Simple analysis of methylmercury in seafood and its application to assessing methylmercury exposure

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Background

1. Methylmercury (MeHg) is an environmental neurotoxicant. Minamata Disease is a toxic nervous disease caused by eating seafood contaminated with MeHg compounds.

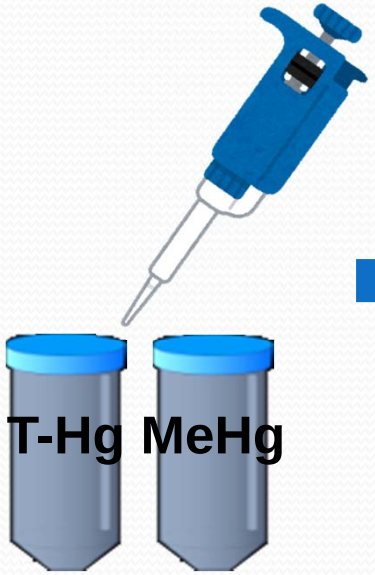
2. People are exposed to MeHg mainly through the consumption of seafood, so determining its concentration in seafood is important for assessing the health risk of MeHg exposure.

3. Analysis of MeHg is difficult, so an easy and cost-effective MeHg analysis method is required in many countries, especially developing countries.

4. Fish consumption is increasing in Vietnam. Most commercial fish and seafood in Vietnam is locally harvested. However, little information is available on how to estimate the health risk of MeHg exposure through fish consumption in Vietnam.

1.

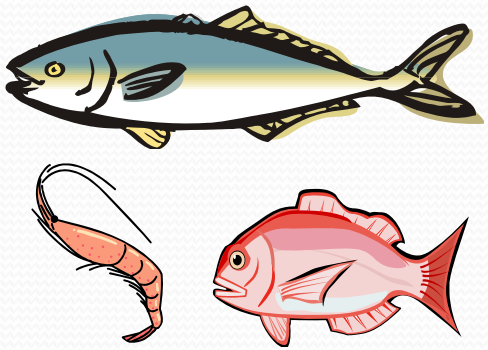
Developing a simpler method for T-Hg and MeHg analysis



1. Simple analysis of total mercury and methylmercury in seafood using heating vaporization atomic absorption spectrometry

2.

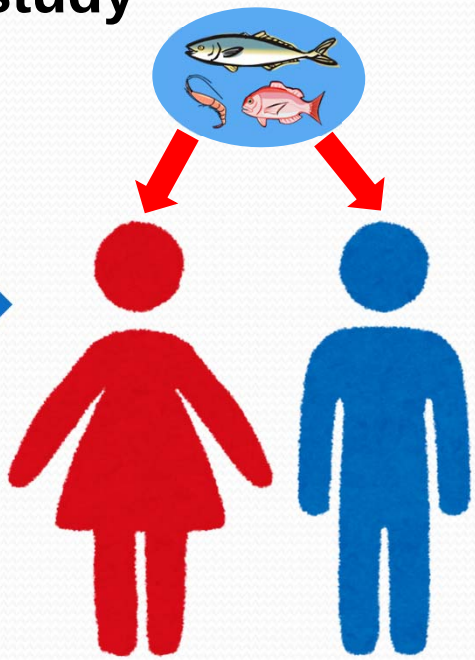
Applying this method to determine T-Hg and MeHg in seafood for assessing MeHg exposure in humans



2. Mercury and selenium levels, and their molar ratios in several species of commercial shrimp in Japan regarding the health risk of methylmercury exposure

3.

Assessing MeHg exposure in humans: an epidemiological study



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

1. Developing a simpler method for T-Hg and MeHg analysis

➢ **Background**:

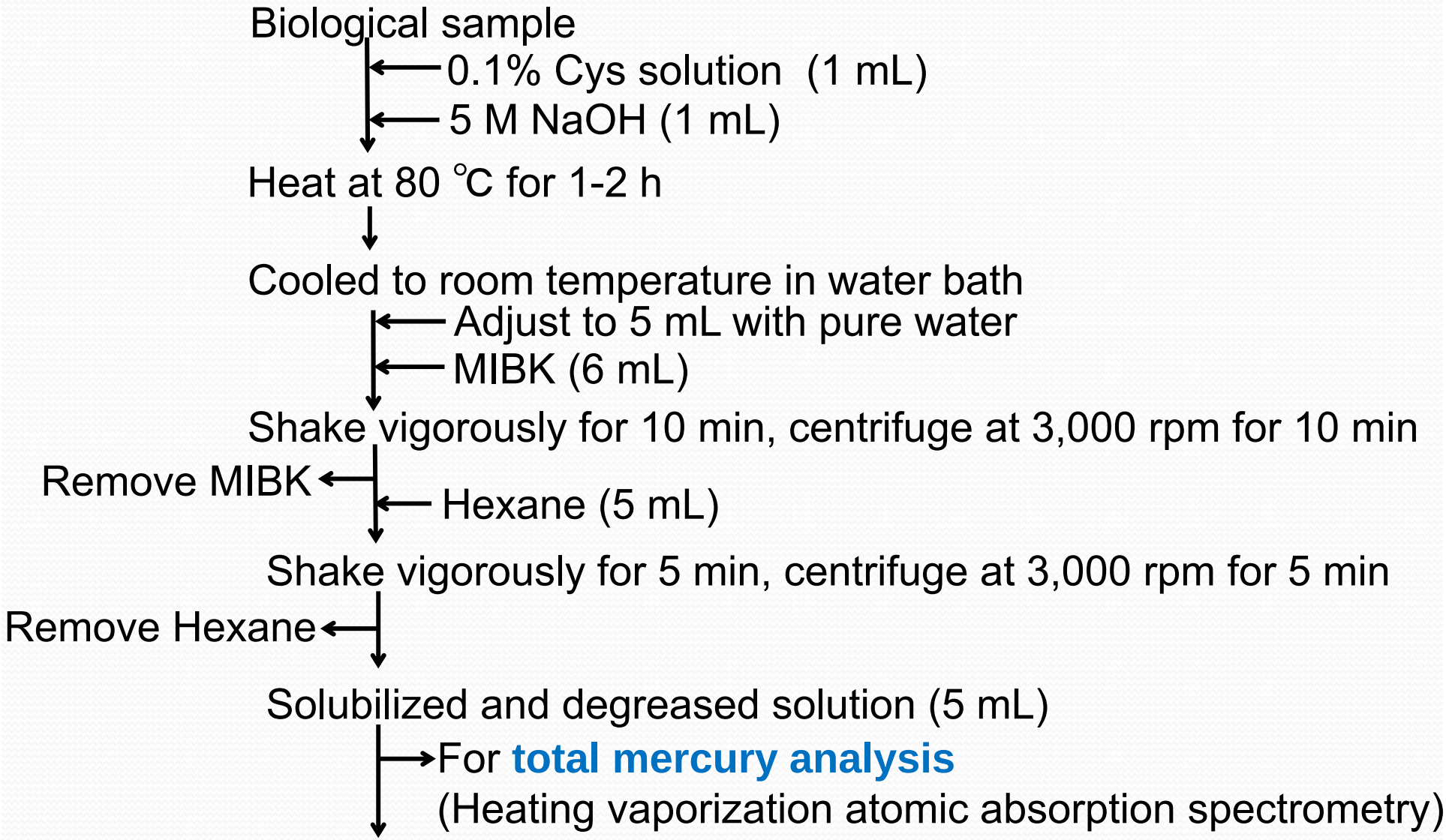
1. In many developing countries, Hg analysis is difficult because it requires expensive equipment, with high running costs, and advanced technology.
2. In many cases, two sets of apparatus and two samples are needed for the analysis of total mercury (T-Hg) and MeHg analysis.
3. Organic mercury in natural biological samples can be assumed to be MeHg, because only MeHg has been detected in natural biological samples, except for human specimens treated with vaccines containing sodium ethylmercuric thiosalicylate.

