Original Article

Hair mercury levels in relation to fish consumption among Vietnamese in Hanoi

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(Received June 23, 2017; Accepted July 30, 2017)

ABSTRACT — People are exposed to methylmercury (MeHg) mainly through fish consumption, which is increasing in Vietnam. However, little information is available on estimating the health risk of MeHg exposure through fish consumption in Vietnam. The present study examined the association between mercury (Hg) levels in hair and selenium (Se) levels in toenails of 196 Vietnamese people and their fish consumption, using a dietary questionnaire to obtain information pertinent for assessing health risk owing to MeHg exposure. The geometric mean of Hg levels in the hair of males and females was 617 ng/g and 575 ng/g, respectively. We found that Hg levels in the hair of 98% of the Vietnamese study subjects were lower than the provisional tolerable weekly intake for MeHg (1.6 µg Hg/kg body weight; which is equivalent to a hair Hg concentration of approximately 2,300 ng/g, with an uncertainty factor of 6.4). There were significant differences in the age-adjusted geometric mean of Hg levels found in hair from females related to their frequency of freshwater fish consumption. The levels of Hg in hair and Se in toenails increased with an increased frequency of marine fish consumption, and both showed a significant positive correlation in subjects who consumed marine fish $\geq$ once/week. This is the first cross-sectional study to investigate the association between hair Hg levels and fish consumption in Vietnam. These findings provide valuable information for future assessments of the health risk of MeHg exposure through fish consumption in Vietnam.

Key words: Methylmercury, Fish consumption, Vietnamese, Selenium

INTRODUCTION

Methylmercury (MeHg) is a well-known neurotoxin (World Health Organization, 2008, 2010). The target organ for MeHg toxicity is the brain, and the developing brain is especially sensitive to MeHg exposure. Hence, fetuses, newborn and young children are especially susceptible to MeHg exposure. Parents, pregnant women, and women of childbearing age should be aware of the potential risk of MeHg. People are primarily exposed to

MeHg through consumption of fish and marine mammals (World Health Organization, 2008, 2010). Consequently, assessment of MeHg exposure is an important public health issue and evaluation of associated health risks is needed, especially for people who consume large amounts of fish and other seafood containing high concentrations of mercury (Hg).

Selenium (Se) is an essential trace nutrient in humans that participates in antioxidant defense mechanisms, thyroid hormone metabolism, and the redox control of intra-

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cellular reactions (Agency for Toxic Substances and Disease Registry, 2003). People consume Se mainly through seafood, with its organic form comprising the greatest proportion of total dietary intake (Agency for Toxic Substances and Disease Registry, 2003; Roman *et al.*, 2014). Selenium plays an important role providing an antagonistic effect on Hg toxicity in various tissues and organs of rodents, fish and marine mammals (Peterson *et al.*, 2009; Khan and Wang, 2009; Sakamoto *et al.*, 2015). The correlation between Se concentrations in human specimens and health risk has been widely studied in the environmental health science of MeHg (Mozaffarian and Rimm, 2006).

Recently, fish consumption is increasing in Vietnam (Food and Agriculture Organization of the United Nations, 2004). The rise in demand for fish and fishery products in Vietnam is caused by two main factors, population and income growth (Food and Agriculture Organization of the United Nations, 2004). Vietnam is ranked sixth place in eight Southeast Asian countries for the consumption of fish and fish products as determined by household surveys (Needham and Funge-Smith, 2015). Most commercial fish and other seafood in Vietnam are locally harvested (Food and Agriculture Organization of the United Nations, 2005). Food and Agriculture Organization of the United Nations (2004) reported that a strong and sustained demand for fish and fishery products was expected from the Vietnamese population. However, little information is available on estimating the health risk of MeHg exposure through fish consumption in Vietnam.

Hair is the preferred biological sample for the assessment of MeHg exposure in many studies because it provides a simple, integrative, and non-invasive sample for estimating long-term average exposure. Hair contains a higher concentration of Hg than blood or urine, allowing easier detection of Hg (Srogi, 2007). Total mercury (T-Hg) in hair is about 250 to 300 times higher than the blood concentration of Hg at the corresponding moment of hair formation (World Health Organization, 2008, 2010). The MeHg usually constitutes at least 80% of T-Hg analyzed in hair from fish consumers (McDowell *et al.*, 2004). Nail clippings also have advantages compared with other biological materials and are thought to reflect long-term exposures (Slotnick and Nriagu, 2006; Koriyama *et al.*, 2008).

