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Comparative Evaluation on Human Infants Dietary Mercury Exposure through Consumption of Fish and Rice Products

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COMPARATIVE EVALUATION ON HUMAN INFANTS DIETARY MERCURY EXPOSURE THROUGH CONSUMPTION OF FISH AND RICE PRODUCTS

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by

Wenbin Cui

2017

To: Dean Michael R. Heithaus
College of Arts, Sciences and Education

This dissertation, written by Wenbin Cui, and entitled Comparative Evaluation on Human Infants Dietary Mercury Exposure through Consumption of Fish and Rice Products, having been approved in respect to style and intellectual content, is referred to you for judgment.

We have read this dissertation and recommend that it be approved.

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Date of Defense: June 27, 2017

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And Dean of the University Graduate School

Florida International University, 2017

DEDICATION

I dedicate this work to my wife Jingyi Zhao, my daughter Rachel Cui, my parents Yongchun Cui and Junping Wang. I also dedicate this work and give special thanks to all my lab mates, faculties and staff of Chemistry department. Without their understanding, encouragement, support and help, the completion of this work would not have been possible.

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ABSTRACT OF THE DISSERTATION

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by

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Miami, Florida

Professor Yong Cai, Major Professor

Human exposure to methylmercury (MeHg) through diets (e.g., fish and rice) is a global health concern. Although MeHg exposure through fish consumption has long been considered the major route of mercury health risks, studies concerning the long-term changes in MeHg exposure from fish remain lacking. In sharply contrast to the fish MeHg issue, the presence of MeHg in rice has only been reported recently and its implications on MeHg exposure, albeit probably important, are still in infancy. Focusing on the discrepancies in the studies of MeHg exposure through fish and rice consumption, this study was aimed to assess the MeHg exposure of human infants through consumption of rice cereals and to evaluate the long-term changes in fish MeHg.

The presence of MeHg in rice prompted the studies on MeHg concentrations and bioaccessibility in rice cereals and potential infant dietary exposure to MeHg through cereal consumption, which is believed to be the first of its kind. The analysis of a variety of infant cereals sampled from the common markets in the United States and China showed that the concentrations of MeHg in the cereals ranged from 0.07 to 13.9 μg/kg with a mean of 1.61 μg/kg. On the basis of these MeHg concentrations, the daily intake

of MeHg through rice cereal consumption for infants was estimated to be 4-122% of the reference dose (RfD). The MeHg bioaccessibility in the cereals, determined using an *in vitro* digestion method, ranged from 25 to 74% with a mean of 48±16%. A further examination on these results, however, revealed the occurrence of MeHg re-adsorption during extraction steps, which leads to the underestimation of MeHg bioaccessibility and warrants cautions to be exercised when using these procedures to evaluate bioaccessibility in general.

The long-term changes in fish MeHg were investigated through conducting a comprehensive data analysis on datasets for the Everglades, a well-studied aquatic ecosystem for Hg contamination. The results showed a clear decline of MeHg in mosquitofish in the Everglades during the past two decades, which was probably related to changes in environmental conditions (e.g., periphyton, dissolve organic matter, and sulfate) instead of mercury deposition.

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ABBREVIATIONS AND ACRONYMS

Chl-a	Chlorophyll a
CV-GCAFS	Cold vaper gas chromatography atomic florescence spectrometry
EDI	Estimated daily intake
GI	Gastrointestinal
Hg	Mercury
Hg^0	Elemental mercury
Hg^{2+}	Mercuric mercury
IHg	Inorganic mercury
IQ	Intelligence quotient
LMB	Largemouth bass
MeHg	Methylmercury
NOM	Natural organic matter
ppm/ppb	Parts per million/billion
Rfd	Reference dose
SRB	Sulfate reducing bacteria
USEPA	United States Environmental Protection Agency
USFDA	United States Food and Drug Administration

Chapter 1. Introduction

1.1 General background of mercury

1.1.1 Sources of mercury to the environment

Mercury (Hg), a heavy metal, is a highly toxic element that is introduced into the environment both naturally and anthropogenically.[1] Natural sources of Hg may include geologic activities such as volcanic and geothermal emissions, volatilization of Hg in marine environments, and emission of Hg from terrestrial environments. Anthropogenic emissions of mercury can result from a variety of activities, including coal burning, waste incineration, and mining. Previous studies suggested that the annual mercury emissions from natural processes were approximately 5,207 Mg, while the anthropogenic emissions were estimated to be 2,035 Mg. It should be borne in mind that high uncertainties are present for the estimation of Hg emissions from both natural and anthropogenic sources, resulting in large variation in the relative importance of these two sources reported in the literature.[2,3] Once released into the environment, mercury can be transported over a long distance in the atmosphere (and hence it is a global contaminant), undergo complicated biogeochemical processes in the environment, accumulate in the food web, and consequently pose severe adverse effects on human and ecosystem health.[4,5] Mercury contamination in various ecosystems has been a global concern for decades.[6-9]

1.1.2 Mercury species in the environment

Mercury can exist in the environment in different forms at three oxidation states, Hg^0 (metallic), Hg^{2+} (mercuric), and Hg^+ (mercurous), with the last one being rare because of its instability.[10-13] In pure form, it is known alternatively as “elemental” or “metallic” mercury and usually expressed as Hg^0. Elemental Hg can be transformed into many inorganic and organic mercury species by undergoing a variety of processes in the

environment.[12] Major inorganic Hg species include mercuric sulfide (HgS), mercuric oxide (HgO), and other mercury salts such as mercuric chloride ($HgCl_2$). In the environment, inorganic mercury may be converted into organic forms such as methylmercury (MeHg). Methylmercury is the most toxic and common organic mercury compound in the biogeochemical cycling of Hg. Methylmercury is primarily formed in the environment by microbial processes in the presence of sulfate reducing or iron reducing bacteria, although abiotic processes, such as chemical methylation of inorganic Hg species, could produce MeHg.[10,11,14]. Other organomercury species occurring in the environment include ethylmercury (EtHg) and phenylmercury (PhHg) and dimethylmercury (DMHg). The occurrence of EtHg in the environment has been reported, suggesting that EtHg could be an important species during Hg biogeochemical cycling, although how EtHg occurs in the environment remains unclear.[15] The cause of PhHg occurrence in the environment is considered to be related to historical discharge.[16]

1.1.3 Exposure and toxicological effects of mercury

All Hg species are toxic, and the toxicological effects of mercury exposure are dependent on the forms of ingested Hg, among other factors. Of the common Hg species, elemental Hg can cross the membranes of cells and enter the circulatory system, then pose adverse effects on the blood cells, the central nervous system, and kidneys. At the same time, elemental Hg also can cross the placenta and accumulate in fetus, affecting the development of nervous system of fetus. The mercuric mercury (Hg^{2+}) usually affects amino acid transfer and accumulates in kidneys and may cause renal damage. Methylmercury, once entering the body, inhibits the formation of microtubule and synthesis of protein in neurons, affects membrane activities, and causes DNA damage.

Severe kidney damage is also the result of MeHg poisoning.[17-19] More importantly, ingested MeHg in pregnant women can be almost completely (95%) absorbed and readily cross the placenta and blood-brain barriers, hence posing severe health risks to fetus.[20]

Historically incidental, acute mercury exposure, e.g., in Iraq and Minamata, Japan, has occurred. Both incidents were the results of consumption of Hg contaminated food, where in Iraq grain treated with MeHg as a fungicide was used to make bread and in Japan seafood in the Minamata Bay contaminated by MeHg from a chemical plant discharge was consumed. The MeHg poisoning occurred in Iraq caused 6530 patients being admitted to hospital and 459 of them died eventually.[21-23] While 2252 patients were officially diagnosed as Minamata disease, 1043 of them died in the tragedy that occured in Minamata city, Japan.[24-27] Nowadays acute MeHg poisoning incidents are rare. Instead, human mercury exposure occurs mainly through consumption of food, in particular fish/shellfish.

1.2 Mercury exposure through fish consumption

1.2.1 Aquatic mercury cycling and mercury bioaccumulation in fish

The major source of Hg in aquatic system is atmospheric Hg deposition.[28] Once entering aquatic ecosystems, Hg exists in different forms and undergoes various chemical and physical transformations.(Figure 1.1)[29] In aquatic system, dissolved Hg^{2+} can be reduced to Hg^0 with the involvement of aquatic microorganism activities or abiotic processes. The formed Hg^0 can then be emitted back to the atmospheric phase of Hg cycling. More importantly and relevant to Hg bioaccumulation and subsequent human exposure, the dissolved Hg can be methylated to MeHg in the aquatic environment, which is mainly driven by the microbial activities with sulfate (and iron) reducing

bacteria (SRB) being considered as the major contributor. The formed MeHg can be readily taken up by aquatic organisms and then bioaccumulated through aquatic food chains.[29] Studies have shown that Mercury in aquatic environment can be transferred into fish through food web, and around 50% ingested methylmercury accumulate in fish muscle tissue.[30,31] By undergoing bioaccumulation, the concentration of MeHg in large predatory fish could reach mg/kg (ppm) levels, leading to human Hg exposure from fish consumption.

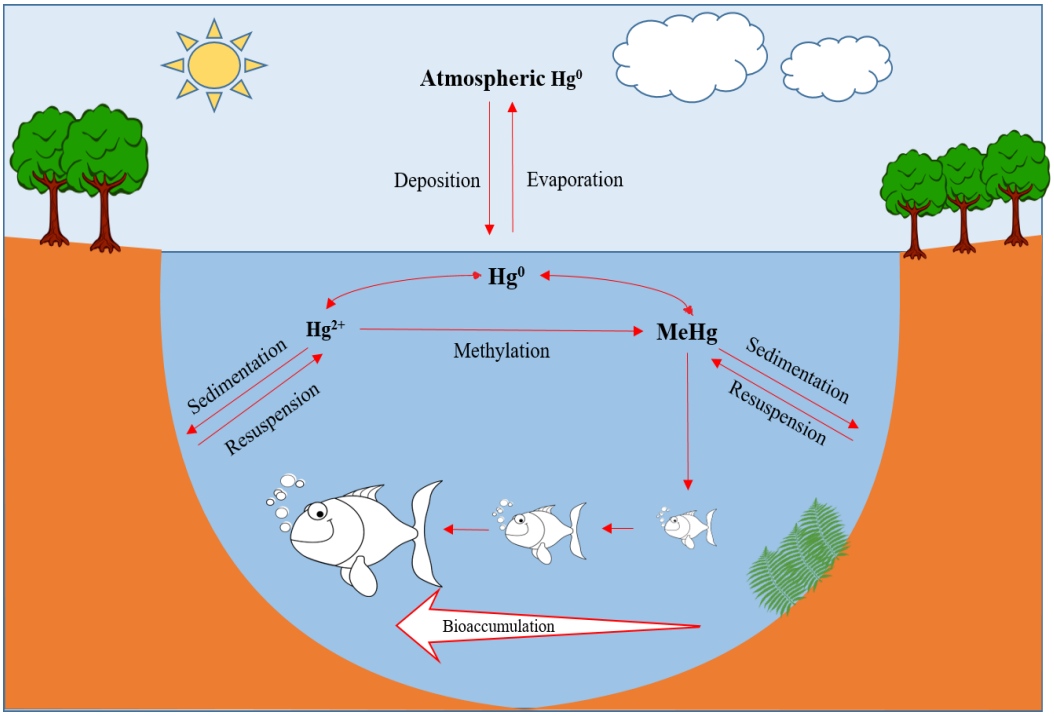


Figure 1.1. Mercury cycling pathways in aquatic environments[28,29]

1.2.2 Mercury concentrations in fish

Typically, as MeHg accumulates in fish tissue, human beings can be exposed to MeHg mainly via fish consumption. According to the United States Environmental Protection Agency (USEPA) nearly all methylmercury exposures in the U.S. occur

through eating fish and shellfish that contain high levels of methylmercury.[32] Some communities eat significantly more quantities of fish than the general population, and thus may be exposed to much greater mercury contamination than the general population. Hence the USEPA suggests issuing a fish consumption advisory when concentrations of MeHg in fish exceed a specific value. The concentrations of Hg in some common fish and shellfish reported by United States Food and Drug Administration are summarized in Table 1.1.[33]

Table 1.1. Reported Hg concentration in fish and shellfish[33]

Species	Mercury concentration, Mean (mg/kg)	Mercury concentration, Median (mg/kg)	Mercury concentration, Min (mg/kg)	Mercury concentration, Max (mg/kg)	Number of samples
Carp	0.11	0.134	ND	0.271	14
Catfish	0.024	0.005	ND	0.314	59
Marlin	0.485	0.39	0.1	0.92	16
Oyster	0.012	ND	ND	0.25	61
Salmon	0.022	0.015	ND	0.19	94
Tilapia	0.013	0.004	ND	0.084	32
Tuna	0.358	0.36	ND	0.82	43
Shark	0.979	0.811	ND	4.54	356
Snapper	0.166	0.113	ND	1.366	67
Swordfish	0.995	0.87	ND	3.22	636
Bass	0.354	0.303	ND	2.18	74
Bluefish	0.368	0.305	0.089	1.452	94
Clam	0.009	0.002	ND	0.028	15

*ND = Not determined

Because of the health risk caused by MeHg intake through eating fish, a great deal of efforts has been made to investigate distribution and magnitude of Hg in fish worldwide during the last few decades. The sources of Hg, Hg accumulation process, concentrations of Hg, MeHg/Hg ratios, and Hg species in fish have been studied by different research groups.[34-42] The MeHg uptake through consumption of fish has been evaluated and

quantified, including the establishment of daily intake dose by governmental and international agencies, with the USEPA reference dose (RfD) of MeHg of 0.1 μg/kg bw/day being widely used.[43]

1.3 Mercury exposure through consumption of rice products

1.3.1 Terrestrial mercury cycling and mercury accumulation in rice

In addition to aquatic cycling and bioaccumulation, terrestrial cycling of Hg, in particular in rice paddy fields, could also result in the production and accumulation of MeHg. Atmospheric Hg can be transferred to terrestrial environments via wet and dry depositions.[44] The reactive gaseous mercury and a portion of Hg on the surface of soil can rapidly volatilize back into the atmosphere,[29] while a large portion of Hg remains in the soil with long retention times.[45]

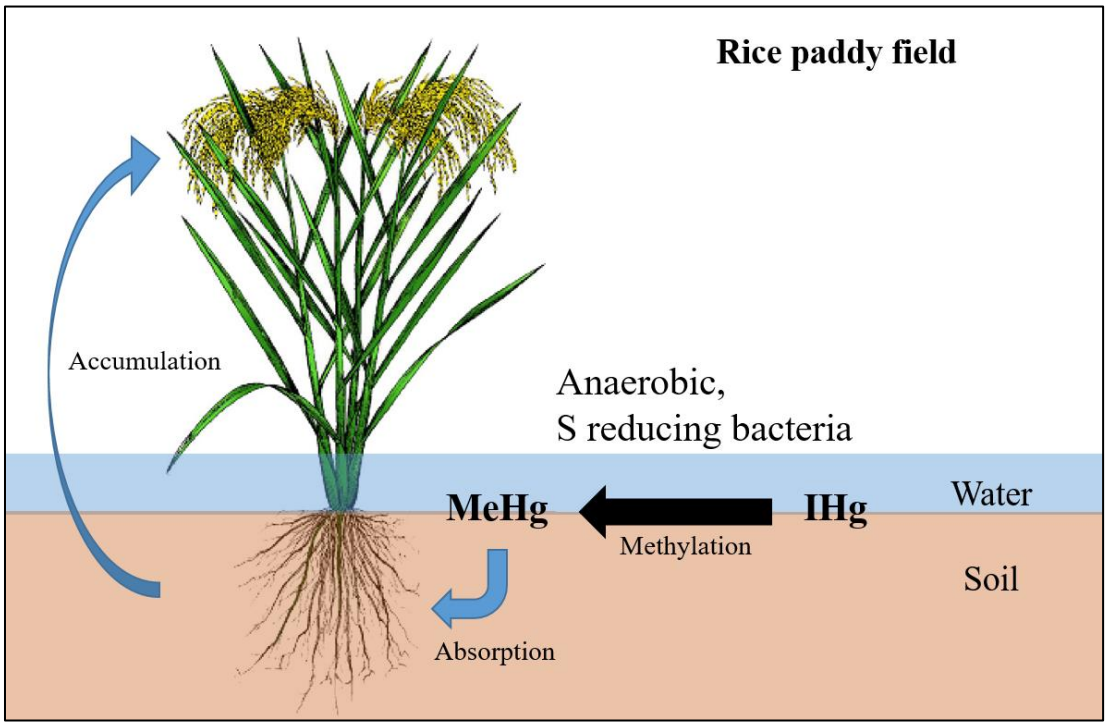


Figure 1.2. Mercury cycling in rice paddy field[28-29,45-51]

The Hg that remains in soil are mainly bound or associated with minerals and organic matter.[46] While some of Hg in soil still can get back to the atmosphere by forming volatile Hg^0 through reduction of Hg^{28} in soil, some Hg could be methylated to form MeHg. The process is especially important for rice paddy fields where the conditions are inductive for Hg methylation, resulting in MeHg production and accumulation in rice. Rice paddy is the dominant agricultural land used for rice plantation, and rice paddy fields also have been considered as important sites for the methylation of Hg in terrestrial ecosystems.[47-51] As the rice paddy has temporarily flooded soil, the methylation of Hg in soil is greatly facilitated in the presence of sulfate reducing bacteria in anaerobic conditions. The formed MeHg can be absorbed by the root and then bioaccumulated through rice plant during the growing season. During the ripening season of rice, MeHg would be readily transferred and accumulated in rice grains.

1.3.2 Mercury in rice and rice products

Although consumption of fish was considered as the only major pathway of MeHg exposure to human beings for decades, recent studies have shown that rice consumption could also be a main pathway of MeHg exposure to human, e.g., in Hg mining areas and in certain inland areas in Southwestern China.[52-54] Over the last decade, studies have confirmed the occurrence of MeHg in rice grain,[47,52,55,56] and found that concentration of MeHg and ratios of MeHg/THg in rice grains are usually much higher than in other plants grown in the same area.[49,57] On the basis of the data reported, the estimated average concentrations of THg and MeHg in rice grains are 8.2 and 2.5 μg/kg for non-polluted sites and 65 and 16 μg/kg for polluted areas (e.g., mercury or gold mining areas), respectively.

The findings of elevated levels of MeHg in rice in certain areas suggest that consumption rice could be a potential MeHg exposure pathway to human which warrants further studies on this potential health risk issue, since rice is one of the most consumed staple food in human diet, sustaining approximately 3 billion people around the world.[58] According to the data obtained so far, the MeHg concentration in rice produced in the US as reported in a few studies are close to that from non-polluted areas.[52] Considering that the MeHg level is relatively low and the average rice ingestion in the US is generally lower than in countries living on rice, the calculation of daily intake of MeHg through consumption of US produced rice indicates that the occurrence of MeHg in rice is probably not a serious concern to adult health. However, great differences in adults and infants must be considered while assessing the potential human exposure to MeHg (see below about infant MeHg exposure).

1.4 Infant exposure to MeHg

When it comes to assess MeHg exposure and potential health risks, adults and infants should be treated differently, as the exposure pathways and the toxicological effects of MeHg are different for them. In fact, infants are much more sensitive to the adverse effects of MeHg exposure because of their high exposure/body weight ratio, and as a result the health risks of MeHg have been predominantly focused on children, infants, and pregnant women.[59] Methylmercury exposure has been previously linked to loss of IQ points, delayed speech and decreased performance in memory function because of the most devastating effects of MeHg are on the developing central nervous system.[60] The MeHg exposure for infants could be through prenatal *in utero* exposure to

maternal MeHg or from postnatal diets, with the former being heavily focused whereas the latter largely overlooked in infant MeHg exposure studies.

1.4.1 Prenatal *in utero* MeHg exposure

Traditionally, prenatal exposure was considered as the main source of accumulation of MeHg in infants. As mentioned above, once being ingested by pregnant women through the consumption of fish, the MeHg can be easily absorbed and readily cross the placenta then transferred to fetus through cord blood. Currently, USEPA has set the reference dose of MeHg as 0.1 μg/kg bw/day, since high level of prenatal MeHg exposure to fetus may cause the cerebral, mental retardation, low birth weight, and early sensorimotor dysfunction.[61] Hence, the best way to prevent fetus being exposed to MeHg seems to be the abandon of consumption of fish containing high levels of MeHg.

1.4.2 Postnatal dietary MeHg exposure

The postnatal pathway for infant MeHg exposure was poorly studied, and only limited information can be found. In previous studies, postnatal exposure was considered primarily related to consumption of breast milk and fish-based food.[62-66] The average concentrations of MeHg in breast milk were found to be 0.17 μg/L (n=182) in Italy, 0.68 μg/L (n=11) in Slovenia, and 0.45 μg/L (n=27) in Japan.[63-67] The mean concentration of MeHg in fish-based infant food reported was 6.1 μg/kg.[68]

Rice cereals have been largely overlooked when considering infant MeHg through diets, probably because of limited information on Hg and MeHg in rice and rice products. Considering the recently reported presence of MeHg in rice, even at elevated levels in some cases, it is intuitive to assume that the MeHg in rice could be transferred to the rice cereals resulting in the occurrence of MeHg in infant cereals. Although there have been

no studies on the presence and concentrations of MeHg in infant rice cereals, previous studies have confirmed the occurrence of varying levels of THg in rice-based baby cereals. On the basis of the results of two studies conducted, the median concentrations of THg in different types of infant cereals sold in Portugal and Spain were reported to be 0.50 (n = 26) μg/kg, ranging from 0.15 to 2.90 μg/kg[69] and 2.61 μg/kg (n = 91), from 0.66 to 5.13 μg/kg, respectively.[70] Hence, the existence of MeHg in rice-based baby cereals should be expected, as a consequence of the MeHg in rice made into cereals. Considering that the reported MeHg concentrations in rice are much higher than those in breast milk and comparable to (for unpolluted areas) or higher than (for polluted areas) MeHg fish-based infant food, it appears necessary to include rice cereals in assessment of infant MeHg exposure.

The increasingly popular rice cereals consumption in the infant diets further warrants the necessity of investigating the extent of MeHg contamination in rice-based baby cereals and the potential exposure of infants to MeHg through cereal consumption. Rice-based cereals have become a vital part of infant diet, as nearly all of infants in US are fed with infant rice cereals.[72] During the last few decades, the bland taste, hypo-allergenic properties of rice and presence of easily digested carbohydrates in rice,[71] have made rice increasingly popular in production of baby foods, especially the ready-to-eat infant cereals, which are the most common first solid food being introduced to American infants.[72] Approximately 81% of infants in the US are introduced to cereals by six months old.[73] Because of the high prevalence of celiac disease (1 in 144) in US, rice-based food became the most safe first solid food fed to infants.[74] The high intake of rice made food is distinct difference between infant and adult diets. Comparing to adults' diet,

infants may consume much higher fractions of rice-based food,[75] which could put infants at higher risks of MeHg exposure than adults. However, the MeHg exposure through consumption of rice cereals, which can be regarded as a new pathway for infants to be exposed to MeHg, has drawn little attention.

Furthermore, although the RfD of MeHg has been established, the RfD set by USEPA only focused on the prenatal MeHg exposure, while the exposure mechanisms of prenatal and postnatal exposure are completely different. Hence, the usage of RfD in assessment of health risk caused by MeHg postnatal exposure may lead to misleading result. However, the postnatal dietary MeHg exposure are severely understudied currently, some of the critical information, for instant, the concentrations, bioaccessibilities, bioavailabilities of MeHg in different types of food except fish, are still missing.