➢ **Purpose**:

To develop a simple cost-effective method for T-Hg and MeHg analysis in biological samples, and to apply it to determining Hg concentration in seafood, which is the main source of MeHg exposure.

Basic protocol for T-Hg and MeHg analysis

Step-1: Solubilization and degreasing of biological samples



Step-2: Extraction and reverse-extraction of the MeHg fraction

Solubilized and degreased solution (2 mL)

← 5 M HBr (2 mL)

← 2 M $CuCl_2$ (0.5 mL)

← Toluene (6 mL)

↓

Shake vigorously for 10 min, centrifuge at 3,000 rpm for 10 min

↓

Toluene layer (5 mL)

← 0.2% Cys-2% NaOAc solution (1 mL)

↓

Shake vigorously for 5 min, centrifuge at 3,000 rpm for 5 min

Remove Toluene ←

↓

Reversely extracted aqueous solution

↓

For **methylmercury analysis**

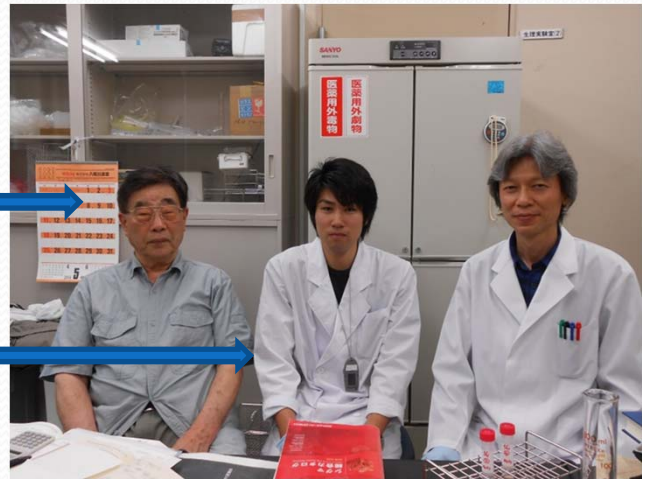
(Heating vaporization atomic absorption spectrometry)

Study contents

1. Recovery test using a MeHg standard working solution
2. Recovery test using a MeHg-spiked seafood (shrimp muscle) homogenate
3. Confirming accuracy of the T-Hg and MeHg analyses using Certified Reference Materials (CRMs - cod fish, sword fish, and hair)
4. Cross checking with another MeHg analytical method (GC-ECD method: published by Dr. Hirokatsu Akagi, NIMD)
5. Applying this method to T-Hg and MeHg analysis in commercial seafood)

Dr. Atsuhiro Nakano
(former department director of Basic Medical Sciences)

Keisuke Yoshimoto
(Prefectural University of Kumamoto)



Summary

1. We have developed a simpler method for determining the T-Hg and MeHg concentrations in common biological samples by using MIBK in the degreasing step.

2. The advantages of this method are:

① a single apparatus is used for determining both T-Hg and MeHg

② both T-Hg and MeHg can be measured using the same biological sample in two consecutive steps

③ only one standard solution needs to be prepared for T-Hg and MeHg in each experiment. Both T-Hg and MeHg can be analyzed using a commercial mercury standard solution (1000 ppm $HgCl_2$), which has the stability required for a calibration curve

④ the protocol is easy and cost-effective compared with other methods such as GC-ICP-MS (gas chromatography-inductively coupled plasma/mass spectrometry) or LC-ICP-MS (liquid chromatography-inductively coupled plasma/mass spectrometry)

This method will be useful for the routine analysis of T-Hg and MeHg in a large number of biological samples such as the tissues of seafood.

2. Applying this method to determine T-Hg and MeHg in seafood for assessing MeHg exposure in humans

- **Background**:

① Regarding shrimp, Japan is one of its largest consumers and its second largest importer in the world (FAO, 2016). However, little information is available on the Hg and Se concentrations in commercial shrimp in Japan.