In the present study, we examined the association between Hg levels in hair or Se levels in toenails and fish consumption using a dietary questionnaire for subjects recruited in Hanoi, to obtain information pertinent for assessing the potential health risk from MeHg exposure in Vietnamese. In addition, T-Hg and MeHg concen-

trations in several commercially canned seafood products in Hanoi were determined for a preliminary survey.

MATERIALS AND METHODS

Sample collection and preparation

A total of 196 Vietnamese people with no occupational exposure to Hg compounds were selected from staff ($n = 98$), and outpatients with acne ($n = 29$), tinea ($n = 29$) or sexually transmitted infections ($n = 40$), at the National Hospital of Dermatology and Venereology in Hanoi from 2015 to 2016. To recruit subjects from wide areas and with various characteristics, we selected a national referral hospital (National Hospital of Dermatology and Venereology), which had outpatients from Hanoi and other provinces in northern Vietnam. All subjects agreed to participate and gave their consent after an explanation of study objectives and methods. Subjects were 16 to 55 years old (mean 27.8 ± 7.4). For hair collection, approximately 50 full-length strands of hair were cut close to the scalp in the occipital area and stored in clean polyethylene bags. Clipped toenail samples were stored in a separate plastic envelope with the identification number of the corresponding subject. Hair and toenail samples were transferred to the National Institute for Minamata Disease, Japan, and stored at room temperature before analysis. This study was approved by the ethics committees of the National Institute for Minamata Disease, Kagoshima University, Prefectural University of Kumamoto (Japan) and National Hospital of Dermatology and Venereology (Vietnam).

Frequency and amount of fish consumption

General information (age, gender, bodyweight, height, occupation, education, marital status, hair treatment, and habits of smoking, alcohol drinking and eating out), frequency of fish consumption and amount of fish consumed per time were estimated for each subject using a food frequency questionnaire (FFQ). The FFQ was used to evaluate the frequency of freshwater and marine fish consumption during the past year. There were three alternatives for each species of fish: ≥ 4 times/week, 1-3 times/week, never or < 1 time/week. Regarding the amount of fish consumed per time, there were two alternatives for each species of fish: ≥ 50 g/time and < 50 g/time. We used food models of fish (50 g) and pictures of fish commonly consumed in Hanoi to increase the accuracy of the survey.

Reagents

Analytical grade reagents were used in this study. Five M sodium hydroxide (NaOH) solution, hydrogen bro-

mide (HBr), copper (II) chloride dihydrate ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$), nitric acid (HNO_3), methyl isobutyl ketone (MIBK: atomic absorption spectrometry grade), hexane (HPLC analysis grade), toluene (HPLC analysis grade) and L-cysteine (Cys) were purchased from Kanto Chemical Co. (Tokyo, Japan). Methylmercury chloride (MeHgCl ; > 98% purity) was obtained from Tokyo Chemical Industry (Tokyo, Japan). Sodium acetate trihydrate (NaOAc : Emsure grade) was purchased from Merck (Darmstadt, Germany). The Hg standard solution (Hg 1000 mg/L, HgCl_2 in 0.1 mol/L HNO_3) was purchased from Wako Pure Chemical Industries (Osaka, Japan). Ultra-pure water was prepared using a Milli-Q system (Merck Millipore, Tokyo, Japan). Cys (0.1%) and Cys (0.2%)-NaOAc (2%) solutions were freshly prepared for each experiment.

Determination of total mercury concentration in hair

The T-Hg in the 3 cm proximal end of scalp hair was determined using direct thermal decomposition Hg analyzers MA-3000 (Nippon Instruments, Tokyo, Japan). Detection was performed using cold-vapor atomic absorption spectroscopy at a wavelength of 253.7 nm. The detection limit of Hg was 0.1 ng. A standard Hg working solution for the calibration curve was prepared using the Hg standard solution (Hg 1,000 mg/L, HgCl_2 in 0.1 mol/L HNO_3) according to the manufacturer's protocol (Nippon Instruments). The certified reference material (CRM) of hair (NIES CRM No.13) was measured as a quality control, and the determined T-Hg level of $4.41 \mu\text{g/g} \pm 0.03 \mu\text{g/g}$ ($n = 5$) was within the certified range of $4.42 \pm 0.20 \mu\text{g/g}$. A coefficient of variation (CV) of 0.7% was obtained from five repeated measurements using standard hair.