1.5 Bioavailability of MeHg in diet

To estimate human infant's dietary exposure to MeHg, not only MeHg concentration but also the bioavailability, which is presently defined as the proportion of an administer dose that reaches the systemic circulation,[76] of MeHg in diet is needed, since only a fraction of ingested Hg could be released from digested food and then be absorbed eventually by human body. In vivo approaches are most commonly used method to determine the bioavailability of contaminant in food. It can provide the most direct data about how much MeHg in different matrices can get into human body. However, considering the complicacy and the high cost of in vivo bioavailability assessments, the *in vitro* gastrointestinal (GI) digestion models were developed for the evaluation the bioaccessibility of MeHg, which defined as the fraction of ingested MeHg that is released

and soluble in GI fluid.[77] The *in vitro* GI digestion model is an easily controlled, reproductive method that simulates the digestion process occurred in digestive track, and then provides the information about how much and where the MeHg in food is solubilized. At present, there is no standard method, hence various models with different experimental designs exist. Some of commonly used methods were summarized in Table 1.2.[78-80]

Table 1.2. Summary of commonly used *in vitro* digestion model[78-80]

Method	Oral digestion	Stomach digestion	Intestine digestion
Simple Bioaccessibility extraction (SBET)	No	pH 1.5 HCl, 1h	No
In vitro digestion model (RIVM)	pH 6.5 Artificial Saliva, 5min	pH 2 Artificial gastric juice, 2h	pH 7 Artificial intestinal juice, 2h
Simulator of Human Intestinal Microbial Ecosystem of infants (SHIME)	No	pH 4 Artificial gastric juice, 3h	pH 6.5 Artificial intestinal juice, 5h
TNO gastrointestinal Model (TIM)	pH 5 Artificial Saliva, 5min	Initial pH 5 Artificial gastric juice, decreasing to pH 3.5, 2.5, 2, after 30, 60, 90 min	pH 7 Artificial intestinal juice, duodenal secretion at 1ml/min, 6h

All the models were designed according to the residence time of food and properties of GI track, such as pH and chemical component in GI track. Efforts have been made to obtain the bioaccessibilities of MeHg in different types of seafood using various *in vitro* digestive models. The results of previous *in vitro* studies indicated that the bioaccessibilities of MeHg in seafood varied dramatically, ranging from 2 to 100%.[78-83] Studies on assessment of MeHg bioaccessibility in rice and rice products remain lacking.

1.6 Research gaps and objectives of this study

It can be seen from the aforementioned previous studies, the two major MeHg exposure pathways are at different stages of research maturity, with MeHg exposure through fish consumption being studied extensively but exposure from rice and rice products receiving much less attention. Nonetheless, important research gaps are still present for these MeHg exposure pathways, as discussed below.

Studies on MeHg in rice and rice products and the related MeHg exposure are still at the early stage, and little quantitative information is available for dietary mercury exposure through consumption of rice and rice products. The relative contribution and importance of mercury exposure through consumption of rice and rice products, in comparison to fish and shellfish, is not clear. On the basis of the limited available data,[52,70,84] we assume that even the MeHg level in rice is lower than MeHg level in fish, rice and rice products still could create health risk to certain people, like infants. To assess the health hazard that may be caused by rice and rice products containing MeHg, more data such as concentrations of MeHg in rice and rice products and daily intake from them are required. In particular, no studies have reported the concentrations of MeHg in infant rice cereals yet, to our best knowledge.

While bioaccessibility of MeHg in fish has been evaluated, no studies have attempted to assess the bioaccessibility of MeHg in rice and rice products. Bioaccessibility should be considered when evaluating human dietary mercury exposure through food consumption, as mercury species are different in different foods and hence so is bioabsorption. The previously determined bioaccessibility of fish MeHg may not be applicable to the MeHg in rice and related products. Moreover, the results of MeHg

bioaccessibility in seafood obtained from different studies varied within a wide range. Even for the same type of fish, the bioaccessible MeHg could be largely different in inter- and intra-studies, with no real rational explanations being given.[50,80,82,85,86]. Therefore, to solve the concerns related to potentially misleading information on MeHg bioaccessibility, studies need to be conducted to more accurately evaluate the bioaccessibility of MeHg in different types of food.

In addition, the established Rfd for daily MeHg intake were all on the basis of epidemiologic studies where fish consumption was the primary MeHg exposure pathway. However, there are two factors, which may lead to MeHg Rfd for fish consumers not applicable for rice consumers, have been ignored. First, the contents in fish and rice are very different, as rice is rich in starch and fish is rich in protein, thus resulting in varying release and absorption efficiency of MeHg in GI track. Second, the MeHg Rfd was set on the basis of the assumption that MeHg may only get into infants through cord blood, which is a totally different exposure pathway than dietary MeHg exposure. The applicability and implications of using previously determined RfD to evaluate the MeHg exposure and the subsequent health risks need to be carefully examined.

As for Hg exposure through fish consumption, extensive studies have been conducted, but what remains lacking is a comprehensive analysis utilizing the databases that have been generated to examine the long-term changes of MeHg in fish and the possible environmental and ecological causes for these changes. Such a comprehensive data analysis on the changes of fish MeHg and exposure requires long-term and large-scale studies on mercury biogeochemical cycling in typical ecosystems. The ecosystem-wide mercury, geochemical, and ecological studies conducted in the Florida Everglades

since 1980s provide an opportunity to examine the changes in fish MeHg and related controlling factors.

The Everglades is one of the largest freshwater wetlands in the world,[87] and it is a subtropical ecosystem located in south Florida. As the input of Hg to the ecosystem is mainly from atmospheric deposition with only limited anthropogenic interferences involved, the studies on Hg in such an ecosystem would be representative. Elevated levels of Hg have been frequently detected in fish of the Everglades since 1980s, making it one of "hot spots" for Hg study. The long-term Hg studies in this system would help to obtain related information to investigate the MeHg level changes and explain the relationship between the MeHg in fish and environmental factors. The Everglades Regional Ecosystem Monitoring and Assessment Program (REMAP), a comprehensive monitoring and research project led by USEPA,[29] was initiated in 1993 to study the source and biogeochemical cycling of Hg in the Everglades. Up to date, four phases have been completed (1995-96, 1999, 2005, 2014), involving approximately 1300 sampling stations. In addition to REMAP, several agencies have also conducted studies dealing with atmospheric mercury deposition, Hg transport and transformation, and Hg concentrations in game fish, wading birds and other large predators. These studies provide timely and critical information needed for a better understanding of the cycling of Hg in the Everglades. A comprehensive analysis on the data produced by these programs will reveal the changes of fish MeHg during the past years and provide information of great importance for a better understanding of the MeHg cycling, transport, transformation, and bioaccumulation in the ecosystem.

Focusing on these research gaps as identified above, the objectives of this study were set for MeHg in rice products and potential MeHg exposure as follows: 1) To determine the levels of both THg and MeHg in common infant cereals representative of different regions of the US and China, two large markets in infant cereal usage; 2) To estimate the daily intake of MeHg through consumption of rice cereals and assess the potential health risks associated with MeHg ingestion through diets for infants; and 3) To investigate the bioaccessibility of MeHg in various infant rice cereal samples and fish that are commonly available on the market by using an *in vitro* digestion model and explore the possible reasons causing variations in MeHg bioaccessibility.

For the Hg in fish study, the objectives were to 1) elucidate the temporal Hg trend in mosquitofish in the past two decades; 2) evaluate the contributions of atmospheric deposition, climate change and ecosystem alteration to these changes; and 3) investigate how Hg in mosquitofish affect the Hg in lager fish at higher trophic level in the Everglades.

Chapter 2. Occurrence of Methylmercury in Rice-based Infant Cereal and Estimation of Daily Dietary Intake of Methylmercury for infants

The work described in this chapter has been submitted to the *Environmental Science & Technology*

Abstract

Recent reports of elevated levels of methylmercury (MeHg) in rice prompted us to reason that the MeHg in rice may be transferred to infant cereals, leading to potential MeHg exposure and health risks of infant cereal consumption. Hence, we determined total mercury (THg) and MeHg levels in 119 infant cereal samples commonly marketed in U.S. and China and estimated daily MeHg intake through cereal consumption to evaluate potential health risks. Concentrations of THg and MeHg in the tested cereal samples ranged from 0.35 to 15.9 μg/kg and from 0.07 to 13.9 μg/kg with means being 2.86 and 1.61 μg/kg, respectively. Rice-based cereals contained significantly higher THg and MeHg than non-rice cereals, indicating that elevated levels of MeHg in rice could indeed be detected in rice cereals. Cereal consumption could be a potential pathway of MeHg exposure for infants, as the estimated MeHg daily intake through cereal consumption amounted to 4-122% of MeHg reference dose (RfD). This postnatal MeHg exposure through cereal consumption, a different pathway than prenatal exposure adopted for RfD calculations, should be further evaluated for its potential health risks.

2.1 Introduction

Mercury (Hg) is a highly toxic element that occurs naturally in the environment and can be present as the result of human activities, such as combustion of fossil fuels. Mercury can exist in the environment in different forms, among which methylmercury (MeHg) is known as the most toxic Hg species produced mainly from microbial methylation of inorganic Hg (IHg) in sediment and soil.[88] Methylmercury has been of

particular interest because of its high neurotoxicity and capability of bioaccumulation through the food chain.[1,89-91]

Methylmercury accumulated in marine and freshwater fish is considered to be the major source of MeHg exposure to human, as MeHg may bio-magnify through food webs and reach mg/kg levels in large predatory fish which are high enough to cause health risks to humans upon consumption.[1,24,92] According to the United States Environmental Protection Agency [29], nearly all human MeHg exposures in the US occur through consumption of fish and shellfish containing high concentrations of MeHg.[32] For particular groups of people, such as fishermen or island residents who eat significantly more fish than general population, they may be exposed to high levels of MeHg.

Research conducted in southwestern China has shown that rice consumption could also be an important pathway of human exposure to MeHg in Hg mining areas as well as in certain inland areas in Southwestern China, where the amount of fish consumption is limited.[48,93] The studies conducted worldwide confirmed the occurrence of MeHg in rice grains.[52] The elevated levels of MeHg in rice are likely because of the enhanced methylation of inorganic Hg under the floored conditions in the rice paddy field. Rice preferably accumulates MeHg in its grains in comparison to inorganic Hg, and concentration of MeHg in rice grains are usually much higher than in other plants grown in the same area.[54,57] The finding that rice grain is possibly a pathway for human exposure to MeHg triggers the alarm and interest in further studies on this potential issue, since rice is one of the most important staple foods in human diets, sustaining approximately 3 billion people around the world.[58] Over the last decade, studies have investigated total Hg (THg) and MeHg levels in rice around the world.[52,56,94] On the basis

of the data reported, the estimated average concentrations of THg and MeHg in rice grains are 8.2 and 2.5 μg/kg for non-polluted sites and 65 and 16 μg/kg for polluted areas (e.g., mercury or gold mining areas), respectively. The Hg levels in rice produced in the US as reported in few studies are close to that from non-polluted areas,[52] and it seems that the occurrence of MeHg in rice is probably not a serious concern to adult health, as the average rice ingestion in the US is generally lower than for people in countries that consume rice as the main part of the diet.

However, as far as risk assessment of the potential human exposure to MeHg is concerned, great differences in adults and children (in particular infants) must be considered. First, because of its bland taste, hypo-allergenic properties and presence of easily digested carbohydrates,[71] rice has been increasingly used in production of baby foods, especially ready-to-eat infant cereals, which are the most common first solid food being fed to American infants.[72] Approximately 81% of infants in the US are introduced to cereals by six months of age,[73] and the high prevalence of celiac disease (1 in 144) also makes the rice-based food the most safe first solid food being fed to infants.[74] Second, compared to adults' diet, infants may consume much higher fractions of rice-based food,[75] which could put infants at higher risks of MeHg exposure than adults. Moreover, infants are much more sensitive to MeHg exposure because of their high exposure/body weight ratio.[59] Since MeHg exposure has been previously linked to loss of IQ points, delayed speech, and decreased performance in memory function because of the most devastating effects of MeHg on the developing central nervous system,[60] increasingly popular rice cereals consumption may lead to MeHg exposure and potential health risks

in infants, which, as a new pathway for infants to be exposed to MeHg, has drawn little attention.

Traditionally, prenatal exposure was considered as the main source of accumulation of MeHg in infants, while postnatal exposure was primarily related to consumption of breast milk and fish-based food.[62-66] However, as a vital part of infant diet, rice-based cereals have been largely neglected because of the perception of low Hg in rice and rice products. As elevated MeHg has been observed in rice and rice-based cereals,[95] it is necessary to investigate the extent of MeHg contamination in rice-based baby cereals and the potential exposure of infants to MeHg through cereal consumption. Therefore, the objectives of this study were to 1) determine the levels of both THg and MeHg in common infant cereals representative of different regions of the US and China, two large markets in infant cereal usage, and 2) estimate the daily intake of MeHg through consumption of rice cereals and assess the potential health risks associated with MeHg ingestion through diets for infants.

2.2 Materials and methods

2.2.1 Infant cereal samples and sample classification

In this study, a total of 119 infant cereal samples were purchased, among which 58 samples were from four big cities in different regions of the U.S. (Miami, FL; New York, NY; San Jose, CA; and Chicago, IL) and 61 samples from four different cities in China (Beijing, Wuhan, Nanjing, and Qingdao). These cities are located geographically in different areas, representing possible rice derived food sources that infants would be exposed to in these two countries. Cereal samples were purchased in local grocery stores

or online and then kept in a cabinet inside a Class 100 clean room (with inlet air purified by gold-coated sand to remove Hg) to avoid contamination.

The cereal samples used here include common brands available in the market, and they were supplied in tin cans, cardboard boxes or plastic containers. The product information provided by the manufacturers about the products includes total fat, carbohydrate and protein amount per serving. The information is used in sample classification and data interpretation without further validation. The contents of carbohydrates, fat and proteins in these samples were in the range of 28.0-86.7%, 0.0-12.6% and 1.9-15%, respectively. For the convenience of discussion, the studied samples were grouped by the type of grain used to make cereals, including rice based (rice only, n = 79), multigrain based (mixture of rice and oat, wheat, corn, rye or quinoa, n = 9) and other grain based (no rice at all, n = 31) cereals according to the ingredients given by the manufacturers. Rice-base cereals were further divided into different subgroups according to manufacturers, including brands A (n = 10), B (n = 5), C (n = 8), D (n = 2), E (n = 2), F (n = 2), G (n = 3), H (n = 6), I (n = 10), J (n = 10), K (n = 2), L (n = 4), and M (n = 3). There are 12 other brands with one sample for each brand, they were classified as group N. These samples were also classified by the locations, including Miami (MIA, n = 15), New York (NY, n = 4), Saint Jose (SJ, n = 4), Chicago (CHI, n = 3), Beijing (BJ, n = 13), Qingdao (QD, n = 13), Nanjing (NJ, n = 6) and Wuhan (WH, n = 21).

2.2.2 Chemicals and reagents

Ultrapure deionized water produced by a Barnstead Nanopure Diamond water purification system with resistivity of 18 MΩ·cm was used for preparing all solutions. American Chemical Society certified grade reagents, such as sulfuric acid, potassium

hydroxide, hydrogen peroxide, copper sulfate, potassium bromide, methylene chloride, and tin chloride, and TraceMetal grade nitric acid and hydrochloric acid were used to minimize the blank problem. All chemicals were supplied by Fisher Scientific, unless otherwise specified. All glassware was soaked in 10% (v/v) nitric acid for overnight, then rinsed with ultrapure deionized water, and baked at 500 ºC in a muffle furnace for 5 h before use.

The acidic potassium bromide solution used for MeHg extraction was prepared by dissolving 180 g of KBr in 250 ml water containing 50 ml concentrated sulfuric acid, and the solutions were mixed up and made up to 1 L using water after cooling down to ambient temperature. Copper sulfate solution (1 M) and citric buffer (1 M) were prepared by adding appropriate amounts of salts in water. Ethylation reagent was prepared by dissolving 1 g of sodium tetraethylborate ($NaBEt_4$, Sigma-Aldrich) in 100 ml of 2 % (w/w) potassium hydroxide. And 40 g of tin chloride was dissolved in 2 L 1 % (v/v) hydrochloric acid to obtain reductant solution used in THg analysis.

Certified 1000 mg/L Hg and 10 mg/L MeHg standard solutions were used for quantification during THg and MeHg analysis. Certified reference materials, GBW10043 (IGGE) rice flour and DORM-2 (National Research Council of Canada), were used for quality control and validation of THg and MeHg determination methods.

2.2.3 Determination of THg and MeHg in cereals

Concentration of THg was analyzed by using the slightly modified method described by Horvat et al.,[96] and MeHg levels in cereal samples were measured using a gas chromatography-atomic fluorescence spectrometry (GC-AFS) (Brooks Rand Automated

MERX) MeHg System, following a extraction method reported previously.[97] The details of sample preparation and analysis methods can be found in supplementary material.

2.2.4 Method validation and quality assurance

Quality control (QC) and quality assurance (QA) were performed following the established procedures.[96] Limit of detection (LOD) of THg and MeHg analysis, estimated by three times standard deviation of 6 replicates of a blank sample, were 0.18 μg/kg and 0.05 μg/kg, respectively. The accuracy of methods was evaluated by spiking reference standard solutions into samples and by analysis of certified reference materials. The mean recoveries of matrix-spiked standards for MeHg and THg analysis were 100.8 ± 30.3% (n = 15) and 102.6 ± 12.7% (n = 13), respectively. The mean recoveries of DORM-2 (Fish protein CRM certified for trace metals) and GBW10043 (rice flour CRM certified for metals) used in MeHg and THg determination were 90.5 ± 27.7% (n = 6) and 96.5 ± 18.2% (n = 6).

2.2.5 Estimation of daily intake of MeHg from rice-based cereal consumption

The daily intake of MeHg was calculated on the basis of the MeHg concentration in food, daily food consumption and body weight (bw) of infants. The equation 2.1 was used in calculation of estimated MeHg daily intake (EDI):

$$EDI = \frac{\sum (C_{MeHg} \times IR)}{bw} \qquad (2.1)$$

Where EDI is given in micrograms per kilogram of body weight per day; C is the concentration of MeHg (μg/kg) and IR is intake rate (g/day).

2.2.6 Data analysis

All data were analyzed using JMP 10.0.0 software (SAS institute Inc., Cary, NC, USA) and Excel 2013 (Microsoft corp., WA, USA). General linear fit, one-way ANOVA,

and box plot were used to identify significant differences ($p < 0.05$) between data from two different groups of samples.

2.3 Results

2.3.1 Hg in infant cereals

The data of Hg levels in rice cereals studied were listed in Table 2.1. Detailed information about all cereal samples, including types of cereals (rice, other grain, and multi grain), concentrations of THg and MeHg, locations purchased, and marketing brands are summarized in Table 2.2.

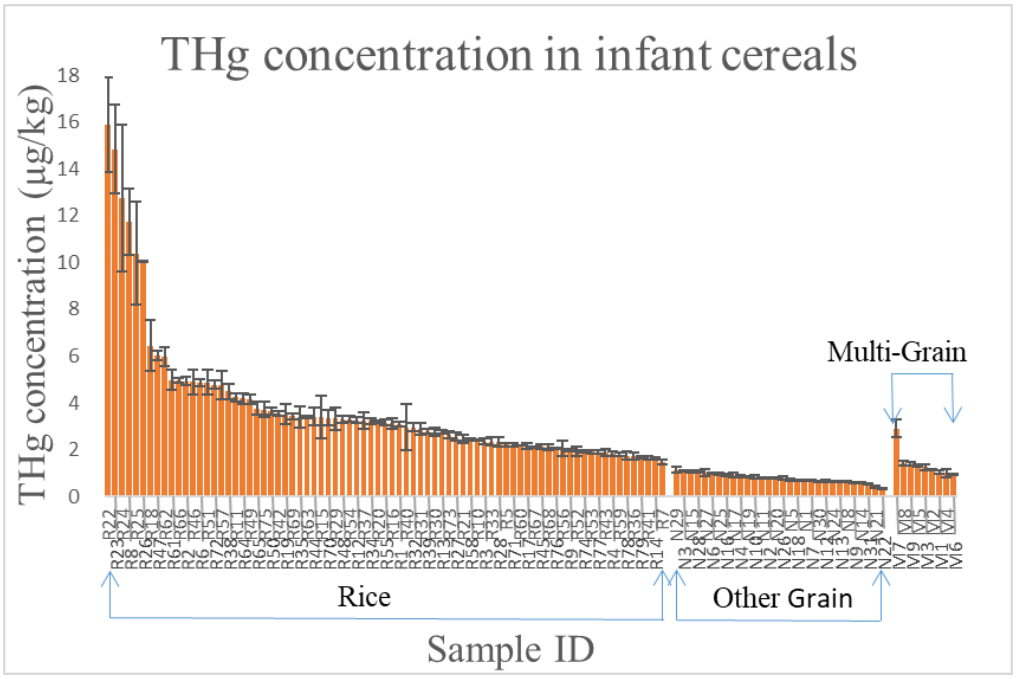
Table 2.1. Hg concentrations (μg/kg) and MeHg/THg ratios in infant cereals.

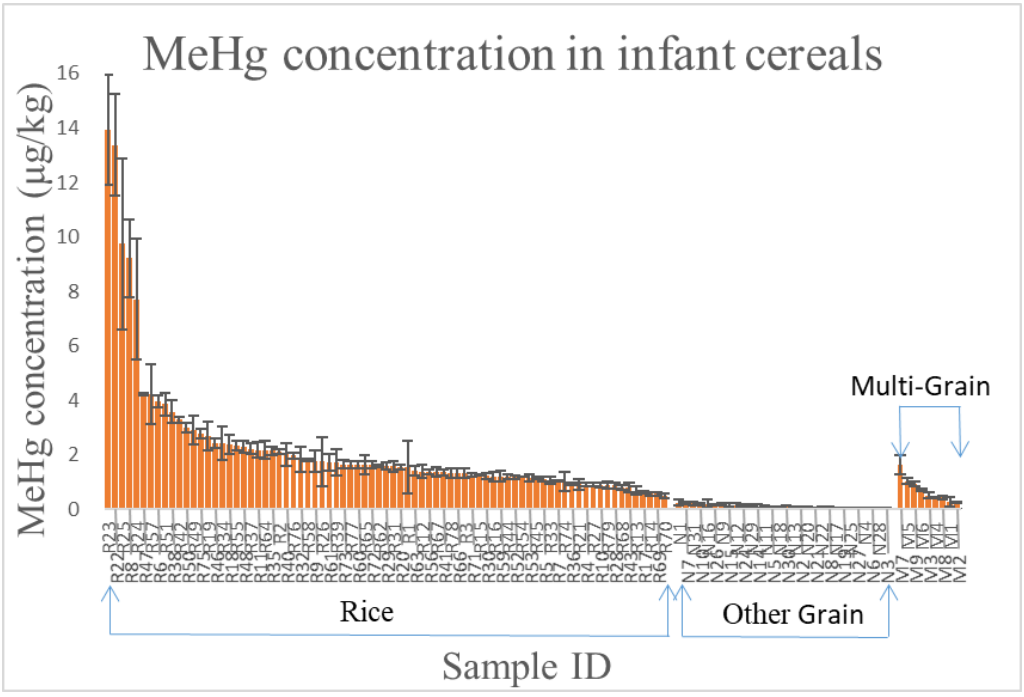
Type	n	THg[a] (μg/kg)	STD	MeHg[a] (μg/kg)	STD	MeHg/THg[b] (%)
Rice	79	3.82 (1.47-15.88)	2.83	2.29 (0.57-13.94)	2.46	57 (16 - 96)
Multi-grain	9	1.38 (0.94-2.91)	0.60	0.70 (0.27-1.64)	0.44	51 (23 - 80)
Other grain	30	0.78 (0.35-1.14)	0.20	0.12 (BDL-0.26)	0.06	15 (3 - 40)

[a]Mercury concentration in different types of cereal, means and ranges were presented
[b]MeHg/THg ratio (w/w) in different types of cereal, means and ranges were presented

The concentrations of THg and MeHg were presented for each group of cereals (rice-based, multigrain, and non-rice) in Fig. 2.1 (within group samples were ranked from high to low on the basis of Hg concentrations). For the group of rice-based cereal samples, the concentrations of THg and MeHg ranged from 1.47 to 15.9 μg/kg with a mean value of 3.81 μg/kg and from 0.51 to 13.9 μg/kg with a mean of 2.28 μg/kg, respectively. For the multi-grain cereal samples, THg and MeHg concentrations were in the ranges of 0.94-

2.91 µg/kg and 0.27-1.64 µg/kg with mean concentrations of 1.37 and 0.70 µg/kg, respectively.