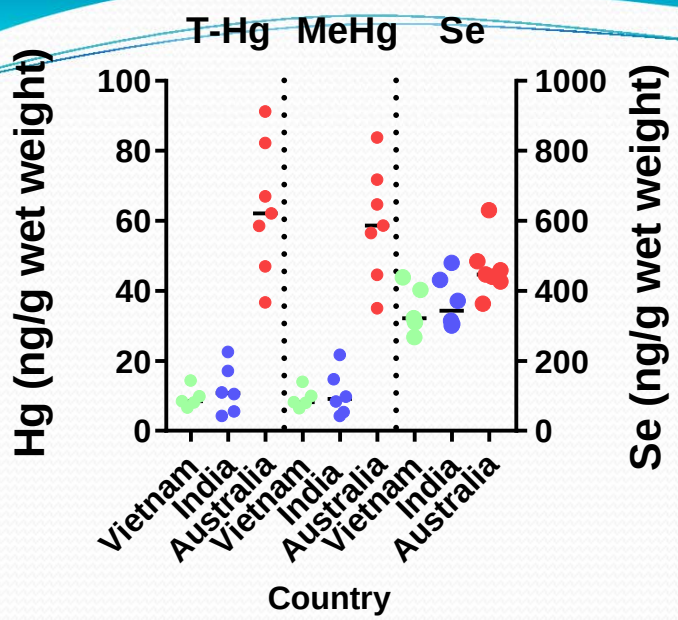
② Se is known to play an important role in the possible antagonistic effects on Hg toxicity.

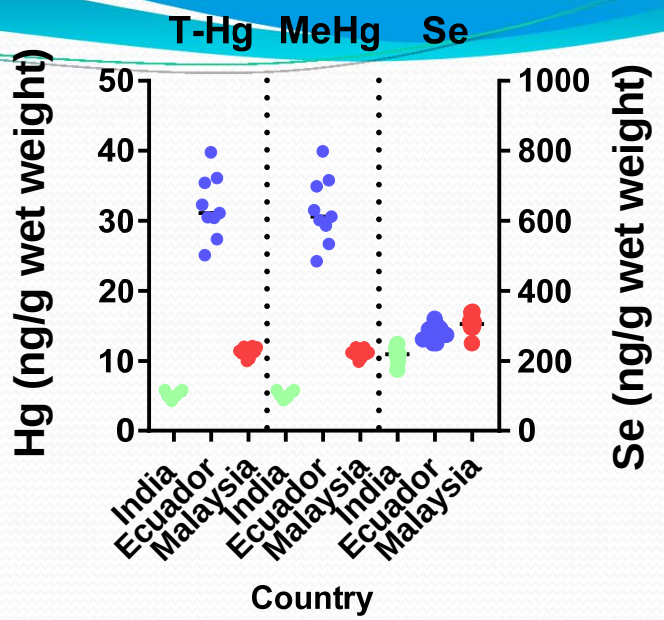
- **Purpose**:

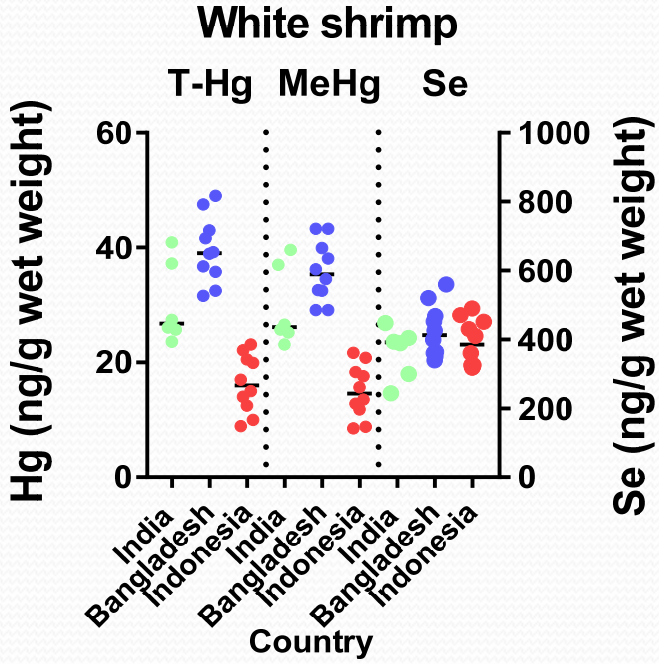
We aim to determine the concentrations of T-Hg, MeHg and Se in the muscles of several species of commercial shrimp available in Kumamoto and Kagoshima prefectures of Japan so that we obtain information for assessing the health risk from MeHg exposure.

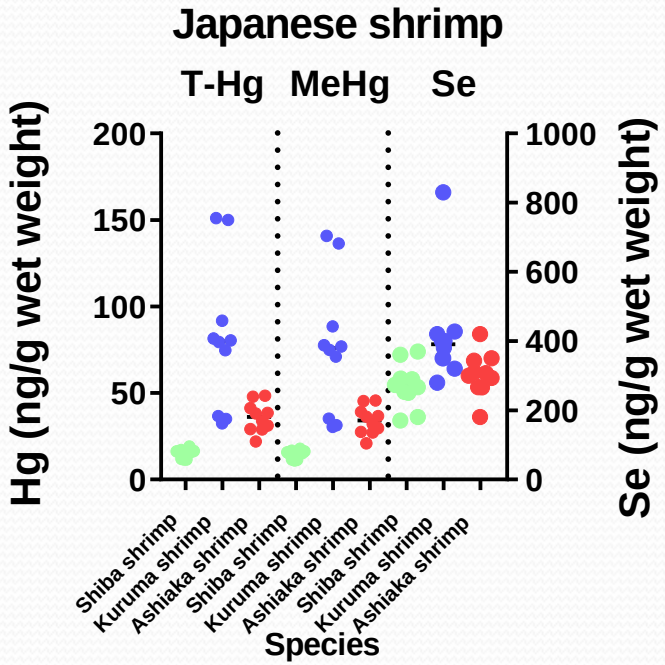
Sample collection and preparation

Species	Scientific name	Country	N
Black tiger shrimp	Penaeus monodon	Vietnam	5
		India	6
		Australia	7
Vannamei shrimp	Litopenaeus vannamei	India	6
		Ecuador	9
		Malaysia	10
White shrimp	Penaeus indicus or Penaeus merguiensis	India	6
		Bangladesh	10
		Indonesia	10
Shiba shrimp	Metapenaeus joyneri	Japan	10
Kuruma shrimp	Marsupenaeus japonicus	Japan	10
Ashiaka shrimp	Penaeus semisulcatus	Japan	10
Red shrimp	Pleoticus muelleri	Argentina	9
Irian tiger shrimp	Penaeus semisulcatus	Indonesia	6