Determination of total mercury and methylmercury in canned fish

Canned fish were obtained from supermarkets in Hanoi, Vietnam. Using company names shown on the label, canned fish samples included: sardines in tomato sauce from companies A, B and C; mackerel in tomato sauce from company D; tuna in oil from companies E and F; yellowfin tuna chunks in vegetable oil from company G, light tuna chunks in oil from company H; tuna in vegetable oil from company I; tuna chunks in brine from company J ($n = 1$ for each). Muscles of canned fish samples were weighed (1 g, wet weight) and transferred to 15 mL polypropylene (PP) tubes then kept at -30°C until analysis.

Extraction of T-Hg and MeHg from canned fish samples followed the protocol described previously (Miyamoto

et al., 2010; Yoshimoto *et al.*, 2016). Briefly, the muscle of canned fish samples was solubilized with 0.1% Cys solution (1 mL) and 5 M NaOH (1 mL), then incubated at 80°C for 1 hr with vortexing every 10 min. After cooling to room temperature, each solubilized sample solution was made up to 5 mL with distilled water. MIBK (6 mL) was then added and the sample solution was shaken at 220 rpm for 10 min. After centrifugation ($1,750 \times g$, 10 min), the upper MIBK layer was removed and the remaining solubilized solution was washed with hexane (5 mL), followed by shaking (220 rpm, 5 min) and centrifugation ($1,750 \times g$, 5 min) to obtain the solubilized and degreased sample solution, which was used for the determination of T-Hg. In the next step, 2 mL of the solubilized and degreased sample solution was added to 2 mL of 5 M HBr, 0.5 mL of 2 M CuCl_2 and 6 mL of toluene, followed by shaking (10 min). After centrifuging of the sample solution ($1,750 \times g$, 10 min), the upper toluene layer (5 mL) was transferred to a new 15 mL PP tube, and 1 mL of Cys (0.2%)-NaOAc (2%) solution was added, followed by shaking (5 min), and centrifugation ($1,750 \times g$, 5 min). After removal of the toluene layer, the reverse-extracted Cys (0.2%)-NaOAc (2%) solution was used to measure MeHg.

The T-Hg and MeHg concentrations in extracts of canned fish samples were determined using direct thermal decomposition Hg analyzers MA-3000 (Nippon Instruments). Total Hg and MeHg were measured in cod fish CRM (National Institute of Advanced Industrial Science and Technology, 2010) for quality control. The mean values ($n = 5$) measured for T-Hg and MeHg in cod fish were $0.62 \mu\text{g/g} \pm 0.01$ and $0.59 \mu\text{g/g} \pm 0.01 \mu\text{g/g}$, respectively, which were within the certified range of $0.61 \pm 0.02 \mu\text{g/g}$ and $0.58 \pm 0.02 \mu\text{g/g}$, respectively. A CV of 0.8% and 0.9% was obtained for T-Hg and MeHg, respectively, after five replicate measurements using cod fish CRM.

Determination of selenium concentration in toenail

A toenail sample was washed by sonication in 0.3% polyoxyethylene lauryl ether and subsequently dried for 12 hr at 80°C . The sample was digested in 10 mL of 35% HNO_3 in a Teflon polytetrafluoroethylene tube in a closed vessel microwave system (Ethos Easy, Milestone Srl, Sorisole, BG, Italy). The concentration of Se was determined with an inductively coupled plasma mass spectrometer (Agilent 7500cs, Agilent Technologies, Tokyo, Japan). Accuracy of the method was assessed using CRM for hair (NIES CRM No.13). Recovery of Se ranged from 92-98% ($n = 3$).

Statistical analysis

Sheehan *et al.* (2014) suggested that population Hg biomarker distributions are often skewed, so the central tendency is best captured by geometric means or medians. As the distribution of Hg concentrations in hair was skewed and had a long upper tail, we calculated the geometric means for hair Hg concentrations and their corresponding 95% confidence intervals (CIs). We used log-transformed Hg values for statistical analyses. A multivariable regression model was used to examine the association of Hg levels with fish consumption and other personal characteristics after adjusting for the effect of age. The associations between log-transformed Hg in hair and age, and log-transformed Hg in hair and Se levels in toenails were assessed using the Spearman rank correlation coefficient. Stata version 14 software (StataCorp LP, College Station, TX, USA) was used for all statistical analyses. Figures were drawn using GraphPad Prism 7 software (Version 7.00, GraphPad Software Inc., La Jolla, CA, USA). *P* values for heterogeneity were obtained using the likelihood ratio test, and *p* values for trend were obtained using the multivariable regression model. All *p* values presented are two-sided and a *p* value of less than 0.05 was considered statistically significant.