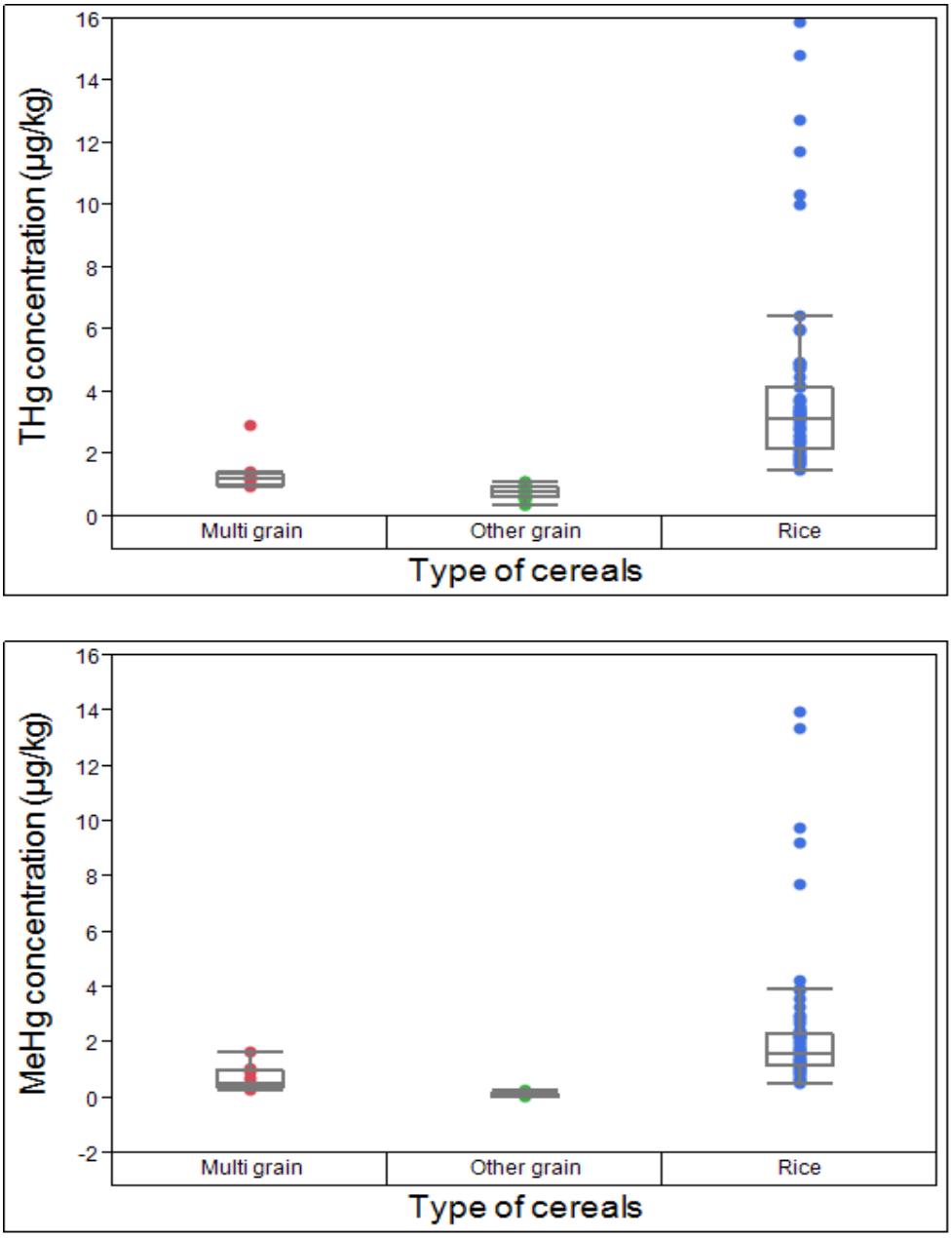


Figure 2.1. MeHg and THg levels in the infant cereal samples tested (µg/kg)

The concentration ranges of THg and MeHg in no rice samples were 0.35-1.14 µg/kg and 0.03-0.26 µg/kg with mean values of 0.78 and 0.11 µg/kg, respectively. Turkey's test on results of Hg determination suggests that THg concentrations in rice-based cereal

samples were significantly higher than those in cereals contain no rice ($p < 0.0001$), and multi-grain cereals ($p = 0.0103$). While the MeHg concentrations in rice cereal were only significantly higher than those in non-rice grain cereal samples ($p < 0.0001$).

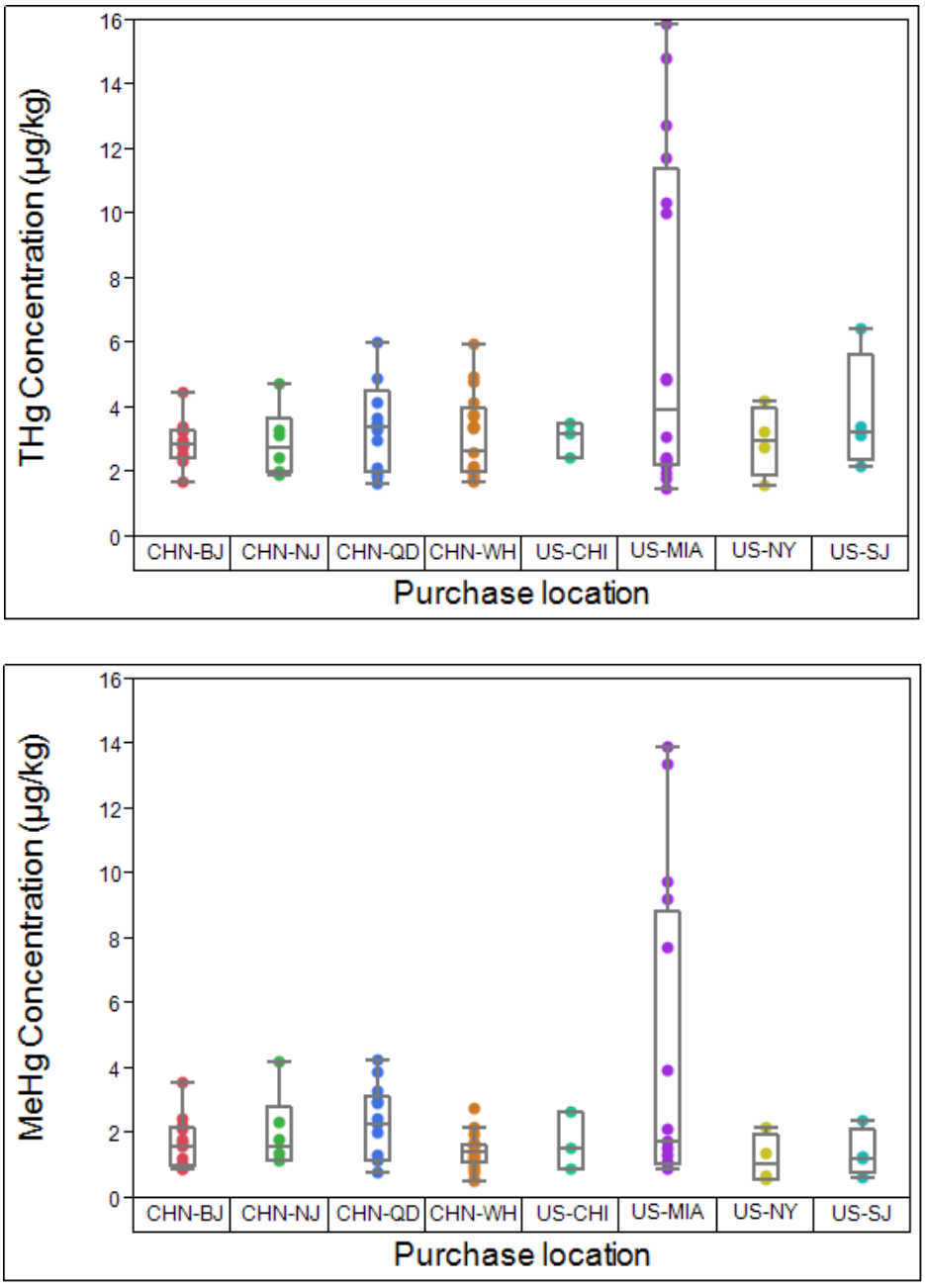


Figure 2.2. MeHg and THg content in rice cereals purchased from different cities

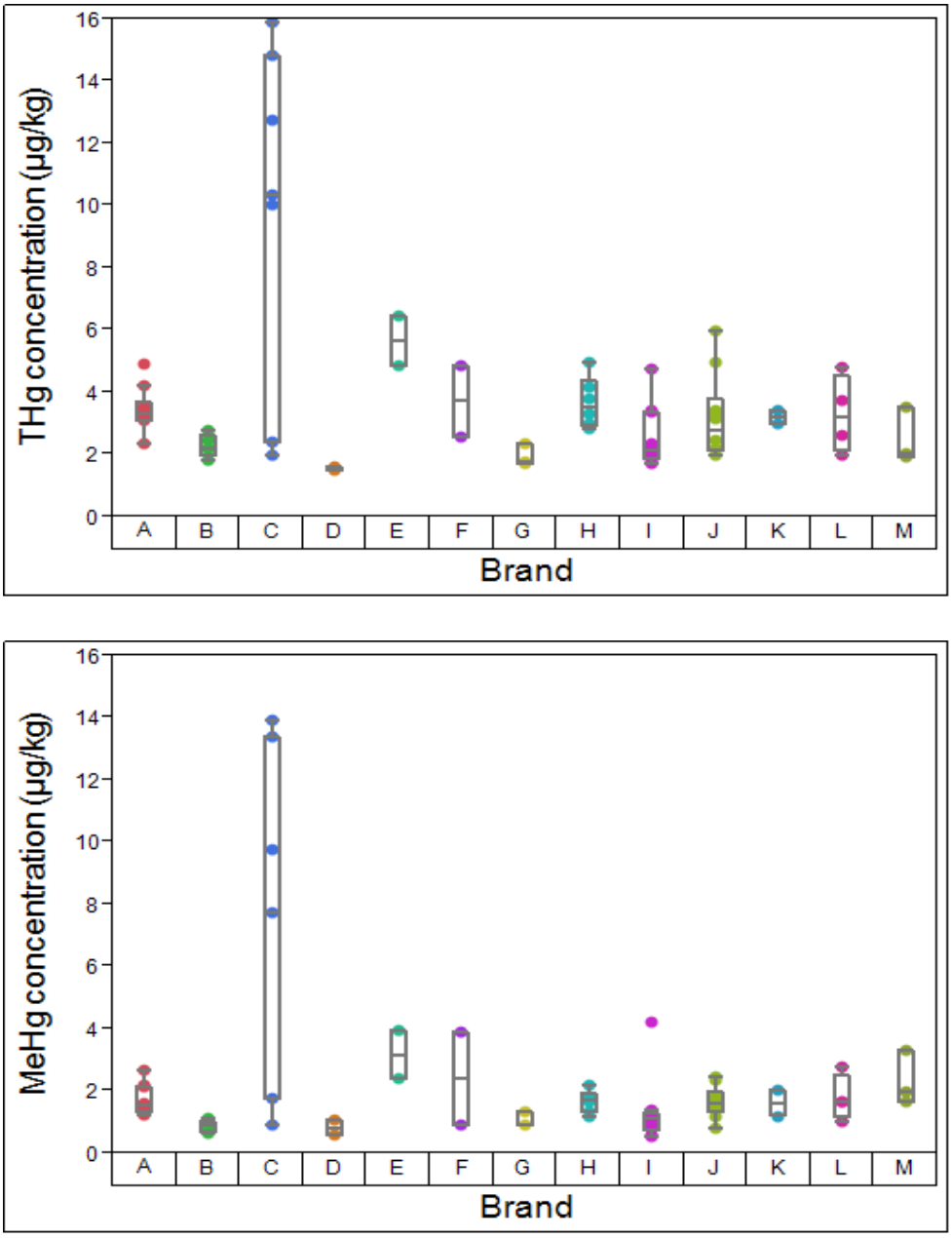


Figure 2.3. MeHg and THg level in different brands of rice cereals

For the group of rice-based cereal samples, statistical analysis was performed to examine the relationship between Hg concentrations and the locations where the samples were purchased (Fig. 2.2). Significant differences were not observed for both MeHg and

THg contents in all cities in both USA and China except Miami ($p = 0.32$ for MeHg and $p = 0.85$ for THg). Five samples from Miami, including 3 samples purchased online (with warehouse locations around Miami), contained distinctly high concentrations of both THg (10.3-15.9 μg/kg) and MeHg (7.72-13.9 μg/kg), resulting in overall mean concentrations (6.44 μg/kg for THg, 4.47 μg/kg for MeHg) being significantly higher than those from other cities (3.14 μg/kg for THg, 1.72 μg/kg for MeHg). Similar statistical analysis was conducted against the samples grouped according to the manufacturers (Fig. 2.3), and lower concentration of both THg and MeHg were observed in rice cereal samples made by manufactures B, D, and G.

2.3.2 Estimation of daily intake of MeHg from rice-based cereal consumption

The dietary intake of MeHg via rice-based cereals was estimated using concentration of MeHg determined in the infant cereal, on the basis of the recommended daily dose of infant cereal for the various stages of infancy (4-5 months: 46.5 g/day, 6-8 months: 67.5 g/day, and 9-11months: 84 g/day) and median value of weight-for-age of infants, which was calculated by averaging the weight-for-age of boys and girls (4-5 months: 5.19 kg, 6-8 months: 8.11 kg, 9-11 months: 8.96 kg and 12-24 months: 10 kg).[75,98] Figure 2.4 presents the estimated dietary intake of MeHg for infants fed on the studied infant cereals with a red line showing the reference daily dose (RfD), which is 0.1 μg/kg/day, set by USEPA. The estimated intake of MeHg of these cereals ranged between 0.004 and 0.123 μg/kg/day with a mean of 0.020 μg/kg/day

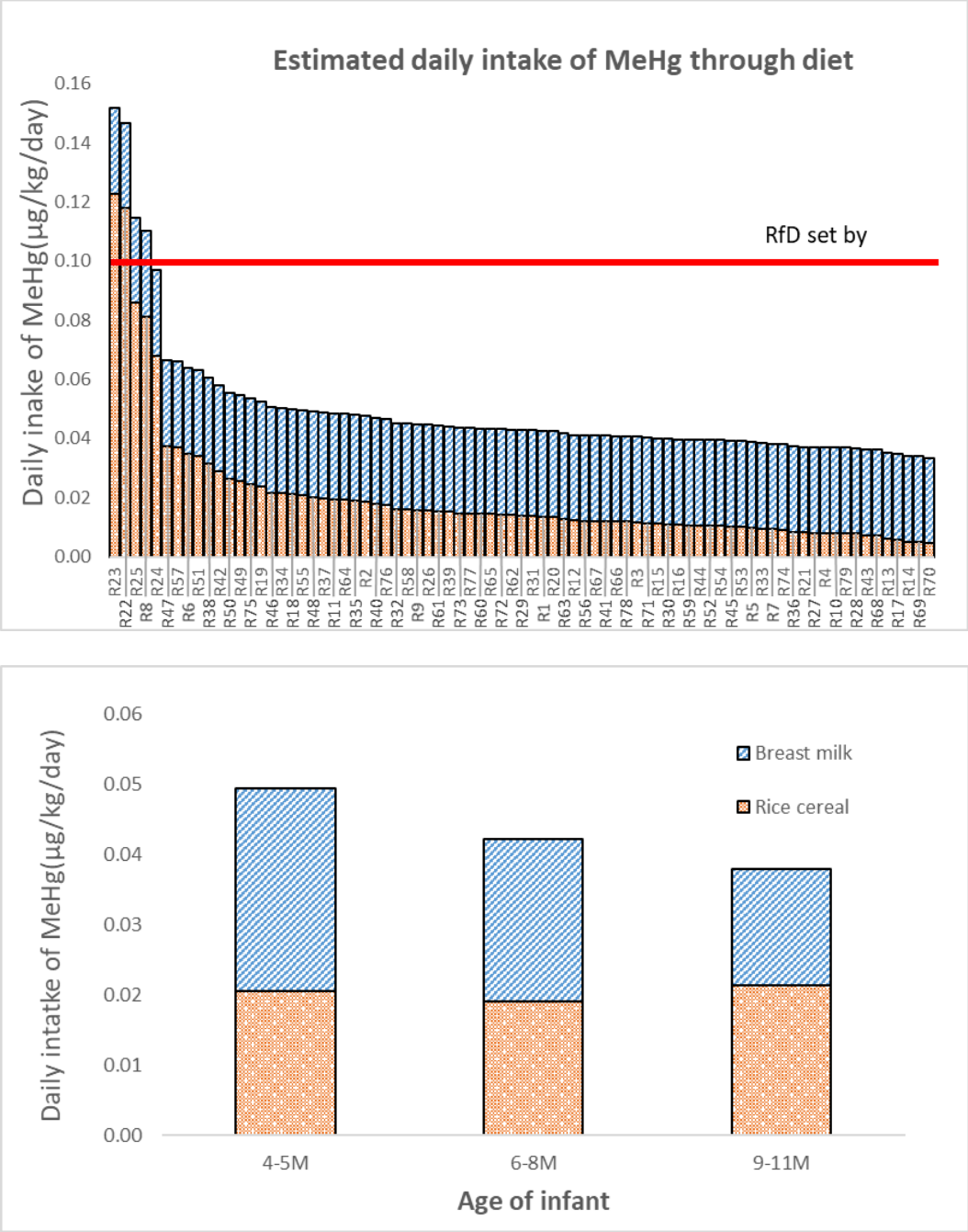


Figure 2.4. Estimation of average dietary MeHg daily intake from cereal consumption (dot) and breastmilk consumption (diagonal) for first year infants

2.4 Discussion

2.4.1 Hg contents in infant cereals

Infant cereal is the traditional choice of the first solid food for infants. It is often mixed with infant formula or breast milk to give a slurry consistency and constitute a basic integral part of an infant diet during the first year of life. Because of the recent finding of elevated MeHg in rice, infant cereals might be potentially considered as a main source of MeHg intake for infants. In this study, mean concentrations of 2.86 ± 2.69 μg/kg for THg and 1.61 ± 2.23 μg/kg for MeHg were found in the cereal samples analyzed. Our THg results seem to agree with previous studies where median concentrations of THg in different types of infant cereals sold in Portugal and Spain were reported to be 0.50 (n = 26) μg/kg, ranging from 0.15 to 2.90 μg/kg[69] and 2.61 μg/kg (n = 91), from 0.66 to 5.13 μg/kg, respectively[70]. As for MeHg, the results could not be compared to the literature, since no previous study has reported the concentrations of MeHg in rice-based infant cereals, to the best of our knowledge.

The potential sources of Hg, especially MeHg, present in the infant cereals may mainly include the raw materials used and the manufacture processes where Hg could be introduced as a contaminant. Although infant cereals may be made from a variety of materials including rice, wheat, oat, corn, rye and quinoa, rice is the most widely used food material for the production of infant cereals and has been used in a large quantity.[99] In comparison to other grains, rice contains higher Hg concentration possibly because of its higher ability to accumulate inorganic Hg as well as MeHg.[50,54,100,101] Recent studies have demonstrated the presence of elevated MeHg in rice grains because of the bioaccumulation of Hg, MeHg in particular, in rice grain.[49,54,102] The MeHg accumulated

in rice grains may end up in the infant cereals when the rice is used for cereal production, resulting higher concentrations of Hg in rice-based cereals compared to other grain based cereals.

The comparison among three groups of cereals (rice-based, non-rice, and multi-grains) showed that THg contents in rice-based cereals were significantly higher than that in cereals containing no rice (Fig. 2.1 and Table 2.1). For multi-grains in which several grains were normally supplemented in addition to rice, THg was also less than that in rice-based cereals, but this difference was not significant. These results indicate that the elevated THg in infant cereals is likely from the rice used for cereal production. Support for this notion came from a line of evidence. First, the determined THg and MeHg contents in rice-based infant cereals in this study are comparable to reported Hg levels in rice samples,[48,51,52,95] suggesting that the source of Hg in cereals could be rice. Second, MeHg levels in rice-based cereals were significantly higher than in cereals containing no rice ($p < 0.0001$), as indicated by comparisons using Turkey's test. The result is strongly indicative of rice being the source of Hg in cereals. Third, the significantly higher MeHg/THg ratios in rice-based cereals (57%) than in non-rice cereals (15%) provided further evidence that rice is the main source of MeHg in infant cereals (Fig. 2.5). Previous studies have shown that rice plants preferably accumulate MeHg in comparison to inorganic Hg, resulting in higher MeHg/THg ratios in rice grains,[49,54] whereas this selective accumulation has not been reported in other plants/grains.[102] The high MeHg/THg ratio also could be the result of removing hull and bran while producing the white rice which was generally used to make rice cereal. Since around 70-80% of inorganic Hg in rice is present in the hull and bran, and this portion of IHg would be

eliminated during the polishing process,[50] whereas MeHg in rice, mainly present in grain, would remain in polished rice. As a result, the MeHg/THg ratio would be higher in white rice and this higher MeHg/THg ratio would be transferred to cereals during cereal production.[50] The loss of IHg during grain processing might also explain large variations in THg contents in cereals observed in this study, as around half of rice based cereal samples have similar levels of THg with multi grain and non-rice cereals.

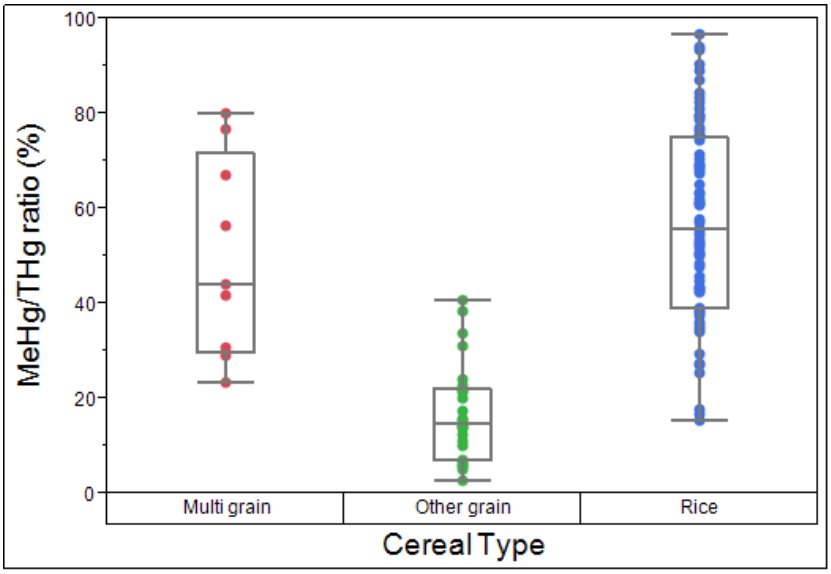


Figure 2.5. Boxplots of MeHg/THg ratios in different types of cereals

Significant differences were not observed while comparing both THg and MeHg contents in the cereal samples purchased from different areas. The result was not unexpected since the locations of purchase are probably not directly linked to the sources of rice grains used for cereal production. It was found that sometimes cereal samples purchased from different cities had the same manufacture product lot numbers. It should be pointed out that 5 of the 15 rice cereal samples from Miami showed distinctly high concentrations of THg and MeHg in comparison with the rest samples from Miami and

from the other cities (R26 was high in THg but low in MeHg) (Fig. 2.2). These 5 samples, produced by the same manufacturer and only were purchased in Miami, were labeled as hot-cereal, meaning that they need to be boiled before serving. These samples were obtained through repeated sampling of this specific type of cereal in the Miami market at different times, and thus they were unlikely manufactured in the same production batch (as indicated by different product lot numbers on the labels). Although the reason why these hot-cereals contain much more MeHg from this manufacturer is unclear, the high Hg contents in all these 5 samples could suggest that this specific type of cereals from this manufacturer could be produced from rice with high levels of Hg and/or be related to manufacturing process.