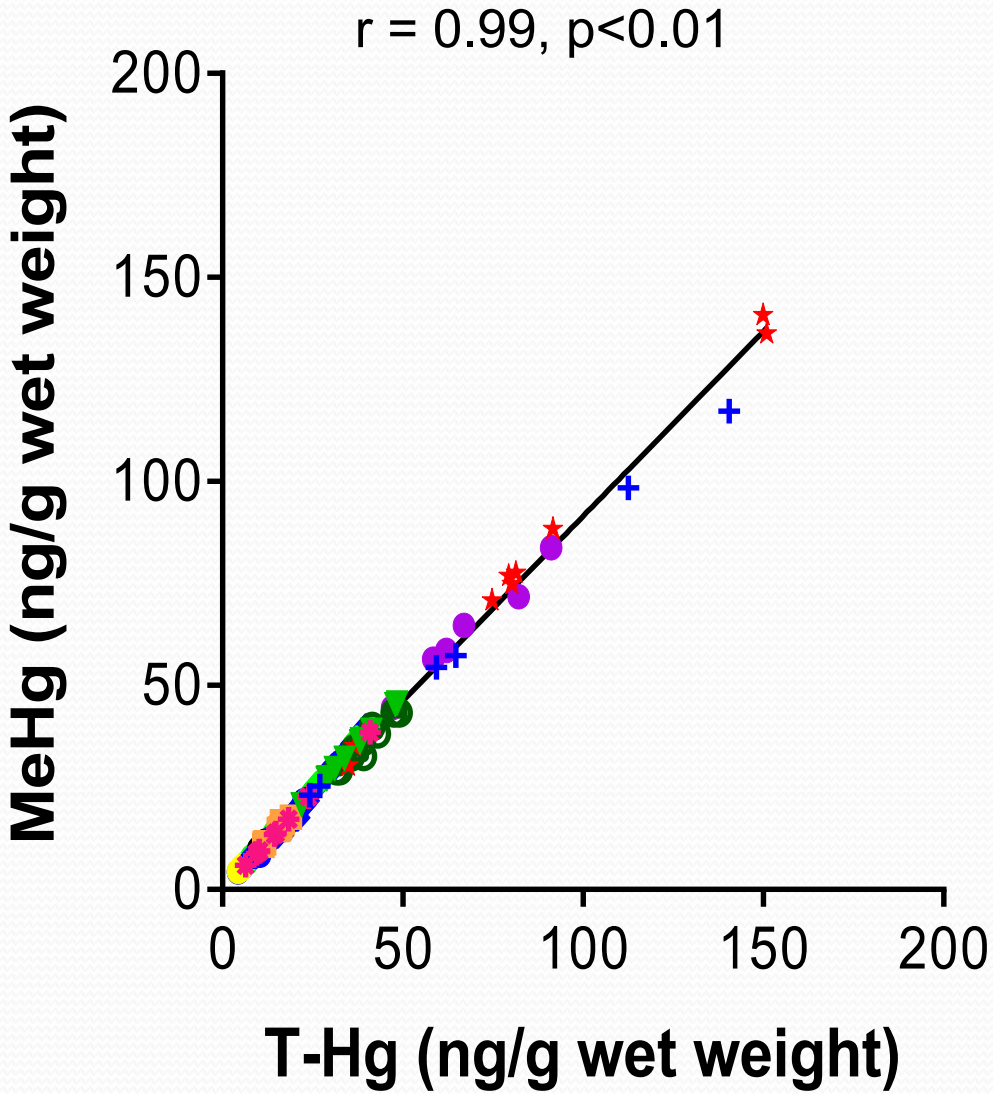

Black tiger shrimp
T-Hg
MeHg
Se
Hg (ng/g wet weight)
Se (ng/g wet weight)
0
20
40
60
80
100
0
200
400
600
800
1000
Vietnam
India
Australia
Country


Vannamei shrimp
T-Hg
MeHg
Se
Hg (ng/g wet weight)
Se (ng/g wet weight)
0
10
20
30
40
50
0
200
400
600
800
1000
India
Ecuador
Malaysia
Country


White shrimp
T-Hg
MeHg
Se
Hg (ng/g wet weight)
Se (ng/g wet weight)
0
20
40
60
0
200
400
600
800
1000
India
Bangladesh
Indonesia
Country

