HUMAN EXPOSURE TO MERCURY AND OTHER ELEMENTS IN EASTERN CHINA

by

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Abstract

Mercury (Hg) contamination is a global issue due to the neurotoxicity of methylmercury, and China is not an exception due to its increasing industrialization. Fish is of the most concern among food sources, in respect to human exposure to mercury. This is because fish accumulates methylmercury through food chain in aquatic systems. The province of Zhejiang is renowned for its Hangzhou cuisine that incorporates freshwater fish. Qiandao Lake, in Zhejiang, was the site of a case study examining the link between the total mercury (THg) concentration in hair samples and fish consumption. A questionnaire survey and hair sampling were carried out on women of childbearing age (17-46 years) from a fishing town by Qiandao Lake. The average hair THg concentration was 0.76±0.51 μg/g dw. The most-frequently consumed species included four species of carp (golden, bighead, silver and predatory), as well as the Mongolian redfin. Hair THg concentrations accumulated rapidly during younger years, reaching a plateau around age 25, implying that the hair mercury concentrations in adult females >25 years can be interpreted as being from environmental exposure. Hair mercury concentrations were positively correlated both with the frequency of fish consumption and the average amount of weekly fish consumption. This indicates that fish consumption is an important contributor to hair mercury concentrations in the absence of occupational or environmental mercury sources. A positive correlation between molar concentrations of selenium and mercury in hair samples was also observed, suggesting a possible antagonistic relationship between those two elements. This is the first study to look at mercury exposure in an eastern China community dependent on freshwater fish, as opposed to marine fish.

This study also compared element trends (Mg, Ca, Cr, Mn, Cu, Zn, As, Se, Pb) in hair samples from three groups: 50 residents from the fishing town by Qiandao Lake (QD), 17 people from Fudan University, Shanghai, China (FU), and 20 people from Queen’s University, Canada (KI). Trends of mean Mg, Cu, Ca, As concentrations among groups are the same (KI>FU>QD).
Mn, Cr, and Hg share the same trends of QD>FU>KI. Se concentrations follow the trend of QD>KI>FU. Strong correlations between Ca and Mg were observed within each group.

Relationships between Se and Hg are clear in QD but not in FU and KI, probably due to the different dietary proportion of fish.
Co-Authorship

This thesis conforms to the manuscript format as outlined by the School of Graduate Studies and Research. The manuscripts being prepared will be co-authored by others as outlined below:


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List of Acronyms

Mg         magnesium
Ca         calcium
Cr         chromium
Mn         Manganese
Ni         Nickel
Cu         copper
Zn         zinc
As         arsenic
Hg         mercury
MeHg       methylmercury
THg        total mercury
Se         selenium
Cd         cadmium
Pb         Lead
Sc         Scandium
In         Indium
bw         body weight
dw         dry weight
ww         wet weight
CTM        Chinese traditional medicine
EDI        estimated daily intake
EPA        Environmental Protection Agency
FU         Fudan University
KI         Queen’s University
NBNA       neonatal behavior neurological assessments
NOAEL      no observable adverse effects level
QD         Qiandao
Rfd        reference dose
JECFA      Joint Expert Committee on Food Additives and Contaminants
WHO        World Health Organization
Chapter 1

General Introduction

1.1 Background

During the past three decades, concerns about environmental mercury contamination have emerged because of its wide distribution and persistence in the environment, as well as its tendency to accumulate in food chains, with possible adverse effects at the top of food webs, especially for humans.

Mercury poisoning through ingestion of contaminated food has been recognized since the accidental Minamata disease happened in Japan in the 1950s and 1960s. Inhabitants around Minamata Bay appeared to be poisoned by consumption of fish and shellfish containing high concentrations of methylmercury (Harada 1995). Mercuric sulphate, a catalyst, for the industrial production of acetaldehyde between 1932 and 1968 was recently proved to be responsible for the outbreak of Minamata Disease (Veiga and Baker 2004). Three epidemics of mercury poisoning happened in Iraq in 1956, 1960 and 1972, which were confirmed to be caused by accidental consumption of alkylmercury treated bread (Bakir et al. 1973). Mercury poisoning incidents also happened in Canada soon after those two episodes. In 1969, mercury from a pulp and paper mill’s chloralkali plant used for reducing chlorine to bleach paper was discharged into a local river in northwestern Ontario, polluting fish and threatening the health of the local population (Lourie
In the 1970s reservoirs created for hydroelectric power in many parts of Canada like Manitoba, Newfoundland and Ontario were found to have fish with elevated mercury concentrations, due to the release of mercury from the underlying rock and soil by bacterial methylation activities (Lourie 2003). Thus, the gradual mercury contamination in many parts of the world raised concerns. Awareness of the relationship between ecosystem and human health and the distribution of mercury in the environment is also growing world-wide (RCEP 2003).

1.1.1 Mercury

Mercury is a naturally occurring metal which is wide-spread and persistent. It is unique because it is the only liquid metal at room temperature. Liquid mercury is volatile, which means it can vaporize into toxic Hg vapor. Different forms of mercury can be poisonous to ecosystems, wildlife and humans at different concentrations. (Lourie 2003; NRC 2000)

1.1.2 Source of mercury in the environment

Both natural and anthropogenic sources contribute to mercury in the environment. Naturally, mercury is released from soil and rocks through weathering, from volcanoes and forest fires, and is found in lakes and oceans (Health Canada 2007). Certain human activities, such as combustion of fossil fuel, roasting and smelting of ores (mainly cinnabar (HgS)), and deforestation leading to soil erosion and lixiviation (the process of soluble substances in the soil being dissolved in water), can also emit mercury into the environment (Roulet et al. 1999). Natural degassing of the Earth’s
crust combined with emissions from burning fossil fuels is estimated to contribute up to 150,000 t of mercury per year (Plant et al. 2004). Most modern metal mining, which in the past caused releases of natural mercury from ore deposits, is now carried out to extremely high environmental standards minimizing mercury pollution (Plant et al. 2004). The main problem of mercury contamination from mining now is related to artisanal gold mining (Porcella et al. 1997). Dental amalgams also contribute to environmental mercury concentrations through the release of mercury vapor during incineration of dental waste, and also human cremation (Bunnell et al. 2007).

1.1.3 Use of mercury

There are three main forms of mercury: elemental (Hg⁰), inorganic (Hg²⁺), and organic compounds (methyl- and ethyl-mercury). Elemental mercury is widely used in gold mining, in chloralkali plants for production of batteries, switches, fluorescent bulbs, as well as measuring and medical devices such as thermometers. Mercury salt is applied for disinfectants, antimicrobials, and electrical equipment, while ethylmercury is used as part of some fungicides and bactericides (EEN 2006a).

1.1.4 Cycling and bioaccumulation of mercury

When mercury is released into the environment from any source, it becomes highly mobile because of its volatility and cycles among the atmosphere, soil, oceans and biota all around the
world. Anthropogenic activities and natural emission from earth’s surface contribute to mercury emission to the atmosphere (Banic et al. 2005). Mercury existing in the atmosphere, mostly in the form of elemental mercury (Hg⁰), is widely believed to transport over long distance and even over international boundaries (Schroeder and Munthe 1998). Water bodies and land can absorb mercury from the atmosphere due to wet and dry deposition of reactive divalent mercury (Hg²⁺), which is the dominant source of mercury in water. Oceans are also a large source of atmospheric mercury owing to biologically mediated reduction of Hg²⁺ (Parsons 2005). Except for deposition, other main natural sources of mercury in water are weathering of minerals, industrial effluents and non-point pollution sources. Inorganic mercury is the predominant form in soil, sediment, surface water, and atmosphere deposition (Burgess 2005). When mercury in the ocean is transformed to Hg²⁺ it can be methylated and become available for uptake by organisms (Booth and Zeller 2005).

Methylmercury is known to be produced in sediments through an anaerobic microbial process, driven by dissimilatory sulfate-reducing bacteria, which can transform inorganic mercury into its organic form (Benoit et al. 2003; Kerin et al. 2006). Some specific strains of dissimilatory iron-reducing bacteria were also demonstrated to have similar capability of methylating mercury (Kerin et al. 2006). Microscopic phytoplankton at the bottom of aquatic food webs take in methylmercury through biological membranes from surface water and sediment (Watras et al. 1998). Experiment studies on sediment cores and introduced hard clams showed that sediment resuspension could be an important factor that influence transferring sediment methylmercury
into organisms (Kim et al. 2006). Once methylmercury gets into the food web, either aquatic or territorial, it builds up through the food web where it steadily transfers from lower trophic levels to upper trophic levels (Watras et al. 1998), a process known as biomagnification. Organisms also accumulate methylmercury with increasing age because of persist; they take up mercury more quickly than they eliminate it (Burgess 2005). This is known as bioaccumulation. Food exposure provides 90% of mercury bioaccumulation in fish bodies (Hall et al. 1997). As a result of the combined functions of biomagnifications and bioaccumulation, many studies showed that the higher trophic level the organism is the greater concentration of methylmercury it seems to have, particularly for fish in aquatic systems (Dorea et al. 2005). Methylmercury is found at the highest concentrations in the top predators of the marine food web chain, such as swordfish, shark and tuna (Burgess 2005), and gets into human bodies when fish is consumed. In addition, methylmercury possibly poses a lower risk on marine system than freshwater systems because it has a lower degradation rate in oceans than in fresh waters (Whalin et al. 2007).

1.1.5 Health effects

Inhalation of mercury vapor via air and ingestion of methylmercury through food are the main pathways of mercury intake for humans. The former is especially significant for mining and gold shop workers who are directly involved in handling metallic mercury (Veiga and Baker 2004), and people who are exposed to dental fillings (EEN 2006a). Inhalation of mercury can be toxic to the respiratory system. Mercury and related soluble compounds, which are formed from mercury oxidation in the lungs, can inhibit enzyme action (Veiga and Baker 2004), and the oxidized
mercury can easily cross the blood-brain barrier, causing impairment of multiple systems that regulate the exchange of metabolic material between the brain and the blood system. The association of these two dysfunctions will affect the metabolism of the nervous system (Veiga and Baker 2004). Mercury vapor can be completely absorbed into the blood and 90% of the absorption is excreted through urine and feces 2-4 days after exposure (WHO 1991), but the interval is long enough for mercury and its oxidized compounds to accumulate in the central nervous system (Mitra 1986; Veiga and Baker 2004). The threshold limit of acceptable vapor mercury concentration is 50,000 ng/m³ (Zhang and Wong 2007). However, the mechanism of methylmercury poisoning is not completely understood yet. What has been found is that methylmercury causes pathological changes in selective areas of the cerebrum, leading to various dysfunctions like chaemia, impairment of visual acuity, as well as sensory disturbance (Eto et al. 2001; Takeuchi 1999; Veiga and Baker 2004). Major symptoms of Hg vapor and methylmercury exposure are listed in Table 1.1.

In terms of reproductive health, methylmercury is stored in women’s bodies and can 1) cross the placenta; 2) affect the fetus during very vulnerable development stages in pregnancy, 3) be transmitted to fetus during pregnancy (Morrissette et al. 2004), and 4) be transported in breast milk (Bose-O'Reilly et al. 2008), so it potentially harms the fetus and infants, which should raise special concerns for childbearing-age women and their children. In addition, young children are under a greater exposure due to higher dietary intake per kg of body weight (EEN 2006b).
1.1.6 Indicators

Health risk assessments would be usually carried out in one of two ways: bio-indicators (biological materials used to monitor the health of environment) or daily intake assessment based on mercury level in fish and frequency and quantity of fish consumption. Considering bio-indicators, several reliable and accurate methods were developed for mercury measurement in human bodies. The most commonly used are blood, urine and hair (US ATSDR 1999). The toenails were also used as a biomarker for mercury measurement in a few studies (Ohno et al. 2007). According to Yoshida and Yamamura’s research (1982), elemental mercury can be detected in urine shortly after exposure to unusually high levels of mercury vapor. This suggests that urine is a good indicator for acute occupational mercury vapor exposure assessment. Since more than 95% of mercury found in blood is in the form of methylmercury, interference from methylmercury exposure can dominate blood analysis when low concentrations of inorganic mercury is measured (Rahman et al. 2000). Therefore, blood, along with hair, is better used to record non-occupational methylmercury exposure, in particular, from fish. Blood can reflect the average exposure within a 50-70 day time period, which is the half-life of methylmercury in blood, while hair reflects the average amount of methylmercury from the growth period of the segment taken for analysis (Mergler et al. 2007). Maternal and cord blood can be used to measure the fetus’s mercury exposure from mothers and the transmission of mercury from mother to infants (Gill et al. 2002).
In this study, hair will be used as the indicator to assess human exposure to mercury and other elements because 1) hair can be collected in a non-invasive manner; 2) it is easy to store at room temperature and does not require special equipment for transportation; 3) up to 100% of total mercury in hair is in the form of methylmercury which can represent the mercury intake from fish consumption; 4) since the growth of hair per month is commonly 1 cm, the length of the segment of hair taken from the end which is closer to the scalp represents the most recent average exposure in the same time frame.

1.1.7 World-wide health assessment

A comparison of studies on hair mercury concentrations from different parts of the world is shown in Table 1.2. However, it is not easy to compare those values since different testing techniques were used, and high-quality controls were not clearly mentioned in some earlier studies. Nevertheless, some obvious conclusions can still be addressed from the commonalities of these studies. Populations of the highest concern are workers and communities in gold-mining areas, people who consume a lot of fish, fishermen and their families, and women of childbearing-age, and children. Occupational factors are proven to have significant influence on one’s mercury burden (Williams et al. 2000). Communities who live around mining areas were also affected since there is no proper separation between working and housing areas in those mining area, leading to exposure for the whole community. Occupational workers who worked with gold-mining processes and their close-by communities were suffering from elevated mercury concentrations in their bodies in Brazil, Cambodia, Tanzania, Ecuador and Philippines.
The elevated mercury burden was most notably in the Amazon region (Dorea et al. 2005). Some of the people living in gold-mining areas have been observed with clinical symptoms of mercury poisoning (Harada et al. 1999).

Mercury contents in hair may be different due to various food sources (Lee et al. 2000). Many studies, both long-term cohort ones and short-term cross-sectional ones, have confirmed that mercury level in hair is positively correlated to fish consumption quantities, and that populations who have a diet rich in fish tend to have higher mercury concentration (Barbosa et al. 2001; Holsbeek et al. 1996; Johnsson et al. 2004; McDowell et al. 2004; Pinheiro et al. 2005; Sarmani and Alakili 2004). With reference to Table 1.2, Polish people have relatively low mercury concentrations most likely due to its much smaller portions of fish in their diet (Kowalski and Wiercinski 2007). Fishermen and their families in some areas were found to have significantly higher concentrations of mercury than reference populations (Williams et al. 2000; Harada et al. 1999). Methylmercury exposure has seasonal variations, and hair mercury level is related to the type of fish species consumed (Dolbec et al. 2001). For the same population, males are usually found to have higher burdens of mercury than females (Lee et al. 2000; Johnsson et al. 2004; Williams et al. 2000), but in some cases there is no significant variation between males and females. The evidence that women have remarkably decreased concentrations of mercury during and after pregnancy and pregnant women have lower mercury level than non-pregnant ones indicated that mercury can be transmitted from mother to fetus through pregnancy (Morrissette et al. 2004; Pinheiro et al. 2005). Mercury concentrations were found to be positively associated
with serum estrone and estradiol levels, indicating possible induction of female hormones by mercury exposure (Agusa et al. 2007). The evidence provided by those studies should be used critically to raise more concerns regarding specific populations, like fishermen, women and children, which has not happened in some areas around the world, and specific advisories/guidelines should be created for them to avoid further poisoning.

The studies mentioned above usually examined either daily intake of mercury or mercury concentration in bio-indicators. However, it is meaningful to test the relationship between the real mercury level in humans and the estimated intake level to see if the estimation is effective. In one recently published study in Japan, the estimated daily intake of mercury showed a significant correlation with total mercury level in hair, toenails and urine (Ohno et al. 2007), which provides strong support for using daily intake estimation.