Comparing the contents of Hg in rice cereals from different manufacturers, no significant differences were found among the most common brands, including brand A with around 80% market share in USA, and brand H, I and J with a combined 75% market share in China,[103,104] while THg and MeHg levels showed significant differences among some brands with small market shares (Fig. 2.3). Several brands, such as B, D, and G, contained lower levels of MeHg, while brand C contained significantly higher concentrations of both THg and MeHg, probably because of varying THg and MeHg levels in the rice used for cereals production and the effect of manufacturing processes. Unfortunately, as we were not able to obtain the rice used for production of cereals from manufacturers, it is difficult to evaluate the effects of manufacturing processes on the MeHg in cereal products. However, as previous studies have shown that cooking processes had limited impact on the MeHg concentration in different types of food,[79,83] the cereal manufacturing processes, mainly the cooking not packing process, may not be

a major factor affecting MeHg in the cereal products. The rice that is used for the cereal production should be considered as a crucial factor, as the source of Hg in rice-based infant cereals could be attributed to rice as discussed previously. Therefore, differences in the Hg contents found among various brands were indicative of the differences in origin of rice used.

2.4.2 Estimation of daily intake of MeHg

The estimated daily intake values of MeHg in this study were compared to the EPA RfD (0.1 μg/kg/day)[43] to evaluate the health risks associated with consumption of rice-based infant cereals (Fig. 2.4). The calculated daily intake of MeHg ranged from 0.004 to 0.122 μg/kg/day, accounting for 4-122% of the RfD, with a mean of 20%. Two of the 79 rice-based infant cereals exceeded the MeHg RfD, suggesting potential MeHg exposure of infants through cereal diet. Considering that cereal diet is not the only MeHg exposure pathway for infants and that breast milk has been regarded as an important source of MeHg in infants,[63,66] we further estimated the MeHg daily intake through both cereals and breastfeeding. The concentrations of MeHg in breast milk were found to be 0.17 μg/L (n = 182) in Italy, 0.68 μg/L (n = 11) in Slovenia, and 0.45 μg/L (n = 27) in Japan.[63-67] On the basis of these reported concentrations and sample sizes we calculated a mean concentration of 0.23 μg/L for MeHg in breast milk. Using the recommended daily intake of human milk according to the EPA handbook for various stages (4-5 months: 126 ml/kg/day, 6-8 months: 101 ml/kg/day, and 9-11 months: 72 ml/kg/day),[105] we estimated MeHg daily intake through breast milk and the overall dietary MeHg intake. The dietary MeHg daily intake through breast milk for infants less than one-year-old ranged from 0.037 to 0.049 μg/kg/day with a mean of 0.043 μg/kg/day, which is 37-49% of the MeHg

RfD (Fig. 2.4). When combining MeHg daily intake through both breast milk and rice cereal, approximately half of calculated daily intake values exceeded 50% of the EPA RfD, while for 5 cereal samples the MeHg intake could be over or close to the RfD value. The daily intake of MeHg through breast milk and cereals for infants seemed to decrease with age (Fig. 2.4), but this should not be interpreted as a decrease in dietary MeHg exposure. For infants of 8-10 months old, fish-based infant food, which have been proven to be higher in MeHg,[68] may be introduced to the diet, leading to increases in MeHg daily intake.

Although we compared the estimated MeHg daily intake of infants through cereal diet to the EPA RfD for evaluation of potential exposure and health risks, it should be borne in mind that there are differences in the calculation of RfD and the scenario of infant exposure to MeHg through ingestion of cereal diet. The RfD was developed on the basis of the evaluation of the neurodevelopmental effects of prenatal MeHg exposure resulting from maternal fish consumption which would expose fetuses to MeHg *in utero*. In establishing the RfD, a benchmark dose level of 11 ppm of Hg in hair was calculated as the lower limit of the 95% confidence interval for the maternal-hair concentration corresponding to a 10% extra risk level.[106] A ratio of 250:1 was used to convert the maternal hair Hg concentration to cord blood Hg concentration, which is 44 μg/L. The benchmark dose level (BMDL) for daily intake of MeHg was calculated as 1.1 μg/kg according to Equation 2.2.

$$\mathrm{BMDL} = \frac{\mathrm{C}\times\mathrm{b}\times\mathrm{V}}{\mathrm{A}\times\mathrm{f}\times\mathrm{bw}} \qquad (2.2)$$

Where C = concentration in cord blood (44 μg/L); b = elimination constant (0.014 day^{-1}); V = volume of blood in body (5 L); A = absorption factor (0.95); F = fraction of daily intake taken up by blood (0.05); bw= body-weight (60 kg)

An uncertainty factor of 10 was then used to calculate RfD.[107]

$$RfD = \frac{BMDL}{UF} = \frac{1.1\ \mu g/kg/day}{10} = 0.1\ \mu g/kg/day$$

The development of RfD did use neurodevelopmental effects of fetus and infant as the sensitive end point for MeHg toxicity, but the RfD was on the basis of maternal intake of MeHg (e.g., through fish consumption) which would be transferred to fetuses *in utero* via cord blood. This is different than postnatal exposure of infants to MeHg through cereal diet, where infant bodies are exposed to MeHg by digesting the food and taking up the MeHg contained in the cereals. As fish consumption was assumed to be a major pathway of MeHg exposure for establishing the RfD,[43] the bioaccessibility and bioavailability of MeHg in rice-based cereals could be different than that in fish because of the different composition of these two types of food. In addition, the elimination rate of MeHg is directly related to exposure frequency,[108] and thus for population group like infants who are exposed to MeHg through diet (breast milk and rice cereal) everyday, a lower elimination rate likely needs to be considered when evaluating health risks. Animal experiments have in fact shown that infants may not be able to eliminate MeHg from their body because of the lack of de-methylation bacteria in gut.[109] All factors mentioned above may greatly influence the RfD calculation.

Considering the difference between the EPA RfD and the postnatal MeHg exposure through cereal diet, caution should be exercised when evaluating the health risks

associated with infant exposure to MeHg in cereals, and simply comparing to the RfD might not provide accurate health risk information. While prenatal MeHg exposure has been extensively studied for its adverse effects on the developing nervous system during fetal, infant, and even childhood stages, postnatal MeHg exposure through diets for children of early ages and the related toxicological effects have received less attention. In a US-based study on 24-month-old children, better Bayley Mental Developmental Index [21] scores were associated with Hg concentration (albeit non-significantly), while increased risk of delayed fine motor skill on the Denver Developmental Screening Test was marginally correlated with higher 3-month postpartum infant hair Hg levels for 26-month-old children in an Italian study.[110,111] As the EPA RfD might not be able to accurately reflect the exposure levels and health risks when infants are exposed to MeHg through diets after birth, it might be informative to compare the results to animal studies when evaluating the health effects of postnatal MeHg exposure. Since MeHg exposure could produce similar neurodevelopmental effects in monkeys and in humans, animal studies using monkeys are particularly relevant in this regard.[112,113] Studies in a cohort of monkeys revealed sensory system impairment and evidence of delayed neurotoxicity, when they were dosed beginning *in utero* and continuing until 4 years of age.[113-115] Reference doses were then derived from these data to evaluate the effects of prenatal and postnatal Hg exposure, suggesting that, for combined *in utero* and postnatal exposure, reference does ranging from 0.01 to 0.05 μg/kg/day could be obtained when an uncertainty factor of 10 was used for extrapolation from animals to humans.[113-115] These results again suggest that the exposure and health effects of postnatal MeHg exposure through diets (e.g., rice cereals, which have received little attention previously) are

probably not evaluated properly if simply comparing to the EPA RfD. Further studies considering the specific characteristics in uptake, metabolism, and excretion of MeHg for infants and children at different ages are warranted for a more precise evaluation on the effects of postnatal diet MeHg exposure during infancy and childhood.

Supplementary Data

Details of Sample preparation and analysis of THg and MeHg in cereals

Digestion of cereal samples for THg analysis was performed using a hot block at 140 ºC for 4 h, following addition of 0.2 g sample to a premixed acid composed of concentrated nitric acid (8 ml) and sulfuric acid (2 ml) in a pre-cleaned 50 ml glass digestion tube. After cooling down, 2 ml of hydrogen peroxide was added into the digestion tubes and the tubes were heated at 95 ºC for 1 h. Five to ten folds dilution was applied with ultrapure deionized water before the samples were analyzed by using a PSA Mercury Analyzer (P S Analytical) in the purge and gold-trap preconcentration mode. Hg concentration was expressed in µg/kg Hg in dry weight.

For MeHg determination, briefly, 0.5 g of cereal sample was digested with 6 ml of $KBr/H_2SO_4/CuSO_4$ solution and the MeHg present in the digest was extracted into 10 ml of CH_2Cl_2. Then, 0.1 to 1 ml of CH_2Cl_2 extract was pipetted into a 40 ml amber glass vial containing 30 ml of ultrapure deionized water. The vial was purged with N_2 to completely volatilize the CH_2Cl_2, leaving MeHg in the aqueous solution. MeHg in the aqueous solution was then derivatized with 150 µl of 1% (w/v) $NaBEt_4$ to convert MeHg to volatile methylethylmercury which was then purged and trapped on a Tenax trap followed by analysis on GC-CVAFS MeHg system.

Table 2.2. General information and mercury levels of the cereal samples

ID	Brand	Location*	Type**	THg(μg/kg)	MeHg(μg/kg)	MeHg/THg (%)	Fat%	Protein%	Carb%
R1	A	MIA	R	3.07±0.46	1.55±0.98	50.5	3.33	6.67	80.0
R2	A	MIA	R	4.92±0.25	2.13±0.09	43.3	3.33	6.67	80.0
R3	A	MIA	R	2.35±0.37	1.34±0.17	57.0	3.33	6.67	80.0
R4	B	MIA	R	1.82±0.05	0.92±0.05	50.5	0.00	6.67	80.0
R5	B	MIA	R	2.21±0.22	1.12±0.10	50.7	0.00	6.67	80.0
R6	E	MIA	R	4.87±0.18	3.95±0.22	81.1	3.13	6.25	81.3
R7	D	MIA	R	1.47±0.29	1.05±0.01	71.4	7.14	7.14	78.6
R8	C	MIA	R	11.74±1.48	9.23±1.43	78.6	4.76	9.52	76.2
R9	C	MIA	R	1.97±0.41	1.78±0.38	90.4	4.76	9.52	81.0
R10	C	MIA	R	2.39±0.04	0.91±0.08	38.1	3.57	7.14	71.4
R11	A	NY	R	4.23±0.37	2.19±0.27	51.8	3.00	7.30	77.8
R12	A	NY	R	3.25±0.06	1.39±0.25	42.8	10.00	6.00	60.0
R13	B	NY	R	2.76±0.67	0.70±0.15	25.4	8.00	5.00	67.0
R14	D	NY	R	1.62±0.27	0.57±0.08	35.2	7.10	7.10	71.4
R15	A	SJ	R	3.39±0.08	1.26±0.02	37.2	3.33	6.67	80.0
R16	A	SJ	R	3.12±0.80	1.22±0.18	39.1	3.33	6.67	80.0
R17	B	SJ	R	2.17±0.10	0.64±0.05	29.5	0.00	6.67	80.0
R18	E	SJ	R	6.43±0.32	2.39±0.34	37.2	3.13	6.25	81.3
R19	A	CHI	R	3.51±0.26	2.68±0.53	76.4	3.33	6.67	80.0
R20	A	CHI	R	3.2±0.51	1.55±0.10	48.4	3.33	6.67	86.7
R21	B	CHI	R	2.44±0.28	0.93±0.20	38.1	0.00	6.67	80.0
R22	C	MIA	R	15.88±2.17	13.38±1.88	84.3	4.76	9.52	76.2
R23	C	MIA	R	14.84±2.19	13.94±2.02	93.9	4.76	9.52	76.2
R24	C	MIA	R	12.73±1.67	7.72±2.20	60.6	4.76	9.52	76.2
R25	C	MIA	R	10.37±1.45	9.75±3.13	94.0	4.76	9.52	76.2
R26	C	MIA	R	10.03±0.63	1.77±0.90	17.6	4.76	9.52	81.0
R27	F	BJ	R	2.55±0.23	0.92±0.08	36.1	8.00	5.00	67.0
R28	G	BJ	R	2.34±0.18	0.89±0.11	38.0	12.50	6.45	57.8
R29	A	BJ	R	3.33±0.12	1.59±0.14	47.7	1.90	6.40	84.5
R30	N	BJ	R	2.76±0.07	1.23±0.11	44.6	2.80	8.00	86.8
R31	N	BJ	R	2.9±0.06	1.58±0.21	54.5	11.60	8.00	77.0
R32	H	BJ	R	2.96±0.18	1.84±0.46	62.2	6.50	1.90	81.5
R33	I	BJ	R	2.34±0.18	1.07±0.14	45.7	5.00	5.38	85.0
R34	J	BJ	R	3.22±0.14	2.43±0.62	75.5	6.00	11.00	79.5
R35	N	BJ	R	3.41±0.23	2.16±0.15	63.3	10.00	5.00	60.0
R36	I	BJ	R	1.71±0.18	0.95±0.04	55.6	5.00	5.38	85.0
R37	N	BJ	R	3.23±0.06	2.23±0.20	69.0	10.00	5.00	28.0

Table 2.2 (continued)									
R38	N	BJ	R	4.49±0.32	3.58±0.42	79.7	2.00	9.00	81.0
R39	H	BJ	R	2.80±0.10	1.72±0.49	61.4	1.90	6.20	84.6
R40	K	QD	R	2.96±0.35	2.03±0.42	68.6	0.60	6.00	85.2
R41	N	QD	R	1.63±0.04	1.36±0.09	83.4	1.00	5.70	76.3
R42	M	QD	R	3.54±0.31	3.30±0.12	93.2	1.55	7.50	85.0
R43	I	QD	R	1.87±0.02	0.81±0.18	43.3	5.00	5.38	85.0
R44	K	QD	R	3.40±0.88	1.20±0.09	35.3	0.60	6.00	85.2
R45	N	QD	R	2.11±0.11	1.16±0.04	55.0	5.00	5.50	68.0
R46	N	QD	R	4.89±0.66	2.45±0.17	50.1	3.00	7.30	77.8
R47	N	QD	R	6.02±0.13	4.24±0.03	70.4	8.20	15.00	67.0
R48	N	QD	R	3.30±0.25	2.29±0.21	69.4	10.00	6.00	60.0
R49	N	QD	R	4.15±0.25	2.92±0.53	70.4	5.00	7.00	87.0
R50	N	QD	R	3.66±0.12	3.01±0.17	82.2	10.00	5.00	28.0
R51	F	QD	R	4.87±0.30	3.87±0.41	79.5	8.00	5.00	67.0
R52	J	QD	R	1.95±0.20	1.19±0.07	61.0	6.00	8.00	82.5
R53	I	NJ	R	1.91±0.01	1.16±0.12	60.7	5.00	5.38	85.0
R54	H	NJ	R	3.30±0.42	1.19±0.00	36.1	6.50	1.90	81.5
R55	J	NJ	R	3.16±0.20	2.35±0.19	74.4	6.00	11.00	79.5
R56	I	NJ	R	2.03±0.30	1.38±0.09	68.0	5.00	5.38	85.0
R57	I	NJ	R	4.75±0.11	4.22±1.09	88.8	5.00	12.00	80.0
R58	J	NJ	R	2.43±0.34	1.83±0.05	75.3	5.00	12.00	80.0
R59	I	WH	R	1.80±0.27	1.21±0.20	67.2	5.00	5.38	85.0
R60	J	WH	R	2.18±0.06	1.64±0.13	75.2	6.00	6.00	84.5
R61	J	WH	R	4.98±0.52	1.73±0.32	34.7	6.00	6.00	84.5
R62	J	WH	R	5.96±0.14	1.60±0.05	26.8	6.00	6.00	84.5
R63	J	WH	R	3.40±0.35	1.44±0.17	42.4	4.00	5.50	86.0
R64	H	WH	R	4.18±0.23	2.19±0.34	52.4	1.90	6.20	84.6
R65	H	WH	R	3.77±0.25	1.64±0.36	43.5	1.90	6.20	84.6
R66	H	WH	R	4.96±0.17	1.35±0.18	27.2	1.90	6.40	84.4
R67	J	WH	R	2.11±0.31	1.37±0.17	64.9	6.00	6.00	84.5
R68	J	WH	R	2.11±0.04	0.81±0.07	38.4	6.00	13.00	75.0
R69	I	WH	R	3.43±0.05	0.57±0.07	16.6	5.00	12.00	80.0
R70	I	WH	R	3.36±0.12	0.51±0.08	15.2	5.00	12.50	79.0
R71	I	WH	R	2.20±0.30	1.27±0.07	57.7	5.00	5.38	85.0
R72	L	WH	R	4.78±0.41	1.63±0.13	34.1	0.50	8.50	78.2
R73	L	WH	R	2.64±0.22	1.66±0.16	62.9	0.50	8.10	79.8
R74	L	WH	R	1.95±0.22	1.03±0.33	52.8	0.60	8.80	81.3
R75	L	WH	R	3.71±0.31	2.78±0.16	74.9	0.60	9.00	80.0
R76	M	WH	R	2.05±0.11	1.98±0.12	96.6	1.55	7.50	85.0

Table 2.2 (continued)									
R77	M	WH	R	1.90±0.08	1.65±0.13	86.8	1.55	7.50	85.0
R78	G	WH	R	1.75±0.19	1.35±0.17	77.1	12.50	6.45	57.8
R79	G	WH	R	1.69±0.10	0.90±0.15	53.3	12.50	6.45	57.8
M1	A	MIA	M	1.04±0.12	0.30±0.17	28.8	6.67	6.67	80.0
M2	A	SJ	M	1.15±0.07	0.27±0.03	23.5	6.67	6.67	80.0
M3	A	NY	M	1.25±0.05	0.52±0.11	41.6	6.67	6.67	80.0
M4	A	CHI	M	1.00±0.00	0.44±0.03	44.0	6.67	6.67	80.0
M5	G	BJ	M	1.30±0.09	1.04±0.11	80.0	12.50	6.78	57.7
M6	H	NJ	M	0.94±0.00	0.72±0.05	76.6	1.50	9.40	80.2
M7	H	WH	M	2.91±0.20	1.64±0.36	56.4	1.50	9.50	80.6
M8	I	WH	M	1.43±0.03	0.44±0.08	30.8	12.50	6.78	57.7
M9	G	WH	M	1.39±0.10	0.93±0.08	66.9	5.00	13.00	79.0
N1	A	MIA	O	0.68±0.06	0.26±0.12	38.2	6.67	13.33	66.7
N2	A	MIA	O	0.80±0.09	0.08±0.01	10.0	3.33	6.67	80.0
N3	A	MIA	O	1.10±0.29	0.03±0.02	2.7	6.67	6.67	73.3
N4	A	MIA	O	0.88±0.11	0.05±0.03	5.7	6.67	6.67	80.0
N5	A	MIA	O	0.72±0.04	0.11±0.01	15.3	6.67	6.67	80.0
N6	A	MIA	O	1.00±0.08	0.05±0.01	5.0	6.67	6.67	73.3
N7	C	MIA	O	0.68±0.03	0.23±0.04	33.8	7.14	14.29	64.3
N8	E	MIA	O	0.63±0.00	0.07±0.05	11.1	6.67	13.33	66.7
N9	D	MIA	O	0.61±0.06	0.19±0.05	31.1	7.14	14.29	64.3
N10	C	MIA	O	0.83±0.04	0.20±0.05	24.1	4.76	9.52	76.2
N11	A	NY	O	0.81±0.02	0.11±0.03	13.6	3.33	6.67	80.0
N12	A	NY	O	0.65±0.02	0.14±0.10	21.5	6.67	13.33	66.7
N13	C	NY	O	0.65±0.02	0.09±0.04	13.8	7.14	14.29	64.3
N14	D	NY	O	0.57±0.04	0.13±0.08	22.8	7.14	14.29	64.3
N15	A	SJ	O	1.08±0.04	0.17±0.05	15.7	6.67	6.67	80.0
N16	A	SJ	O	0.94±0.06	0.20±0.15	21.3	6.67	6.67	80.0
N17	A	SJ	O	0.90±0.10	0.06±0.02	6.7	6.67	6.67	73.3
N18	A	SJ	O	0.70±0.06	0.10±0.02	14.3	3.33	6.67	80.0
N19	A	SJ	O	0.84±0.06	0.06±0.02	7.1	6.67	6.67	73.3
N20	B	SJ	O	0.80±0.15	0.08±0.02	10.0	6.67	13.33	73.3
N21	E	SJ	O	0.40±0.09	0.07±0.01	17.5	6.67	13.33	66.7
N22	C	SJ	O	0.35±0.01	0.07±0.02	20.0	7.14	14.29	64.3
N24	A	CHI	O	0.65±0.04	0.14±0.05	21.5	6.67	13.33	66.7
N25	B	CHI	O	0.98±0.12	0.06±0.02	6.1	6.67	13.33	73.3
N26	C	CHI	O	0.79±0.19	0.19±0.02	24.1	7.14	14.29	64.3
N27	C	CHI	O	1.04±0.19	0.06±0.01	5.8	3.57	14.29	71.4

Table 2.2 (continued)									
N28	D	CHI	O	1.08±0.09	0.03±0.05	2.8	7.14	14.29	64.3
N29	N	BJ	O	1.14±0.03	0.14±0.04	12.3	6.30	13.30	71.0
N30	N	BJ	O	0.66±0.10	0.10±0.07	15.2	5.30	8.60	79.4
N31	G	WH	O	0.54±0.00	0.22±0.06	40.7	12.60	7.12	57.5

***Sampling location: MIA = Miami, NY = New York, SJ = Saint Jose, CHI = Chicago, BJ = Beijing, QD = Qingdao, NJ = Nanjing, WH = Wuhan.**

****Type: R = Rice, M = Multi-grain, O = Other-grain**

Chapter 3. Determination of Bioaccessibility of Methylmercury in Rice-based Infant Cereals using an *In vitro* Digestion Model

Abstract

Considerable levels of methylmercury (MeHg) has been found in rice for over a decade. Studies conducted in our group have suggested that elevated levels of MeHg exist in rice products, hence consumption of rice products may pose a potential health risk to human beings, especially human infants who consume abundant rice cereals and are much more sensitive to the toxic effect of MeHg. Effort was made to assess the potential risk caused by consuming rice cereals for infants, however the crucial information, such as bioavailability or bioaccessibility of MeHg in rice cereals was still missing. The present study used an *in vitro* gastrointestinal extraction method to determine the bioaccessibility of MeHg in rice-based infant cereals and to examine the factors controlling the MeHg bioaccessibility. The bioaccessibilities of MeHg in studied rice cereals ranged from 25% to 74%. Compared to the bioaccessibilities of MeHg in different types of cooked fish, which was traditionally considered as the main MeHg exposure pathway to human beings, no significant differences were observed. Further experiments were conducted to identify the factors influence MeHg bioaccessibility in individual sample. The results indicated the re-adsorption of bioaccessible MeHg in digestive juice was mainly responsible for the variety of MeHg bioaccessibility in food samples. After taking the portion of re-adsorbed MeHg into account, the bioaccessibility of MeHg in all measured rice cereal and fish samples were close to 100%. The finding of this study suggested that we may have been misled by the results from previous studies, which may lead to the neglect of the potential risk of MeHg exposure through the consumption of rice products.