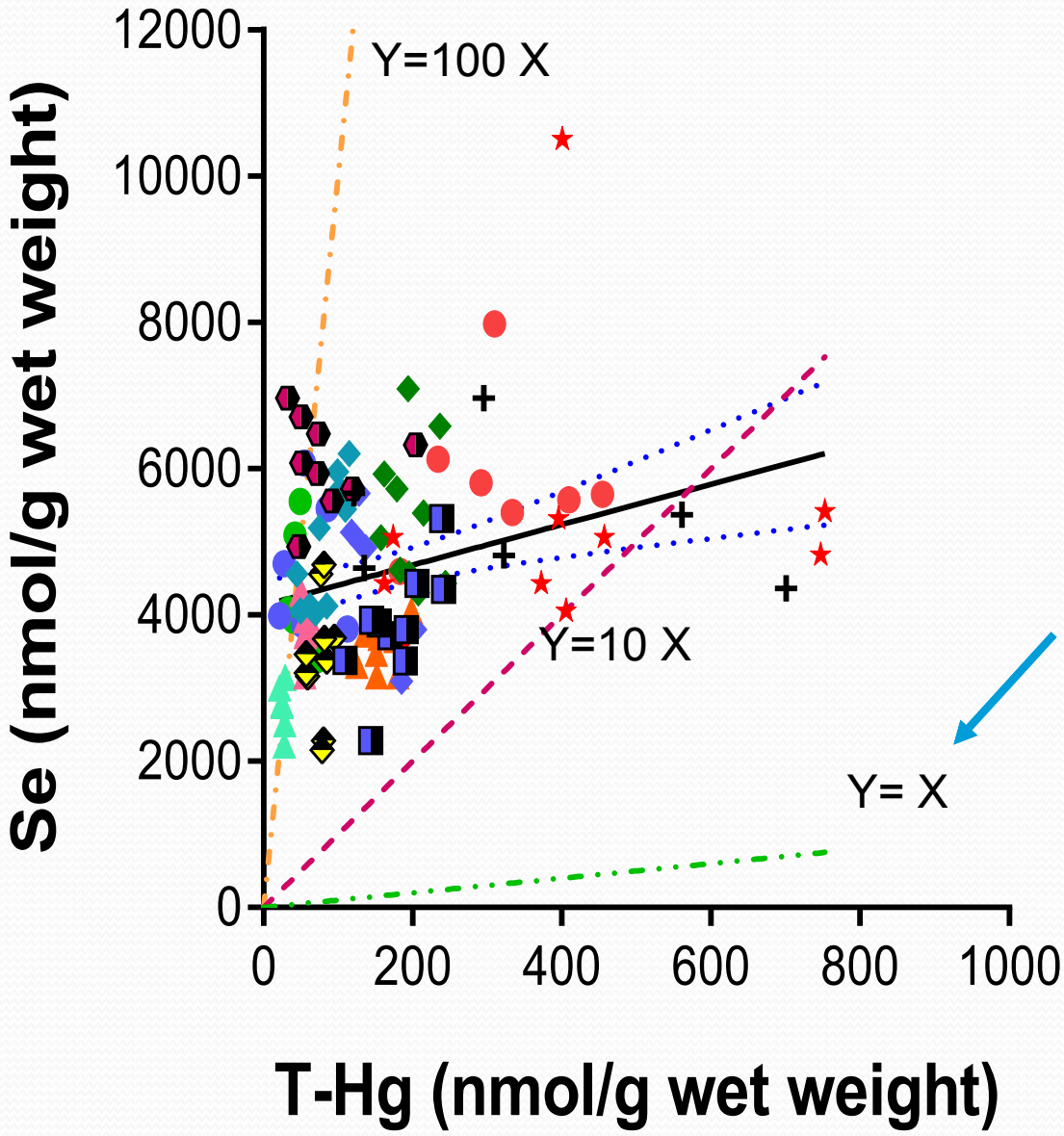

Japanese shrimp
T-Hg
MeHg
Se
Hg (ng/g wet weight)
Se (ng/g wet weight)
0
50
100
150
200
0
200
400
600
800
1000
Shiba shrimp
Kuruma shrimp
Ashiaka shrimp
Species

Relationship between T-Hg and MeHg concentration in the muscles of shrimp marketed in Japan

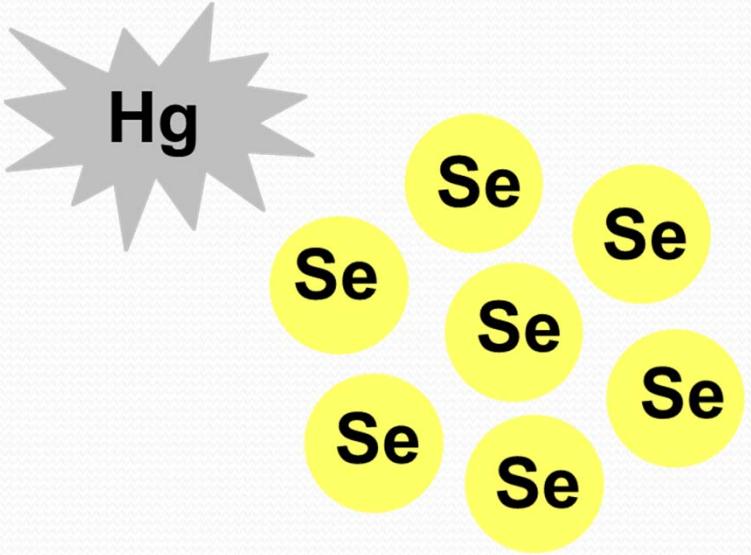


The T-Hg and MeHg concentrations in the shrimp muscle showed a significant positive correlation (r=0.99, p<0.01). The proportion of MeHg as a percentage of T-Hg ranged from 90% to 99%.

Relationship between T-Hg and Se molar concentrations in the muscles of shrimp marketed in Japan



All data were above the line for Y (Se) = X (T-Hg), with the Se/Hg molar ratios being greater than one (16-106), indicating that Hg toxicity may decrease in the presence of relatively high concentrations of Se.