RESULTS

Relationship between mercury concentrations in hair and personal characteristics

Figure 1 shows the distribution of Hg concentrations in hair for males and females. The distributions of Hg concentrations in hair from both males and females were skewed and had long upper tails. The geometric means of Hg levels in males and female were 617 ng/g and 575 ng/g, respectively. The present study found no significant difference in Hg concentrations in hair from males and females. In addition, there was no significant difference in hair Hg concentrations among staff and outpatients with acne, tinea or sexually transmitted infections (data not shown). The highest geometric mean of Hg levels in hair was found in staff (603 ng/g), and the lowest Hg level was found in outpatients with acne (562 ng/g).

Figure 2 shows the relationships between age and log-transformed Hg concentrations in hair for males and females. There were significant positive correlations between log-transformed Hg concentrations in hair and age for males ($r = 0.37$, $p < 0.01$) and females ($r = 0.33$, $p < 0.01$). The log-transformed Hg concentrations in hair tended to increase with age.

The age-adjusted geometric means of Hg levels in hair from males and females grouped by personal character-

Fig. 1. Distribution histograms of mercury concentrations in hair of males and females.

Males: $n = 99$

Females: $n = 97$

istics are shown in Table 1. In the male population, there were no significant associations between the age-adjusted geometric mean of Hg levels and other characteristics. In contrast, female samples exhibited significant differences

Hair mercury levels related to fish consumption in Vietnamese

Fig. 2. Relationship between age and log-transformed mercury concentrations in hair of males and females.
 a. Relationship between age and log-transformed mercury concentrations in hair of males.
 ○: Male: $n = 99$, $r = 0.37$, $p < 0.01$;
 b. Relationship between age and log-transformed mercury concentrations in hair of females.
 ▲: Female: $n = 97$, $r = 0.33$, $p < 0.01$.

in the age-adjusted geometric means of Hg levels grouped by occupation and the frequency of eating out. Females grouped as “factory workers and construction workers” and “farmers and craftspeople” had higher Hg levels in hair than those grouped as “state officers and retired state officers” or “lecturers and students”. A multivariate regression model adjusting for the effect of age showed that females who ate out < 3 times/week had higher Hg levels in hair than those who ate out ≥ 3 times/week.

Relationship between mercury concentrations in hair and fish consumption

Table 2 shows the age-adjusted geometric mean of Hg levels in hair from males and females grouped by frequency and amount of fish consumption. The female population showed a significant difference in the age-adjusted geometric mean of Hg levels among all subgroups: frequency of freshwater fish consumption ($p = 0.027$); frequency of marine fish consumption ($p = 0.017$); amount of freshwater fish consumption ($p = 0.022$) and amount of marine fish consumption ($p = 0.033$). The male population showed significant differences in the age-adjusted geometric mean of hair Hg levels related to their frequency of freshwater fish consumption ($p = 0.019$).

To investigate the effect of freshwater fish consumption on hair Hg levels, the age-adjusted geometric mean

of Hg levels in hair from males and females was grouped by a frequency of marine fish consumption of “never or < 1 time/week”, as shown in Table 3. After limiting the subjects who had a frequency of marine fish consumption grouped as “never or < 1 time/week”, there were no significant differences in the age-adjusted geometric means of hair Hg levels between males grouped according to the frequency or amount of freshwater fish consumption. However, there were significant differences in the age-adjusted geometric mean of Hg levels in hair from females grouped by the frequency of freshwater fish consumption ($p = 0.016$). Females who had a higher fish consumption frequency showed higher age-adjusted geometric mean Hg levels in hair.

Relationship between mercury concentrations in hair and selenium concentrations in toenails

Figure 3 shows the relationships between log-transformed Hg concentrations in hair and Se concentrations in toenails in groups defined by the frequency of freshwater fish and marine fish consumption. There was no significant correlation between log-transformed Hg concentrations in hair and Se concentrations in toenails in subjects that consumed freshwater fish. However, log-transformed Hg concentrations in hair and Se concentrations in toenails tended to increase with the increased frequency of

Table 1. Age-adjusted geometric mean of mercury levels (ng/g) in hair from males and females in relation to personal characteristics.