1.2 Gaps and problems

Concerns regarding fish consumption as a major source of mercury intake for humans have arisen since the Minamata disease in Japan decades ago. In China, mercury poisoning has been a concern mostly in mining areas where poisoning syndromes have appeared, like north-east and south-west provinces. However, the actual degree of contamination and mercury exposure to humans are still under quantified, especially for the east part of China, despite the obvious importance of the topic. A preliminary study by Prof. Yuxiang Wang (Queen's Biology) and Dr. Linda Campbell raised potential issues relating to fish mercury contamination of Qiandao Lake,
which is the largest freshwater reservoir in Zhejiang province, east China. Since 2005, they have been collaborating on a preliminary study of various Chinese lakes through a class module on metal and mercury contamination in Wang’s annual field course to Zhejiang, China. In brief, the class sampled fish across a series of lakes from Taihu in Shanghai to the Yangtze River near Chongqing. It was predicted that fish from Tai Lake would have elevated mercury and metal concentrations because it is very heavily industrialized with weakly-enforced regulations. However, fish from Qiandao Lake, a remote 50-year old reservoir, had mercury concentrations at least an order of magnitude higher than for fish from Taihu Lake. In fact, many fish from Qiandao significantly exceeded Chinese consumption limits (0.3 μg/g), with many fish having over 1 μg/g mercury. Moreover, according to a dietary patterns survey conducted in Zhejiang Province in 2000, the proportion of fish consumption among women in Zhejiang gradually increased approximately three-fold from 1982 to 2000, with the amount being more than double of the 2000 National goal of ideal fish consumption (Zhang et al. 2002). Fish from Qiandao Lake are highly desirable and popular with human fish consumers, and have a very high social importance in the local culture and tourism industry. Accordingly, those facts have highlighted some areas of concern that require further investigation, such as: 1) Where is the mercury from? 2) Were those fish with high mercury actually from Qiandao Lake? 3) How much fish are regularly consumed by local people, who might eat fish with high mercury? 4) Does the population have a high risk of mercury poisoning? 5) Is their mercury exposure related to fish consumption? The last three questions were addressed step by step in this study.
1.3 Study Objectives and study design

The major objective of this study was to determine if consumption of fish specifically from Qiandao Lake is a potentially important human health risk factor by means of field sample/questionnaire collection and laboratory analysis. A cross-sectional epidemiological field survey focusing on fish consuming habits was conducted at Chun’An, which is a small town located at Qiandao Lake, to collect information on possible mercury exposure. A questionnaire was designed referring to our own preliminary study at Queen’s and various questionnaires used by other scientists (EQI 2008; Feng et al. 2008; Knobeloch et al. 2005; Weihe et al. 2005). Hair samples were collected at the same time from the same individuals who filled in survey questionnaires, reflecting the actual exposure for the test period. Both questionnaires and hair samples were brought back to Canada for laboratory mercury measurement and data analysis. Correlations of questionnaire information and laboratory results were analyzed to measure the impact of fish consumption on mercury concentration for the specific population at Chun’An. In addition, to supplement information on the overall health status to the mercury study, the concentration of eleven other elements (Mg, Ca, Cr, Mn, Ni, Cu, Zn, As, Se, Cd, Pb) in hair samples were measured using an inductively coupled plasma mass spectrometry and element trends between participants in China and Canada were compared.

1.4 Thesis structure

This thesis is composed of five chapters. Chapter 1 is a general introduction of relevant background knowledge of mercury, specific to health perspectives, to provide the necessary
background to understand this study as well as to point out the existing gaps and study objectives. It is followed by a first manuscript focusing on the evaluation of mercury concentration in hair samples from a lakeside county at Qiandao Lake in China and the relationship between age, mercury and fish consumption. Chapter 3 is a second manuscript which contains the measurement of eleven selected elements in hair samples from two regions in China and one region in Canada as well as the comparison of metal trends among these three regions. A literature review of research achievements on the potential human exposure to foodstuff and Chinese traditional medicine and human mercury burdens specifically in China composes in Chapter 4. The review follows after the two manuscripts because the data in the literature were compared with our own data obtained from this study as presented in Chapter 2 and 3. The Chapter 5 summarizes the strengths and limitations of this study with suggestions for future research.
### Tables and Figures

Table 1.1 Health effects of mercury and methylmercury to humans

<table>
<thead>
<tr>
<th>Affected organs</th>
<th>Exposure</th>
<th>Symptoms</th>
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<tbody>
<tr>
<td>Hg vapor</td>
<td>brain</td>
<td>short term: chest pain, dyspnoea, cough, haemoptysis</td>
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<tr>
<td></td>
<td></td>
<td>long term: interstitial pneumonia, chemical bronchitis, pneumonitis</td>
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<td>lung: pulmonary fibrosis</td>
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<td></td>
<td>respiratory tract</td>
<td>long term: fatigue, irritability, loss of memory, vivid dreams, depression, muscular tremors</td>
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<tr>
<td>MeHg</td>
<td>brain</td>
<td>short term: muscular atrophy, mental disturbance</td>
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<td></td>
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<td>long term: reduced visual perception, cerebellar ataxia, disequilibria, impairment of gait and speech, sensory disturbance</td>
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<td>(Holsbeek et al. 1996)</td>
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References

Agusa, T., Kunito, T., Iwata, H., Monirith, I., Chamnan, C., Tana, T. S., et al. (2007). Mercury in hair and blood from residents of Phnom Penh (Cambodia) and possible effect on serum hormone levels. *Chemosphere, 68*(3), 590-596.


Chapter 2

Total mercury in human hair and relation to freshwater fish consumption in an eastern China lakeside community

Abstract

Mercury contamination is a global issue due to the neurotoxicity of methylmercury, and China is not an exception due to increasing industrialization. Fish is considered as important source of mercury exposure for fish-eating population. This study looked at the total mercury (THg) concentration in hair samples from women of childbearing age from a fishing town by the Qiandao Lake, Zhejiang. The province of Zhejiang in eastern China is renowned for its Hangzhou cuisine which incorporates freshwater fish and biota. Fifty females between 17 and 46 years old were included the survey, which also included assessment of dietary habits and inclusion of fish in their diet. The average THg concentrations in hair was $0.76 \pm 0.51 \, \mu g/g$ (0.18-3.04, dry weight), with only one sample exceeding the World Health Organization’s recommended limit for hair (2.0 $\mu g/g$). Within this group, the most-frequently consumed species included four species of carp (golden, bighead, silver and predatory) as well as the Mongolian redfin. Hair THg concentrations accumulated rapidly during younger years, reaching a plateau around age 25, implying that the hair mercury concentrations in adult females $>25$ years can be interpreted as being from environmental exposure, not age-related accumulation. Mercury concentration was positively correlated ($p=0.002$) with the frequency of fish consumption and the average amount of weekly fish consumption. This indicates that fish consumption is an important contributor to hair
mercury concentrations in absence of occupational or environmental Mercury sources. A positive correlation ($p<0.001$) between molar concentrations of selenium and mercury in all hair samples was also observed, suggesting possible antagonistic relationship between those two elements. The estimated daily intake (EDI) of mercury from fish consumption ranged from 0.02 to 0.25 μg/kg.bw. A positive correlation was found between EDI and hair mercury ($p=0.022$), confirming the input of fish consumption on hair mercury concentrations. This is the first study to look at mercury exposure in an eastern China community dependent on freshwater fish, as opposed to marine fish.

**Key words**

Questionnaire, female, age, fish consumption preference, estimated daily intake

**2.1 Introduction**

During the past three decades, global concerns about environmental mercury (Hg) contamination have increased, in part because of its ubiquitous distribution and persistence in the environment as well as its tendency to accumulate in food webs, with possible adverse effects on top predators, especially humans (Parsons, & Percival, 2005). Mercury is neurotoxic, especially in the developing brain (US EPA 2001). Fish consumption has been known to be an important source of mercury to humans globally (Harada, 1995), including China (Cheng et al., 2009) because up to 100% of total mercury in fish is methylmercury (MeHg). In China, research on mercury poisoning has been mostly concentrated in mercury and gold mining areas in the north-east.
provinces and south-west provinces where high mercury loads in humans have been observed, (see Chapter 4). There were also a few studies of eastern coastal areas looking at fish consumption as a source of mercury to humans (Cheng et al., 2009; Gao et al., 2007). However, the actual degree of contamination and mercury exposure to humans in eastern China is still poorly characterized.

The province of Zhejiang in eastern China is renowned for its HangZhou cuisine, which emphasizes freshwater fish and biota (A. Z. Zhang, Wu, & MacLennan, 2004). According to a survey of dietary patterns conducted in Zhejiang Province in 2000, the proportion of fish consumption of women in urban areas in the province of Zhejiang gradually increased three-fold from 1982 (7.9 kg/year) to 2000 (22.3 kg/year), which was more than double the 2000 national goal (9 kg/year) for ideal fish consumption (M. Zhang, Binns, & Lee, 2002). Qiandao Lake, a remote 30-year old reservoir located in the province of Zhejiang is a popular tourist destination and renowned for its beauty and the quality of its fish, and is one of the four major freshwater fish cultivating areas (County annals of Chun’An 1990). Fish, especially bighead carp (Hypophthalmichthys nobilis R.), from Qiandao Lake are regarded as highly desirable and popular, and they play an important role in the local culture and tourism industry. In this study, Qiandao Lake is used as a case study to examine the relationship between fish consumption and human mercury burden in eastern China.
Initial data indicate that fish from Qiandao Lake have mercury concentrations at least an order of magnitude higher than for fish from Tai Lake near Shanghai (Cole, 2007). In fact, many fish from Qiandao Lake significantly exceed Chinese consumption limits (0.3 μg/g), with many fish having over 1 μg/g mercury (Cole 2007). After a local hydropower station was established at Xin’An Jiang in 1959, the whole area below 108 meters in Chun’An formed the 20.9 thousand-acre Xin’An Jiang Reservoir. In 1984, this area was named “Qiandao Lake” by State Council of the People's Republic of China, and is considered as a national landscape and is famous for its scenery (County annals of Chun’An 1990). There are 83 fish species in Qiandao Lake, with the most valuable species including Chinese perch (*Siniperca chuatsi* B.), predatory carp (*Chanodichthys erythropterus* B.), and amur catfish (*Silurus asotus* L.). Women are considered a vulnerable population because mercury can be transmitted to fetuses through the placenta during pregnancy or transported to infants by breast-feeding (Bose-O'Reilly, Lettmeier, Roider, Siebert, & Drasch, 2008; Morrissette et al., 2004). In this study, human hair was used as a bio-indicator to assess mercury exposure and burden in women of child-bearing age (17-46 years). Human hair is known to be an effective indicator for human exposure to mercury through dietary intake because methylmercury in hair is at least 150 orders of magnitude higher than corresponding concentration in blood (Gill, Schwartz, & Bigras, 2002). Methylmercury is the major form of mercury intake from fish, and previous studies have shown that total mercury in hair is about 80-85% methylmercury on average. Hair can be collected easily non-lethally and non-invasively, it is easy to store and transport and can be analyzed using standardized methods. A cross-sectional study including hair sampling and questionnaire surveys were conducted by the investigators, in
order to measure the total mercury concentrations in hair from target subjects and to determine the relationship between fish consumption and mercury burdens in residents who consume fish primarily from Qiandao Lake.

2.2 Methodology

2.2.1 Study design and population

In October 2008, a cross-sectional study design was implemented, including questionnaire surveys and hair sample collection in the Chun’An a lakeside fishing community (population 45,3000). The target subjects were females who had been continuously living in this region for a minimum of 3 months prior to the study. That period was the summer time at the sampling location which probably meant similar availabilities of fish amount and species from the market. The survey was carried out at a local barbershop, where approximately 100 females customers to the barbershop between 17 to 46 years old, were approached, with 50 women agreeing to participate. Time constraints and culture sensitivities prohibiting some people from contributing hair samples, prevented us from collecting a larger sample size. Participants were asked to read an information sheet about the study (translated into Chinese from English and proofread by two Chinese native speakers) and oral consent was obtained before participating. For participants who were not able to read, the investigator read aloud in Chinese and then the questions were answered orally. Each participant was assigned a unique number that corresponded to the questionnaire and hair sample to ensure anonymity throughout the study. Questionnaires were
collected from the same people who donated a hair sample. Hair samples were taken back to Canada for laboratory analysis of mercury and other elements.

The questionnaire was based on various questionnaires used by other scientists (X. Feng et al., 2008; Knobeloch, Anderson, Imma, Peters, & Smith, 2005; Weihe, Grandjean, & Jorgensen, 2005), and refined through a hair sampling study at Queen’s University. Personal information such as age, body weight, career and education level were collected from each participant. Each participant was asked to estimate their frequency of fish-consumption and provide a weekly intake estimate of fish during the past 3 months prior to the survey with the help of pictures representing typical serving portions of fish. The frequency of consumption of specific fish species available from local fish market, such as yellow catfish, bighead carp, silver carp, and chinese perch, were also included in the survey. Note that the consumption of Chinese perch and big-eye perch was combined because subjects could not distinguish the exact species of fish they ate. There were two missing observations for weekly mass of fish consumed because two participants did not fill the answers. In addition, participants were asked about other possibilities that may affect mercury concentrations in hair, hair colour (dying, bleaching), shampoo, hair dryers and skin-whitening (lightening) products, thermometer breakages, and consumption of certain types of Chinese traditional medicines known to include mercury, and 99.5% of responses were obtained in this part.
2.2.2 Hair collection and storage

Single-use nitrex gloves were used when collecting the hair sample with clean stainless steel barber scissors. A bundle of hair strands of approximately 0.5 cm in diameter was isolated by a plastic paper clip, and then cut as closely as possible to the scalp in the occipital region. After cutting, the hair sample was directly placed into a plastic Ziploc bag stored at room temperature before taken back to Queen’s University, Canada.

2.2.3 Laboratory Analysis

Assuming hair growth of 1 cm per month, about 3 cm of each hair sample from the scalp end was used for analysis, which corresponds to the time period of fish-eating habits questioned in the survey (Gill et al., 2002). If the hair was shorter than 3 cm, then the whole sample was used. Hair samples were rinsed with pure acetone and cleaned with ultra-purified water using Milli-Q A10 (Millipore, Molsheim, France) to remove external contaminations. Approximately 0.05 g of hair from each sample was weighed, cut into small pieces and digested with nitric acid and hydrogen peroxide H$_2$O$_2$ (1:1, 5ml) in a MARS Microwave Digestion System (CEM, Matthews, USA) for one hour (digestion temperature: 180°C; time: 15 mins).

Mercury analysis was performed by a cold vapor atomic fluorescence spectrometry (CVAFS; Tekran 2600, Toronto, Canada), based on the US EPA (United States Environmental Protection Agency) Method 1631. Hair mercury was analyzed in batches of 25 samples using a batch-specific standard calibration curve. Daily quality control (QC) included wash station blanks,
calibration blanks, and method blanks, as well as initial precision recovery (IPR, 5.0 pg/g Hg) initially and operation precision recovery (OPR, 5.0 pg/g Hg) after every 10 samples. Duplicate samples (a second aliquot of hair processed through the entire analysis process) were measured every 20 samples and confirmed to be within the range of 20%. Human hair certified reference material CRM 397 (BCR, Brussels, Belgium) was included in every run, and the recoveries ranged from 86%-96% (CRM value 12.3 ± 0.5 μg/g). The sample detection limit (DL) was 0.55 pg/g, ascertained by testing 8 Millipore water samples. The Method DL was 0.69 pg/g, calculated by analyzing 8 method blank solutions which underwent the entire analysis process and the reporting reliable detection limit (RDL) was 1.38 pg/g, but none of the samples fell below the RDL.

2.2.4 Statistical Analysis

Normality was assessed by the Shapiro-Wilk test, with log-10 transformed mercury hair data having a normal distribution. Relationships between mercury, age and fish consumption were tested by Pearson’s correlation coefficient, and simple and multiple regression analyses. One-tailed t-tests were used to analyze whether the groups of subjects who had hair treatment (dying/bleaching, hairdryer, usage of shampoo) and who used skin whitening (lightening) cream had higher mercury concentration than the other groups who did not. One tailed t-test was used to test whether the group of subjects who used skin whitening cream had higher consumption level of fish than the group who did not use the cream, while two-tailed test was used to test the difference of age between these two groups. Regarding the relationship between molar
concentrations of selenium and mercury in hair samples, simple regression analysis was applied because the residual distribution did not display signs of unequal variance or trends even though Se data was not well normally distributed. The same idea applied to the relationship between EDI and hair mercury level. A p-value of less than 0.05 was considered to be statistically significant. Statistical analyses were performed on JMP (version 6.0). Figures were plotted on SigmaPlot (version 11.0).