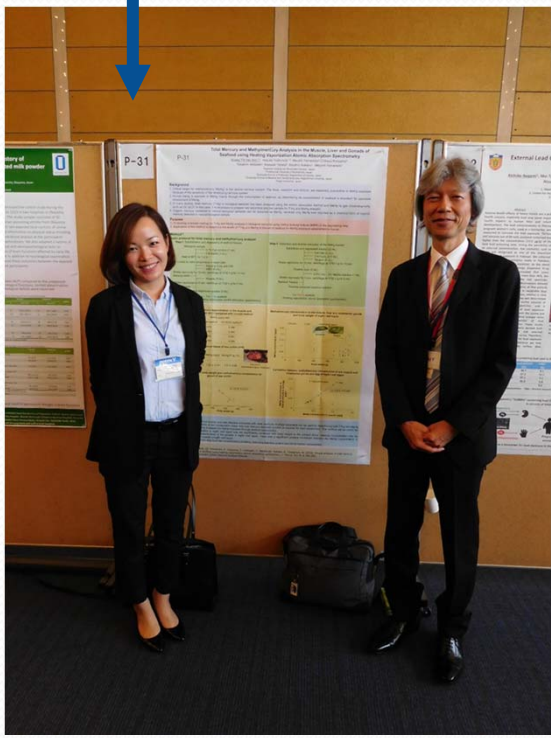
Summary

1. We applied the method we had developed to analyze T-Hg and MeHg levels in imported and domestically-produced commercial shrimp in Japan. The levels of T-Hg and MeHg levels in commercial shrimp in Japan were lower than the regulated Japanese level of 300 ng/g for MeHg in fish.

2. The average Se/Hg molar ratios in the muscle of commercial shrimp were relatively high in the range of 16-106.

This survey suggests that shrimp commercially available in Japan would not pose a particularly high risk to consumers regarding MeHg exposure.

Van Anh Thi Hoang
(from Vietnam;
Prefectural University
of Kumamoto)



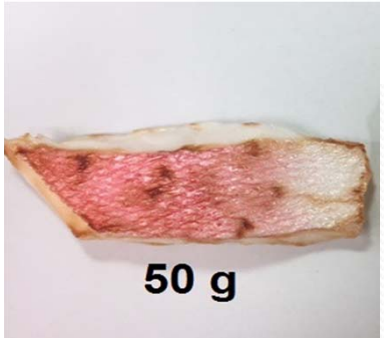
3. MeHg exposure assessment in humans: ~Epidemiological study in Hanoi, Vietnam~

➢ **Background:**

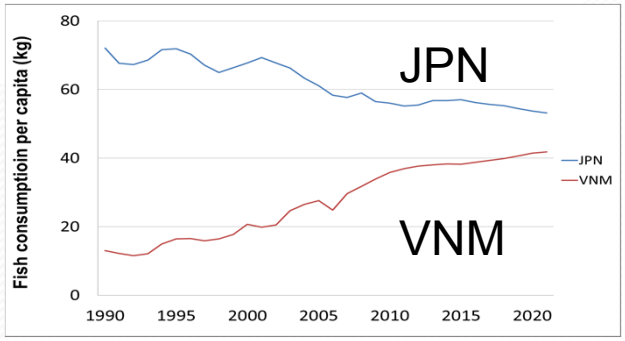
Fish consumption is increasing in Vietnam. However, little information is available on estimating the health risk of MeHg exposure through fish consumption in Vietnam.

➢ **Purpose:**

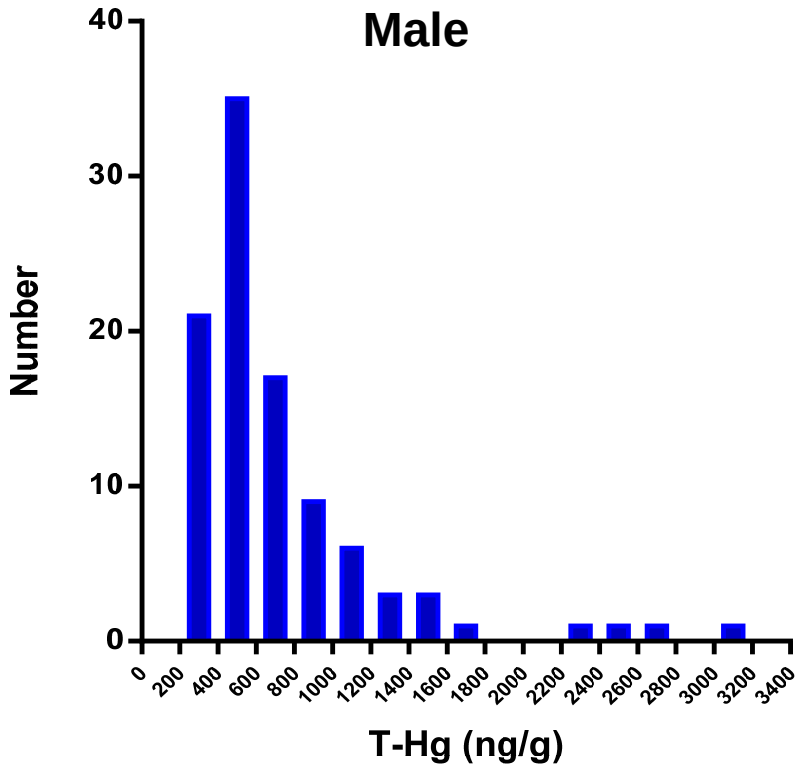
To examine the association between Hg levels in the hair and Se levels in the toenails of 196 Vietnamese people and their fish consumption in Hanoi, using a food frequency questionnaire (FFQ) and food model to obtain information pertinent to assessing the health risk from MeHg exposure.