3.1 Introduction

Methylmercury (MeHg) is known as a highly neurotoxic contaminant. Upon entering the human body, MeHg will be absorbed rapidly in gastrointestinal (GI) track, then distributed through the body. It can cross the blood-brain barrier, to affect the brain, which will result in severe health problems including loss of IQ, delayed speech and decreased performance in memory function and even death at high dose of MeHg.[60,116] Traditionally, fish consumption was considered as the major pathway of MeHg exposure to human beings since MeHg could be readily bioaccumulated along the aquatic food chain resulting in high levels of MeHg present in fish.[117] The well-known Minamata disease, which is a fetal neurological disorder, is one of the most notorious examples of mercury poisoning resulting from the consumption of fish and shellfish containing high levels of MeHg.[24] Although nowadays incidents involving extremely high MeHg exposure like in Minamata disease are rare, the MeHg levels present in a variety of fishes grown in many natural environments are still a worldwide health concern. The chronic exposure of human beings to MeHg through consumption of fish and shellfish has been the major health risks associated with MeHg, in particular to pregnant women and children.

Recently, scientists worldwide continued to report the occurrence of MeHg in rice, even at elevated levels in some cases, while the concentrations of MeHg in other grains such as wheat and oatmeal were almost undetectable.[49,52,94,118] The finding of elevated level of MeHg in rice is because the physicochemical condition of rice paddy has been proven to be able to facilitate methylation of inorganic Hg (IHg) in paddy water and soil. Rothenberg et al. summarized the results of previous studies, which have shown that the

average concentrations of MeHg in rice grains are 2.5 μg/kg for non-polluted sites and 16 μg/kg for polluted areas (e.g., mercury or gold mining areas), respectively. The findings of MeHg occurrence in rice have raised the issue of rice consumption being an exposure pathway of MeHg, in addition to fish consumption, in particular in the inland areas where fish consumption is limited and/or rice and soil are heavily contaminated by Hg. It also should be noted that the MeHg concentrations in rice produced in most studied areas are close to that from non-polluted areas,[52] and it seems that the occurrence of such levels of MeHg in rice probably has limited impact on adult's health, especially if rice is not overwhelmingly consumed as the predominant diet.

When assessing the potential human exposure to MeHg and the associated health risks, differences in adults and infants must be considered since they have different susceptibility to MeHg toxicity and different diets. In fact, the exposure of infants to MeHg has been of particular concern with regard to MeHg health issue, as developing nervous system is a more sensitive target organ than the adult brain and MeHg exposure could lead to various adverse health effects to infant development.[116] Traditionally, prenatal exposure was considered as the main source of occurrence of MeHg in infants. Methylmercury accumulated in human infants was mainly considered being transferred from pregnant women to fetus via cord blood during the pregnancy after intake of seafood containing MeHg by expectant mother. Postnatal MeHg exposure through diets is also a potential pathway for infants, albeit being understudied, as limited studies have shown that the consumption of breast milk and fish based infant food may related to MeHg exposure to human infants.[62-66]

The postnatal MeHg exposure for infants through consumption of rice and rice cereals has been largely overlooked. The recent findings of MeHg presence in rice may suggest that the MeHg in rice could be transferred to rice products such as infant cereals and therefore consumption of rice cereals could be a potential pathway for infant MeHg exposure. A limited number of studies on samples of rice-based baby cereal, which is the most common solid food introduced to human infants, have shown that the concentrations of both THg and MeHg in rice-based baby cereals are close to the concentrations of THg and MeHg in rice,[69,70,95] suggesting that the THg and MeHg in rice remained in rice cereals after production. The results of our previous work indicated that the concentrations of MeHg in rice-based baby cereals ranged from 0.51 to 13.9 μg/kg with a mean of 2.28 μg/kg. The estimated daily intake of MeHg for infants through consumption of rice-based baby cereals on the basis of the measured MeHg level in rice-based baby cereals ranged from 4% to 123% of MeHg reference dose (RfD), which is calculated on the basis of data related to fish consumption, set by United States Environmental Protection Agency (USEPA).[29]

Our previous estimations on infant MeHg daily intake through consumption of rice-based cereals were on the basis of the total MeHg concentrations determined for the cereals. To accurately assess exposure of MeHg from rice-based baby cereals, information on bioavailability of MeHg is needed, as it is possible not all MeHg is available for absorption. Since the calculated RfD is directly related to MeHg bioavailability, while the different properties and contents of two types of foods mean the possible different MeHg bioaccessibilities in these foods. Usually, *In vitro* digestion approaches using an enzymatic digestion is a simple, feasible and widely used method for

assessing bioaccessibility, which is defined as the maximum value of bioavailability. In this method, samples are incubated in artificial gastrointestinal fluid containing enzymes, including amylase, pepsin, pancreatin and bile extract, to simulate the digestion process in human body. Then the MeHg recovered from the gastrointestinal fluid are identified as the bioaccessible MeHg, while the MeHg in digestive residue are taken as unbioaccessible. At present, there are various models with different experimental designs exist. In general, an *in vitro* method is considered valid if the its result matched that obtained from *in vivo* models. However, high cost and possible ethical dilemma caused by *in vivo* experiments have limited the its availability of data, hence in the most case, the selection of *in vitro* digestion models is based on the content of food. Different methods have been used to digest fish and rice samples to evaluate the bioaccessible metal including arsenic, cadmium and lead.[18-21] Despite various digestion times applied according to purposes of studies, the enzyme used in most case are amylase, pepsin, lipase, pancreatin and bile extract. Efforts have also been made to assess the bioaccessibilities of MeHg in seafood using *in vitro* digestive models as well. The results of previous *in vitro* studies indicated that the overall mean bioaccessibilities of MeHg in different seafood ranged from 2% to 100%.[22-27]

Unfortunately, there is still a knowledge gap in understanding the percentage of bioaccessible MeHg in infant rice cereals. To our best knowledge, there have been no studies reported on how much MeHg from rice cereals is released and absorbed by the digestive system. Moreover, the results of MeHg bioaccessibility in seafood obtained from different studies varied within a wide range. Even the bioaccessible MeHg in the same type of fish could vary largely in inter-and intra-studies, while no real rational

explanation was given, suggesting the necessity of investigating MeHg bioaccessibility and the controlling factors from the food and from the methods used.[50,80,82,85,86] Therefore, in this study, we focused on investigation of the bioaccessibility of MeHg in various infant rice cereal samples and fish that commonly available on the market by using an *in vitro* digestion model. Meanwhile, the possible reasons which result in variations in bioaccessibilities of MeHg in infant rice cereals and fish were investigated. Knowing bioaccessible fraction of MeHg in rice cereal and fish, together with the controlling factors on variety of MeHg bioaccessibility in those samples, will advance our understanding of the impact of dietary MeHg on infant's health and provide accurate data to perform health risk assessment for infant MeHg exposure.

3.2 Materials and methods

3.2.1 Reagents and materials

Ultrapure deionized water produced by a Barnstead Nanopure Diamond water purification system with resistivity of 18 MΩ·cm was used for preparing all solutions. TraceMetal grade nitric acid, hydrochloric acid and ACS certified grade reagents, including potassium hydroxide, copper sulfate, sulfuric acid, potassium bromide, methylene chloride, hydrogen peroxide, and tin chloride were used in pretreatment and analysis of samples. All chemicals were supplied by Fisher Scientific, unless otherwise specified. Overnight 10% (v/v) nitric acid soaked and muffle furnace baked glassware were used to get rid of influence of residual Hg during experiments

The acidic potassium bromide solution used for MeHg extraction was prepared by dissolving 180 g of KBr in 50 ml sulfuric acid in 100 ml water, and the solutions were mixed up and made up to 1 L using water after cooling to ambient temperature. Copper

sulfate solution (1 M) and citric buffer (1 M) were prepared by adding appropriate amounts of salts in water. The Ethylation reagent was prepared by dissolving 1 g of sodium tetraethylborate (NaBEt4, Sigma-Aldrich) in 100 ml of 2 % (w/w) potassium hydroxide. A proper amount of tin chloride was dissolved in 1 % (v/v) hydrochloric acid to obtain 2% (w/v) reductant solution used in THg analysis. Certified 10 ppm MeHg standard solutions were used for quantification during MeHg analysis. Certified reference materials, DORM-2 (National Research Council of Canada), was used for quality control and validation of MeHg determination methods.

3.2.2 Sampling and preparation of rice cereal and fish samples

In this study, a total of 21 infant rice cereal samples were purchased in local grocery stores and then kept in a cabinet inside a Class 100 clean room (with inlet air purified by gold-coated sand to remove Hg) to avoid contamination. Three different type of fish samples, including Tuna, Tilapia, and Salmon, were purchased from the local market, and frozen in a freezer at low temperature (-14 ºC).

To prepare sample for *in vitro* digestion, approximately 1.0 g of rice cereal sample and 4.0 ml of ultra-pure water was placed into a 50-ml centrifuge tube, then was stored in fume hood until digestion. The fish fillets were steam-cooked for 15 minutes using a rice cooker. Then the cooked fish samples were blended using a blender to obtain the homogenized samples. Approximately 1.0 g of blended fish sample was added into 50 ml centrifuge tube followed by adding 4.0 ml of ultra-pure water, then was stored in fume hood until digestion.

3.2.3 *In vitro* artificial gastrointestinal fluid digestion

During the experiment, the prepared fish and rice cereal samples were digested using artificial digestive fluid, which contained inorganic and organic salt, and various enzymes. Then the portion of MeHg in liquid phase, which was considered as bioaccessible MeHg, and the MeHg remained in residue, which was treated as unbioaccessible MeHg, were measured respectively to determine the MeHg bioaccessibility in samples. The experimental setup of the *in vitro* digestion approach used has been described previously. [122] This model has been successfully used in assessment of different contaminant in various types of food. Artificial digestive fluids, including saliva, gastric juice, intestinal juice and bile, were prepared by following the method described by Versantvoort et al.[122] The extraction was started by adding 6 ml artificial saliva fluid into the prepared rice cereal and fish samples, and the tube was capped and placed in a water bath shaker (37 ºC, 100 rpm) to mimic digestion in mouth. After 5 mins, 12 ml prepared gastric fluid was added into sample and then digestion for another 2 hours to simulate the digestion in stomach. Eventually, 12 ml of intestinal juice with 6 ml of bile were added in sample, and pH value was adjusted to 6.8-7.0 using 1% (w/v) KOH solution. The tube was shaken for another 2 hours to mimic intestinal digestion. Following these steps of digestion, the sample was centrifuged at 7000 rpm for 10 minutes to separate the solution from the residual solids both of which were subject to MeHg analysis.

3.2.4 Determination of MeHg content

The MeHg levels in cereal, fish samples, residue and supernatant of digested samples were measured using a gas chromatography-atomic fluorescence spectrometry (GC-AFS)

(Brooks Rand Automated MERX) MeHg System, following a modified extraction method reported previously.[97] Briefly, 0.5 g of cereal or fish sample or 3 ml supernatant or entire residue of digested samples was digested with 6 ml of $KBr/H_2SO_4/CuSO_4$ solution and the MeHg present in the digest was extracted into 10 ml of CH_2Cl_2. Then, 0.1 to 1 ml of CH_2Cl_2 extract was pipetted into a 40-ml amber glass vial containing 30 ml of ultrapure deionized water. The vial was purged with N_2 to completely volatilize the CH_2Cl_2, leaving MeHg in the aqueous solution. MeHg in the aqueous solution was then derivatized with 150 µl of 1% (w/v) NaBEt4 to convert MeHg to volatile methylethylmercury which was then purged and trapped on a Tenax trap followed by analysis on GC-CVAFS MeHg system.

3.3 Results and discussion

3.3.1 Bioaccessibility of MeHg in rice cereals and possible affecting factors

The concentrations of THg and MeHg in 21 infant cereal samples studied ranged from 2.0 to 15.9 µg/kg and 1.2 to 13.9 µg/kg with a mean value of 6.0 ± 4.2 µg/kg and 4.5 ± 3.7 µg/kg, respectively. The 21 rice cereal samples involved in this study included various brands from different locations. The results obtained in this study were compared with the studies about MeHg in rice and our previous study on MeHg in rice-based cereals. To our best knowledge, there is no other group who has conducted the study on MeHg in rice-based baby cereals, except for our previous work in which we determined THg and MeHg in a large number of infant cereal samples commonly available in the main markets in China and USA. The range of MeHg concentration is comparable to the concentrations of MeHg in rice from unpolluted area and rice-based baby cereals purchased in both China and USA.[52] The results indicated that rice MeHg was transferred

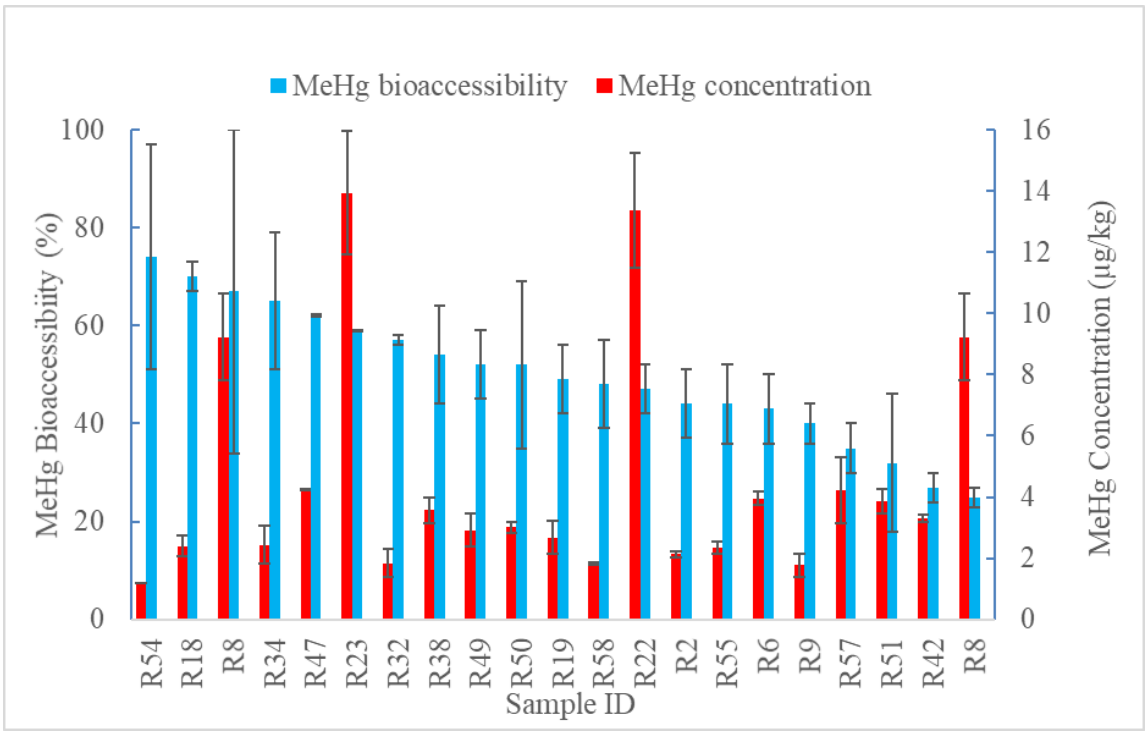
into infant cereals after cooking and packing process, while the concentration did not changed drastically.

To estimate the bioaccessibility of MeHg in rice cereal samples, the sum of MeHg extracted by artificial gastrointestinal fluid was compared to the total MeHg found through acidic KBr extraction. The MeHg was assumed to be dissolved in gastrointestinal fluid before it can be absorbed by the gastrointestinal track. Detailed sample information, including Hg concentration, bioaccessibilities of MeHg in rice cereals were summarized in Table 3.1.

Table 3.1. Hg concentrations and bioaccessibilities of infant rice cereals and fish

Sample ID	MeHg concentration (μg/kg, n=3)	THg concentration (μg/kg, n=3)	Bioaccessibility (%, n = 3)
R54	1.24 ± 0.08	3.30 ± 0.42	74 ± 23
R18	2.39 ± 0.34	6.43 ± 0.32	70 ± 3
R8	9.23 ± 1.43	11.74 ± 1.48	67 ± 33
R34	2.43 ± 0.62	3.22 ± 0.14	65 ± 14
R47	4.24 ± 0.03	6.02 ± 0.13	62 ± 0.2
R23	13.9 ± 2.02	14.8 ± 2.19	59 ± 19
R32	1.84 ± 0.46	2.96 ± 0.18	57 ± 10
R38	3.58 ± 0.42	4.49 ± 0.32	54 ± 12
R49	2.92 ± 0.53	4.15 ± 0.25	52 ± 7
R50	3.01 ± 0.17	3.66 ± 0.12	52 ± 17
R19	2.68 ± 0.53	3.51 ± 0.26	49 ± 7
R58	1.83 ± 0.05	2.43 ± 0.34	48 ± 9
R22	13.4 ± 1.88	15.9 ± 2.17	47 ± 5
R2	2.13 ± 0.09	4.92 ± 0.25	44 ± 7
R55	2.35 ± 0.19	3.16 ± 0.20	44 ± 8
R6	3.95 ± 0.22	4.87 ± 0.18	43 ± 7
R9	1.78 ± 0.38	1.97 ± 0.41	40 ± 4
R57	4.22 ± 1.09	4.75 ± 0.11	35 ± 5
R51	3.87 ± 0.41	4.87 ± 0.30	32 ± 14
R42	3.30 ± 0.12	3.54 ± 0.31	27 ± 3
R8	9.23 ± 1.43	11.7 ± 1.48	25 ± 2
Tilapia	14.3 ± 4.50	15.5 ± 2.30	68 ± 15
Salmon	4.99 ± 1.16	5.59 ± 0.87	47 ± 8
Tuna	131 ± 5.76	148 ± 4.26	70 ± 20

The results of MeHg analysis suggest that the bioaccessible portion of MeHg in 21 infant cereal samples ranged from 25 to 74%, with a median of 49% and a mean of 50%. And as shown in the Figure 3.1, the bioaccessibilities of MeHg in infant rice cereals ranged widely, while the same phenomenon was also observed in the previous studies on MeHg bioaccessibilities in fish. No correlation between the content, including protein, fat, carbohydrate, or Hg concentration, and MeHg bioaccesibility was found in this study. The other possible explanations proposed by other groups include the differences in properties of individual samples, digestion ability of enzyme used, and protein denaturation caused by storage of individual samples. However, samples in this study were kept under the same storage condition, and even bioaccessibility of MeHg in same samples made in different locations (same product with different lot number) showed significant differences. Furthermore, as shown in the previous studies, even protein denaturation occurred during the cooking of fish was unable to affect the MeHg speciation and concentrations, hence the protein denaturation mentioned above may not be considered as the major causes of varying bioaccessibilities of MeHg in rice cereal samples. The digestive abilities of enzymes used were suggested to be a possible reason that has caused varying MeHg bioaccessibilities in same type of fish. The reason was considered because MeHg in fish is mainly present as the form of MeHg-thiol complex,[81,85,123] and the proteins need to be broken down through the involvement of enzymes to release MeHg and make MeHg becoming bioaccessible.



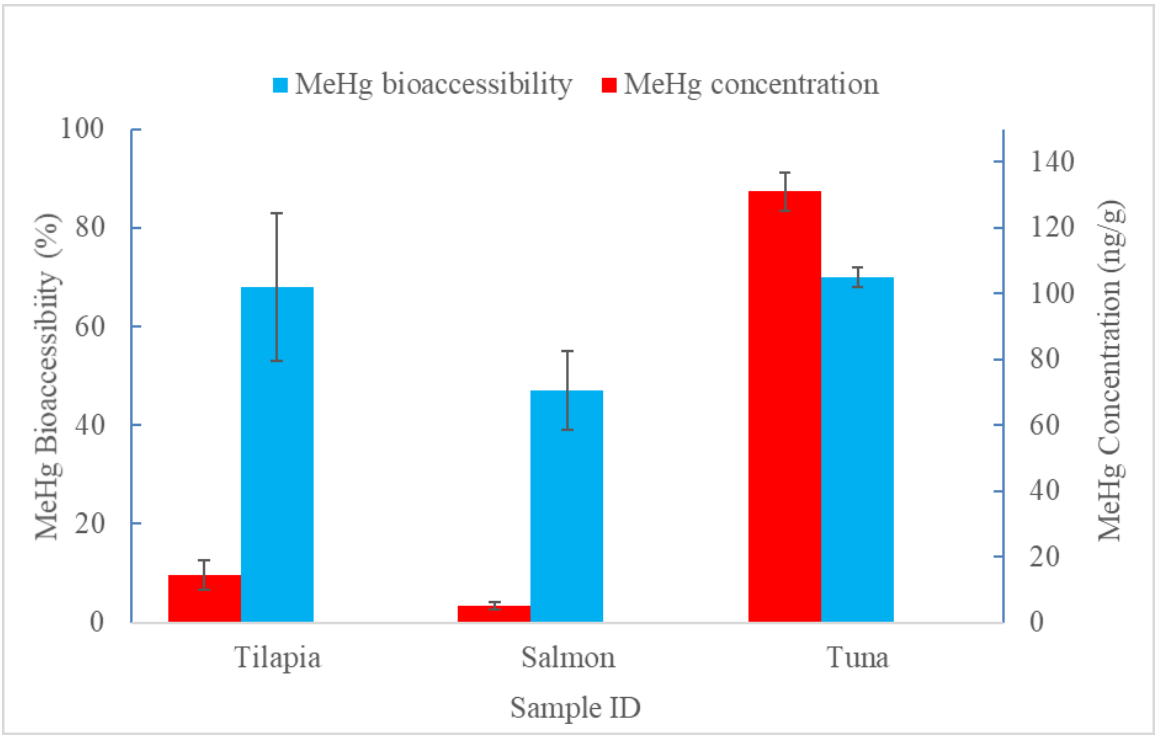


Figure 3.1. MeHg levels and bioaccessibilities in infant rice cereal and fish

But even in the previous study on MeHg bioaccessibility in same type of fish using same enzyme, significant differences in MeHg bioaccessibilities were observed in individual samples,[86] suggesting the limited influence of digestion abilities of enzyme on MeHg bioaccessibility in fish tissue.

More importantly, the bioaccessibility of MeHg in food samples were expected to raise if more enzymes were used and digestion times were increased, on the assumption that various enzymes used and longer digestion would both help with the protein digestion, which means more MeHg would be released. However, in this study, it has been observed that the bioaccessibility of MeHg in rice cereal samples under gastric digestion were higher than that under gastrointestinal conditions (where more types of enzymes were present in this dual-step digestion). These results indicated that no more MeHg was released after samples were further digested during intestinal digestive phase even when more enzymes were added (shown in Fig. 3.2).

