81			

a. Predictors: (Constant), Amount of Hg intake from Shellfish/week, Amount of Hg intake from Canned Mackerel/week, Amount of Hg intake from Fresh tuna/week, Amount of Hg intake from Reef fish/week

b. Dependent Variable: Total [Hg] in Hair (ug/g)

8.3 (d) Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	3.592	.436		8.245	.000
	Amount of Hg intake from Fresh tuna/week	3.106	.803	.390	3.866	.000
	Amount of Hg intake from Reef fish/week	-1.259	.819	-.156	-1.537	.128
	Amount of Hg intake from Canned Mackerel/week	-1.384	.676	-.207	-2.048	.044
	Amount of Hg intake from Shellfish/week	-1.149	2.012	-.058	-.571	.570

a. Dependent Variable: Total [Hg] in Hair (ug/g)

Appendix 8.4

8.4 (a) Descriptive Statistics

	Mean	Std. Deviation	N
Total hair Hg concentration (ug/g)	1.6017	.9657	29
Calculated amount of Hg intake from reef fish/week	.3506	.2962	29
Calculated amount of Hg intake from canned mackerel/week	.3911	.5333	29

8.4 (b) Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.172[a]	.030	-.045	.9872	.030	.398	2	26	.676

a. Predictors: (Constant), Calculated amount of Hg intake from canned mackerel/week, Calculated amount of Hg intake from reef fish/week

b. Dependent Variable: Total hair Hg concentration (ug/g)

8.4 (c) ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	.776	2	.388	.398	.676[a]
	Residual	25.338	26	.975		
	Total	26.114	28			

a. Predictors: (Constant), Calculated amount of Hg intake from canned mackerel/week, Calculated amount of Hg intake from reef fish/week

b. Dependent Variable: Total hair Hg concentration (ug/g)

8.4 (d) Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	1.509	.331		4.551	.000
	Calculated amount of Hg intake from reef fish/week	-7.41E-02	.635	-.023	-.117	.908
	Calculated amount of Hg intake from canned mackerel/week	.304	.353	.168	.863	.396

a. Dependent Variable: Total hair Hg concentration (ug/g)

Appendix 8.5

8.5 (a) Descriptive Statistics

	Mean	Std. Deviation	N
Total hair Hg concentration (ug/g)	4.8970	3.1290	43
Calculated amount of Hg intake from predatory fish/week	.6604	1.1047	43
Calculated amount of Hg intake from fresh tuna/week	.2647	.4668	43
Calculated amount of Hg intake from reef fish/week	.3261	.4142	43

8.5 (b) Model Summary[b]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.423[a]	.179	.115	2.9429	.179	2.827	3	39	.051

a. Predictors: (Constant), Calculated amount of Hg intake from reef fish/week, Calculated amount of Hg intake from predatory fish/week, Calculated amount of Hg intake from fresh tuna/week

b. Dependent Variable: Total hair Hg concentration (ug/g)

8.5 (c) ANOVA[b]

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	73.446	3	24.482	2.827	.051[a]
	Residual	337.763	39	8.661		
	Total	411.210	42			

a. Predictors: (Constant), Calculated amount of Hg intake from reef fish/week, Calculated amount of Hg intake from predatory fish/week, Calculated amount of Hg intake from fresh tuna/week

b. Dependent Variable: Total hair Hg concentration (ug/g)

8.5 (d) Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	5.167	.654		7.901	.000
	Calculated amount of Hg intake from predatory fish/week	9.109E-03	.454	.003	.020	.984
	Calculated amount of Hg intake from fresh tuna/week	1.956	1.076	.292	1.818	.077
	Calculated amount of Hg intake from reef fish/week	-2.435	1.101	-.322	-2.211	.033

a. Dependent Variable: Total hair Hg concentration (ug/g)

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