2.3 Results and Discussion

2.3.1 Characteristics of subjects and concentrations of mercury in human hair

The survey of the 50 participants in this study indicated that all women were between ages of 17 and 46 years, and the education level of the majority of subjects was high school equivalence or lower. 14% of subjects held college degrees and 6% had bachelor degrees. Occupations were diverse, for example, self-employed (small business, n=7), salespeople (n=6), high school students (n=5), office clerk (n=3), waitress (n=2), accountant (n=1), tour guide (n=1) and housewife (n=1), mostly in low and middle income classes.

The hair mercury concentrations ranged from 0.18 to 3.04 μg/g dw (Table 2.1), with the average and standard deviation being 0.76 ± 0.51 μg/g while the median was 0.66 μg/g (Table 2.1). About 68% of the women had mercury concentrations no higher than 1.0 ug/g dw (Figure 2.1), and about 20% of women had mercury concentrations above 1.5 ng/g (Figure 2.1). Only one
participant had mercury concentrations exceeding the WHO’s recommended limit for human hair samples (Figure 2.1). Mean hair mercury was highest among women who consumed fish on a daily basis and for those who consumed >250 g fish / week (Table 2.1).

The US Environmental Protection Agency (EPA) and Health Canada have recommendations on hair mercury concentrations of 1.5 and 6 μg/g, respectively (Morrissette et al., 2004). Normal level and threshold level provided by World Health Organization (WHO) for total mercury in hair are 2.0 μg/g and 10.0 μg/g, respectively (Al-Majed & Preston, 2000). The mercury content for 92% of the subjects in our study was below 1.5 μg/g, and only one subject had hair mercury concentrations over the normal level (2.0 μg/g). This subject (3.04 μg/g hair mercury) consumed, on average, greater than 250 g fish per week. None of the subjects had hair mercury content exceeding the Health Canada recommendation (6 μg/g) nor the threshold level (10 μg/g) recommended by WHO.

All subjects had hair total mercury below the WHO’s “No Observable Adverse Effect Level” (NOAEL, 50 μg/g). The NOAEL value is based on neurotoxicity data from three major poisoning events in Japan and Iraq, indicating the subjects in this study may not be at severe risk of mercury poisoning. Two of 50 subjects were pregnant at the time of survey, and their hair mercury concentrations were 0.55 μg/g and 0.42 μg/g, respectively. Our ability to statistically assess the difference between pregnant and non-pregnant groups is limited, but those two women were among those that have THg less than NOAEL.
The mean value of total hair mercury (0.76 μg/g) from our study group is lower than those reported for other fish-eating female populations around the world, including Swedish women (0.8 μg/g, 32 women), Cambodian women (geometric-mean 5.1 μg/g, 50 women), and women at Rio Negro in Brazil (6.5-32.6 μg/g with a median of 18.3 μg/g, 41 women), but a bit higher than the US cohort (maternal hair mercury 0.55 μg/g, 135 women) where women have lower weekly fish servings (US: our study region=1.2:1.38 fish meals per week) (Agusa et al., 2007; Dorea, Barbosa, Ferrari, & De Souza, 2003; Johnsson, Sallsten, Schutz, Sjors, & Barregard, 2004; Oken et al., 2005). The hair mercury concentrations in our study are even lower than those reported from other areas in China. Hair mercury from 64 women and men in Harbin in the northeastern area of China were observed of elevated mercury concentrations with mean mercury value of 1.69 μg/g (range 0.11-36.36 μg/g) in hair (Q. Y. Feng, Suzuki, & Hisashige, 1998). Hair mercury for 108 men and women from Wujiazhan also in northeastern China had an average of 3.41 μg/g THg (L. Zhang & Wang, 2006). Studies closest to Qiandao Hu in east China were mostly from cities and villages along the coastal areas. Hair mercury for 59 women from Zhoushan, Zhejiang, had an average of 2.3 μg/g (Cheng et al., 2009), higher than seen for a similar number of women in our study.

Since our survey covered only a few occupations mostly of relatively lower social-class, this study is not a cross-section of the entire female population of Chun’An. It is known that women of a higher socio-economic bracket may consume more expensive fish such as yellow catfish and
Chinese perch, which have higher mercury concentrations due to their high trophic position in the lake. In addition, women from fishing families or women who sell fish in local market were not directly included in the study, and these subjects may be more likely to have a higher fish consumption rate and higher mercury exposure. A larger cross-sectional study incorporating the entire socio-economic structure of the community may be helpful to understand the true health risk from mercury exposure from consuming fish. However, our study is the first to assess mercury in hair from people who predominantly consume freshwater fish in eastern China, and our results show that there is a direct relationship between fish consumption and mercury uptake. Monitoring total mercury in fish on a regular basis will help us to protect people from health risk due to exposure to elevated mercury in diet.

2.3.2 Fish consumption preferences

According to our survey, all subjects ate fish in the past 3 months, with 5 people consuming on a daily basis (Figure 2.2). The most-frequently consumed species were golden carp, silver carp, bighead carp, Mongolian redfin, and predatory carp (Figure 2.2), which more than 10% of subjects consumed at least once a week. The next category includes species that 0-10% of subjects consumed at least once a week, such as grass carp, yellow catfish, perch, snake head, Amur catfish and eel (Figure 2.2). All the other species that were reported never to be consumed on a weekly basis were classified as non-frequently consumed species.
Among the first two categories, Mongolian redfin, predatory carp, yellow catfish (0.68 μg/g dw) and snakehead are wild piscivorous species known to have elevated mercury concentrations. Total mercury in bighead carp and grass carp, both detritivores and planktivores from our study region were lower with 0.244 and 0.219 μg/g dw respectively. According to our previous studies, wild fish usually have much higher total mercury concentrations than farmed species (Cole, 2007), implying that further investigation on fish contamination in both wild and farmed species is in need to further compile advisories on which species are safe to consume and the appropriate proportion of consumption.

2.3.3 Mercury and age

Reviewing various studies around the world, the relationship between age and hair mercury appears to be conflicting. In Bangladesh and Kuwait, no correlation was observed between age and hair mercury (Al-Majed & Preston, 2000; Holsbeek, Das, & Joiris, 1996). However, Dorea and his colleagues (2003) found a positive correlation between hair mercury and women’s age in Brazil. In Cambodia, weak positive correlations between mercury and age were seen in males but not in females (Agusa et al., 2007). However, those studies attempted to fit linear regressions to the data. We hereby brought up an hypothesis that mercury may not accumulate in hair linearly as observed in our study, which probably explains the existence of the conflicting results.

The survey asked about average amount of fish consumed weekly in four categories (Grade 1: 0-50g; Grade 2: 50-100g; Grade 3: 100-250g; Grade 4: greater than 250g). Attempting to fit a
linear regression on this data gives a weakly positive regression (log-10 hair THg = -
1.085+0.030(age); n=50, $R^2=0.136$, $p=0.008$). Since the subjects in ‘Grade 2’ category had a good
distribution of age, we used this group to examine the distribution of mercury content as a
function of age and fitted a hyperbola curve into the scattered dots of hair mercury versus age
(Log-10 Hair THg = $-0.093(Age)/(Age-13.930)$; n=22; $R^2=0.328$; $p=0.0053$), as shown in
Figure 2.3. The fitted curve was similar after the outlier was removed (Log-10 Hair THg = $-
0.112(Age)/(Age-13.316)$; n=21; $R^2=0.428$, $p=0.0013$). According to the fitted dose-response
curve shown in Figure 2.3, mercury accumulated rapidly in the 2\textsuperscript{nd} and 3\textsuperscript{rd} decades, but became
steady after age 25. After age 25, the mercury concentration in hair appears to be entirely due to
how much mercury the person is being exposed to through diet, not body burden accumulation
over time. Trends in the other three categories appear to follow the approximate same curve
although data for those groups were insufficient to fit curves.

2.3.4 Mercury and fish consumption

Figure 2.4 shows a positive linear relationship between hair mercury and average weekly
consumption mass (Log-10 Hair THg = $-0.480+0.114($weekly mass$)$; $R^2=0.153$, $p=0.0059$; 95% CI
on b: (-0.681, -0.284); 95% CI on a: (0.036, 0.188)). Multiple-regression models indicate that the
frequency of fish consumption is significantly correlated with hair mercury concentrations, but
less significantly as the average weekly fish consumption mass (Log-10 Hair THg = -
0.782+0.135($frequency$)+0.082($weekly mass$)$; n=48 with two missing observations, $R^2=0.235$, F
ratio = 6.918, $p=0.0024$). Age was also entered in a multiple regression model along with
frequency of fish consumption and average amount of weekly fish consumption, but it was not important at contributing to hair mercury concentrations, which further confirms our previous hypothesis that hair mercury may not accumulate along with age, assuming consistent fish consumption over time.

None of the subjects in our survey indicated that they worked in industrial plants or mining-related activities, and there was no report of chronic elevated occupational or household mercury exposure. This indicates that fish consumption is probably the primary source of mercury exposure for the study group. Our findings were consistent with other studies on fish-eating populations around the world (Al-Majed & Preston, 2000; Cheng et al., 2009; Holsbeek et al., 1996).

2.3.5 Other factors

Skin whitening products from developing countries is a recognized source of chronic mercury poisoning, because some brands contain mercuric chloride (HgCl), although many brands use other whitening chemicals. For example, a woman from Indonesia was reported with severe nephritic syndrome after regular usage of skin whitening cream that contained a mercury level of almost 2000 times the allowable limit (Soo et al., 2003). In our survey, a weak but significant difference of hair mercury concentrations was observed in the two groups of women who used skin whitening products (n=23) and who did not (one-tailed t-test, n=27, p=0.021). There is also a statistically significant difference of average weekly fish consumption (one-tailed t-test, p=0.020), but no difference in age (two-tailed t-test, p=0.187) between those two groups. The difference between those two groups could be possibly owing to both fish consumption usage of skin
whitening products, but the weak correlation indicates that fish consumption may still be the primary source of mercury to the subjects. One-tailed t-tests indicate lack of significance for usage of hair dye, hair dryer or shampoo.

Not include in our survey but deserving of follow-up are two potential mitigating factors: green tea and number of children. Researchers found evidence that tea-drinking group excreted more methylmercury through blood than non-tea drinkers with approximately same levels of fish consumption (Canuel et al., 2006). The numbers of children that the subjects had may also affect the hair mercury concentrations. Evidence showed that women have remarkably decreased concentrations of mercury during and after pregnancy, implying that the mercury burden in the mothers was transmitted to fetus (Morrissette et al. 2004).

2.3.6 Mercury and selenium

Selenium (Se) and Hg are known antagonists in mammalian biota. Selenium is incorporated in Se-glutathione which is able to interact with Hg to form a Hg-Se protein compound. A one-to-one or greater Se: Hg molar ratio is often integrated to mean that detoxification or binding of Hg has taken place, although a lower molar ratio often indicates the possibility of Hg toxicity (Khan & Wang, 2009). Using Se data obtained from a separate ICP-MS method development study which used portions of the same hair samples on ICP-MS (see Chapter 3), we were able to assess the relationship between mercury and selenium. A positive correlation linking log-10 transformed molar concentrations of mercury and selenium (Figure 2.5) was observed for the hair samples (Log-10 molar Se =-6.213+0.198 (Log-10 molar Hg); $R^2=0.305, p<0.0001$). If a 1:1 molar ratio of Se: Hg mean that mercury is biologically inert (Pinheiro et al., 2005), a ratio lower than 1.0, as
the slope ratio in Figure 2.5, may imply that the toxicity of mercury might be partially alleviated by selenium in the study group.

2.3.7 Estimation of daily intake of Hg

Given the increased concern about mercury exposure through fish consumption, estimated daily intake (EDI) assessments were applied to estimate the risk of fish consumption to the study group. For each subject, the estimated average daily consumption amount was computed by dividing the average weekly consumption amount obtained from questionnaires by 7 days. The Chinese consumption limit for fish $0.3 \, \mu g/g$ was used as the assumption of the average mercury concentration in fish species. The body weight of each subject was known from the questionnaire survey. Hg intake from the consumption of fish for each subject ($\mu g/kg\text{.day}$) was estimated by multiplying $0.3 \, \mu g/g$ by average daily consumption amount (g/day) and then dividing by individual body weight.

The EDI in our study ranged from 0.02 to 0.25 $\mu g/kg\text{.bw}$, with a mean value of 0.11 $\mu g/kg\text{.bw}$. The Joint Committee of FAO/WHO also established regulatory guidelines regarding dietary mercury intake. It recommended a provisional tolerable weekly intake (PTWI) of $1.6 \, \mu g/kg \text{ body weight (bw)}$ for methylmercury, equivalent to a daily intake limit of $0.23 \, \mu g/kg\text{.bw}$ (Booth & Zeller, 2005). Based on this criteria, EDI from only one subject exceeded this limit assuming that the average mercury level in fish is $0.3 \, \mu g/g$. However, since their hair mercury concentrations were well below the NOAEL ($50 \, \mu g/g$), it is not currently a health concern. In fact, more precise
estimations of EDI can be made using data of mercury concentration in fish species from the study region, which is currently being carried out by colleagues in our lab. Eleven species, which were consumed most-frequently and moderately, can be selected from the groups of fish. The average mercury concentrations in selected fish species are then estimated from fish mercury level in selected species and the percentage of subjects who reported eating each species, using the methodology developed by Luk and Au-Yeung (Luk & Wai, 2006).

A positive correlation was found between hair mercury in the log scale and EDI (Figure 2.6; Log-10 EDI= -0.983+0.374(Log-10 THg); $R^2=0.109$, $p=0.022$). It confirms that the hair mercury level can be explained by fish consumption. Similar trends between hair mercury and estimated methylmercury intake were found by Holsbeek using data from Liverpool, Papua New Guinea, and the Seychelles and by Kojima and Araki in Japanese people (Holsbeek et al., 1996).

In conclusion, the female residents sampled at the lakeside county of Qiandao Lake had hair mercury concentrations in safe range compared with the NOAEL. Hair mercury concentrations in 98% of the subjects were lower than the normal level set up by WHO, which may not pose a health risk of mercury exposure for now. Mercury did not accumulate with increasing age after 25 years of age. Fish consumption was a major source of mercury exposure in this area. Nevertheless, the sampled subjects may not be representative of the whole population in the study region. Thus, further investigation on fish contamination and human exposure is needed.
### Tables and Figures

Table 2.1 Mercury concentrations in women at Chun’An county in categories of fish consumption frequency and average weekly fish consumption amount (n=50)

<table>
<thead>
<tr>
<th>category</th>
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<th>hair mercury concentration(μg/g)</th>
<th>X ± SD</th>
<th>5-95% CI of mean</th>
<th>range</th>
<th>median</th>
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<tr>
<td>all</td>
<td>50</td>
<td>0.76 ± 0.51</td>
<td>0.61-0.90</td>
<td>0.18-3.04</td>
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<td>Frequency of freshwater fish and sea foods</td>
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<td>0.32-0.66</td>
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<td>once a day</td>
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<tr>
<td>50-100g</td>
<td>22</td>
<td>0.64 ± 0.38</td>
<td>0.48-0.81</td>
<td>0.19-1.89</td>
<td>0.59</td>
<td></td>
</tr>
<tr>
<td>100-250g</td>
<td>13</td>
<td>0.69 ± 0.40</td>
<td>0.45-0.93</td>
<td>0.18-1.59</td>
<td>0.63</td>
<td></td>
</tr>
<tr>
<td>greater than 250g</td>
<td>8</td>
<td>1.33 ± 0.76</td>
<td>0.69-1.97</td>
<td>0.73-3.04</td>
<td>1.12</td>
<td></td>
</tr>
</tbody>
</table>

CI: confidence interval
Figure 2.1 Distribution of total mercury in hair from 50 women of 17-46 years old at Chun’An county
Figure 2.2 Consumption frequency of 16 species represented by the percentage of subjects who consume each species at certain consumption frequency by women of 17-46 years old at Chun’An county (n=50)

A=at least once a week; B=at least once a month; C=at least once a year; D=never; E=have no idea
GO=golden carp; SI=Silver carp; BI=Big head carp; MO=Mongolian refin; PR=predatory carp
GR=grass carp; YE=yellow catfish; SN=snake head; AM=amur catfish; PE=perch; EE=eel;
CH=channel catfish; BL=bluntnose black-bream; ST=sturgeon; BLG=bluegill; SU=sucker
Figure 2.3 Relationship between age and hair mercury by women who consume on average 50-100g fish per week (n=22)
Figure 2.4 Relationship between hair mercury and average weekly consumption amount of fish from women at Chun’An county (n=50)
Figure 2.5 Relationship between mercury and selenium in human hair from women at Chun’An county (n=50)
Figure 2.6 Relationship between EDI and hair mercury from women at Chun’An county (n=50)
References

Agusa, T., Kunito, T., Iwata, H., Monirith, I., Chamnan, C., Tana, T. S., et al. (2007). Mercury in hair and blood from residents of Phnom Penh (Cambodia) and possible effect on serum hormone levels. Chemosphere, 68(3), 590-596.