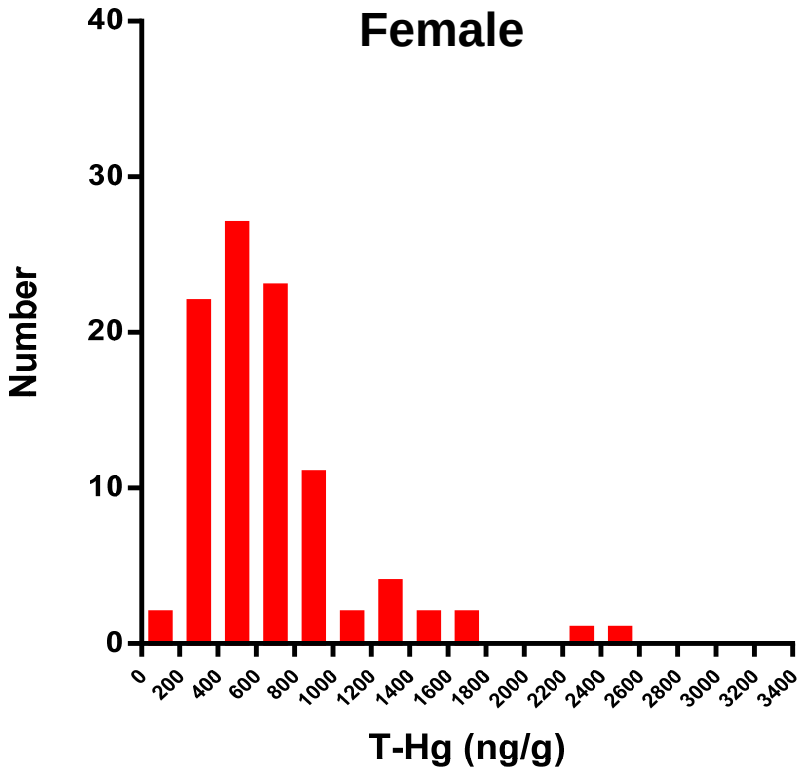


Source: OECD-FAO, 2012



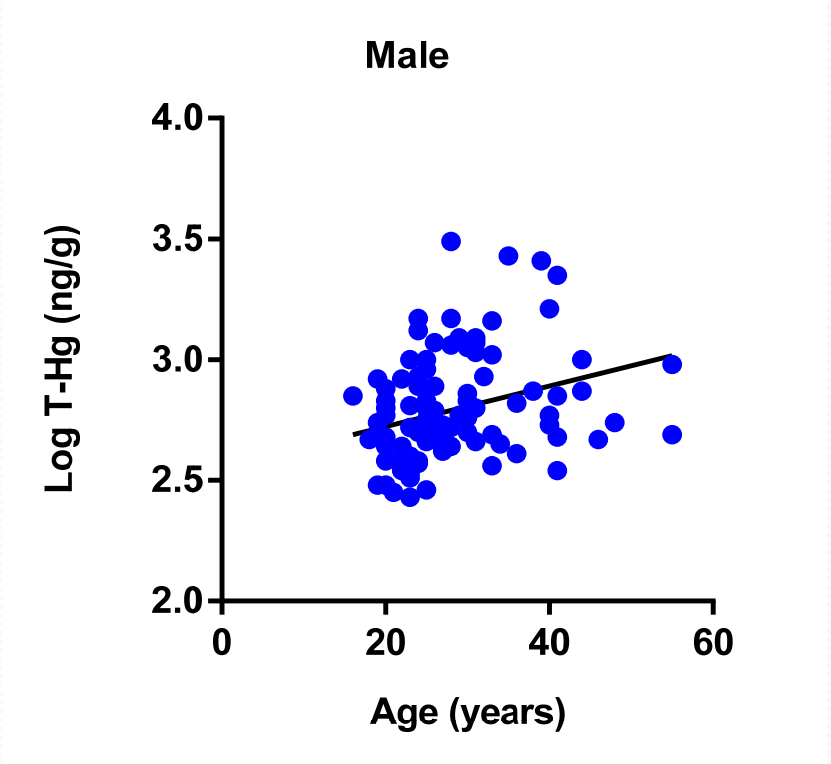
Distribution histograms of Hg concentrations in the hair of males (n=99) and females (n=97)

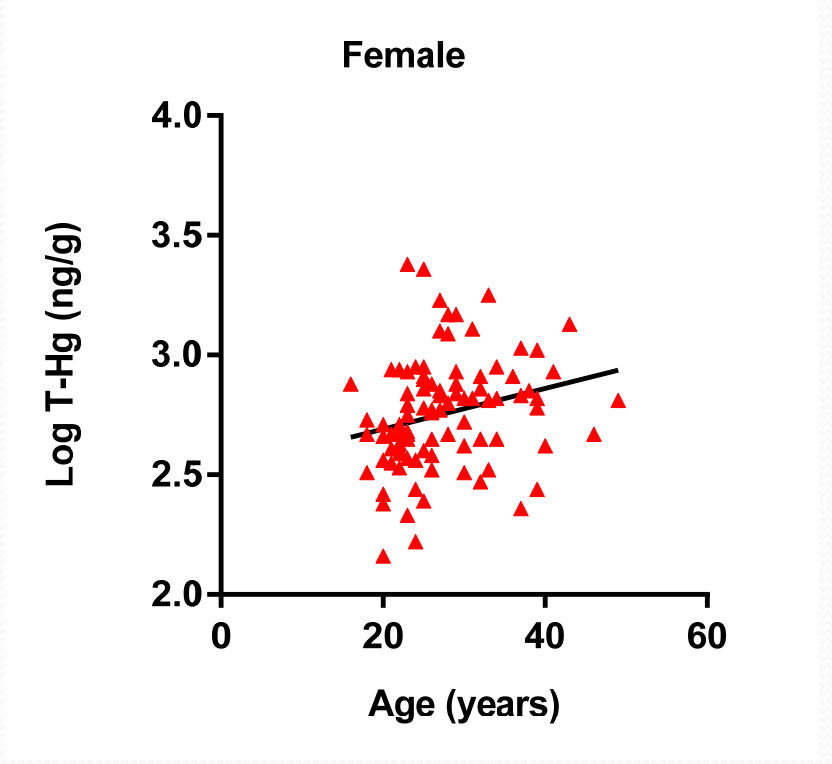




The geometric means of Hg levels in the hair of males and females were 617 ng/g and 575 ng/g , respectively. Hg levels in the hair of 98% of the Vietnamese participants were lower than the provisional tolerable weekly intake for MeHg (JECFA). These values were lower than those in the hair of Japanese men (2.5 ppm=2500 ng/g) and women (1.6 ppm=1600 ng/g), respectively.