Item	Male				Female			
	N	GM** (ng/g)	95% CI	p	N	GM** (ng/g)	95% CI	p
Total	99	617	550-676		97	575	513-631	
Occupation								
State officer and state officer retired	23	603	501-692	0.534	38	562	479-646	0.009*
Lecturer and student	23	603	537-692		24	562	501-631	
Factory workers and construction workers	18	617	562-692		20	575	501-646	
Farmer and craftspeople	12	631	562-724		5	589	490-692	
Others	23	646	537-776		10	589	457-759	
Age								
16-24	40	490	372-646	0.051	39	479	355-661	0.011*
25-29	25	617	257-676		28	575	513-631	
30-39	21	759	589-955		25	676	479-955	
40-55	13	933	575-1479		5	794	427-1514	
Education								
Less than high school	13	617	490-794	0.356	6	525	372-741	0.896
High school	19	617	550-708		15	550	468-646	
Degree/diploma	67	617	550-692		76	575	513-646	
Marital status								
Single	50	589	501-692	0.433	46	537	447-646	0.46
Married	49	646	550-759		51	589	501-708	
Smoke								
Smoker	32	589	501-708	0.57	1	562	N/A	N/A
Non-smoker	67	631	562-708		96	562	513-631	
Hair treatment								
Treatment	4	513	302-851	0.431	62	550	479-631	0.468
Non-treatment	95	631	562-692		35	603	501-708	
Alcohol								
Drinker	84	617	550-692	0.844	11	457	339-631	0.158
Non-drinker	15	631	490-813		86	589	525-646	
Eat out								
Less than 3 times/week	65	603	525-676	0.322	74	617	550-692	0.001*
3 or more than 3 times/week	34	661	562-794		23	437	355-537	

*Significant difference. Males: n = 99; Females: n = 97. ** Geometric mean

marine fish consumption, and showed a positive correlation in subjects that consumed marine fish $\geq$ once/week ($r = 0.37$, $p = 0.047$).

Species of consumed fish and mercury concentrations in canned fish

Table 4 summarizes the species of fish commonly consumed by males and females examined in Hanoi. During one year, 93 males and 96 females consumed freshwater fish. The freshwater fishes consumed by males and females were common carp, roho labeo and tilapia. During one year, 56 males and 63 females consumed marine fish. Males typically consumed mackerel, salmon and scad, whereas females usually consumed basa, mackerel

and scad. Tuna was the second lowest and least consumed fish species in the male and female populations, respectively.

Table 5 summarizes the Hg concentrations determined in the muscle of canned fish purchased in Hanoi. The highest Hg concentration was found in yellowfin tuna chunks in vegetable oil (T-Hg: 622.9 ng/g; MeHg: 589.5 ng/g) and the lowest Hg concentration was found in the muscle of sardines in tomato sauce from company C (T-Hg: 5.1 ng/g; MeHg: 5.1 ng/g). The values of MeHg as a percentage of T-Hg in all canned fish ranged from 90.4% to 100%.

Hair mercury levels related to fish consumption in Vietnamese

Table 2. Age-adjusted geometric mean of mercury levels (ng/g) in hair from males and females grouped by frequency and amount of fish consumption.

Item		Male					Female				
		N	GM** (ng/g)	95% CI	P values for		N	GM** (ng/g)	95% CI	P values for	
					Heterogeneity	Trend				Heterogeneity	Trend
Frequency of consumption	Freshwater fish										
	Never or < 1 time/week	16	513	417-617	0.019*	0.028*	7	417	324-537	0.027*	0.009*
	1-3 times/week	62	617	550-676			70	550	490-603		
	≥ 4 times/week	21	741	617-891			20	724	589-871		
	Marine fish										
	Never or < 1 time/week	69	589	525-661	0.163	0.117	58	525	457-603	0.017*	0.062
Amount of consumption	1-3 times/week	24	676	589-776			36	631	537-724		
	≥ 4 times/week	6	776	575-1023			3	759	550-1023		
	Freshwater fish										
	Never or < 50 g/time	25	617	513-759	0.963		14	427	324-562	0.022*	
	≥ 50 g/time	74	617	550-692			83	603	537-661		
	Marine fish										
	Never or < 50 g/time	59	603	537-692	0.642		50	513	437-589	0.033*	
	≥ 50 g/time	40	631	550-741			47	631	550-741		

*Significant difference. Males: n = 99; Females: n = 97. ** Geometric mean

Table 3. Age-adjusted geometric mean of mercury levels (ng/g) in hair from subjects with a frequency of marine fish consumption never or < once/week.