Chapter 3

Comparison of element trends in human hairs from volunteers in east China and Kingston, Canada

Abstract

This study analyzed metal concentrations in human hair to compare elements trends among different groups of people. Hair samples collected from three different groups, 50 residents at a lakeside county in Qiandao Lake, eastern China (QD), 17 people from Fudan University, Shanghai, China (FU), and 20 people from Queen’s University, Canada (KI). Determination of Mg, Ca, Cr, Mn, Ni, Cu, Zn, As, Se, Cd, Pb was performed by inductively coupled plasma mass spectrometry (ICP-MS). The results showed that the three groups have the same order of concentrations of selected major elements (Ca>Zn>Mg>Cu) but have different orders of other selected elements. Trends of average Mg, Cu, Ca, As concentrations were the same among groups (KI>FU>QD). Mn, Cr, and Hg shared the same trends of QD>FU>KI. Se concentrations followed the trend of QD>KI>FU. Strong correlations between Ca and Mg were observed within each group. Relationships between Se and Hg were clear in QD but not in FU and KI, probably due to the different proportion of fish in diet.

Keywords

ICP-MS, magnesium, calcium, selenium, arsenic, lead, mercury
3.1 Introduction

Essential and non-essential elements in human body have a direct or indirect influence on normal functioning. Magnesium (Mg) are calcium (Ca), are major elements and zinc (Zn), copper (Cu), manganese (Mn), and chromium (Cr) are trace elements that are essential to modulate the proper function of metabolism (Griffith 1995). Conversely, arsenic (As), lead (Pb), and mercury (Hg) are toxic elements and are associated with various clinical symptoms under high body burden. Meanwhile, selenium is essential at low concentrations but toxic at high concentrations. In our study, we measured the metal concentrations of the above mentioned elements in hair samples to compare the elements trends among the selected Chinese and Canadian populations.

The hair samples were from three sources, female residents from a lakeside county at Qiandao Lake in eastern China (QD), students from Fudan University, Shanghai, China (FU), and students and faculty members from Queen’s University in Ontario, Canada (KI). The hair samples from the latter two regions were from females and males with an approximately equal sex ratio. These three regions share the similarity of having fish in their dietary pattern but with different proportion and meal size (Morrissette et al., 2004; Zhang, Binns, & Lee, 2002). At the same time, these three groups have different culture, food source and style, and other exposures.

Inductively coupled plasma mass spectrometry (ICP-MS) has been developed extensively as a simple, rapid, and efficient tool, for determination of element concentrations for blood, urine and hair. Hair can be collected in a non-invasive manner; it is easy to store at room temperature
and it does not require special equipment for transportation. Since the growth of hair per month is commonly 1 cm, the length of the segment of hair taken from the end which is closer to the scalp represents the most recent average exposure in the same time frame. Therefore, hair was used as the bio-indicator for exposure and tested on ICP-MS to present element concentrations in the study group.

3.2 Methodology

During March and October 2008, hair samples were collected from the three study groups. Twenty hair samples were collected from students and faculty members of Queen’s University with a mix of sex (Kingston, Canada). Fifty hair samples of only females were from another study specifically on mercury exposure in eastern China. 17 hair samples were from students from Fudan University with a mix of sex (Shanghai, China). Study ethics, hair collection procedures and the digestion process of samples followed the methods as described in Chapter 2. Determination of Mg, Ca, Cr, Mn, Ni, Cu, Zn, As, Se, Cd, Pb was performed by ICP-MS.

3.2.1 Instrumentation

A Varian 820-MS ICP-MS instrument equipped with collision reaction interface (CRI) was used to analyze the digested hair samples. The sample introduction system consisted of a peristaltic pump, a Micromist nebulizer and a Scott type spray chamber. Hydrogen gas was introduced into the skimmer cone to remove spectroscopic interferences. Scandium (Sc) and Indium (In) were
used as internal standards to correct for instrumental signal drifting over a long running time.

25Mg, 43Ca, 52Cr, 55Mn, 60Ni, 65Cu, 68Zn, 75As, 77Se, 78Se, 111Cd, 206Pb, 45Sc and 115In were the monitored isotopes. The data acquisition was carried out in steady-state, peak-hopping with three points per peak, 10 scans per replicate and 5 replicates per sample and 0.025 a.m spacing. A dwell time of 100,000 µs was configured for Se, Cd and As to increase limit of detection; while the rest of the elements used a dwell time of 10,000 µs. The optimized operating conditions are listed in Table 3.1.

### 3.2.2 Quantification strategies

External calibration was used to assess the elemental concentrations in digested hair samples. Standard metal solutions (1000 µg/ml, 4%HNO₃) for ICP-AES & ICP-MS from SCP Science (Baie D'Urfé, Québec, Canada) were used as the metal source. A series of multi-elemental standard solutions covering the published range of concentration (Goulle et al., 2005) of different elements in hair samples were prepared by sequential dilution with doubly ultra-purified water (Millipore A10, 18.2 MΩ) from the standard metal solutions into desired concentrations. Trace-metal nitric acid (2%) was added to stabilize the metal ions and matrix-match the digested hair samples. The standards were run in increasing concentrations beginning with a blank that has the same constitution except the metal ions.

The calibration curves with blank subtraction for each element were obtained by linear regression. The limit of detection (LOD) was calculated as three times the standard deviation of
blanks and the limit of quantification (LOQ) was determined as ten times the standard deviation of blanks as actually applied detection limit (n = 10, Table 3.2). The accuracy of the zinc (Zn), selenium (Se), cadmium (Cd), mercury (Hg) and lead (Pb) determination in human hair was ensured by using the certified reference material CRM 397 (BCR, Brussels, Belgium; Table 3.3), since this hair reference material only has these elements certified.

3.2.3 Statistical analysis

Descriptive data were used to characterize the metal concentrations of the three study groups. The normality of data was tested by a Shapiro-Wilk Test. As most of the variables did not show a normal distribution and the non-parametric Kruskal-Wallis test (chi-square) was used to test the difference of element concentrations among study groups. The correlation of pairs of elements was examined by the non-parametric Spearman’s test (Spearman’s $\rho$). 0.05 was considered as statistically important. Values less than LOQ were substituted with zero because it was not possible to fabricate less-than values with robust distribution methods due to small sample size and a large proportion of less-than values. Since the same hair samples were also analyzed for mercury in another study, mercury was included in the comparison of this study (see Chapter 2). Statistical analyses were performed on JMP (version 6.0). Figures were plotted on SigmaPlot (version 11.0).
3.3 Results and Discussion

3.3.1 Metal concentrations

LOQ of ICP-MS analysis ranged from 0.088 to 12.17 ng/g depending on the specific element. Compared with Goulle’s study validating reference values of multi-elements with ICP-MS (Goulle et al., 2005), our detection limits for Cr, Mn, Ni, Cu, Zn, Ar, Se, Pb were approximately 10-1000 times lower. All measured values of Mg, Ca, Cr, Mn, Cu, Zn, As, and Se were above LOQ. But 41%, 34.5%, and 21% of Ni, Cd, and Pb were respectively less-than LOQ, as indicated in Table 3.2. These less-than values were substituted to zero. However, Ni and Cd were not compared among groups in the following analysis because of a large proportion of less-than values, which might generate bias.

The mean and standard deviation values of eleven metal concentrations (μg/g, dry weight) in hair samples from three study groups, QD, FU and KI were summarized in Table 3.4. The mean concentration of elements in hair from the three sample regions were following the same order of four major elements: Ca>Zn>Mg>Cu. For the other elements, the order in QD was Cr> As> Se>Hg>Pb>Mn, and the order in FU was Pb>Cr>As>Hg>Se>Mn while the order in KI was As>Se>Pb>Hg>Mn>Cr.

Compared with other studies which analyzed multi-elements, the exact concentrations of those elements vary among studies (Goulle et al., 2005; Moreda-Pineiro et al., 2007; Rodrigues, Nunes, Batista, De Souza, & Barbosa, 2008), when dealing with different populations. That might
be due to different inhalation and ingestion possibilities, such as dietary pattern, source of food and drinking-water, as well as occupational exposures. However, the range of each elements from QD, FU, KI in our study fell into approximately the same order of magnitude.

Qin recommended upper limits for Pb, As, and Hg for Chinese people based on a previous study on body burden of metals and estimation of dietary intake (Qin, 2004), which was 10.0, 1.0, and 1.5 μg/g respectively. Pb concentrations of all analyzed hair samples from FU and QD were below the limit. Though 7.5% of measured mercury concentrations were over Qin’s limit, 98.5% of them were still under the normal level (2 μg/g) recommended by WHO (Al-Majed & Preston, 2000). That means the sampled subjects were still with a safe range of Pb and Hg concentrations. However, there is concern for several subjects with elevated As burden in QD and FU groups. The highest concentration of As was observed of 25.95 μg/g as compared to Qin’s limit. Another study from Taiwan analyzed Ca, Cu, Mg, Zn in females of similar age range as our study (Wang, Chang, Zeng, & Lin, 2005). The mean Ca concentrations of QD and FU in our study were approximately 2-3 times lower than the Taiwan adults females, and Mg concentration were approximately 3-6 times lower, while Cu and Zn were roughly in the same range. Accordingly, the metal concentrations may differ greatly in different populations. In our study, since the technique of ICP-MS on monitoring metal in human hair has been proven to be an effective tool in many studies (Gouille et al., 2005) and the results of quality assurance parameters in our study were also good, the measurement of the ten elements (Mg, Ca, Cr, Mn, Cu, Zn, As, Se, Pb) can serve as a reference range of multi-elements in hair but only for people from the same region and
possibly with similar dietary patterns and occupational exposures.

### 3.3.2 Comparison of elements among groups

Median, 25-75% quartile range, error bars and outliers within 5-95% percentile of each element among study groups are shown by grouping charts of elements with similar trends (Figure 3.1, 3.2). Other elements with different trends were given together in Figure 3.3.

According to the results of Kruskal-Wallis test, similar trends of elements compared among groups were found (Chi-square and p-value shown in Table 3.5). There is a significant difference among KI, QD and FD for Mg, Ca, Cu and As, and KI is significantly higher from QD and FU with no difference between QD and FU. Significant difference was observed for Mn, Cr, Hg with the same order of median as QD>FU>KI, and Se with an order of median as QD>KI>FU. As for Pb, there was no significant difference among three study groups. Concentrations of Zn are similar among groups. The results of Kruskal-Wallis test was also compared with the results of ANOVA and only a difference was observed for Pb. The results by ANOVA indicated that Pb from FU was significantly higher than QD and KI with no difference between QD and KI.

The differences of Mg, Ca, Cu among groups are probably due to different dietary patterns and intake of mineral supplements. Compared to powdered skim milk, small fish have five fold more Cu, two fold higher Ca, and two orders of magnitudes more of Zn, and Mn (Larsen, Thilsted, Kongsbak, & Hansen, 2000). Zinc concentrations are known to be significantly higher
in protein-rich food like fish than in low-protein food (Terres et al., 2001). Therefore, fish is possibly playing important roles on the concentration of these elements. Fish is a primary source of selenium and mercury (Dorea, Moreira, East, & Barbosa, 1998), the trends of these two elements in our study may be explained by the different amount of fish consumption and the mercury concentrations in fish mostly consumed. Economic fish sampled in Shanghai were reported of low concentration of mercury (see Chapter 4). However, since there was insufficient information on the dietary pattern, mercury measurements on major dietary sources, and occupational exposures among FU and KI group, it is impossible to identify the exact causes of the mercury and selenium trends.

The arsenic concentrations in the KI group is fairly high, with 100% over the normal range (0.08-0.25 μg/g) (Saad & Hassanien, 2001). Industrial exposure, food and beverage, and groundwater are the primary sources of arsenic to humans, while groundwater is considered a serious problem in many countries all over the world. Compared with other regions of the world, Canada has a relatively high concentration of arsenic (2.5%) in its native silver and nickel-cobalt arsenide bearing deposit (Mandal & Suzuki, 2002). Arsenic poisoning happened in Ontario and Nova Scotia, Canada, decades ago due to inhalation of contaminated air from lead smelters and the usage of well water containing large amounts of arsenic, respectively (Mandal & Suzuki, 2002). Elevated arsenic concentrations were also observed in municipal water supplies recently in British Columbia, Canada (Mandal & Suzuki, 2002). The usage of certain types of arsenic-containing insecticides and herbicides may also spread the arsenic in the environment. The
exposure pathway of KI group were not clear in this study and further investigation on arsenic concentrations in those sources are needed.

### 3.3.3 Correlations between two elements

In order to test the relationship between pairs of analyzed elements, the Spearman’s $\rho$ and corresponding p-value were given in Table 3.6. Positive correlation was found between Mg-Ca, Cr-Hg, Cr-Mn, Cu-Mg, Zn-Mg, Ca-Zn, Cu-As, Se-Hg, Se-Cr. Negative correlation was also found between Mg-Hg, Cu-Hg, Cu-Cr, Cu-Mn, As-Hg, Se-Mg, Se-Cu, Pb-Hg.

The correlations of metal pairs found above by Spearman’s test were considering all the data from the three study regions as a whole group, we were also interested in learning if the same relationships between metal pairs also exist within each study group. Mg-Ca, Se-Hg, and Ca-Zn are taken as examples because of their strong correlations. The positive trends between Mg and Ca observed overall were also seen within each study group but with different slopes (Figure 3.4), indicating the ubiquity of the relationship.

Se and Hg are known antagonists in mammalian biota and Se can reduce the toxicity of Hg (Dorea et al., 1998). A one-to-one or greater Se: Hg molar ratio is often assumed that detoxification or binding of Hg has taken place, although a lower molar ratio often indicates the possibility of Hg toxicity (Khan & Wang, 2009). Therefore, Se and Hg concentrations in our study were transformed into molar numbers per gram hair. The linear trend was clear in QD but
the trend was not so clear in FU and KI (Figure 3.5). This is possibly because the proportion of fish in the subjects’ diet vary as Se and Hg in hair are known to be mostly from dietary intake.

The relationship of Ca-Zn were different among three study groups (Figure 3.6), even though statistical correlation was observed when considering all data as a whole group, implying that there in fact may not be a relation between Ca and Zn. The same idea may apply to the other metal pairs which are not further investigated in our study.