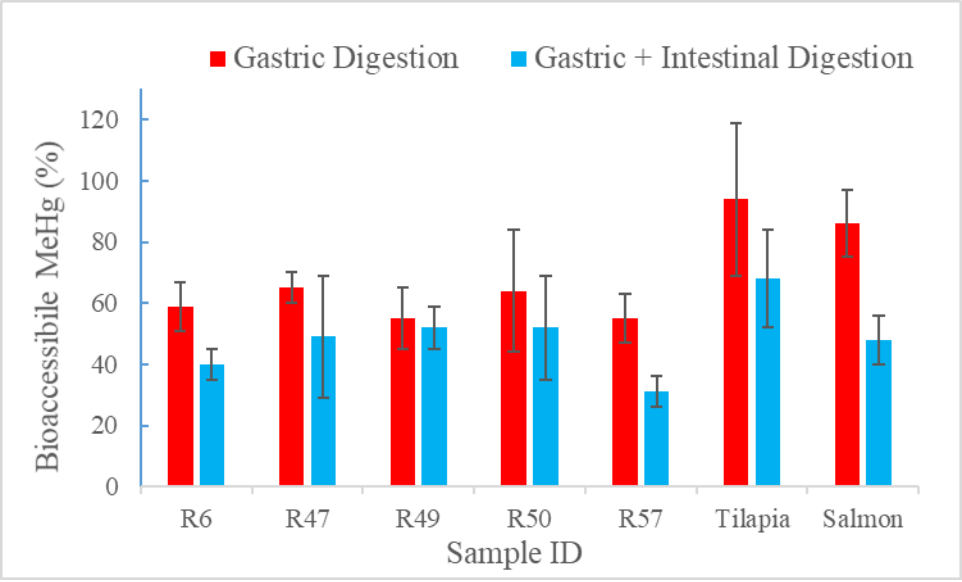


Figure 3.2. MeHg bioaccessibility in dual step digestion

It is worth noting that in the study as we increased the total digestion time from 1 h (0.5 h gastric followed by 0.5 h intestinal digestion) to 8 h (4 h gastric followed by 4h intestinal digestion), the bioaccessible MeHg showed a tendency to reduce. (Shown in Fig. 3.3)

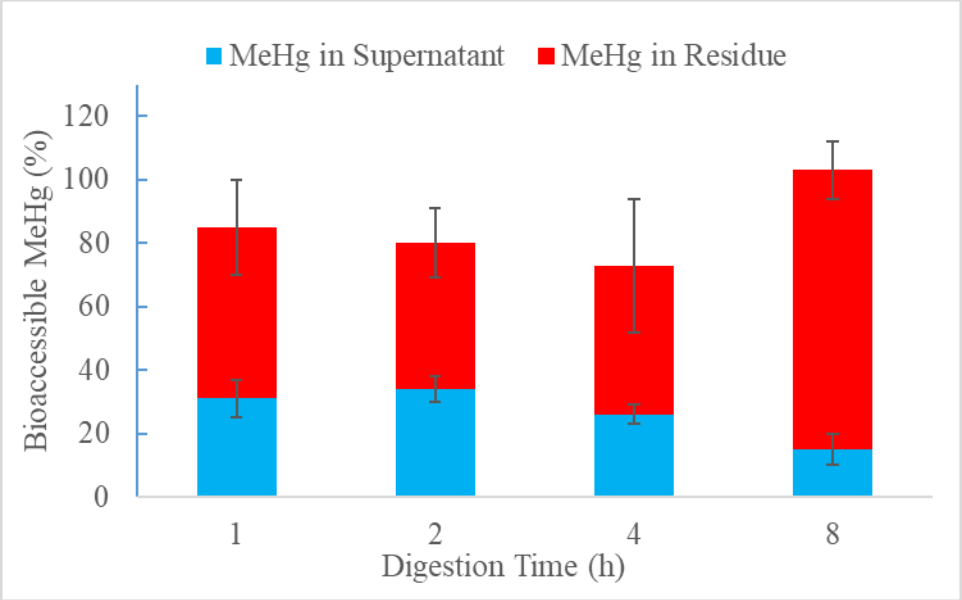


Figure 3.3. MeHg measured in supernatant and residue of rice cereal samples under different digestion time

For instance, the bioaccessibility of MeHg in sample R57 dropped from 35% (30 min for both gastric and intestinal digestion) to 15% (240 min for both gastric and intestinal digestion). These results could suggest that during the digestion process, most of the MeHg in rice cereals have been released from food matrix into solution phase after gastric and intestinal digestion, but some of the released MeHg might be re-adsorbed on the residual solids resistive to the digestion. The result may be particularly the case for the differences in MeHg bioaccessibility between gastric and gastrointestinal digestions as a consequences of increased pH values (from 2 to 7, from gastric to intestinal digestion

phase), because previous studies have shown that the distribution of MeHg and MeHg complexion in solution strongly related to the pH.[124] As the pH of solution increased, the portion of MeHg and MeHg-protein complexion in liquid phase of solution rich in particles reduced,[125] since the availability of HS^- group on particles, which can be strongly bound with MeHg, decreased. Hence, in the case, the re-adsorption of bioaccessible MeHg on digestive residue may have occurred.

3.3.2 Effect of re-adsorption on MeHg bioaccessibility

To confirm the occurrence of re-adsorption of MeHg onto the residues post digestion, the proper amount of MeHgCl standard solution was spiked into the gastrointestinal digested rice cereal samples to examine the adsorption of the spiked MeHg. The results showed that around 64% of spiked MeHg standard was adsorbed on the digestion residue. Moreover, the results of multiple steps extraction experiments, where the digestion residue was repeatedly extracted, provided further evidence for the occurrence of MeHg adsorption. In the experiments, the residues of gastric and intestinal digestion were extracted using neutral intestinal fluid (pH =7, no enzyme) and acidic gastric fluid (pH=2, no enzyme) in sequence.

In Figure 3.4, the repetitive extraction results showed that only a small portion of MeHg on the residue was released under the neutral condition (intestinal fluid extraction), while a significantly larger portion of MeHg was eluted under acidic condition (gastric fluid extraction). In addition, there was still detectable portion of bioaccessible MeHg being eluted during the second acidic juice extraction of acidic fluid eluted residues. After combining all portions of dissolved MeHg in each extractant, the amount was close to the total MeHg present in rice-based baby cereal samples. The

results of experiments could suggest that almost 100% of MeHg in rice-based baby cereals were probably bioaccessible, and the distribution of bioaccessible MeHg in liquid phase was strongly affected by pH of digestive juice. The adsorption of bioaccessible MeHg on residue occurred from pH 2 to pH 7, while a significantly larger fraction of bioaccessible MeHg was adsorbed under neutral condition than that under acidic condition. The reason was because, as mentioned above, the distribution of MeHg-thiol complex in liquid phase decreased as pH increased from 2 to 7, hence resulting in occurrence of larger portion of released MeHg-thiol complex being re-adsorbed on the surface of residue.

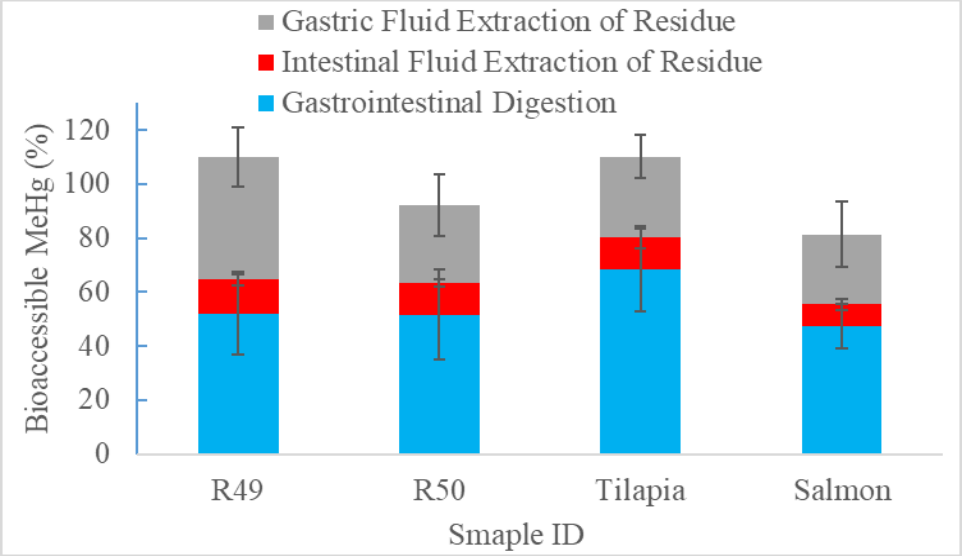


Figure 3.4. MeHg bioaccessibility determined using multiple steps extraction

In addition, pH changes may alter the binding of MeHg with the functional groups (thiol) in residue and thus affect MeHg re-adsorption on residue. The sulfur (thiol) containing residue under neutral condition may bind bioaccessible MeHg, since the digestive residue of rice contains thiol containing peptides. The high pH value could

promote the dissociation of thiol groups,[126,127] resulting in the combination between $MeHg^+$ and thiolates. In fact, previous studies have shown that the adding of thiol containing reagent indeed affected the bioaccessibility of MeHg in fish and seafood.[81,128] It also needs to be pointed out that the distribution of bioaccessible MeHg is a dynamic distribution process, hence the re-adsorbed MeHg should be considered as bioaccessible. Because the absorption of MeHg in gastrointestinal track is a dynamic process as well, the adsorbed MeHg and MeHg-thiol complex may be released back to digestive juice as the dissolved MeHg was absorbed by GI-track.

3.3.3 Effect of speciation on MeHg bioaccessibility

In the interpretation of possible mechanism about re-adsorption of bioaccessible MeHg released from rice cereals, the speciation of MeHg was found be related to how MeHg was adsorbed on the digestive residue. Hence the speciation of MeHg in rice cereal samples was expected to be correlated to the MeHg bioaccessibilities. The experiments were conducted to confirm the hypothesis. As shown in Fig. 3.5, in the most cases, extraction of un-digested rice cereal samples using only gastric juice containing no enzyme had a result with high MeHg bioaccessibility, which strongly suggested the facility of releasing of MeHg in rice cereal under acidic condition. The result was not surprising because of the presence of Cl^- ions in the acidic gastric fluid that may promote the release of MeHg from MeHg-protein complexation because of the relatively high concentration of Cl^- and its high binding constant with MeHg.

As the MeHg bioaccessibility varies with samples, Figure 3.5 showed that the gastrointestinal digestion may increase the bioaccessibility of MeHg in some of cereal

samples, such as sample R49. Part of MeHg in undigested R49 was released into digestion fluid after extraction using gastric fluid containing no enzyme.

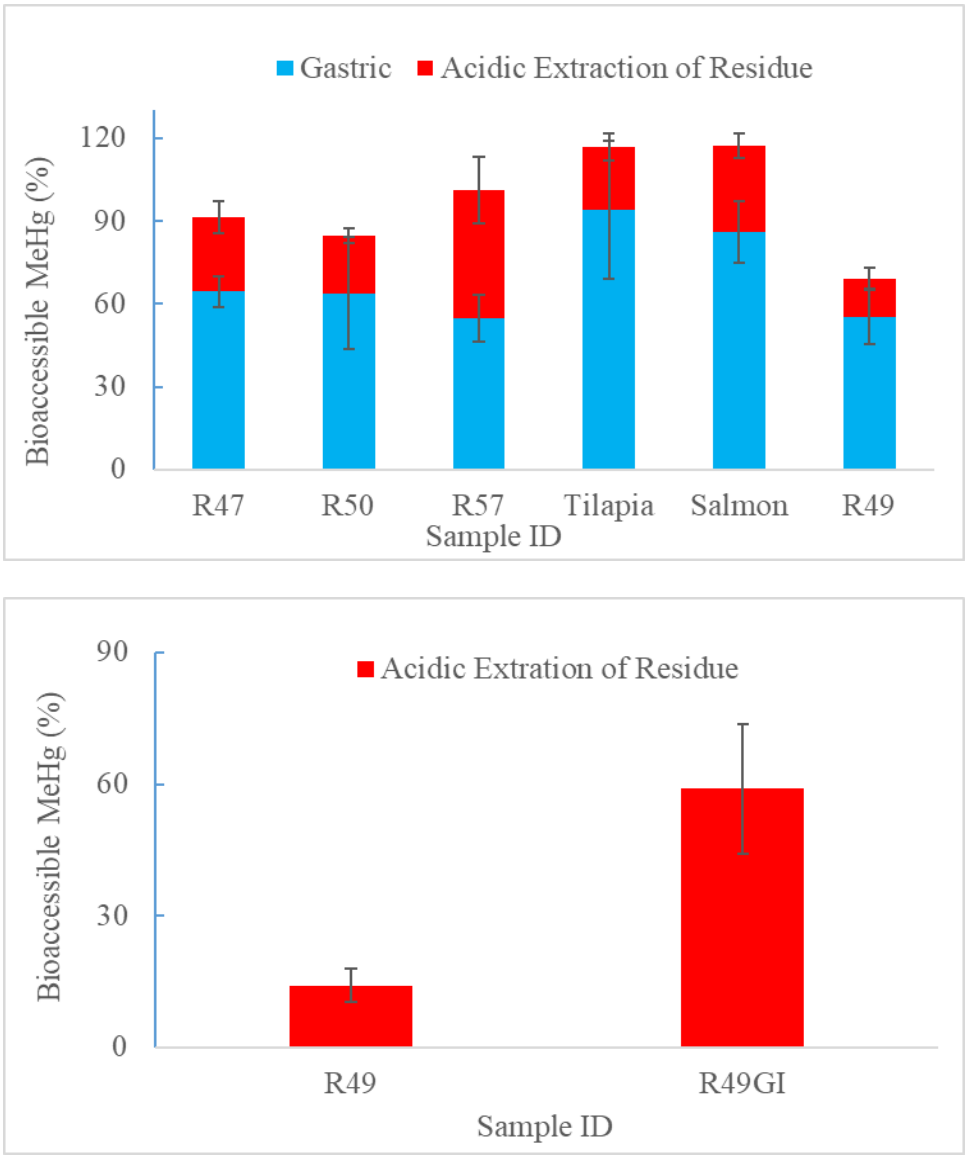


Figure 3.5. Bioaccessible MeHg in supernatant and residue of undigested samples

However, continually using gastric fluid without enzyme was not able to elute more MeHg from the residue, while large portion of MeHg in residue was eluted using gastric fluid without enzyme only after the sample going through entire gastrointestinal

digestion. The results indicated that the presence of enzymes helped with the breakdown of proteins and the release of MeHg. The results also indicated that not only the adsorption of MeHg on residue, but also the speciation of MeHg in sample have an influence on the MeHg bioaccessibility, since only a portion of MeHg in rice cereal samples was bioaccessible while the rest portions were not until the contents were enzymatically digested.

3.3.4 Effect of food matrix on MeHg bioaccessibility

The finding of re-adsorption of bioaccessible MeHg in rice-based baby cereal samples during *in vitro* extraction procedures could also be used to explain the varying bioaccessibilities of MeHg in fish observed in previous studies where similar procedures were used. To verify our hypothesis, experiments were conducted to investigate the effect of re-adsorption on MeHg bioaccessibility in different food matrix. First, the concentrations of MeHg in fish were determined. Compared to rice cereals samples, the MeHg concentration in fish samples varied widely among individuals, and freshwater fish contain less MeHg than saltwater fish do, which matched previous data as well.[129-131] Then the bioaccessible MeHg in the fish samples were determined, which ranged from 47% to 80% (shown in Table 3.1 and Fig. 3.1), using the gastrointestinal fluid extraction. The results were comparable with those observed in previous studies conducted by other groups.[80,82,83,131] At last, the multiple steps extraction experiments were conducted on the digested residues of fish samples. Compared to the rice cereal experiments, the similar results were obtained in the fish experiments. As shown in the Figure 3.4 and 3.5, a considerable portion of MeHg was eluted from the residues of digested and undigested fish samples, and bioaccessible MeHg in both fish samples were close to 100%. The

results were expected since the previous studies have pointed out that the MeHg in both rice and fish mainly exists in the forms of CH_3HgCl and CH_3Hg-thiol complex,[50,85] therefore as both food could be completely digested, the amount of bioaccessible MeHg should expected to be the same.

3.4 Conclusions

The present study used an *in vitro* gastrointestinal extraction method to determine the bioaccessibility of MeHg in rice-based infant cereals and to examine the factors controlling the MeHg bioaccessibility. The fractions of bioaccessible MeHg in rice cereals varied from 25 to 74% following the conventional gastric and intestinal extractions. However, a further examination on the results when considering the effects of various factors on MeHg bioaccessibility suggests the occurrence of re-adsorption of the MeHg initially released into the solution phase. After taking the portion of re-adsorbed MeHg into account, the bioaccessibility of MeHg in all measured rice cereal and fish samples were close to 100%. These results indicated that the amount of MeHg that can be released from food matrix into gastrointestinal track could be underestimated, if not considering carefully the extraction procedures used. Results of previous studies using *in vitro* digestion models indicated the bioaccessibilities of MeHg in fish varied widely and were relatively low in some cases, but this study suggests that the re-adsorption of MeHg on the residues should be taken into account when explaining these results. The assessment of potential health risk caused by MeHg exposure through fish and rice products consumption could be influenced by using underestimated data while calculating the daily intake of MeHg through diet. In addition, in this study, the digestive ability of enzymes was also suggested to be related to the MeHg bioaccessibility,

providing important data for further studies on the factors affecting MeHg bioaccessibility in different types of foods.

Chapter 4. Decadal Variations of Mercury in Mosquito Fish in the Everglades and Relation to Changes in Atmospheric Hg Deposition and Ecosystem Alteration

Abstract

The finding of elevated methylmercury (MeHg) in wildlife in the Florida Everglades in last nineteen eighties resulted in great concern about the impact of this highly toxic mercury (Hg) species to this ecosystem. Since then, great efforts have been made to identify the source, fate, transport of Hg, as well as the production and transformation of MeHg. However, a comprehensive analysis utilizing various databases has not been conducted. By performing the statistical analysis of data obtained from various data bases, the temporal trends of Hg contamination in the food web across different trophic levels over recent years were investigated. In this study, a clear decline of Hg in mosquitofish was observed while the similar trend has not been clearly observed for Hg in large fish at high trophic levels. Then the analysis on the data of Hg in environmental matrices including air, surface water, and periphyton was carried out. The result of data analysis suggested that the periphyton possibly play a dominant role in controlling Hg in mosquitofish by affecting the production of MeHg, while the other parameters such as dissolved carbon (DOC) and sulfate in water may have influence on Hg in mosquitofish by affecting the Hg in periphyton. However, more work should be performed in this field in future for the purpose of resolving the Hg problem in the Everglades as well as the other similar systems.

4.1 Introduction

Mercury (Hg) is a worldwide contaminant and it has drawn great public concerns because of its prevalent existence, widespread distribution via the atmospheric transport, high toxicity and biomagnified to high concentrations in organisms at high trophic levels.

Mercury in the environment originates from both natural (e.g., volcanoes,[45] forest fires,[132-134] oceanic and terrestrial emission, and natural degassing of the earth's crust) and anthropogenic sources (e.g., mining, mineral processing, chlor-alkali production and combustion of fossil fuels).[41] The major form of Hg emitted into the environment is inorganic Hg (Hg^0 and Hg^{2+}). After entering the aquatic environments, the inorganic Hg can be transformed into a more toxic form, methylmercury (MeHg), which can be bioaccumulated and biomagnified through the food chain. With the increase in the anthropogenic emission of Hg in the past several centuries, elevated Hg has been frequently detected in the fish of a large number of aquatic ecosystems.[135,136]

Since the realization of the risk of Hg to human health, a variety of measures (e.g., closing the Hg mining, removal from many consumer items, and improving emission control) have been and are being implemented to minimize the use and release of mercury, reducing the emission of Hg into the environment around the world since 1980s.[137] Accordingly, the decrease in the emission of Hg has been reported in some regions, especially in Europe and Northern America.[138] Although fish could present a rapid response to Hg wet deposition[139], debatable results have been observed on the variation of Hg in fish. Both increase and decrease in fish Hg have been found in the northern America and Europe lakes, where the input of Hg has decreased dramatically.[140-147] These inconsistent findings could be caused by two reasons. Firstly, it is a complicated course for the inputted inorganic Hg finally being accumulated in fish as MeHg, involving a number of transport and transformation processes (e.g., adsorption/desorption, methylation/demethylation, reduction/oxidation and bioaccumulation). As these processes are controlled by many environmental factors, e.g.,

natural organic matter, sulfate temperature. These factors, rather than Hg emission, may dominantly control the long-term variation of fish Hg in aquatic environments. In fact, climate changes (e.g., global warming)[148,149]and ecosystem variations (e.g., fish body condition,[150] eutrophication) has been deemed to result in the variation of fish Hg in a variety of aquatic ecosystems. Secondly, large uncertainties exist for the long-term trend of fish Hg, in particularly for large fish, because of the limitations of fish amount per angler, sampling sites, and big individual differences.[151] Small size fish could be a better indicator for studying the response of high trophic species to Hg emission and ecosystem alteration because of the virtues of less individual differences, ease of getting samples, and possibility of getting samples at more sites.

The Everglades is a subtropical wetland ecosystem located in the south of Florida. Elevated Hg has been frequently detected in fish, birds, and other animals of the Everglades since 1980s,[152] promoting the long-term Hg studies in this system. Hg flux input into the Everglades (>90% from atmospheric deposition) was much less than that in highly Hg contaminated system.[138,153-155] However, Hg in fish were determined to be much higher in this ecosystem, which could be caused by the high natural organic matter (NOM) concentrations,[156] quick methylation in the periphyton, water, and sediment,[157] and slow photodegradation in water.[158] Despite these studies on Hg distribution and cycling, there is a lack of knowledge on the long-term variation in fish Hg in the Everglades. A significant change in atmospheric deposition of Hg has been observed in many regions, including the United States,[159] whose effects on Everglades should be clarified. In addition, Comprehensive Everglades Restoration Plan (CERP), the largest environmental restoration project in the world, was launched in 2000. This project has

changed and is being changing the topography, water chemistry, and ecosystem of the Everglades.[160] These changes in ecosystem may also alter Hg cycling in Florida Everglades.

Eastern mosquitofish (*Gambusia Holbrooki*) is a kind of small fish in the Everglades. In consideration of its ubiquitous existence and ease to get sufficient samples at each site, eastern mosquitofish could serve as a good indicator for studying the response of fish to external changes of Hg. The objectives of this study were to elucidate the temporal trend in mosquitofish Hg in the past two decades, and to evaluate the contributions of atmospheric deposition, and ecosystem alteration to these changes.

4.2 Materials and methods

4.2.1 Data source

This major data base utilized in this study (mosquitofish Hg data and various environmental parameters) was obtained by the USEPA REMAP program (four phases, 1995-1996, 1999, 2005, and 2014). Wet atmospheric deposition of Hg into the Everglades was from the National Atmospheric Deposition Program. Mosquitofish Hg data at 12 sites from different programs collected by South Florida Water Management District (DBHYDRO database) were also included for the purpose of validation.

4.2.2 Sampling and sample analysis

Four phases of sampling with a large number of sampling sites (covering the entire freshwater Everglades, including water conservation areas 1, 2, and 3 (WCA-1, WCA-2, and WCA-3), and Everglades National Park (ENP), from North to South) have been conducted in the Everglades (1995-96, 1999, 2005, and 2014), supported by the USEPA REMAP program. Detailed sampling procedures and analytical procedures for MeHg,

total Hg (THg) and other ancillary parameters and QA/QC of Hg analysis can be found in the report of USEPA REMAP program.

4.2.3 Measurement of MeHg in the porewater of sediment/floc and absorbed water of periphyton

MeHg concentrations in the porewater of sediment/floc and absorbed water of periphyton were collected at four sites and 6 sites of the Everglades in October 2010 and April 2012, respectively. Sediment and floc samples were centrifuged at 4000 rpm to obtain the porewater. MeHg in periphyton adsorbed water was collected by gently hand-squeezing the periphyton.