Item		Male					Female				
		N	GM** (ng/g)	95% CI	P values for		N	GM** (ng/g)	95% CI	P values for	
					Heterogeneity	Trend				Heterogeneity	Trend
Frequency of consumption	Freshwater fish										
	Never or < 1 time/week	13	513	407-646	0.218	0.18	7	380	282-501	0.016*	0.015*
	1-3 times/week	45	589	525-661			39	501	447-575		
	≥ 4 times/week	11	676	537-851			12	676	525-871		
Amount of consumption	Freshwater fish										
	Never or < 50 g/time	18	617	501-776	0.507		11	417	302-562	0.098	
	≥ 50 g/time	51	575	501-646			47	550	468-631		

*Significant difference; n = 127. **Geometric mean

DISCUSSION

To the best of our knowledge, this is the first cross-sectional study to demonstrate the association between hair Hg levels and fish consumption using a dietary questionnaire to obtain information pertinent for assessing the health risk from MeHg exposure in Vietnam. In the present study, Hg levels in the hair of 98% of Vietnamese participants were lower than the provisional tolerable weekly intake (PTWI) for MeHg (1.6 µg Hg/kg body weight, which is equivalent to hair Hg levels of approximately 2,300 ng/g, with an uncertainty factor of 6.4) (Joint FAO/WHO Expert Committee on Food Additives, 2003; Sakamoto *et al.*, 2012). There was no significant

difference in Hg concentrations in hair from males and females. Males and females also had no significant difference in the frequency of fish consumption, which may explain the similar levels of Hg in hair from both sexes.

In the present study, we calculated daily average MeHg intakes using the age-adjusted geometric mean of Hg levels in hair from males and females. The calculated daily average MeHg intakes for males and females (0.062 and 0.058 µg/kg per day, respectively) were lower than the EPA reference dose for MeHg of 0.1 µg/kg per day (United States Environmental Protection Agency, 1997; National Research Council, 2000). This suggests that males and females in the present study will not have an appreciable risk of deleterious effects from MeHg expo-

Fig. 3. Relationship between selenium concentrations in toenail and log-transformed mercury concentrations in hair. *Frequency of freshwater fish consumption:* $n = 86$; a. $\circ$: ≥ 1 time/week: $r = 0.15$, $p = 0.20$; b. $\blacktriangle$: Never or < 1 time/week: $r = 0.15$, $p = 0.68$. *Frequency of marine fish consumption:* $n = 86$; c. $\circ$: ≥ 1 time/week: $r = 0.37$, $p = 0.047$; d. $\blacktriangle$: Never or < 1 time/week: $r = 0.13$, $p = 0.36$

Table 4. Fish species mostly commonly consumed by males and females in Hanoi, Vietnam.

Species	Scientific name	Male		Female	
		N	%	N	%
Freshwater fish	Total	93	100	96	100
	Tilapia	72	77.4	76	79.2
	Common carp	71	76.3	70	72.9
	Roho labeo	51	54.8	53	55.2
Marine fish	Total	56	100	63	100
	Mackerel	38	67.9	39	61.9
	Scad	22	39.3	25	39.7
	Salmon	17	30.4	16	25.4
	Tuna	13	23.2	8	12.7
	Basa	9	16.1	20	31.7

Table 5. Mercury concentrations (ng/g) in canned fish marketed in Hanoi, Vietnam.

Type	Company	T-Hg (ng/g wet weight)	MeHg (ng/g wet weight)	MeHg/T-Hg (%)
Sardines in tomato sauce	A	81.6	73.8	90.4
Sardines in tomato sauce	B	7.1	6.8	95.8
Sardines in tomato sauce	C	5.1	5.1	100
Mackerel in tomato sauce	D	8.1	8.1	100
Tuna in oil	E	76.5	76.1	99.5
Tuna in oil	F	54.4	52.9	97.2
Yellow fin tuna chunks in vegetable oil	G	622.9	589.5	94.6
Light tuna chunks in oil	H	63.2	62.5	98.9
Tuna in vegetable oil	I	29.7	29.6	99.7
Tuna chunks in brine	J	78.3	76.1	97.2

n = 1 for each type of canned fish examined

sure through fish consumption.