3.4 Conclusion

In our study, the concentration of Mg, Ca, Cr, Mn, Cu, Zn, As, Se, Pb were measured and the trends of those elements among three study groups were discussed. The measured concentrations were mostly in agreement with other studies but only As in KI is rather high compared with literature reports. The results showed that the three groups have the same order of concentrations of selected major elements (Ca>Zn>Mg>Cu) but with a different order of other selected elements. Trends of average Mg, Cu, Ca, As concentrations are the same among groups (KI>FU>QD). Mn, Cr, and Hg share the same trends of QD>FU>KI. Se concentrations follow the trend of QD>KI>FU. Strong correlations between Ca and Mg were observed within each group. Relationships between Se and Hg are clear in QD but not in FU and KI, probably due to the different importance of fish in diet.
Tables and Figures

Table 3.1 Optimized operating conditions for elemental analysis of digested hair samples on Varian 820-ICP-MS

<table>
<thead>
<tr>
<th>Flow Parameters (L min⁻¹)</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma Flow</td>
<td>18.0</td>
</tr>
<tr>
<td>Auxiliary Flow</td>
<td>1.80</td>
</tr>
<tr>
<td>Sheath Gas</td>
<td>0.15</td>
</tr>
<tr>
<td>Nebulizer Flow</td>
<td>0.90</td>
</tr>
<tr>
<td>Sampling Depth (mm)</td>
<td>6.8</td>
</tr>
<tr>
<td>RF Power (kW)</td>
<td>1.40</td>
</tr>
<tr>
<td>Pump Rate (mL min⁻¹)</td>
<td>0.5</td>
</tr>
<tr>
<td>Spray chamber temperature</td>
<td>3 °C</td>
</tr>
</tbody>
</table>

Table 3.2 Hair multi-element analytical validation (ng/g)

<table>
<thead>
<tr>
<th></th>
<th>R² for calibration curve</th>
<th>LOD</th>
<th>LOQ</th>
</tr>
</thead>
<tbody>
<tr>
<td>magnesium</td>
<td>1.0000</td>
<td>0.17</td>
<td>0.55</td>
</tr>
<tr>
<td>calcium</td>
<td>1.0000</td>
<td>3.68</td>
<td>12.27</td>
</tr>
<tr>
<td>chromium</td>
<td>1.0000</td>
<td>0.006</td>
<td>0.021</td>
</tr>
<tr>
<td>manganese</td>
<td>0.9999</td>
<td>0.002</td>
<td>0.005</td>
</tr>
<tr>
<td>nickel</td>
<td>0.9945</td>
<td>0.18</td>
<td>0.61</td>
</tr>
<tr>
<td>copper</td>
<td>0.9999</td>
<td>0.072</td>
<td>0.24</td>
</tr>
<tr>
<td>zinc</td>
<td>1.0000</td>
<td>0.026</td>
<td>0.088</td>
</tr>
<tr>
<td>arsenic</td>
<td>0.9972</td>
<td>0.055</td>
<td>0.18</td>
</tr>
<tr>
<td>selenium</td>
<td>1.0000</td>
<td>0.028</td>
<td>0.092</td>
</tr>
<tr>
<td>cadmium</td>
<td>0.9999</td>
<td>0.0007</td>
<td>0.002</td>
</tr>
<tr>
<td>lead</td>
<td>0.9982</td>
<td>0.003</td>
<td>0.010</td>
</tr>
</tbody>
</table>
### Table 3.3 Analysis results for certified reference material CRM 397 (μg/g, dw)

<table>
<thead>
<tr>
<th>Element</th>
<th>Certified Value (dw, ppm)</th>
<th>Test Value (dw, ppm)</th>
<th>Instrument</th>
</tr>
</thead>
<tbody>
<tr>
<td>zinc</td>
<td>199 ± 5</td>
<td>197.7 ± 3.8</td>
<td>ICP-MS</td>
</tr>
<tr>
<td>selenium</td>
<td>2.00 ± 0.08</td>
<td>2.24 ± 0.09</td>
<td>ICP-MS</td>
</tr>
<tr>
<td>cadmium</td>
<td>0.521 ± 0.024</td>
<td>0.525 ± 0.022</td>
<td>ICP-MS</td>
</tr>
<tr>
<td>lead</td>
<td>33.0 ± 1.2</td>
<td>38.3 ± 1.2</td>
<td>ICP-MS</td>
</tr>
</tbody>
</table>

### Table 3.4 Mean and standard deviation of elements in hair samples from China and Canada (n=87, μg/g, dw)

<table>
<thead>
<tr>
<th>Element</th>
<th>Mean ± SD</th>
<th>QD (n=50)</th>
<th>FU (n=17)</th>
<th>KI (n=20)</th>
<th>Reference Range (μg/g) (Goule et al., 2005)</th>
<th>Normal Threshold for Chinese People (Qin, 2004)</th>
</tr>
</thead>
<tbody>
<tr>
<td>magnesium</td>
<td>40.0 ± 35.2</td>
<td>40.1 ± 22.3</td>
<td>69.9 ± 48.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>calcium</td>
<td>620 ± 447</td>
<td>421 ± 249</td>
<td>917 ± 604</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>chromium</td>
<td>2.81 ± 3.40</td>
<td>1.37 ± 4.04</td>
<td>0.17 ± 0.14</td>
<td>0.11-0.52</td>
<td></td>
<td></td>
</tr>
<tr>
<td>manganese</td>
<td>0.41 ± 0.24</td>
<td>0.49 ± 0.61</td>
<td>0.19 ± 0.36</td>
<td>0.016-0.57</td>
<td></td>
<td></td>
</tr>
<tr>
<td>nickel</td>
<td>0.89 ± 1.20</td>
<td>70% less-than</td>
<td>45% less-than</td>
<td>0.08-0.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>copper</td>
<td>9.98 ± 2.18</td>
<td>13.40 ± 4.53</td>
<td>51.12 ± 43.75</td>
<td>9.0-61.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>zinc</td>
<td>196 ± 89</td>
<td>178 ± 47</td>
<td>219 ± 119</td>
<td>129-209</td>
<td></td>
<td></td>
</tr>
<tr>
<td>arsenic</td>
<td>1.67 ± 1.81</td>
<td>1.19 ± 1.37</td>
<td>4.97 ± 2.20</td>
<td>0.03-0.08</td>
<td></td>
<td></td>
</tr>
<tr>
<td>selenium</td>
<td>1.00 ± 0.13</td>
<td>0.52 ± 0.07</td>
<td>0.75 ± 0.15</td>
<td>0.37-1.37</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cadmium</td>
<td>0.04 ± 0.04</td>
<td>41% less-than</td>
<td>90% less-than</td>
<td>0.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mercury</td>
<td>0.76 ± 0.51</td>
<td>0.58 ± 0.54</td>
<td>0.37 ± 0.37</td>
<td>1.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>lead</td>
<td>0.42 ± 0.57</td>
<td>1.50 ± 1.82</td>
<td>0.57 ± 0.80</td>
<td>0.13-4.57</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.5 Test results of comparison of concentrations of eleven elements in hair among QD, FU, KI groups (n=87)

<table>
<thead>
<tr>
<th>Element</th>
<th>Chi square</th>
<th>p-value</th>
<th>order of location</th>
<th>R square</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>magnesium</td>
<td>11.6010</td>
<td>0.003</td>
<td>KI&gt;Fu=QD</td>
<td>0.1076</td>
<td>0.0084</td>
</tr>
<tr>
<td>calcium</td>
<td>10.6762</td>
<td>0.0048</td>
<td>KI&gt;FU=QD</td>
<td>0.1174</td>
<td>0.0053</td>
</tr>
<tr>
<td>chromium</td>
<td>47.8329</td>
<td>&lt;0.0001</td>
<td>QD&gt;FU&gt;KI</td>
<td>0.1148</td>
<td>0.0060</td>
</tr>
<tr>
<td>manganese</td>
<td>21.3978</td>
<td>&lt;0.0001</td>
<td>QD&gt;FU&gt;KI</td>
<td>0.0831</td>
<td>0.0262</td>
</tr>
<tr>
<td>copper</td>
<td>47.8094</td>
<td>&lt;0.0001</td>
<td>KI&gt;FU=QD</td>
<td>0.4049</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>zinc</td>
<td>2.8397</td>
<td>0.2417</td>
<td>QD=FU=KI</td>
<td>0.0224</td>
<td>0.3869</td>
</tr>
<tr>
<td>arsenic</td>
<td>28.6952</td>
<td>&lt;0.0001</td>
<td>KI&gt;FU=QD</td>
<td>0.3919</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>selenium</td>
<td>56.3279</td>
<td>&lt;0.0001</td>
<td>QD&gt;KI&gt;FU</td>
<td>0.7173</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>mercury</td>
<td>17.3319</td>
<td>0.0002</td>
<td>QD&gt;FU&gt;KI</td>
<td>0.1018</td>
<td>0.0110</td>
</tr>
<tr>
<td>lead</td>
<td>5.6490</td>
<td>0.0593</td>
<td>QD=FU=KI</td>
<td>0.1560</td>
<td>0.0008</td>
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Table 3.6 Spearman’s $\rho$ and corresponding p-value between pairs of elements

<table>
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<tr>
<th></th>
<th>Ca</th>
<th>Cr</th>
<th>Mn</th>
<th>Cu</th>
<th>Zn</th>
<th>As</th>
<th>Se</th>
<th>Hg</th>
<th>Pb</th>
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<tbody>
<tr>
<td>Mg</td>
<td>0.8185</td>
<td>-0.1193</td>
<td>0.0071</td>
<td>0.3528</td>
<td>0.4718</td>
<td>0.1687</td>
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<tr>
<td>p=</td>
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<td>0.2711</td>
<td>0.9480</td>
<td>0.0008</td>
<td>&lt;0.0001</td>
<td>0.1273</td>
<td>0.0133</td>
<td>0.0232</td>
<td>0.0989</td>
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<td>Ca</td>
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<td>0.0357</td>
<td>0.1275</td>
<td>0.4154</td>
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<tr>
<td>p=</td>
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<td>0.7428</td>
<td>0.2393</td>
<td>&lt;0.0001</td>
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<td>0.8793</td>
<td>0.3347</td>
<td>0.3991</td>
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</tr>
<tr>
<td>Cr</td>
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<td>0.0136</td>
<td>-0.1853</td>
<td>0.4151</td>
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<td>Mn</td>
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<td>0.0813</td>
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</table>
Figure 3.1 Variability of Mg (a), Ca (b), Cu (c) and As (d) with similar trends in three study groups (QD: n=50, FU: n=17; KI: n=20)
Figure 3.2 Variability of Mn (a), Hg (b) and Cr (c) with similar in three study groups (QD: n=50, FU: n=17; KI: n=20)
Figure 3.3 Variability of Zn (a), Se (b), Pb (c) with different trends among three study groups
(QD: n=50, FU: n=17; KI: n=20)
Figure 3.4 Correlation of Mg-Ca within three study groups (QD: n=50, FU: n=17; KI: n=20; μg/g)
Figure 3.5 Correlation of Se-Hg within three study groups (QD: n=50, FU: n=17; KI: n=20; mol/g)
Figure 3.6 Correlation of Ca-Zn within three study groups (QD: n=50, FU: n=17; KI: n=20; μg/g)
References


Chapter 4

Review of foodstuffs and medicines as a source of mercury to humans and health assessment in China

Abstract

Mercury, a commonly known neurotoxin, is able to cause a series of adverse effects on humans under acute high exposure or long-term exposure. Based on literature in the past decade, this article focuses on the status of mercury contamination in foodstuff, especially fish, and Chinese traditional medicine, compiles existing knowledge on mercury burdens in human bodies within China, and identified data limitations and knowledge gaps. Mercury concentrations in most freshwater fish were below the maximum allowable limit set by Ministry of Health of China (0.3 ug/g), with a few exceptions, such as some specific species from northeastern China and Guizhou province. Among other investigated aquatic foods, only riversnails were reported to have slightly excess mercury. No significant mercury contamination was found in cereals and other foodstuff which compose major parts of people’s diet in Urumchi (Sinkiang province) and Qingdao (Shandong province), except for duck eggs from Zhaoyuan (Heilong Jiang province). With respect to human exposure to mercury, sampled individuals contained lower hair mercury concentration than the threshold level recommended by WHO (10.0 ug/g), with a few exceptions in the northwestern China. In terms of the two most concerned areas in China, the mercury concentrations in both fish and human showed a corresponding decline in northeastern China, while rice, instead of fish, was found to place a significant role of mercury intake in Guizhou,
southwest China. Relationship between maternal individuals and newborns were not well studied within China. More research needs to be carried out to form a whole picture of mercury contamination in China, especially more efforts from human health perspectives.

**Key words**

Methylmercury, fish, aquatic food, cereals, estimated daily intake, hair mercury, China

### 4.1 Introduction

Mercury (Hg) is a naturally occurring metal that cycles among the atmosphere, soil, oceans and biota all around the world. Over the past thirty years, concerns about environmental mercury contamination have emerged due to wide distribution and persistence in the environment. In particular, methylmercury (MeHg) is the form that is most toxic. It has the greatest potential to affect the central nervous system and can cause cardiovascular disease with long-term exposure (EEN, 2006). In addition, mercury bioaccumulates in food chains as methylmercury. Biomagnification of methylmercury to elevated concentrations pose potential adverse effects to animals and humans at the top of the food chain. Mercury toxicity through ingestion of contaminated food was firstly recognized in the Minamata incidents in Japan in the 1950s and 1960s (Harada, 1995).

Since mercury has been widely utilized in both industrial and household products, humans are exposed to it in a variety of ways. The most common forms are elemental mercury, also known as
mercury vapor, and methylmercury. Elevated exposure to elemental mercury vapors from ambient air was common for people working in the environment with high mercury concentrations, known as occupational exposure (Health Canada, 2007). Occupational exposure was reported for chloralkali plants, mercury mines, mercury-based gold extraction, thermometer factories, dental clinics with poor mercury handling practices and production of mercury-based chemicals (US ATSDR, 1999). Non-occupational sources of human exposure include applications of dental fillings, vaccines containing thimerosal, or leaching from mercury-containing products (Clarkson, Magos, & Myers, 2003; Marques, Dorea, Fonseca, Bastos, & Malm, 2007). In terms of methylmercury exposure, diet including drinking water and food are key exposure routes considered for the general population (UNEP, 2002). Seafood, especially fish, can be the primary dietary source of non-occupational mercury intake for the general population (WHO, 2004). Mercury accumulates in edible fish tissue and is ingested by humans when fish is consumed. Fish species, food web length, trophic level, age, sex, size of the fish, and habitat are important variables for mercury concentrations in fish (Muir, Shearer, Van Oostdam, Donaldson, & Furgal, 2005; L. Zhang & Wong, 2007). The quantity and species of fish consumed have effects on mercury intake levels. Human populations which consume a high amount of fish, local catch and market fish account for the predominant sources of methylmercury (Mergler et al., 2007).

Waste produced in urban and industrial areas, sewage irrigation, sludge application and mining and smelting operations for metallic ores are the most dominant sources of release of Hg
into the environment in China, especially to the aquatic system (H. Wang & Stuanes, 2003). This indicates that foodstuff may be contaminated by environmental mercury which deposits from the atmosphere into the biosphere. Zhang and Wong (2007) reviewed the status of mercury contamination in different ecological compartments in China, and its potential environmental and human health impacts. The objective of this review was to examine more closely mercury contamination in foodstuff in China, particularly in fish, and compare those to compiled data on human mercury exposure indices, particularly hair analyses. Mercury contamination in Chinese traditional medicine (CTM) will also be touched upon since some of the CTM which contains mercury plays important roles in the domestic and international CTM market.

4.2 Methodology

Literature on mercury concentrations in foodstuffs and human hair in various locations around China were compiled by using appropriate search terms on Web of Science and the Chinese Journal Full Text Database (CJFD) indexing services. Only studies with high quality in reporting and presenting of data were included. The criteria was set that studies should have the following information: 1) the location and species of fish; 2) description of sample preparation; 3) approaches of sample digestion and analytical instrument; and 4) description of quality assurance/quality control (QA/QC) approaches. Studies that lacked more than one of the above criteria were excluded from the review due to inadequate information. Data prior to 1997 were not included because methodologies and QA/QC were not clearly described and the Hg values reported were highly suspect.
Among those high quality papers screened for review, various analytical techniques, digestion process, QA/QC approaches and statistical methods were used, and must be acknowledged. The most common techniques for total mercury measurements were cold-vapor atomic absorption spectrometry (CV-AAS; Shi, Liu, Ma, 2006; L. Zhang, Wang, Shao, 2005) and cold-vapor atomic fluorescence spectrometry (CV-AFS; Chen et al., 2007; Su, Qi, Jiang, Ma & Ni, 2007; P. Zhang et al., 2004), while acid digestion was the most common approach (Su, Qi, Jiang, Ma & Ni, 2007; L. Zhang, Wang & Shao, 2005). QA/QC included calibration blanks, methods blanks, and replicates. However, detection limit was rarely mentioned (Chen et al., 2007), while standard water samples from the National Bureau of Standard were commonly used to replace certified reference materials (CRM) of fish and hair (Su, Qi, Jiang, Ma & Ni, 2007). IAEA H-8 and GBW 08571 were used by Zhang et al. as fish CRM (2004). BCR 40 from commission of the European Communities and NIES No.13 from Japan were used as hair CRM in Song’s study (Song, Yang, Li, Cheng & Wang, 2007). In order to compare data from across studies, total mercury (THg) concentrations were converted to standard units, ug/g wet weight in fish and foodstuffs and ug/g dry weight in human hair (Table 4.1). Dry weight (dw) THg concentrations in fish were converted to wet weight values using the factor 0.31 (Ramlal et al., 2003). A correction factor of 0.90: dry: wet weight was applied to hair samples, which was determined by using weights of hair samples from different persons before and after complete drying.
4.3 Sources of Exposure

4.3.1 Fish

Fish is considered a popular dietary component for many communities in China and globally. Fish are nutritious and rich in high-quality protein, minerals, vitamins and beneficial fats, however, these benefits have been balanced by mercury contamination (Dorea, de Souza, Rodrigues, Ferrari, & Barbosa, 2005; Morrissette et al., 2004). The Ministry of Health of China has regulations on maximum allowable concentrations of mercury in fish and other foodstuff as listed in Table 4.2 (GB2962-94 1994). The enforced standard in China is lower than all the other international criteria on allowable limits of mercury concentrations in fish generated by the U.S., Canada and WHO, as shown in Table 4.3. Although they have strict regulation on mercury in fish, mercury concentrations above the regulatory limits have been observed many times, even in remote regions away from anthropogenic or natural sources of mercury, particularly in species occupying high trophic levels (Health Canada, 2007). The Joint Committee of FAO/WHO also established regulatory guidelines regarding dietary mercury intake. It recommended a provisional tolerable weekly intake (PTWI) of 1.6 μg/kg body weight (bw) for methylmercury (Booth & Zeller, 2005).