4.2.4 Data analysis

The Mann-Kendall test was performed to determine if there is a significant decreasing or increasing trend for a certain variable by JMP (SAS Institute, Inc., Version 10.0.0 for Windows). Spearman's correlation test was applied to determine the correlation between Hg in and environmental factors. PCA and CA analyses were performed by SPSS (Version 19 for Windows, SPSS Inc., Chicago, IL) to identify the factors controlling Hg in mosquitofish and MeHg in periphyton.

4.3 Results and discussion

4.3.1 Decadal variation of mercury in Everglades mosquitofish from 1995 to 2014

A long-term field monitoring program with a large number of sampling sites was conducted in the Everglades by the USEPA (USEPA-REMAP) since 1995. Mosquitofish Hg measured in this program was utilized as an indicator of the long-term trend of Hg in Everglades fish. As shown in Fig. 4.1, a decline in mosquitofish Hg was observed from 1996 to 2014 in dry season and from 1999-2014 in wet season (Mann Whitney test, $p <$

0.01), also indicated by the significant decrease in both mean and median values (Fig. 4.1).

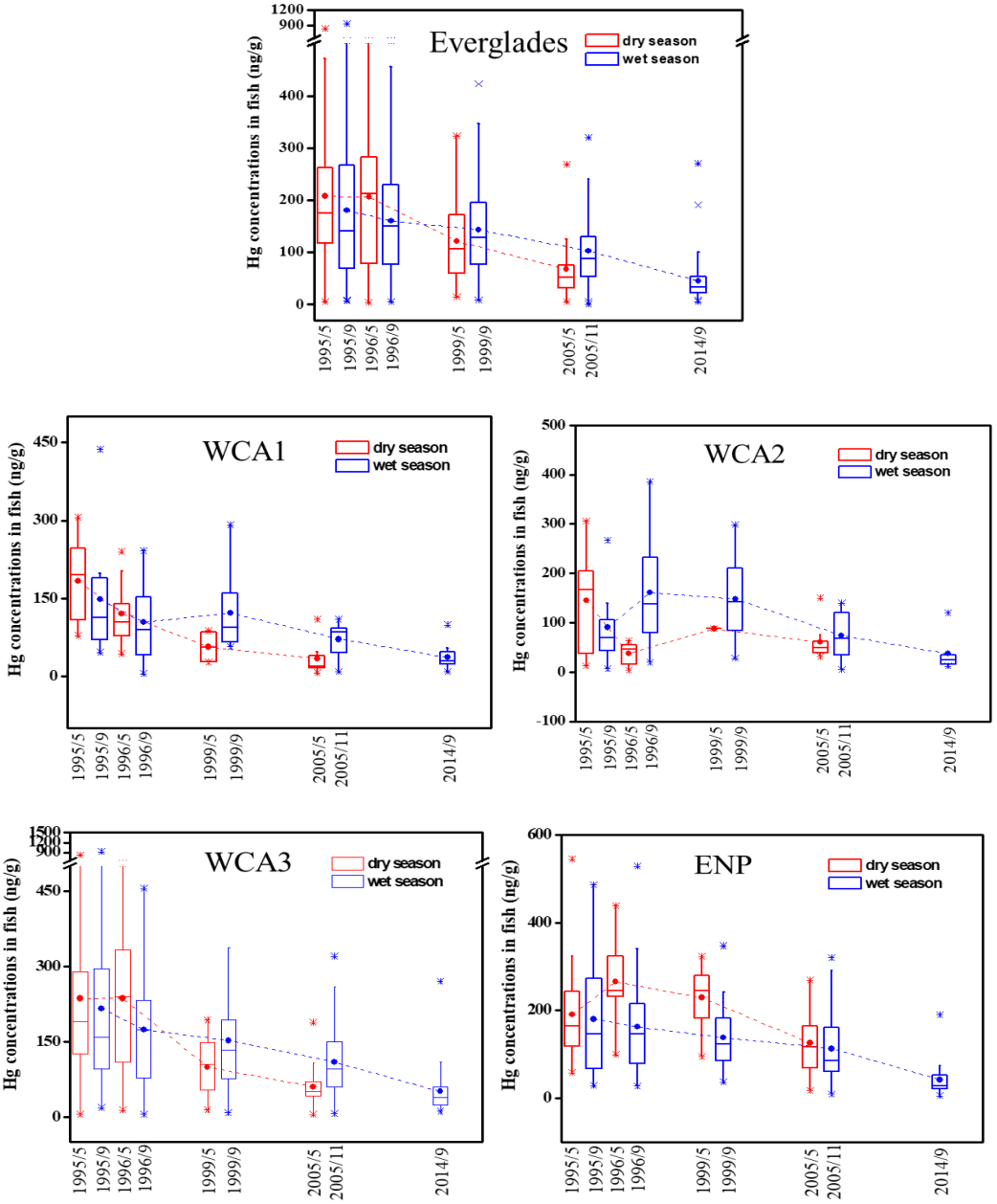


Figure 4.1. Long-term variation in the mosquito fish Hg in the Everglades, and WCA 1, WCA 2, WCA 3 and ENP parts of the Everglades from 1995-2014

In addition, the decreasing rate in dry season (~7%/year) was slightly higher than that in wet season (~4%/year). Significant decrease in mosquitofish Hg was observed at all the four parts of the Everglades. The decreasing rate in dry season and wet season was 8.5%/year and 3.8%/year, 4.1%/year and 3.6%/year, 7.9%/year and 3.8%/year, 4.0 %/year and 3.8 %/year for WCA 1, WCA 2, WCA 3 and ENP, respectively. Similar decreasing trend was also observed at 8 of 13 sites monitored by other projects (data obtained from the DBHYDRO) (Fig. 4.2). All these results suggest that Hg in Everglades mosquitofish declined in the past two decades.

Compared to the result of Hg in mosquitofish, the Hg level in fish at higher trophic level didn't show the similar trend. As shown in Figure 4.3, the Hg concentration in both sunfish and LMB didn't change too much during the last two decades among all over the Everglades area. The results of Mann-Kendall test ($p > 0.05$) performed on the Hg in both sunfish and LMB collected from all 11 sampling sites indicated that no significant decline in sunfish Hg or LMB Hg was observed. These inconsistent results could be due to several reasons. Firstly, duet to the difficulty of sampling, it is a big challenge to get sufficient large fish samples at a large number of sites, which is expected to result in large errors for analyzing the trend of Hg in large fish. Secondly, small fish are expected to response much quicker to ecosystem alteration in comparison to large fish due to their short generation time. In addition, as the mosquitofish only accounts for small part of sunfish and LMB diet, hence the change in mosquitofish Hg is not big enough to cause Hg level changes in sunfish and LMB.

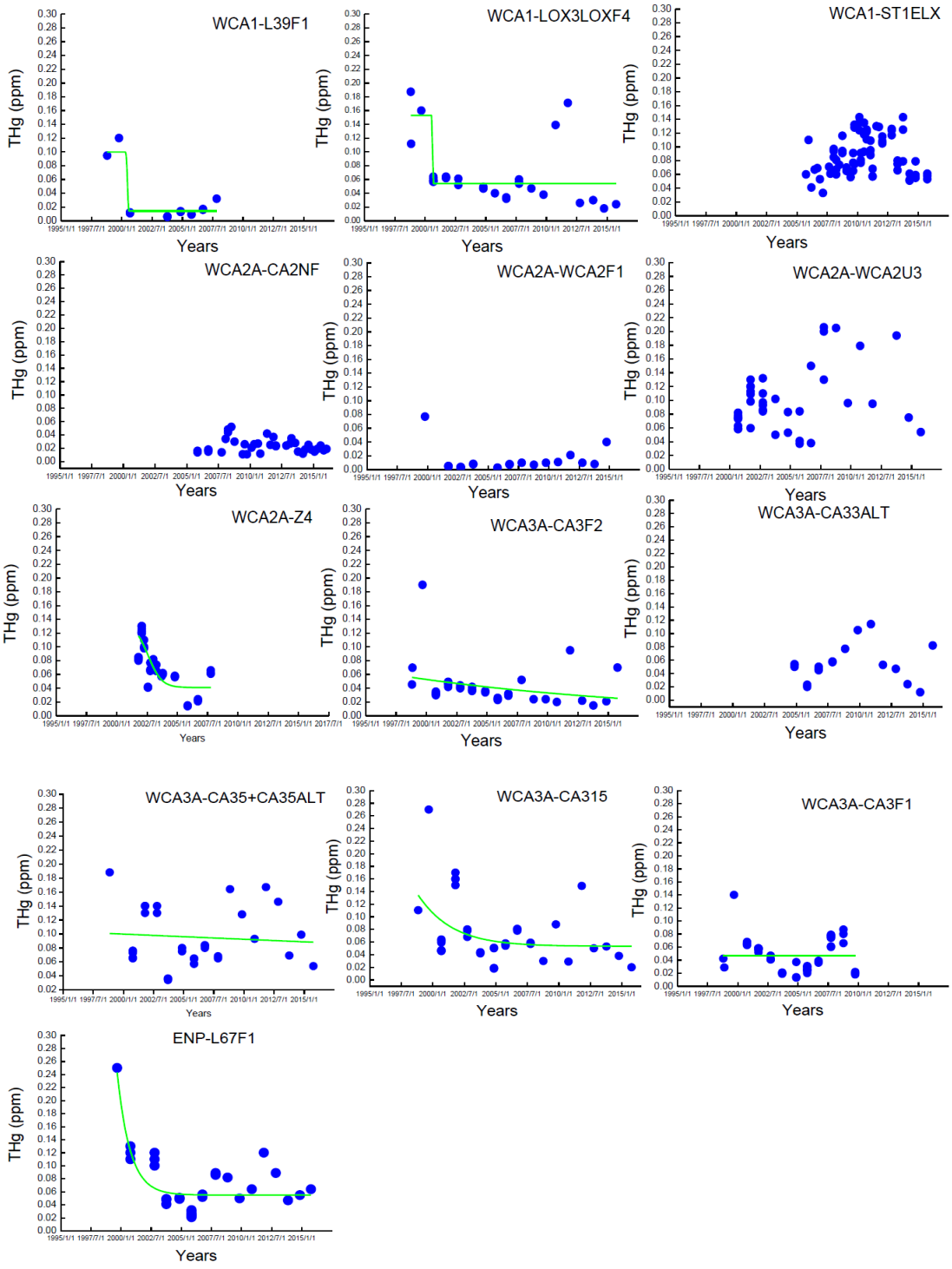


Figure 4.2. Long-term variation in the mosquito fish Hg from 1995-2014 (data obtained from SFWD DBHYDRO data)

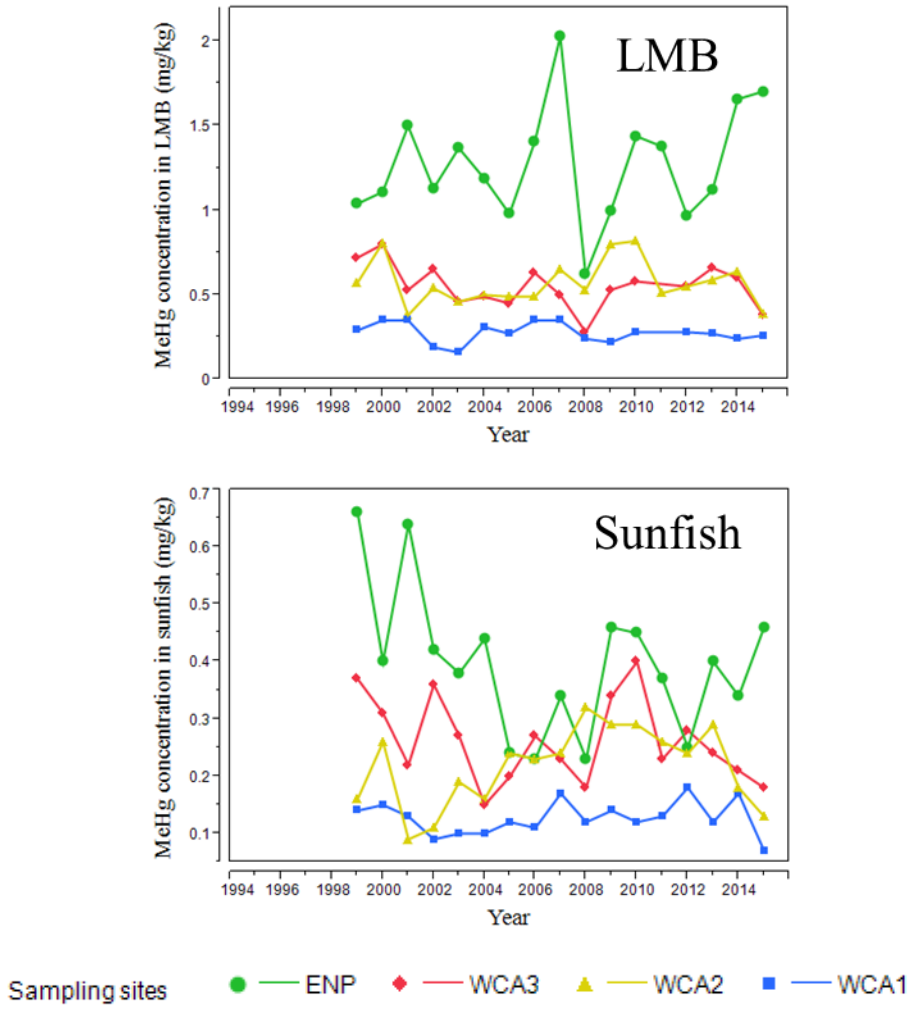


Figure 4.3 Long-term variation in the large bass and sunfish Hg from 1999-2015

Inorganic Hg is the major form of Hg inputted into aquatic environments, whereas most proportion of Hg in mosquitofish is in the form of MeHg (> 95%).[157]Mercury in fish are controlled by a variety of processes, including the input of Hg, and a number of transport and transformation processes (e.g., adsorption/desorption, methylation/demethylation, reduction/oxidation and bioaccumulation). Decrease in fish Hg has been observed in a variety of ecosystems in the past few decades. Most of them

were deemed to be caused by the decrease in Hg discharge,[141] while climate changes (e.g., global warming)[148,149]and ecosystem variations (e.g., fish body condition,[150] eutrophication) were also thought to be potential reasons.

In the Everglades, atmospheric deposition is the major source of inorganic Hg, accounting for > 95% of Hg inputted into the system.[152] The decrease in mosquitofish Hg could be because of the change in atmospheric Hg deposition or ecosystem alteration in the Everglades. These possible explanations were further tested in later sections.

4.3.2 Change in Hg atmospheric deposition into Everglades and its effects on mosquitofish Hg

Wet deposition of Hg was monitored at four sites of Everglades (NADP project data) since 1996. As shown in Figure 4.4, no significant change was observed on the input of Hg from wet deposition in both wet season and dry season in the past twenty years Mann-Kendall test, $p > 0.1$). Although there is lack of long-term monitoring of atmospheric Hg concentrations and Hg dry deposition in the Everglades and south Florida, atmospheric Hg concentrations in United States were observed to be relatively stable from 2008-2015,[159] indicating that dry deposition in the Everglades may be also stable during this period. In addition, no significant drop was observed in water and sediment Hg (Mann-Kendall test, $p > 0.1$), further proving that the atmospheric deposition of Hg changed insignificantly during the past twenty years. This indicates that the significant decrease in mosquitofish Hg in the past twenty years should not be because of the change in atmospheric deposition in the Everglades.

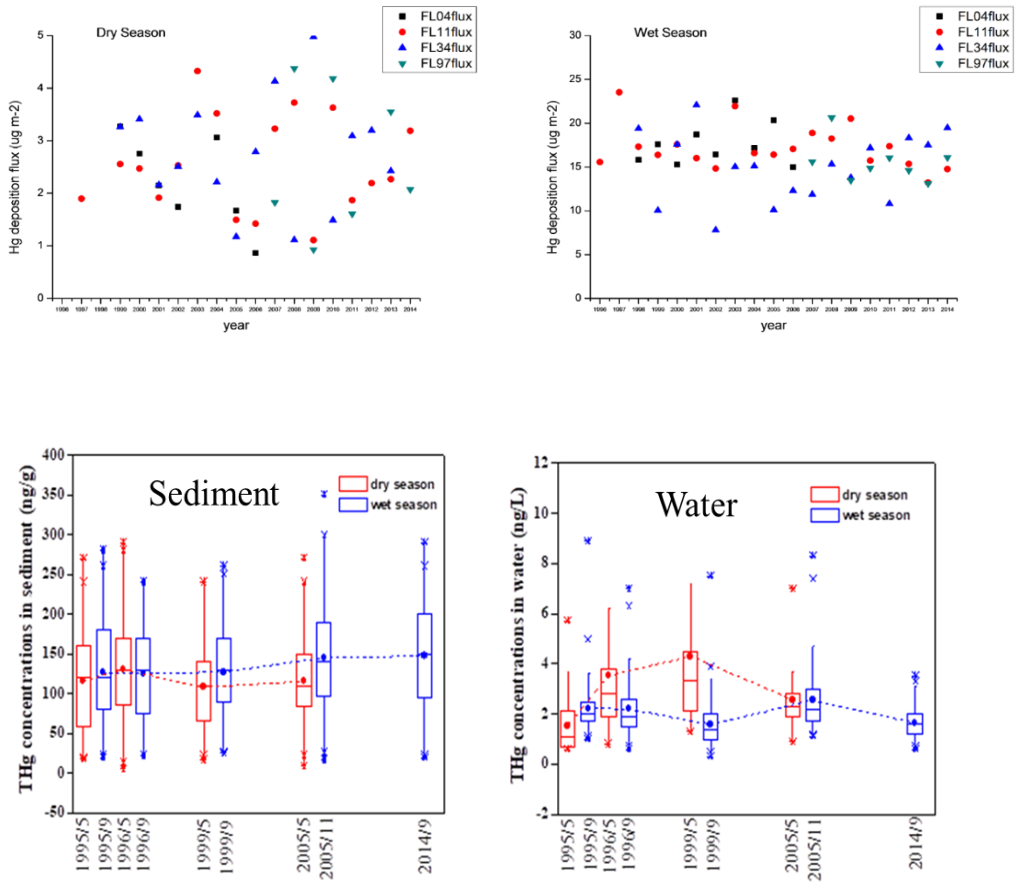


Figure 4.4. Wet deposition of Hg into the Florida Everglades and THg in Everglades sediment and water

Variation in atmospheric deposition of Hg has been deemed to be the major factor controlling Hg levels in fish in a number of aquatic ecosystems.[141,161] Diverse trend has been observed on the atmospheric deposition flux of Hg in the world, owing to the complicated trend in Hg emission (e.g., increase in Asia and Africa, and decrease in Europe and North America) in the pasted several decades.[159] Both local and regional sources contribute significantly to Hg in the South Florida.[156] Since most local sources of Hg have been terminated before 1990s and a relative stable Hg concentration may exist in

the atmosphere in the past two decades, it is reasonable to obtain a flat change in Hg atmospheric deposition in the Everglades.

By excluding the contribution of atmospheric deposition to the quick drop in mosquitofish Hg, it is reasonable to assume that this phenomenon may be caused by the alteration in ecosystem (e.g., less production of MeHg in the Everglades).

4.3.3 Ecosystem alteration in the Everglades and its effects on mosquitofish Hg

MeHg is the major form of Hg specie in mosquitofish[157] and MeHg in fish is from other compartments (e.g., periphyton, floc, water), other than production in fish body. MeHg in the Everglades mainly produced in the soil and periphyton. As for the long-term variation of MeHg in soil and periphyton (Fig. 4.5), no significant change was detected in the soil MeHg of Everglades; however, MeHg in the water and periphyton decreased dramatically from 1995-2014, especially in the dry season. Periphyton and water MeHg decreased at a rate of 6.7% and 3.8% in dry season, and 0.7 % and 2.8 % in wet season, respectively. Periphyton and sediment are the major source of MeHg in the Everglades.[160] As periphyton was one of the major sources of MeHg in Everglades water, it is reasonable to assume that the decline in mosquitofish Hg may be caused by the decrease in periphyton MeHg. This opinion was further supported by analyzing the relations of mosquitofish Hg with THg and MeHg in water, soil, floc, and periphyton of the Everglades. As shown in Table 1, mosquitofish Hg exhibited a good relation with MeHg in periphyton ($R = 0.44$, $p < 0.01$) and water ($R = 0.42$, $p < 0.01$) and THg in periphyton ($R = 0.35$, $p < 0.01$), while weak relation was observed on mosquitofish Hg and THg and MeHg in sediment and floc and THg in water.

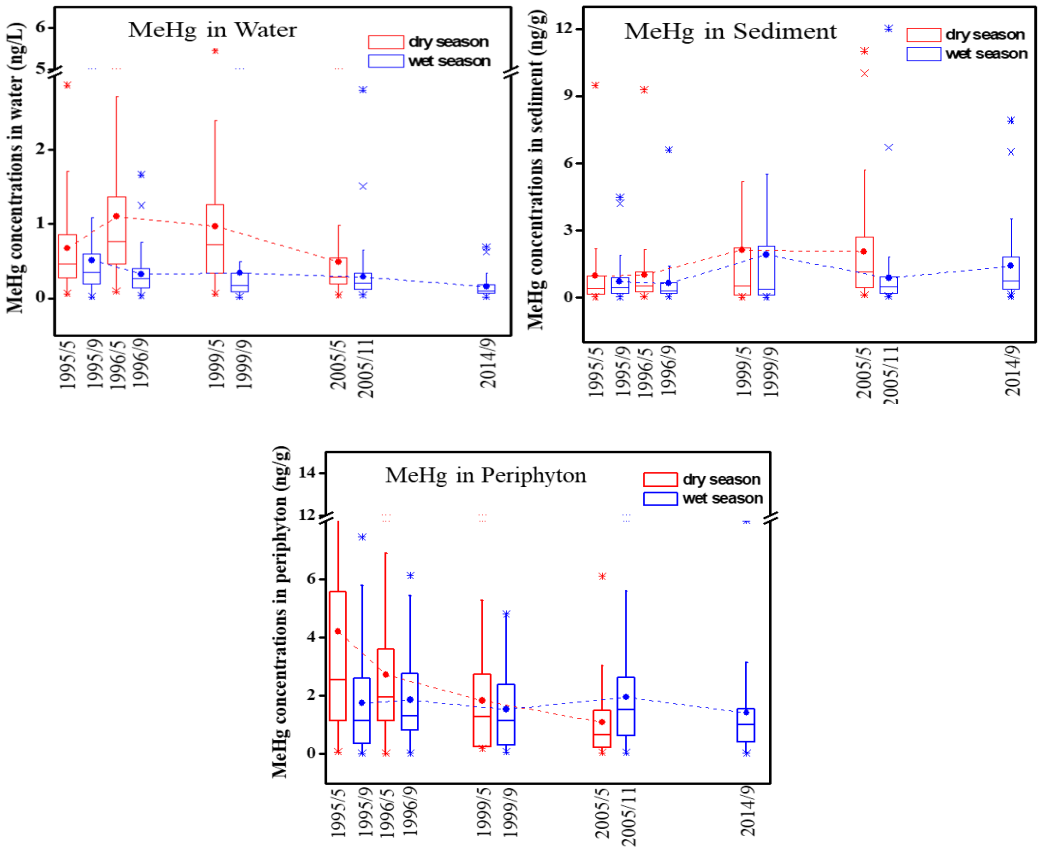


Figure 4.5. Long-term variation in MeHg in Everglades water, sediment, and periphyton from 1995-2014 (EPA-REMAP data).