We found that Hg levels in hair tended to increase with age for both males and females, consistent with the previously reported correlation between Hg levels in hair and age (National Research Council, 2000; McDowell *et al.*, 2004). Fish consumption differs with age and older age groups of both males and females eat more fish than younger age groups (Weichselbaum *et al.*, 2013; Otsuka *et al.*, 2014). The present findings were consistent with the reported Hg exposure through the consumption of freshwater and marine fish in Pakistan (Shah *et al.*, 2016) and Hg contamination in human hair and fish in Cambodia (Agusa *et al.*, 2005).

In the present study, the age-adjusted geometric means for Hg levels in hair were significantly different between participants grouped by distinct occupations, and between females grouped by the frequency of eating out. Mercury levels in hair were previously correlated with the frequency of fish consumption (McDowell *et al.*, 2004) and species of fish consumption (Burger, 2000). Hence, differences in the frequency and species of fish consumption may explain the different Hg levels in hair found in different occupation groups and in females that ate out more frequently.

Yasutake *et al.* (2003) reported that the treatment of hair samples with a lotion for artificial waving caused a 30% reduction in Hg levels. In the present study, we found no significant difference between age-adjusted Hg levels in hair from subjects who had hair treatment compared with non-treatment in both males and females. This finding agreed with the report that no difference was found in hair Hg levels among hair treatments and untreated groups in research on hair Hg levels in U.S children and women of childbearing age (McDowell *et al.*, 2004).

In the present study, there was a significant difference in hair Hg levels between freshwater and marine fish con-

sumption. However, inhabitants of Hanoi eat both freshwater and marine fish. Therefore, when subjects were limited to those who consumed marine fish never or < once/week, we found higher Hg levels in the hair of females who had a higher frequency of freshwater fish consumption. Other confounding factors, such as the pattern of fish consumption and dietary interactions (Chapman and Chan, 2000; Bradley *et al.*, 2017), may also explain differences in the hair Hg levels of females in this study. However, the current questionnaire did not include details of such factors, and further studies are necessary to fully explain the current observations.

We found a significant positive correlation between hair Hg levels and toenail Se levels in subjects who consumed marine fish ≥ 1 time/week. Past studies showed that levels of Hg in hair and Se in toenails were correlated with blood Hg and Se levels, respectively (Thomson, 2004; World Health Organization, 2008). Furthermore, levels of both Hg and Se in human blood were related to fish consumption (Burger and Gochfeld, 2013). Thomson (2004) reviewed the interaction between Se and heavy metals and found a strong interaction between Se and Hg in marine foods. Burger and Gochfeld (2013) concluded that the mean and median of Se/Hg molar ratios were higher for marine fish than for all freshwater fish examined. Therefore, a significant positive correlation between hair Hg levels and toenail Se levels may relate to consumption of marine fish.

In the current study, freshwater fish commonly consumed by males and females included common carp, roho labeo and tilapia, whereas basa, mackerel, salmon and scad were the marine fish typically consumed by both males and females. These fish species are reported to have low levels of Hg (< 100 ng/g), except for carp with Hg levels in the intermediate range 100-500 ng/g (Ministry of Health, Labour and Welfare, 2003; World

Health Organization, 2010; U.S. Food and Drug Administration, 2014). In contrast, tuna have high levels of Hg (World Health Organization, 2010) and was one of the least consumed fish by Vietnamese males and females. Therefore, low Hg levels in the hair of Vietnamese participants may reflect the consumption of fish with low Hg levels, and relatively low consumption of high Hg fish such as tuna.

We determined T-Hg and MeHg concentrations in commercial canned seafood purchased in Hanoi to survey the Hg concentration in seafood in Vietnam. Canned seafood is a known source of MeHg exposure (Boadi *et al.*, 2011; Siedlikowski *et al.*, 2016), and canned tuna contains high concentrations of Hg (Alcala-Orozco *et al.*, 2017). In all samples tested, only Hg concentrations in the muscle of yellowfin tuna exceeded the maximum level allowed by WHO, 500 ng/g for MeHg in all types of fish (United Nations Environment Programme, 2002), and permitted by Japanese regulations, 400 ng/g for T-Hg and 300 ng/g for MeHg (Notice Kannyu No.99, 1973). The Hg concentrations in remaining tuna products were lower than the range (100-200 ng/g) reported by the FDA (Burger and Gochfeld, 2004; U.S. Food and Drug Administration, 2001, 2014). The Hg concentrations in the muscle of mackerels were lower than the levels previously reported (jack mackerel, Orleans: 61 ng/g and jack jurel mackerel, Chicken of the Sea: 50 ng/g) by Shim *et al.* (2004). Similarly, Hg concentrations in sardines were lower than the Hg levels in canned sardines (117 ng/g, 141 ng/g, and 143 ng/g) reported by Boadi *et al.* (2011). The current study found that the percentage of MeHg in T-Hg in canned fish samples ranged from 90.4% to 100%, which was consistent with levels found in fish, squid and shrimp in previous studies (Bloom, 1992; Yoshimoto *et al.*, 2016; Hoang *et al.*, 2017).