According to a dietary survey conducted in the province of Zhejiang, China, fish is an important part of the cuisine (M. Zhang, Binns, & Lee, 2002). Data over the past decade for mercury concentrations in various economic fish species from water bodies around China is compiled in Table 4. Figure 1 shows the locations of different study areas reviewed in this article.
Based on the fish data, exposure calculations were conducted following the protocols by Health Canada (Health Canada, 1995). Daily intakes of mercury were estimated using the following formula:

\[
\text{EDI} = \frac{C \times CR \times EF}{BW}
\]

EDI (=Estimated daily intake) is the amount of total mercury (THg) that enter the body for each kilogram of body weight per day (μg/kg/day).

C is the concentration of THg in fish.

CR is the amount of fish that is consumed per year.

EF indicates how often fish is consumed during a year. The 2000 national goal of ideal fish consumption is adapted for the results of CR \times EF. In this context, CR \times EF = 9 kg/year = 24.66 g/day (M. Zhang, Binns, & Lee, 2002).

BW is the average body weight of target population in kilograms (kg). The average body weight for the Chinese population is provided by China Population & Development Research Center (CPDRC, 2001).

Compared with the tolerable daily intake (TDI) index provided by the Joint European Committee on Food Additives, which is 0.71 μg/kg bw /day, all the EDI in Table 4.4 are far below this level but females are under greater risk due to their lower body weight, assuming females generally consume similar amounts of fish as males.
Briefly, most of the economic fish sampled in the above areas were below the Chinese maximum allowable limit (0.3 μg/g) with a few of them approaching the threshold. A study carried out at Yangtze River 10 years ago, from which fish are very popular for Chinese consumers, examined the reservoir effect after the Three-Gorges was dammed and compared mercury accumulation between reservoirs and rivers. Among 1076 carp samples from 21 reservoirs and 9 sub-streams of Yangtze River, most had average mercury concentrations below 0.3 μg/g with one exception, Dahuahe Reservoir (average, 2.463 μg/g). They also provided evidence that mercury concentrations in fish from reservoirs were commonly higher than those from rivers (Xu et al., 1998). In central China, a survey on urban and suburban lakes around Wuhan city provided evidence that fish from urban areas had higher concentrations of mercury than those from suburban areas in Hubei province (Su, Qi, Jiang, Ma & Ni, 2007). Seafood from Ningbo, Zhejiang, had mercury concentrations below 0.3 μg/g but the concentration tended to increase four-fold from 1997 to 2000, which may have been caused by increased discharge of contaminants into aquatic systems (F. Ye, 2002). In northeast China, mercury in fish was tracked in the Songhua River and the Di’er Songhua River through the past two decades. Mercury concentration in catfish from Songhua River dropped from 0.6 μg/g in the early 1980s to 0.216 μg/g in 2003, with similar trends being observed in other fish species (D. Feng, Ma, Bai & Xu, 2004). However, fish from the Zhaoyuan section of Songhua River showed some fluctuations (Ren, Shi, Guan & Fan, 2001). The peak concentration of mercury in fish from the Di’er Sonhua River was found between 1973-1976 (0.73 μg/g), followed by a gradual decline to 0.087 μg/g in 2004, although the speed of mercury elimination was slower than predicted by some scientists (L.
In the southwest of China, specifically in Guizhou province, most of the fish species didn’t exceed the 0.3 μg/g maximum allowable limit with a few exceptions according to the most recent data on fish (Rustadbakken et al., 2009).

Overall, no large surveys in China have been conducted for mercury concentrations in commercial fish and no advisories for fish consumption have been established, even though mercury pollution in fish has been tracked in certain areas such as the northeast. There is a need to generate awareness of potential health risks associated with consumption of fish with elevated mercury and to provide Chinese people with information on the comparative distribution of chemicals and nutrients in fish to help formulate dietary recommendations.

### 4.3.2 Other aquatic foodstuff

Samples of river-snail (average THg: 0.159 μg/g, wet weight) from Yangtze River, Jiangsu section, had slightly higher concentrations of mercury than fish (0.075 μg/g, wet weight) collected from the same area, but both of them were still below the standard threshold for fish enforced by the Ministry of Health of China (Chen et al., 2007). Water caltrop, lotus root, and duck from Nansi Lake, Shandong province, showed no significant mercury contamination except for river-snails (1.340 μg/g; Shi, Liu & Ma, 2006), which further confirmed that river-snail might be another important source of mercury intake, especially due to its popularity as a snack food. The specific uptake pathway of mercury for the river snail is currently unknown. Studies in other systems have demonstrated that sediment dwelling organisms generally have higher
concentrations because they are filter-feeding, and processing large amount of water and accumulating pollutants. This may also explain the elevated mercury concentration in ricer snail.

4.3.3 Cereals and other foodstuff

Except for aquatic food, other sources of food with elevated mercury concentrations were also reported around the world. Some meat of terrestrial animals, such as Alaskan reindeer (Duffy, Duffy, Finstad, & Gerlach, 2005), as well as cereal grains, chicken and pork were reported to contain methylmercury (Mergler et al., 2007). Some communities also have high methylmercury exposure due to the consumption of fish-eating marine mammals, like whale meat (Booth & Zeller, 2005; Weihe, Grandjean, & Jorgensen, 2005). Within China, there are some special possible exposure sources owing to the dietary culture (mercury concentrations refer to Table 4.5).

Rice cultivated in mercury contaminated areas in the province of Guizhou contained high concentrations of methylmercury (L. Zhang & Wong, 2007). No significant contamination was found in chicken, pork, lamb, or duck in Urumchi, Sinkiang (Yan, Guo, Pang, Zhang & Wang, 2005), nor from rice, fruit, meat, vegetables and egg from markets at Qingdao (C. Zhang 2001). In comparison, tissue and internal organs from ducks and their eggs sampled from Zhaoyuan of Songhua River were reported to have mercury concentrations over the standard threshold (0.05 μg/g) as shown in Table 4.2 (Ren, Shi, Guan & Fan, 2001).
4.3.4 Chinese traditional medicine

It has been recognized since the 1990s that Chinese traditional medicine (CTM) constitutes a substantial exposure pathway. CTM with mercury and other metal components has been used in China for a long time, and is commonly used for many illnesses such as acariasis (a rash caused by mites, sometimes with a papillae), insomnia, and the sequelae of cerebrovascular disease (D. Wang & Zhu, 2007). In recent years, there has been increased concern over the regulation of metal components in CTM as a result of observed the CTM-related mercury poisonings (Sun, Zou & Liu, 2006). Accordingly, the need for quality control has become more and more apparent, especially with the growth of the CTM industry (Z. Li, 2005).

Several factors contribute to the mercury content in CTMs. Firstly, certain types of CTM contain mercury as their main component. For example, cinnabar (HgS) was first recorded as a medicine in the earliest Chinese medical book *Shen Nong's Herbal* more than 2000 years ago (Liang & Cai, 2001). More than 30 types of CTMs, widely applied for rheumatism, skin illness and venereal disease (D. Wang & Zhu, 2007), contain cinnabar and are taken orally or used externally (Liang & Cai, 2001). Moreover, where mercury is not the primary ingredient, many traditionally used herbs can take up contamination from their surrounding environment (H. Zhang, Zhao & Ni, 2003). It has been demonstrated that the same herbal species of CTM can have varying concentrations of mercury based on where they were produced (Sheng, Lai, Zhang & Liu, 2007; Zhao et al., 2005). Other factors such as processing, storage, transportation, and preparation of CTM can also influence the concentration of mercury in CTMs (Z. Li, 2005). Nearly a score of
CTM products were found to have excessive mercury according to investigations within China (Z. Li, 2005; Zhao et al., 2005; H. Zhang, Zhao & Ni, 2003), while a survey conducted in Singapore, between 1990-1997 on CTM products, reported that 42 different products were found to contain excessive mercury, lead, and arsenic (Koh & Woo, 2000).

Domestically, although the State Food and Drug Administration in China has generated strict regulations on the concentrations of different toxic elements in CTM, many problems existed regarding the management of the Chinese medicine market, for instance, contaminated growing environment of herbal medicines, bioaccumulation of toxic elements during growing process, and inappropriate processing techniques. Moreover, some people in Europe and North America have started to value herbal remedies in recent decades, though many existing problems hold back its way to world-wide application. For example, ingredients in CTMs are often undeclared on product label and unlicensed products with high concentrations of mercury still reach the UK market (MHRA, 2004).

4.4 Health assessment in China

A few studies reported on hair mercury concentrations for general populations in many Chinese cities (Table 4.6). The US Environmental Protection Agency (EPA) and Health Canada have recommendations on hair mercury concentrations of 1.5 and 6 μg/g respectively (Morrissette et al., 2004). Normal level and threshold level provided by WHO for mercury in hair are 2.0 μg/g and 10.0 μg/g, respectively (Al-Majed & Preston, 2000). The range of total mercury
concentrations in hair samples listed in Table 4.6 are mostly lower than the level recommended by Health Canada (6.0 μg/g) and the threshold level provided by WHO (10.0 μg/g), with several individuals from Di’er Songhua River of extremely high hair mercury concentrations. This indicates that health risks from mercury poisoning still exist, especially for northeastern China. However, the integrity of this data cannot currently be confirmed as there was very limited statistical data presented in the literature. For example, in most instances, there was no standard deviation reported. Therefore these data must be interpreted with caution and should be updated to reflect current concentrations. The hair mercury data from our study at the area of Qiandao Lake was compared with literature data (Table 4.6) and it showed that the hair mercury concentrations from our study were relatively lower than most other regions across China, perhaps due to the exclusion of subjects who worked in fish markets or were from fish-families. They were also lower than the population at Ningbo of the same province but who may consume a bigger proportion of marine fish.

Studies have shown that two regions in China have significant concentrations of mercury contamination and exposure relative to the other regions. The province of Guizhou, located in southwestern China, has the highest average Hg content in coal (1.1 μg/g), much higher than the world average of 0.1 μg/g (Zheng, Liu, & Chou, 2007), leading to a greater contribution of mercury released into the environment. Twelve large mercury deposits have been discovered in Guizhou so far (Feng et al., 2008). This equates to an approximate 100 fold more atmospheric mercury released around power plants than in Sweden, and an estimated daily intake of 0.142-
0.161 μg/kg/day through inhalation for residents. This is important considering a value of 0.1 μg/kg/day poses a potential for development of adverse effects on human bodies (L. Zhang & Wong, 2007). Rice from one local village was found to have extremely high concentrations of up to 569 μg/g total mercury which was also reflected in hair mercury concentrations from local residents because of rice consumption (Feng et al., 2008). A longitudinal study on hair samples from residents in a mercury contaminated area around an acetate acid plant at Guizhou in 1996 and 2001, before and after the improvement of production techniques, found that hair mercury concentration in 1996 ranged from 0.20 to 11.90 μg/g with approximately 10% of the hair samples over the WHO threshold level (10 μg/g). In 2001 the average mercury concentration in hair samples collected from the same population dropped to 0.86 μg/g with only 8.75% of the hair samples having mercury in excess of the WHO normal level (2 μg/g; L. Liu, Qu & Shen, 2003).

The other area of concern is in northeastern China, which includes the provinces of Liaoning, Jilin and Hei Longjiang. Reports of high concentrations of contamination from mercury and methylmercury have been prevalent since the 1970s due to widespread industrial activities beginning in the 1930s (L. Zhang & Wang, 2006). In areas of the Songhua River and Di’er Songhua River, mercury concentrations in local fishermen had reached the threshold of mercury poisoning observed from Minamata disease in Japan during the early 1980s (Wu, Yang, Ma, Yu & Wang, 2007; L. Zhang & Wang, 2006). Since 1982, the subsequent funding from local governments have aided in controlling the anthropogenic sources and recovery of those areas. Due to the termination of waste discharge and elimination of industrial plants since 1982, a study
on mercury concentrations in 1992 showed that surface water, soil, foodstuff, fish and local residents’ mercury burdens declined significantly to relatively safe levels within the Songhua River region (L. Ye et al., 2005). The range of averages of total mercury in hair decreased to 0.16-4.18 μg/g in the Songhua River region, and declined from historically 26.24-118.30 μg/g to 2.205 (0.110-116.634) μg/g in the Di’er Songhua River region until early 2000s, though some of them are still under risk of mercury poisoning (D. Feng, Ma, Bai & Xu, 2004; L. Zhang, Wang & Shao, 2005; L. Zhang & Wang, 2006).

Few studies on dose-response relationships between fish consumption rates and adverse health effects have been conducted in China. One study conducted in Hong Kong initially provided evidence that sub-fertile males tended to have much higher concentrations of mercury in hair than those who were more fertile, and males were under remarkably greater burdens of mercury than women (Dickman, Leung, & Leong, 1998). Another recent study at Zhoushan, Zhejiang, using neonatal behavior neurological assessments (NBNA), provided further support for the hypothesis that prenatal methylmercury exposure can adversely affect neurodevelopment (Gao et al., 2007).

Except for frequent fish consumers and workers with occupational exposure to mercury, women of child-bearing age, pregnant women and infants are also considered as vulnerable populations to mercury exposure. Methylmercury can harm fetus and infant by means of transference from the mother(Morrissette et al., 2004), which should raise special concerns for
women of childbearing-age and children. Based on an extensive analysis, which was a benchmark dose analysis on three longitudinal studies at Sechelle Islands, Faroe Islands and New Zealand, the US. Environment Protection Agency (EPA) derived a reference dose (RfD) of 5.8 μg/L for Hg in cord blood (Mergler et al., 2007). A recent survey was conducted on 1057 mother–infant pairs in Hong Kong. Of the 1057 cord blood samples collected, 78.4% had mercury concentrations higher than 5.8 μg/L (Fok et al., 2007). In another survey of neonates at Zhoushan in the province of Zhejiang, the average mercury concentrations in cord blood were lower than the concentrations from subjects of Hong Kong, with 69.9% of all the cord blood samples had mercury concentrations at or above EPA RfD (Gao et al., 2007). The average mercury concentration (1.93 μg/L) in 63 cord blood samples from Wuxi city, central eastern China, was below the EPA RfD, indicating a relatively safe situation (Chen et al., 2007).

The Joint Expert Committee on Food Additives and Contaminants (JECFA), under FAO/WHO, developed a Benchmark dose limit of 12 μg/g in maternal hair based on a different selection of studies and bioindicators, and suggested a steady-state daily ingestion of methylmercury at 1.5 μg/kg bw per day for women of childbearing-age in order to avoid adverse effects on offspring (WHO, 2004). A study conducted at Luzhou in the province of Sichuan, reported relatively low mean concentrations of maternal hair and neonate’s hair (0.10 μg/g and 0.20 μg/g respectively) compared with the JECFA benchmark (Chen et al., 2004). The FDA and the EPA, as well as Health Canada, have made recommendations for selecting and eating fish,
particularly as it applies to women and young children (EPA, 2004). However, to date there are no such advisories available in China.