Principal component analysis (PCA), and multiple regression analyses also showed that Hg in mosquitofish have the best correlation with MeHg and THg in periphyton, in comparison to Hg in the other matrices and related environmental parameters (Fig. 4.6). These results indicate that the decrease in periphyton (food source for mosquitofish and major sources of MeHg in Everglades water) MeHg may be the reason of dramatic decline in Everglades mosquitofish Hg.

Sediment methylation has been thought to be major source of MeHg in aquatic environments. Our pervious study160 found that both soil (sediment in other systems)

and periphyton are the major source of MeHg in the Florida Everglades. The net production of MeHg in the soil was calculated to be much larger than that in periphyton. However, a large portion of produced MeHg may be adsorbed on solid phase which cannot be diffused into the water body.

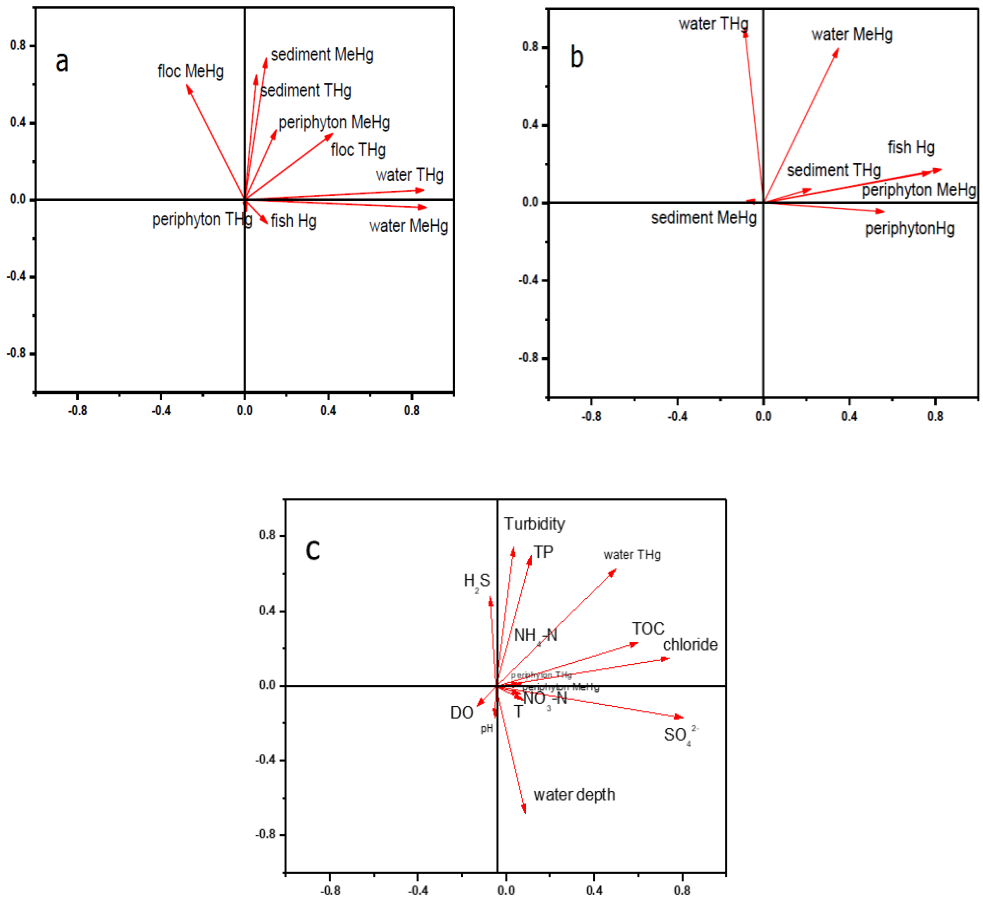


Figure 4.6. PCA of Hg in fish with Hg in other matrices (a, floc included, b, floc not included) and MeHg in periphyton with environmental factors (c).

This could be particularly true in the Everglades, where soil has a much higher content of organic matter and could sequester more MeHg. To test this hypothesis, MeHg in porewater of soil/floc and surface water adsorbed by periphyton was measured and

compared with MeHg in the water body. As shown in Fig. 4.7, MeHg in soil/floc porewater was comparable to or even lower than that in associated overlying water, indicating that most MeHg in surface water may not be from the sediment phase. MeHg adsorbed on periphyton was higher than that in surface water, indicating a significant diffusion of MeHg from periphyton to water body. These results suggest that periphyton may be a more important source for MeHg in comparison to soil/floc although the production of MeHg in soil was higher than that in periphyton. This was consistent with the statistical analysis on the basis of the monitoring data from 1995-2014, indicated by the better correlation of MeHg in water with MeHg in periphyton (R = 0.53) in comparison to MeHg in the soil (R = 0.13).

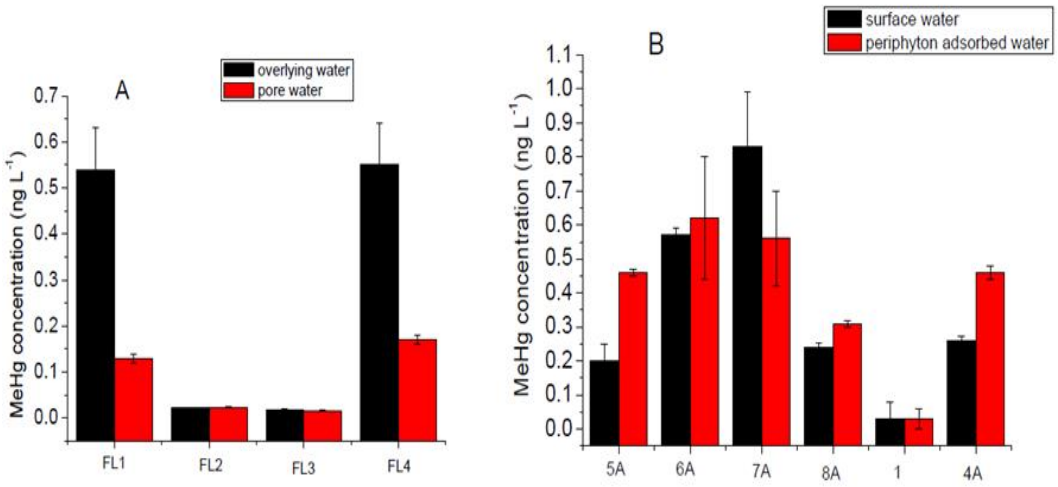


Figure 4.7. MeHg concentrations in Everglades soil/floc porewater, periphyton adsorbed water, surface water, and surface water.

A variety of processes could affect the level of Hg in fish in aquatic environment, including the deposition of Hg^{139}, and changes in environmental factors that can

significantly affect MeHg production or fish biomass or bioaccumulation of Hg via food chain. In this study, it was found that the decrease in MeHg periphyton may result in the dramatic drop in mosquitofish Hg since 1995. Most previous studies reported that Hg in fish was controlled by Hg atmospheric deposition,[141] climate changes,[148,149] fish body condition,[150] etc. This finding gives a new insight on the factors controlling Hg in fish. Periphyton is the major primary producer in the Everglades. As a kind of important food for mosquitofish, the decrease in periphyton MeHg in periphyton would directly result in the decrease in fish MeHg. In addition, periphyton is a major source of MeHg in water. The decrease in MeHg production by periphyton can cause the decrease in water MeHg and cause the less acquisition of MeHg by mosquitofish through the bioaccumulation from water via the phytoplankton chain. The levels of MeHg in periphyton are affected by both net production of MeHg by periphyton, which are controlled by a variety of environmental factors that controls periphyton composition and MeHg methylation, e.g., THg levels, phosphorus, pH, dissolved oxygen (DO), sulfate, etc. The changes in these environmental factors will be further investigated and their possible effects on periphyton MeHg levels were tested.

4.3.4 Possible reasons of the decrease in periphyton MeHg from 1995 to 2014

To elucidate the reason of the decrease in periphyton MeHg since 1995, in periphyton, relations of periphyton MeHg with a variety of environmental parameters, including THg in periphyton, THg in water and periphyton, DO, pH, Temperature, water depth, sulfate, H_2S, Turbidity, total organic matter (TOC), total phosphorus [58], NO_3^-, Cl^-, ash free dry weight (AFDW) of periphyton, and chlorophyll a (Chl-a), were first investigated to identify the major factors controlling MeHg levels in periphyton.

Periphyton MeHg presented a positive relation with THg in water and periphyton, water depth, TP, TOC and SO_4^{2-}, H_2S, and Cl^-, and a negative relation with DO, pH, Temperature, and NO_3^- ($p < 0.05$) (Table 4.1). The other factors had an insignificant relation with MeHg in periphyton.

Table 4.1. Correlation analysis and multiple regression analysis of Hg in mosquitofish and MeHg periphyton with environmental parameters.

	Spearman's analysis(R)		Multiple regression(β)			
	Fish Hg	Periphyton MeHg		Fish Hg	Fish Hg (w/o floc)	Periphyton MeHg
Water THg	0.09*	0.16**		0.04	0.03	0.03
Water MeHg	0.42**	0.53*		0.07	0.1	
Sediment THg	0.11**	0.48**		0.07	0.03	
Sediment MeHg	0.01	0.30**		-0.13	-0.15	
Periphyton THg	0.35**	0.39**		-0.01	0.13	0.25
Periphyton MeHg	0.44**	--		0.32	0.52	
Floc THg	0.14*	0.17**		-0.23	--	
Floc MeHg	0.05	0.26**		-0.21	--	
DO	0.04	-0.20**				0.05
pH	0.02	-0.27**				-0.1
T	0.02	-0.12**				-0.02
Water Depth	0.06	0.22**				0.17
SO_4^{2-}	0.03	0.24**				-0.33
H_2S	0.35**	0.20**				-0.25
Turbidity	0.06	0.15**				-0.1
TOC	-0.02	0.36**				0.42
TP	0.04	0.30**				0.22
SRP	-0.29**	0.05				
NO_3^--N	-0.12*	-0.14*				0.01
Chloride	0.09	0.17**				0.04
AFDW	0.08	0.01				
Chl-a	-0.21**	0.1				
			r^2	0.13	0.37	
			p	<0.01	<0.0001	

Several commonly used multivariate statistical analyses (PCA, CA, and multiple regression analysis) (Fig. 4.6 and Table 4.1) were then performed to estimate the relative importance of these factors in periphyton MeHg. These analyses showed that periphyton THg, TP, TOC, water depth, H_2S, and SO_4^{2-} are the primary controlling factors for MeHg in periphyton.

For these important factors, THg decreased at a rate of 7.2% in dry season and 5.2% in wet season, (Fig. 4.8) in good consistence with the long-term change in mosquitofish Hg. TP concentrations were observed to decrease at a rate of 2.4% in wet season and 4.9% in dry season during this period. Sulfate in water decreased at a rate of 2.1% in dry season and 2.2% in wet season. Significant decrease was also observed on water depth. TOC presented a negligible change from 1995 to 2014, indicating that it may not be the reason of decrease in mosquitofish Hg during this period.

As the precursor of Hg methylation, the decrease in periphyton THg and sulfate is expected to inhibit the methylation of Hg in the periphyton, resulting in the decrease in periphyton MeHg. This was partially proved by the decrease in H_2S in surface water, which is the product of sulfate reduction. The decrease in TP is expected to change both the biomass and microbial community of periphyton, which may subsequently affect the uptake of inorganic Hg and production of methylmercury by periphyton. Water depth is another important parameter that would affect the periphyton as well as the ecosystem.

Comprehensive Everglades Restoration Plan (CERP), the largest environmental restoration project in the world, was launched in 2000. This project has changed and is changing the topography, water chemistry, and ecosystem of the Everglades.[162]

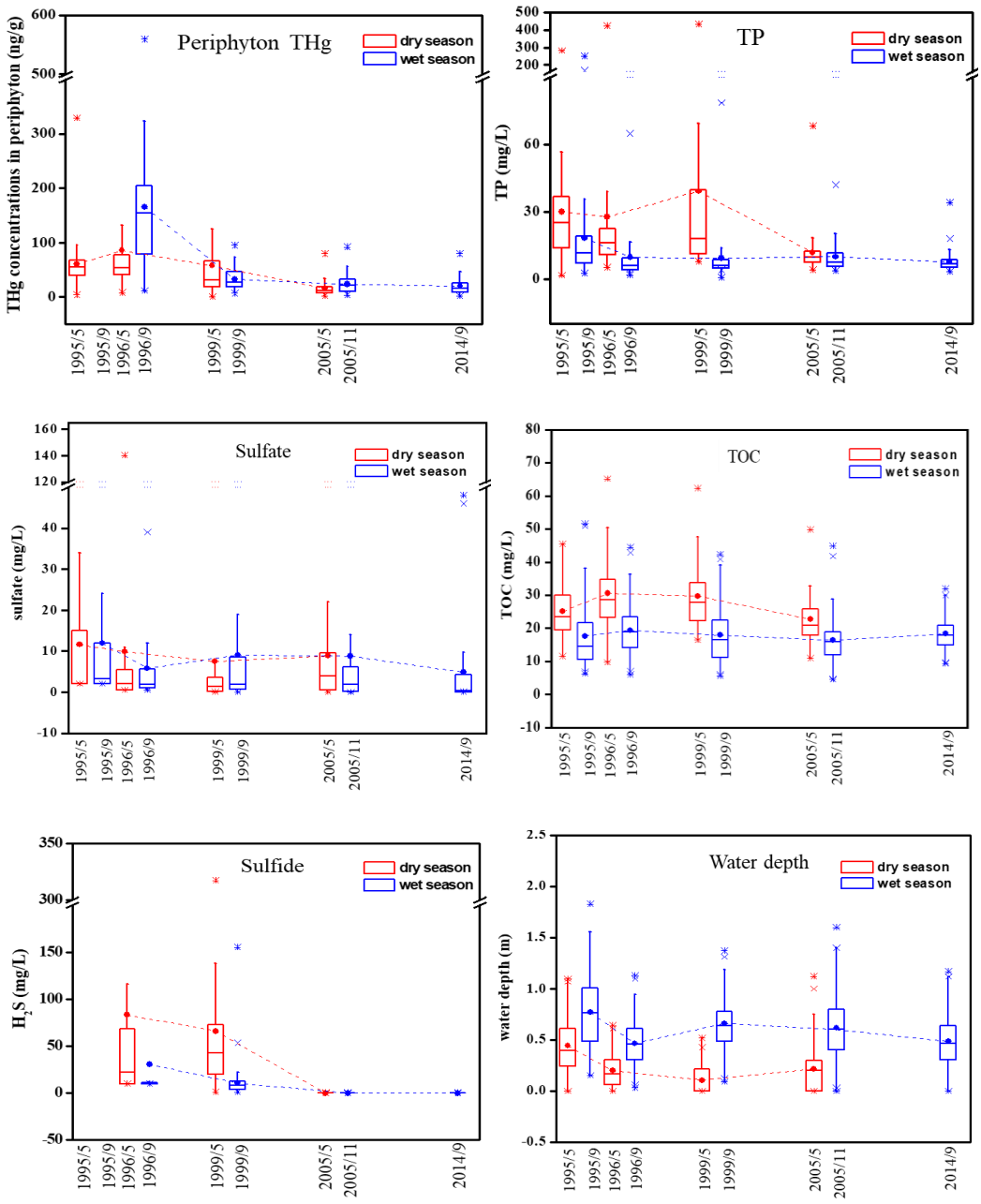


Figure 4.8. Long-term variation in Everglades periphyton THg, and TP, SO_4^{2-}, TOC, H_2S, water depth in Everglades water from 1995-2014 (EPA-REMAP data).

A variety of measures have been implemented in the northern farming area, reducing the discharge of phosphorus and sulfate into the Everglades. As a major biological

nutrient for both plant and microorganisms, the decrease in TP would result in the change in periphyton biomass and periphyton composition and microbial structure in periphyton, which may further result in the decrease in THg uptake and MeHg production in periphyton. Sulfate is the necessary reactant for Hg methylation by SRB, the decrease in sulfate may affect the SRB in the periphyton. Hydrologic condition is another important factor for periphyton biomass and composition. The continuing getting drying since 1995 is expected to change periphyton significantly during this period. These changes in periphyton by ecosystem alteration could then affect the production of MeHg in periphyton, resulting in the decline in mosquitofish Hg. It should be noted that the changes in periphyton, hydrologic condition, and environmental factors may also have significant influence on mosquitofish biomass. The biomass of mosquitofish as well as small fishes has been observed to significantly decrease from 1995 to 2014 (Joel Drexler, unpublished data). According to the biodilution theory, Hg in mosquitofish is expected to decrease accordingly. This suggests that the change in mosquitofish biomass should not be the dominant reason of the dramatic drop in mosquitofish Hg.

4.4 Conclusions

In this study, a clear decreasing trend was observed on mosquitofish Hg, implying a possible turning-good pattern for Hg problem in the Everglades. Periphyton was found to possibly play a dominant role in controlling Hg in mosquitofish by affecting the production of MeHg. The results of statistical analyses performed on the data of MeHg in different matrices and environmental parameters supported the hypothesis. Besides, no clearly trend of Hg level in large fish at high trophic level was observed in this study. The observation was considered being related to the difficulty of sampling, which leads to the

sampling on limited number of sites then further results in large errors for data analyzing the trend of Hg in large fish. In addition, the lag-effect caused by the relative long life of large fish may also contribute to the errors occurred in trend analysis. By performing the study, data from various databases were intergraded and the factors affecting Hg in fish were studied. However, as mosquitofish only account for part of small fish in the Everglades, there is a need to monitor the variation of other small fishes in the Everglades. In addition, efforts should be made on monitoring long-term variation in large fish Hg at sufficient sites for the purpose of a more accurate assessment of Hg in fish in the Everglades.

Chapter 5. Summary and Future work

5.1 Summary

In the study MeHg in rice cereal part, considerable levels of MeHg and THg were found in the studied infant cereal samples including commonly available cereals in the market of 8 major cities in US and China. The concentrations of MeHg in rice-based infant cereals were significantly higher than those in cereals containing no rice, suggesting that the source of MeHg in infant cereals could be the rice used to make cereals. The estimated daily intake of MeHg for infants through diet (including rice cereals and breast milk) could exceed or be close to RfD set by USEPA, but further studies are required for more precisely evaluate the effects of postnatal diet MeHg exposure during infancy and childhood. The results provide an important data towards the understanding and assessment of MeHg exposure and health risks caused by consumption of rice products for infants.

The results of bioaccessibility study indicated the amount of MeHg can be released from food matrix into gastrointestinal track was underestimated. After taking the portion of re-adsorbed MeHg into account, the bioaccessibility of MeHg in all measured rice cereal and fish samples were close to 100% instead of mean value approximately 50% reported by previous studies. Therefore, the assessment of potential health risk caused by MeHg exposure through fish and rice product consumption could be influenced by using underestimated data while calculating the daily intake of MeHg through diet. In addition, the presence of enzyme, as well as MeHg speciation in digested samples also have been proven to be related to the measured MeHg bioaccessibility, which also provided valuable data for a further study on the factors affecting MeHg bioaccessibility.

In Chapter 4, Hg decline was observed in mosquitofish, while the similar trend has not been clearly observed for Hg in fish at high tropic level. The low frequency of sampling or the lagged response of fish at high trophic level to Hg variation in prey fish were proposed as the possible reason of the observation. Beyond that, by performing the statistical analysis on the data obtained during the last two decades, periphyton was found to possibly play a dominant role in controlling Hg in mosquitofish by affecting the production of MeHg in this study. However, it is obvious that data collected at more sites and higher frequency are still needed for a more accurate evaluation of importance of Hg in periphyton on Hg control in Everglades water.

5.2 Future work

In the second and third part of this dissertation, the concentrations of MeHg in rice-base infant cereals were determined and then the daily intake of MeHg through the consumption of rice-base infant cereals for babies was estimated. In addition, the bioaccessibility of MeHg in rice-base infant cereals was investigated to better estimate the risk of exposure to MeHg via food consumption. However, this assessment is on the basis of the assumption that all solubilized MeHg can eventually be absorbed, which could overestimate the risk. In future work, the absorption efficiency of bioaccessible MeHg should be determined to refine the results. In this study, we found that MeHg bioaccessibility in both rice and fish depended on the speciation of MeHg. However, due to the lack of a sufficient analyzing method, the speciation of MeHg in food samples was not determined, limiting our understanding on the speciation of MeHg bioaccessible and the transformation of MeHg species during cooking and digestion. MeHg speciation in raw fish and rice samples as well as in cooked and digested samples should be

determined in future work to better understand the bioaccessibility and transformations of MeHg in foods.

For study on fish Hg variation in the Everglades, a clear decreasing trend was observed on mosquitofish Hg, implying a possible turning-good pattern for Hg problem in the Everglades. However, similar trend has not been clearly observed for Hg in large fish and predators at high tropic level, e.g., largemouth bass, wading bird, etc. These inconsistent results could be due to several reasons. Firstly, duet to the difficulty of sampling, it is a big challenge to get sufficient large fish samples at a large number of sites, which is expected to result in large errors for analyzing the trend of Hg in large fish. Secondly, small fish are expected to response much quicker to ecosystem alteration in comparison to large fish due to their short generation time. Thus, there may be a lag-effect for large fish. Efforts should be made on monitoring long-term variation in large fish Hg at sufficient sites in the Everglades. Besides, only mosquitofish Hg (as an indicator of small fishes) were monitored in previously performed EPA-REMAP project. As mosquitofish only account for part of small fish in the Everglades, there is a need to monitor the variation of other small fishes in the Everglades. As a system with a small quantity of local anthropogenic discharge of Hg, it is almost an unrealistic mission to largely decrease the discharge of Hg into the Everglades at the near future. However, there is an urgent need to reduce Hg levels in Everglades fish for both the ecosystem and human health. This study indicates that ecosystem alteration could be an effective measure to conquer the problem of Hg pollution in the Everglades. However, it should be noted that this should be on the basis of clearly understanding the major processes of MeHg cycling in the Everglades (methylation/demethylation, benthic layer-water and

periphyton-water diffusion fluxes, bioaccumulation, etc.). Otherwise, it will be impossible to sufficiently evaluate the effectiveness of implemented measures. More work should be performed on this field in future for the purpose of resolving the Hg problem in the Everglades as well as the other similar systems.

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PUBLICATIONS AND PRESENTATIONS

Wenbin Cui, Guangliang Liu, Mayara Bezerra, et al. Occurrence of Methylmercury in Rice-base Infant Cereals and Estimation of Daily Dietary Intake of Methylmercury for Infants. (submitted)

Wenbin Cui, Michael Ojeda, Guangliang Liu, et al. Determination of Bioaccessibility of Methylmercury in Rice-based Infant Cereals using *In vitro* Digestion Model. (to be submitted)

Yanbin Li, Wenbin Cui, Guangliang Liu, et al. Decadal Variations of Mercury in Mosquito Fish in the Everglades and Relation to Changes in Atmospheric Mercury Deposition and Ecosystem Alteration. (to be submitted)

Wenbin Cui, Guangliang Liu, Ping Jiang, et al. Methylmercury in Food Webs in the Everglades: Temporal Variations over the Last Two Decades. The Greater Everglades Ecosystem Restoration (GEER) Science Conference. Coral Springs, FL; April 21-23, 2015. (Poster)

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Wenbin Cui, Guangliang Liu, Yong Cai. Occurrence of Methylmercury in Rice-base Infant Cereals and Estimation of Daily Dietary Intake of Methylmercury for Infants. 252nd National ACS meeting, Philadelphia, PA, August 21-25, 2016. (Poster)

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Wenbin Cui, Michael Ojeda, Guangliang Liu, et al. Determination of Methylmercury Bioaccessibility in Rice Products and Fish using *In vitro* Digestion Model. Florida International University Graduate Student Appreciation Week Scholarly Forum, Miami, FL, March 27-31, 2017. (Oral)

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