Fish processing in Vietnam plays an important role in producing high value products and contributing to export development. The main Vietnamese fish products include fresh, frozen, dried and canned, as well as fish sauce or paste; frozen fish are the main product, followed by dried fish and fish sauce or paste (Food and Agriculture Organization of the United Nations, 2004). In addition, fish supplies in Vietnam moved from freshwater fishing to freshwater aquaculture or cage culture, which made fish more available for consumption (Food and Agriculture Organization of the United Nations, 2004). Thong and Olsen (2012) reported that fish consumption in Vietnam was related to the quality, price and availability of fish. Therefore, the increased fish consumption in Vietnam may be related to the increased availability, better quality and reduced price of fish.

Recently, we reported that T-Hg, MeHg and Se concentrations in commercial shrimp in Japan, which included Vietnamese black tiger shrimp, were lower than the Japanese regulated level (Notice Kannyu No.99, 1973) of 300 ng/g for MeHg in fish (Hoang *et al.*, 2017). Moreover, the average Se/Hg molar ratios in the muscle of commercial shrimp were comparatively high (in the range of 16-106). Other studies reported that Se may counteract Hg toxicity and Se/Hg molar ratios above one may increase the protective effect of Se against MeHg exposure (Peterson *et al.*, 2009; Khan and Wang, 2009). We showed that shrimp commercially available in Japan do not pose a particularly high risk regarding MeHg exposure to consumers. Further survey analysis of MeHg and Se concentrations in commercial fresh seafood in Vietnam is warranted for proper assessment of health risk due to MeHg exposure.

The strengths of the present study are: (1) this is the first cross-sectional study of the association between hair Hg levels and Vietnamese fish consumption, using a dietary questionnaire to obtain information for assessing the health risk from MeHg exposure in Vietnam; (2) we demonstrated that the age-adjusted geometric mean level of Hg in hair from Vietnamese females was related to freshwater fish consumption; (3) we found significant positive correlations between hair Hg levels and toenail Se levels among subjects who consumed marine fish $\geq$ once/week. Limitations of this study are: (1) we did not find the main reason for the significant difference in the age-adjusted Hg levels in hair of Vietnamese females related to the frequency of freshwater fish consumption; (2) we have not analyzed Hg levels in fresh fish and other seafood from Vietnam, and further studies are required to fully explain the current findings.

In conclusion, the present study has demonstrated that Hg levels in the hair of 98% of the Vietnamese participants were lower than the PTWI for MeHg at 1.6 μg Hg/kg body weight, with an uncertainty factor of 6.4 (Joint FAO/WHO Expert Committee on Food Additives, 2003). A significant difference in the age-adjusted geometric mean of Hg levels in hair from females was related to the frequency of freshwater fish consumption. In addition, Hg levels in hair and Se levels in toenails increased with the increased frequency of marine fish consumption, and showed a significant positive correlation among subjects who consumed marine fish $\geq$ once/week. The values of MeHg as a percentage of T-Hg in canned fish purchased in Vietnam ranged from 90.4% to 100%.

ACKNOWLEDGMENTS

The authors are grateful to Drs. Le Huu Doanh, Le Huyen My, Tran Cam Van, Dinh Huu Nghi, Hoang Thi Ngoc Ly, Nguyen Thi Tuyen, Than Trong Tuy, Nguyen Thi Huyen Thuong, and Tran Thi Thanh Tam (National Hospital of Dermatology and Venereology) for their supports in the collection of human samples. We also thank Ms. Hazuki Kashiwagi and Megumi Kudo (National Institute for Minamata Disease) and Ms. Yukiko Uchiyama (Prefectural University of Kumamoto) for their technical assistance. This work was supported by the International Postgraduate Scholarship for Research on Mercury from the Prefectural University of Kumamoto, and a grant from the National Institute for Minamata Disease and Kagoshima University. We thank Charles Allan, PhD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

Conflict of interest---- The authors declare that there is no conflict of interest.

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