4.5 Conclusion

In conclusion, the lack of comparable, reliable, and up-to-date, long-term monitoring study data pertaining to mercury contamination in China suggests that a nation-wide survey should be undertaken. Within hotspot areas of increased contamination, such as the province of Guizhou, more extensive studies are merited in order to protect the local inhabitants. To date, though evidently a potential cause for concern, mercury is not acknowledged in the annual report of the State EPA of China.
### Tables and Figures

#### Table 4.1 Standard units used in this review

<table>
<thead>
<tr>
<th>THg in fish</th>
<th>THg in human hair</th>
<th>THg in blood</th>
<th>Tolerable Daily Intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>standard units</td>
<td>μg/g</td>
<td>μg/g</td>
<td>μg/L</td>
</tr>
</tbody>
</table>

#### Table 4.2 Maximum allowable concentrations of mercury in foodstuff in China (μg/g)

<table>
<thead>
<tr>
<th>Sample</th>
<th>Maximum mercury concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>rice and other grain products</td>
<td>≤0.02</td>
</tr>
<tr>
<td>potato, vegetable and fruit</td>
<td>≤0.01</td>
</tr>
<tr>
<td>milk and milk products</td>
<td>≤0.01</td>
</tr>
<tr>
<td>meat, egg and egg products</td>
<td>≤0.005</td>
</tr>
<tr>
<td>Fish and other aquatic product</td>
<td>≤0.3</td>
</tr>
</tbody>
</table>

#### Table 4.3 Criteria on mercury in fish by difference organizations (μg/g)

<table>
<thead>
<tr>
<th>Organizations</th>
<th>mercury</th>
<th>methylmercury</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>FAO/WHO¹</td>
<td>0.4 (maximum allowable limit)</td>
<td></td>
<td>(WHO, 1989)</td>
</tr>
<tr>
<td>FDA²</td>
<td>1 (action level)</td>
<td>0.5 (advisory consumption level)</td>
<td>(US EPA, 2001)</td>
</tr>
<tr>
<td>CFIA³</td>
<td>0.5 *</td>
<td></td>
<td>(Health Canada, 2007)</td>
</tr>
</tbody>
</table>

1 the Joint Food and Agriculture Organization/World Health Organization
2 the U.S. Food and Drug Administration
3 the Canadian Food Inspection Agency
*applied on all commercially-sold fish except three piscivorous fish shark, swordfish, and fresh/frozen tuna
Table 4.4 Total mercury concentrations in economic freshwater fish from different parts of China (μg/g, wet weight)

<table>
<thead>
<tr>
<th>Geographic Region</th>
<th>location</th>
<th>Provinces</th>
<th>n of samples</th>
<th>average THg /range</th>
<th>EDI for male μg/kg bw</th>
<th>EDI for female μg/kg bw</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Northeast China</td>
<td>Di’er Songhua River</td>
<td>①</td>
<td>158</td>
<td>0.087</td>
<td>0.0317</td>
<td>0.0360</td>
<td>Zhang, Wang &amp; Shao, 2005</td>
</tr>
<tr>
<td></td>
<td>Songhua River (Zhaoyuan)</td>
<td>①</td>
<td>0.275</td>
<td>0.100</td>
<td>0.114</td>
<td></td>
<td>Ren, Shi, Guan &amp; Fan, 2001</td>
</tr>
<tr>
<td></td>
<td>Songhua River (Harbin)</td>
<td>①</td>
<td>0.085</td>
<td>0.0310</td>
<td>0.0352</td>
<td></td>
<td>Z.Li, Lv &amp; Cheng, 2001</td>
</tr>
<tr>
<td></td>
<td>Yalv River</td>
<td>①</td>
<td>0.01-0.29</td>
<td>0.00364 -0.106</td>
<td>0.00414 -0.120</td>
<td></td>
<td>Q. Liu, 1999a</td>
</tr>
<tr>
<td>Central &amp; east China</td>
<td>Shanghai</td>
<td>②</td>
<td>0.0002-0.0025</td>
<td>0.0000729-0.000911</td>
<td>0.0000828-0.00103</td>
<td></td>
<td>F. Wang, 2007</td>
</tr>
<tr>
<td></td>
<td>Lakes in Wuhan</td>
<td>③</td>
<td>0.051-0.199</td>
<td>0.0186-0.0725</td>
<td>0.0211-0.0823</td>
<td></td>
<td>Su, Qi, Jiang, Ma &amp; Ni, 2007</td>
</tr>
<tr>
<td></td>
<td>Yangtze River</td>
<td>②</td>
<td>0.075</td>
<td>0.0273</td>
<td>0.0310</td>
<td></td>
<td>Chen et al., 2007</td>
</tr>
<tr>
<td></td>
<td>Ningbo</td>
<td>⑤</td>
<td>0.218*</td>
<td>0.0794</td>
<td>0.0902</td>
<td></td>
<td>F. Ye, 2002</td>
</tr>
<tr>
<td>North China</td>
<td>Nansi Lake</td>
<td>⑥</td>
<td>413</td>
<td>0.056-0.282</td>
<td>0.0204 -0.103</td>
<td>0.0232-0.117</td>
<td>Shi, Liu, Ma., 2006</td>
</tr>
<tr>
<td></td>
<td>Tai’an</td>
<td>⑥</td>
<td>0.018-0.235</td>
<td>0.00656-0.0856</td>
<td>0.00745-0.0972</td>
<td></td>
<td>Zhi, Liu, Zhu &amp; Yang, 2005</td>
</tr>
<tr>
<td></td>
<td>Beijing</td>
<td>②</td>
<td>0.050 in tissue</td>
<td>0.0182</td>
<td>0.0207</td>
<td></td>
<td>P. Zhang et al., 2004</td>
</tr>
<tr>
<td></td>
<td>Qingdao</td>
<td>⑥</td>
<td>0.231**</td>
<td>0.0841</td>
<td>0.0956</td>
<td></td>
<td>C. Zhang, 2002</td>
</tr>
<tr>
<td></td>
<td>An‘di Reservoir</td>
<td>⑥</td>
<td>0.072-0.517</td>
<td>0.0262-0.188</td>
<td>0.0298-0.214</td>
<td></td>
<td>Y. Liu, 1999b</td>
</tr>
<tr>
<td>West China</td>
<td>Urumchi</td>
<td>⑥</td>
<td>32</td>
<td>0.049</td>
<td>0.0178</td>
<td>0.0203</td>
<td>Yan, Guo, Pang, Zhang &amp; Wang, 2005</td>
</tr>
<tr>
<td></td>
<td>Yinchuan</td>
<td>⑨</td>
<td>156</td>
<td>&lt;0.0007</td>
<td>&lt;0.000255</td>
<td>0.00029</td>
<td>H. Zhang, Ni &amp; Li, 1997</td>
</tr>
<tr>
<td>Southwest China</td>
<td>Wanshan</td>
<td>⑩</td>
<td>19</td>
<td>0.312</td>
<td>0.114</td>
<td>0.129</td>
<td>P. Zhang et al., 2004</td>
</tr>
</tbody>
</table>

*location of provinces refer to Figure 4.1

**mercury concentration in seafood

***standard threshold : see Table 4.2
<table>
<thead>
<tr>
<th>Location</th>
<th>Provinces*</th>
<th>Food item</th>
<th>Average THg (μg/g)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wanshan</td>
<td>○</td>
<td>cereals</td>
<td>up to 569</td>
<td>Horvat et al., 2003</td>
</tr>
<tr>
<td>Urumchi</td>
<td>○</td>
<td>lamb</td>
<td>0.010±0.007</td>
<td>Yan, Guo, Pang, Zhang &amp; Wang, 2005</td>
</tr>
<tr>
<td></td>
<td></td>
<td>beef</td>
<td>0.013±0.008</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>pork</td>
<td>0.010±0.008</td>
<td></td>
</tr>
<tr>
<td>Qingdao</td>
<td>○</td>
<td>Cereals</td>
<td>0.010±0.002</td>
<td>C. Zhang, 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>vegetables</td>
<td>0.007±0.002</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>fruit</td>
<td>0.008±0.001</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>meat</td>
<td>0.015±0.003</td>
<td></td>
</tr>
<tr>
<td>Nansi Lake</td>
<td>○</td>
<td>river-snail</td>
<td>0.134±0.158</td>
<td>Shi, Liu, Ma, 2006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>water caltrop</td>
<td>0.012±0.086</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>lotus</td>
<td>0.009±0.007</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>ducks</td>
<td>0.042±0.035</td>
<td></td>
</tr>
<tr>
<td>Yangtze River</td>
<td>○</td>
<td>river-snail</td>
<td>0.159</td>
<td>Chen et al., 2007</td>
</tr>
<tr>
<td>Zhaoyuan</td>
<td>○</td>
<td>Tissue and organs from ducks</td>
<td>0.068-0.121</td>
<td>Ren, Shi, Guan &amp; Fan, 2001</td>
</tr>
<tr>
<td></td>
<td></td>
<td>duck eggs</td>
<td>0.053</td>
<td></td>
</tr>
</tbody>
</table>

*Location of provinces refers to Figure 4.1

**standard threshold : see Table 4.2
Table 4.6 Total mercury concentrations in human hair at different cities of China (μg/g)

<table>
<thead>
<tr>
<th>Cities/areas</th>
<th>Provinces</th>
<th>n</th>
<th>Range (mean) of</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>THg(μg/g)</td>
<td></td>
</tr>
<tr>
<td>Baotou</td>
<td>②</td>
<td>207</td>
<td>0-0.652</td>
<td>Kang, Yang &amp; He, 1994</td>
</tr>
<tr>
<td>Taiyuan</td>
<td>③</td>
<td>143</td>
<td>0-0.66</td>
<td>Cao 2002</td>
</tr>
<tr>
<td>Qiandao</td>
<td>⑤</td>
<td>50</td>
<td>0.18-3.04 (0.76)</td>
<td>see Chapter 2</td>
</tr>
<tr>
<td>Guizhou</td>
<td>⑩</td>
<td>80</td>
<td>0.31-3.42 (0.96)</td>
<td>L. Liu, Qu &amp; Shen, 2003</td>
</tr>
<tr>
<td>Ningbo</td>
<td>⑤</td>
<td>262</td>
<td>0.241-6.435 (1.306)</td>
<td>Song, Yang, Li, Cheng &amp; Wang, 2007</td>
</tr>
<tr>
<td>Liaohe Drainage area</td>
<td>⑬</td>
<td>96</td>
<td>0.42-10.60 (1.25)</td>
<td>Wu, Yang, Ma, Yu &amp; Wang, 2007</td>
</tr>
<tr>
<td>Tieling, Liaoning</td>
<td>⑬</td>
<td>50</td>
<td>0.34-4.86 (0.87)</td>
<td>X. Liu, Cheng, Liu &amp; Hachiya, 2009</td>
</tr>
<tr>
<td>Songhua River</td>
<td>⑦</td>
<td>106</td>
<td>0.17-4.64</td>
<td>D. Feng, Ma, Bai &amp; Xu, 2004</td>
</tr>
<tr>
<td>Di’er Songhua River</td>
<td>⑦</td>
<td>250</td>
<td>0.122-129.693 (2.450)</td>
<td>L. Zhang, Wang &amp; Shao, 2005</td>
</tr>
</tbody>
</table>

Figure 4.1 Map of China with reviewed study areas

Legend
1- Heilongjiang
2- Shanghai
3- Hubei
4- Jiangsu
5- Zhejiang
6- Shandong
7- Beijing
8- Sinkiang
9- Ningxia
10- Guizhou
11- Liaoning
12- Inner Mongolia
13- Shanxi
References


Chapter 5

Strengths, limitations and future work

5.1 Strengths

- Other studies on mercury exposure and hair did not always include a corresponding questionnaire. In our mercury study, a questionnaire was conducted along with a hair sample collection to understand the potential sources of the hair mercury levels including fish consumption patterns. To enhance the effectiveness of the questionnaire, a preliminary study at Queen’s University was carried out before the sampling in China to practise and improve the survey questions and strategies. A pre-survey on fish species at a local fish market at the lakeside town of Qiandao Lake was also conducted so that the fish species included in the questionnaire survey were actually available for participants to consume from the local market.

- Biases were minimized.
  - Firstly, participants from the Qiandao area were not aware that the survey was about mercury exposure and that fish consumption might be related to mercury burden in their bodies, so that their choices of fish consumption frequency and weekly fish consumption were likely not affected by their knowledge of mercury exposure.
  - Secondly, pictures of fish portion size and fish species were provided to participants when they were completing the questionnaire, which helped them to choose answers of
weekly fish consumption mass and consumption frequency of individual fish species as close to the fact as possible.

- The external contamination of hair samples was minimized to make sure the measurement of mercury and elements concentrations actually represented the elements in hair samples.
  - Based on the occupations of participants reported in the survey, none had occupational exposure of mercury vapor in industrial areas during the three months prior to the survey.
  - All hair samples were washed with shampoo before cutting at the sampling site, and then washed with pure acetone once and purified water twice to remove possible external contamination.
- Rather than using cheaper and easier-obtained certified materials of fish tissue or liver, certified material of human hair (BCR 397) was used in our study to better assure the quality of instrument analysis on metal concentrations because hair is the material that was analyzed in our study.
- Previous studies had conflicting results on the relationship between age and mercury concentrations when trying to fit linear regressions. In Chapter 2, we found that a hyperbola curve fitted the relationship between age and mercury level. Since we saw a linear increase of mercury with fish consumption but not after age 25, we concluded that fish consumption is an important contributor of mercury burdens in the population surveyed. Therefore, the observation that mercury does not accumulate after age 25 is very important for supporting this argument.
5.2 Limitations

- In our study, the sample size at Qiandao area was limited due to culture issues and political sensitivity. As well, small numbers in some sub-groups weakened the statistical strength of data analysis.

- With regard to the survey at Qiandao area, females who were working in the fish market and who were from fishing families were not included in our survey, but they would also be important to study since they probably tend to consume more fish and they may probably have different preference of fish consumption due to their accessibilities to fish.

- Since the sampling at Qiandao area was carried out mainly at a local barbershop, the samples were considered as “convenience sample”. The social level of people who went to the barbershop depended on the level of the barbershop (cost, decoration, numbers of male and female barbers). The sampled group may not be able to represent the whole female population of the sampling town due to the choice of barbershop.

- As for the multi-element study, the gender composition of participants among three groups were different. It is more appropriate to either compare only female data, or compare a mixture of male and females.
5.3 Future work

- Because of the limited occupations of participants at Qiandao area and small sample size, the sampled subjects likely do not represent the whole population in that area. A future study on a larger scale with a large sample and a reasonable composition of occupations may be needed to understand the prevalence of mercury for that area.

- Further investigation of mercury concentrations in fish samples from Qiandao Lake is important, especially for those most-preferred species, in order to understand which fish species from Qiandao Lake are safe to consume and to generate advisories on fish consumption. Future data on mercury concentration in fish from the same region along with our fish consumption frequency data can be used to estimate daily intake of mercury for the population surveyed, in order to predict the risk associated with fish consumption in the area of Qiandao Lake.
Appendix A Ethics clearance and Package of GREB application

Any research project involving human subjects must go through ethics approval of one of the Ethics Boards prior to the start of the project. My research was submitted to the General Research Ethics Boars (GREB). The application package included the following items:

- Research proposal
- Information sheet
- Consent form (English copy and translated copy in Mandarin)
- Questionnaire sample
Research Proposal

Objectives:

1. Validate our hypothesis that people who consume relatively high amount of fish from Qiandao Lake will have corresponding higher mercury concentrations in their hair.

2. Prove whether consumption of fish from Qiandao Lake is a potentially important health risk factor.

3. Compare the differences of hair mercury level between participants from Fudan University and Queen’s University.

Brief description of research:

Mercury (Hg) is of particular importance because it is a neurotoxic metal with profound implications for healthy development of children and health of adults. With the rapid economic development, mercury and metal contamination has become among the pressing serious environmental pollution issues, not only locally, but also globally through atmospheric emissions and trade. However the actual degree of contamination and mercury exposure to humans in China are still underquantified, despite the obvious importance of the topic, Dr. Linda Campbell, my graduate supervisor, in collaboration with Dr. Yuxiang Wang (Biology) had investigated mercury trends in fish species from lakes across eastern China, particularly those consumed by humans. Since fish consumption is one of the key pathways for humans to be exposed to mercury, we plan to assess the importance of fish as a vector of mercury to humans, using Qiandao Lake, east China, as a case study. Since dietary Hg is incorporated into growing hair, which can be collected
in a non-invasive manner, mercury concentrations in hair can be used to indicate the mercury exposure to humans on a population level. Pre-testing of those methods is still ongoing and has been successful so far. We propose to recruit participants from local residents in several towns which are close to Qiandao Lake as well as participants from both Fudan University and Queen’s University. Questionnaires on dietary consumption of fish, gender, age, and potential mercury exposure will be collected and small discreet clippings of head hair samples will be sampled from the participants. Hg concentration in hair will be measured in Dr. Campbell's laboratory or in the Analytical Services Unit using Cold-vapour atomic fluorescence spectrometry (CV-AFS), and the mercury data evaluated against the questionnaire responses. The hypothesis being tested here is that people who consume relatively high amount of fish, particularly wild species, will have higher mercury concentrations in their hair as well. The rationale is that if we can use non-invasive hair samples to assess the exposure to mercury and correlate that to fish and environmental exposure, it would enable a baseline evaluation of the importance of fish as a vector of mercury to humans.

Conclusively, on one hand we would like to prove by this study that whether fish eating is a potentially important health risk factor for local residents who consume fish from Qiandao Lake frequently. On the other hand, we would like to see whether there are significant differences of hair mercury concentrations between Shanghai China and Kingston Canada due to different fish consumption patterns.

**Proposed research methodology: whole process**

1. Recruit 1-3 student volunteers from Fudan University. They will be trained in investigation
methods, hair collection methods and confidentiality issues

2. The student volunteers will be assigned to different locations to carry out questionnaire surveys and hair sample collection.

3. After the consent information has been interpreted to participants, they will be asked them to fill out a questionnaire (attached) related to dietary fish consumption, possible mercury exposure and hair treatment.

4. Analyse the hair samples submitted by each participant to quantify mercury concentrations.

5. Code the survey results into a spreadsheet and enter the corresponding mercury concentration for each volunteer. (Only the unique research ID will be used during the data analyses. The names of each volunteer will be kept in a separate file to ensure anonymity throughout the process.)

6. The data analyses of the coded survey results and the mercury concentrations will be undertaken to test the hypothesis that elevated fish consumption will correspond with higher mercury concentrations in hair.

7. Our contact person in Fudan University will distribute the results of hair analysis and interpret it to each participant by mail or phone, whichever the participant prefers.

8. Any presentations of the results will collate the data in such way that the identity of all participants will be kept strictly anonymous.

Proposed research methodology: hair samples

1. A small sample of hair (0.4 g) from each participant will be cut carefully from a discreet location, using ultra-trace metal handling techniques. To keep the personal information
confidential, each sample will be marked with a unique number and the name of each participant will not be shown on the sample or any other forms that may be visible to others.

2. Laboratory analyses: We will be following the AN2600-08 Tekran method developed for preparation and chemical digestion of hair and fur samples in order to extract mercury in an analyzable form (protocol available on request). This method is adapted from the U.S. Environmental Protection Agency Method 1631 which is an internationally accepted standard for mercury analyses. The samples will be digested and extracted in Dr. Campbell’s clean room laboratory.

3. Cold vapour atomic fluorescence spectrometry (CVAFS) will be used to analyse the extracted mercury in each digestate. The CV-AFS equipment is located in Dr. Linda Campbell’s clean room laboratory (Biosci Complex 3206).
Information Sheet

This study, *Assessing human exposure from mercury in fish from east China lakes*, is being conducted by Tian Fang (Masters of Environmental Studies graduate student) and Dr. Linda Campbell in the School of Environmental Studies at Queen’s University, as a part of Tian’s Masters of Environmental Studies research project.

This study is to examine the human uptake of mercury related to fish consumption and environmental mercury exposure. You will be asked to fill in a brief questionnaire about your dietary fish consumption, possible mercury exposure and hair treatment. Then a small sample of your hair will be carefully cut from a discreet location to be analyzed for mercury. We have a sample on hand if you would like to see how much hair needed for Hg analyses. The entire session should last no more than 15 minutes.

There are no known physical, psychological, economic, or social risks associated with this study. Your participation in this procedure is completely voluntary and you may withdraw from this study at any time. You are not obliged to answer any questions that make you feel uncomfortable. If you do withdraw from this study, the samples and data collected from you will be destroyed. The result of mercury level in your hair will be sent to you in half a year since you donate your hair by phone, email or mail.
To maintain an approximately balanced sample set in terms of age and gender of volunteers participating in this study, the information we will be recording about you is your age, gender, and questions about your fish consumption, possible daily mercury exposure and hair treatment on the questionnaire, and mercury concentration in your hair sample. The hair sample will be used for no other purpose than the mercury measurement. While measuring the hair sample for mercury in the lab, the hair sample will be destroyed. After the study has been completed, all remaining hair samples will be destroyed and no other further analyses will be done on them.

The only individuals who will have access to this information are the researchers, Tian Fang, Dr. Linda Campbell, directly involved with this project at Queen’s University, and the contact person from School of Public Health, Fudan University. Your confidentiality is guaranteed and the data from your participation will not be connected to your name in any publication. If you wish, we will share with you the data obtained from your participation and privately discuss the interpretation of your result with you.

If you would like further information about the study, or have additional questions or concerns, please feel free to contact any of the researchers: Tian Fang, email: 6tf3@queensu.ca, or Dr. Linda Campbell, email: linda.campbell@queensu.ca, or (the contact person)
This study has been approved by the Queen’s University General Research Ethics Board. You may also contact the Chair of the Queen's University General Research Ethics Board, Dr. Joan Stevenson, (613) 533-6081, email: chair.GREB@queensu.ca.
Consent Form

I, _____, have volunteered to participate in the study titled, *Assessing human exposure from mercury in fish from east China lakes.*

I have read the information sheet and understand what is required for participation in the study. I have had my questions answered to my satisfaction. I understand that this is a survey on assessing human exposure to environmental mercury by testing mercury concentration in human hair samples. I understand that I will fill in the questionnaire and then submit a small sample of my hair. I understand that my hair sample will be destroyed during mercury measurement. I understand that my participation in the study is completely voluntary and that I am free to withdraw at any time. I also understand that my confidentiality will be protected throughout the study, and that the information I provide will be available only to the principal investigator (Dr. Linda Campbell) and the researcher (Tian Fang).

I am willing to fill out the questionnaire: Yes____   No___

I am willing to have my hair sampled: Yes____   No___

Should I have further questions, I understand that I can contact any of the following individuals:

Tian Fang; email: 6tf3@queensu.ca; Dr. Linda Campbell; email: linda.campbell@queensu.ca; or (information from the contact person)
Please print your name above the line

______________________________

Please sign your name above the line                     Date

❖ Which way do you prefer to be contacted for your hair mercury result? Please circle
A. By telephone              B. By email             C. By mail

_____________________________                        _______________________________
Telephone number                                                   E-mail

_____________________________
Mailing Address

(please sign two forms, one is for you and the other one is for investigators)
评估中国东部湖泊地区人类所受食用鱼含汞之影响的研究

同意书

我，______，自愿参加研究项目标题为“评估中国东部湖泊地区人类所受食用鱼含汞之影响的研究”所开展的调研工作。

我已读过此研究项目的说明书，并已确知对自愿参加者的要求。我对此研究项目存在的疑虑也已得到了令人满意的解答。我了解，这是一项通过检测头发样品中的汞浓度来评估人类受汞暴露环境影响程度的调查。我了解，作为志愿者参加此项研究，我需要填写一份问卷调查表并提供少量头发作为样品。我了解，我的头发样品在汞浓度测量过程中会被毁坏。我了解，作为志愿者参加此项研究，我是完全自愿的，并且可以在任何时候自由退出。我也了解，我个人资料的私密性在整个研究过程中都会受到保护，我个人提供的一切信息只有主调查人（皇后大学环境研究学院 Linda Campbell 博士）, 研究员（皇后大学环境研究学院硕士学生方湉）和复旦大学的联络人（to be added）可以获得。

我愿意填写问卷调查表：是 [ ]  否 [ ]
我愿意提供头发样品：是 [ ]  否 [ ]

如果我还有疑虑，我知道可以与以下任何一位研究人员联系：方湉，电子邮件 6tf3@queensu.ca，Linda Campbell 博士，电子邮件 linda.campbell@queensu.ca，或者。。。。

请在横线上用印刷体写出您的名字

请在横线上签名

签名日期

您希望用什么方式与您联系以便告知您的头发样品检测结果？（请圈出您的答案）
A. 电话  B. 电子邮件  C. 邮递信件
您的电话号码

您的电子邮件地址

您的邮政地址及邮编
Questionnaire

<table>
<thead>
<tr>
<th>Gender</th>
<th>Weight(kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>Education Level</td>
</tr>
</tbody>
</table>

In the following questions, seafood refers to aquatic animals like fish, shellfish, octopus and squid

Are you currently pregnant?
☐ Yes ☐ No ☐ N/A

Do you eat freshwater fish?
☐ Yes ☐ No

If YES, for how many years have you eaten freshwater fish? (please circle)
A. Less than 1 year  B. 1-5 years  C.5-10 years  D. more than ten years

Do you know that the fish you eat is farmed, stocked or wild? (please circle)
A. Farmed fish
B. Stocked fish
C. Wild fish
D. Don’t know

Do you eat seafood?
☐ Yes ☐ No

If YES, for how many years have you eaten sea food? (please circle)
A. Less than 1 year  B. 1-5 years  C.5-10 years  D. more than ten years

In the past 2 months, how frequently do you eat fish, on average? (please circle)
A. At least once a day  
B. At least once a week  
C. At least once a month  
D. Never  

In the past 2 months, how much fish did you eat per week, on average? (please circle)  
A. 0-50g  
B. 50-100 g  
C. 100-250g  
D. More than 250 g  

In the past 2 months, what is the frequency that you eat those fish in the following list, on average?  
Do you eat them at home or in the restaurant?  

<table>
<thead>
<tr>
<th></th>
<th>How frequently do you have them?</th>
<th>Where did you have them?</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>At least once a week</td>
<td>At least once a month</td>
</tr>
<tr>
<td>Yellow catfish</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Big head carp</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Silver carp</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Grass carp</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Amur Catfish</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Black carp</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Predatory carp</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Gold fish</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Mongolian refin</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Channel catfish</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Chinese perch</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Eel</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Sturgeon</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Sucker</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Bluegill</td>
<td>□</td>
<td>□</td>
</tr>
<tr>
<td>Questions</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------</td>
<td>-----</td>
<td>----</td>
</tr>
<tr>
<td>Within the last 2 months, have you handled mercury from broken thermometers or electric switches?</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Within the last 2 months, have you cleaned up broken fluorescent light tubes or energy-saving light bulbs?</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Within in the last 2 months, have you used Chinese traditional medicines which may contain mercury as one of the ingredients?</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Within the last 2 months, have you had your hair colored?</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Do you blow-dry or straighten / curl with a hot iron on a daily or weekly basis?</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Do you use store-brought shampoo and conditioner hair products on a daily or weekly basis?</td>
<td>☐</td>
<td>☐</td>
</tr>
<tr>
<td>Within the last 2 months, have you used skin lightening or whitening creams?</td>
<td>☐</td>
<td>☐</td>
</tr>
</tbody>
</table>
Appendix B Detailed Mercury Laboratory Analysis

Labware

Microwave teflon vessels for digestion, amber bottles for sample storage, testing tubes and other labwares were soaked in 10% HNO₃ acid bath at least overnight at room temperature. They were rinsed six times with ultra-purified trace metal quality water before and after acid bath.

Volumetric flasks were filled with approximately 10% HNO₃, rinsed six times with DIW before use. Caps for testing tubes without liners were cleaned as described for labware. Caps for amber bottle with liners were rinsed with DI water for six times. Clean caps were stored in sealed plastic bags for next use.

Reagents

All reagents were selected with the highest (mercury free) quality practical. Deionized water generated from Millipore A10 water purification system (Millipore, Bedford, USA) and trace metal chemicals were used to prepare solutions. The Nitric Acid (HNO₃) and Hydrochloride (HCl) were produced by Fisher Science) of trace metals grade.

To prepare the bromine mono-chloride solution, KBr and KBrO₃ were placed in two separated glass weighing bottles and heated in a mercury free oven at 180°C for at least 8 hours to reduce mercury content. 11g of KBr and 15g of KBrO₃ were dissolved in approximately 200ml DI water in a 1L Teflon bottle, adding 800ml concentrated HCl slowly and carefully into the
solution when fume generated and a bright orange color formed. Then fill the bottle up to 1L.

Tightly cap the bottle until there was no more fume and the bottle cooled down to room temperature. The solution was stored in a sealed plastic bag at room temperature in the clean lab.

A 3% Stannous Chloride (SnCl\textsubscript{2}) solution was prepared by weighing 30g of SnCl\textsubscript{2} crystals into an amber bottle, adding 10ml concentrated HCl to dissolve the SnCl\textsubscript{2}, and making up to 1L with DI water. Then the SnCl\textsubscript{2} was purged overnight for at least 4 hours with mercury free argon gas. The SnCl\textsubscript{2} solution was made one day before analysis in the clean lab.

A 30% Hydroxylamine hydrochloride (HH) solution was made by weighing 30.0g of NH\textsubscript{2}OH.HCl in to a glass beaker, adding 0.05ml 3%SnCl\textsubscript{2} and 30ml DI water to dissolve the HH, transferring the solution into a 100ml volumetric flask, and then adding DI water to the volumetric. The HH solution was transferred into an amber bottle and purged for at least 2 hours with mercury free argon gas. The HH solution was made right on the day of analysis in the clean lab.

**Sample preparation**

Our target was to know the mercury accumulation in hair during the past 3 month before the hair was taken. Since hair usually grows 1 cm for 1 month, about 3 cm of each hair sample from the end which was closer to scalp was used for analysis. If the hair was shorter than 3cm, the whole sample was used. Hair samples were rinsed with acetone and cleaned with Millipore water to
remove external contaminations before digestion. To ensure complete digestion of hair sample, each to-be-analyzed hair sample was cut into small pieces of about 0.2~0.5cm. At the time of analysis, approximately 0.05g hair of each sample was accurately weighed with a 6-decimal analytical balance, and directly placed into microwave vessel. 5ml HNO₃ was added and the cap was tightly screwed on the vessel. The vessels were place on a microwave rack for digestion at 180°C, 1600W for 15 minutes. After cooling for 30 minutes, 0.5ml H₂O₂ was added and mixed, and then the digestion process continued for another 15 minutes. The applied power for digestion depended on the number of vessels which were treated each time. The solution was transferred into a 50ml volumetric flask and filled up to 50ml after it was allowed to cool to below 70°C. 25ml of this solution was transferred to 60ml PE bottles waiting for ICP-MS analysis. 0.25ml BrCl was added into the rest solution and filled up to 50ml again, then the solution was put into amber glass bottle, capped and allowed to sit overnight in the fridge to convert all forms of Hg into Hg(II), waiting for CVAFS analysis. Before CVAFS analysis, samples need to be dilute properly as well as adding HH to destroy excess BrCl at the day of analysis. The sample was analyzed on a cold vapor atomic fluorescence spectrometry. Before ICP-MS analysis, internal standard (Sn) need to be added.

**Instrumentation and its principle**

Mercury analysis was performed by a cold vapor atomic fluorescence spectrometry (CVAFS) was used to analyze liquid samples. The method was based on USEPA（United States Environmental Protection Agency）method 1631 with minor modifications. Organic component in a sample was
digested through microwave digestion process. Nitric acid and Hydrogen peroxide were used to extract all forms of Hg from sample solution. Then BrCl acted as an oxidizer to transform all formations of Hg to Hg(II), and HH was then used to react with excess halogens. By adding SnCl₂, Hg(II) was reduced to Hg(0) which is volatile. The Hg vapor was pushed into a gold trap by mercury free argon gas through a phase separator. Hg from this gold trap was extremely desorbed onto a second gold trap where the Hg was carried to the cell of CVFAS for detection.

**QA/QC**

Instrument performance, primarily sensitivity was assessed prior to the analysis of any samples. At the beginning of each analysis, new calibrations were analyzed.

*Purges* was made of 10% BrCl to clean out Hg leftover from previous analysis in the CVFAS system. *Wash station blanks* were DIW which were used to clean out the system after each sample was tested. *Calibration blanks* with 0.5% BrCl were analyzed to track contamination from the CVFAS system. *Six standards* (0.5, 1.0, 5.0, 25.0, 50.0, 100.0 ppt of Hg) were analyzed. The recoveries of standards were used to test the sensitivity of CVAFS. *IPR 5.0 and OPR5.0* consisted of 5.0ppt of Hg which provided information on how much was taken over from the previous sample. *Method blanks* consisted of the same amount of acid extraction reagent through the sample preparation procedure and treated the same as samples. Analysis of these tracked information on contamination caused by labware and reagents used in the sample preparation steps. Certificate reference material (CRM) (BCR397) was also analyzed to certify the methods.
CRM and method blanks usually have 2-3 duplicates which were scattered throughout the analysis to ensure the consistency of the system.

The order of analysis was as follows: 4 purges, 6 wash station blanks, 4 calibration blanks, 6 matrix standards, IPR5.0, CRM, method blank, 20 samples, OPR5.0, method blank, another 20 samples, CRM, method blank, OPR5.0, 2 purges and 2 wash station blanks to clean up the system.