Relationship between age and T-Hg concentrations in the hair of males (n=99) and females (n=97)





- The Hg concentrations in the hair of the Vietnamese participants tended to increase with age in both male and female.
- Fish consumption varies with age, and older age groups of both males and females may eat more fish than younger age groups.

Age-adjusted geometric mean of Hg levels in the 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		38	562	479–646	
Lecturer and student	23	603	537–692		24	562	501–631	
Factory worker and house maker	18	617	562–692	0.534	20	575	501–646	0.009*
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		39	479	355–661	
25–29	25	617	257–676	0.051	28	575	513–631	0.011*
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		6	525	372–741	
High school	19	617	550–708	0.356	15	550	468–646	0.896
Degree/diploma	67	617	550–692		76	575	513–646	

Age-adjusted geometric mean of Hg levels in the 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**	
Marital status								
Single	50	589	501–692	0.433	46	537	447–646	0.460
Married	49	646	550–759		51	589	501–708	
Smoke								
Smoker	32	589	501–708	0.570	1	562	195–1585	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	
Eating 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	

Age-adjusted geometric mean of Hg levels in the hair of males(n=99) by frequency and amount of fish consumption

	Item	Male				
					P values for	
		N	GM (ng/g)	95% CI	Hetero geneity	Trend
Frequency of consumption	**Freshwater fish**					
	Never or <1 time/week	16	513	417–617		
	1–3 times/week	62	617	550–676	0.019*	0.028*
	≥ 4 times/week	21	741	617–891		
	Marine fish					
	Never or <1 time/week	69	589	525–661		
	1–3 times/week	24	676	589–776	0.163	0.117
	≥ 4 times/week	6	776	575–1023		
Amount of consumption	**Freshwater fish**					
	Never or < 50 g/time	25	617	513–759	0.963	
	≥ 50 g/time	74	617	550–692		
	Marine fish					
	Never or < 50 g/time	59	603	537–692	0.642	
	≥ 50 g/time	40	631	550–741		

Age-adjusted geometric mean of Hg levels in the hair of females (n=97) by frequency and amount of fish consumption

Item		Female				
					P values for	
		N	GM (ng/g)	95% CI	Hetero geneity	Trend
Frequency of consumption	**Freshwater fish**					
	Never or <1 time/week	7	417	324–537		
	1–3 times/week	70	550	490–603	0.027*	0.009*
	≥ 4 times/week	20	724	589–871		
	Marine fish					
	Never or <1 time/week	58	525	457–603		
	1–3 times/week	36	631	537–724	0.017*	0.062
	≥ 4 times/week	3	759	550–1023		
Amount of consumption	**Freshwater fish**					
	Never or < 50 g/time	14	427	324–562	0.022*	
	≥ 50 g/time	83	603	537–661		
	Marine fish					
	Never or < 50 g/time	50	513	437–589	0.033*	
	≥ 50 g/time	47	631	550–741		

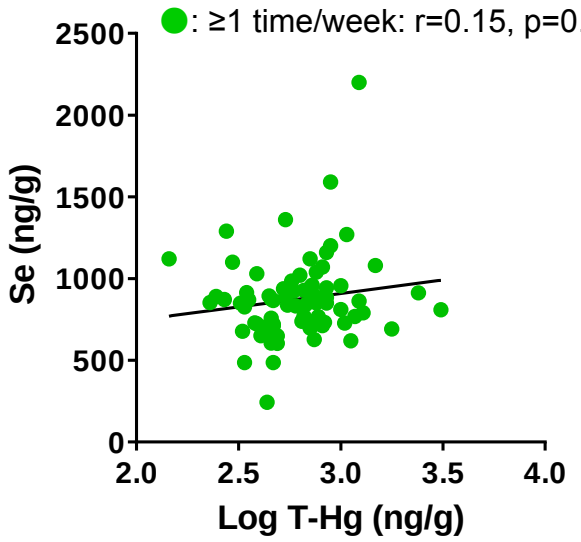
Age-adjusted geometric mean of Hg levels in the hair of subjects with a frequency of marine fish consumption of never or <one/week

Item		Male				
					P values for	
		N	GM** (ng/g)	95% CI	Heterogeneity	trend
Frequency of consumption	**Freshwater fish**					
	Never or <1 time/week	13	513	407–646		
	1–3 times/week	45	589	525–661	0.218	0.180
	≥ 4 times/week	11	676	537–851		
Amount of consumption	**Freshwater fish**					
	Never or < 50 g/time	18	617	501–776	0.507	
	≥ 50 g/time	51	575	501–646		

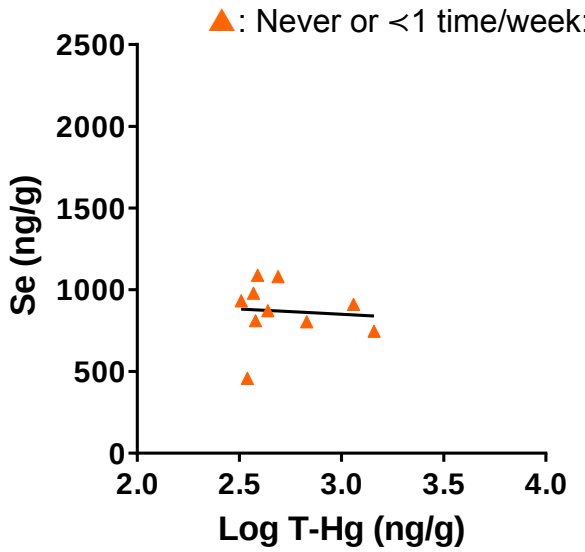
Item		Female				
					P values for	
		N	GM** (ng/g)	95% CI	Heterogeneity	trend
Frequency of consumption	**Freshwater fish**					
	Never or <1 time/week	7	380	282–501		
	1–3 times/week	39	501	447–575	0.016*	0.015*
	≥ 4 times/week	12	676	525–871		
Amount of consumption	**Freshwater fish**					
	Never or < 50 g/time	11	417	302–562	0.098	
	≥ 50 g/time	47	550	468–631		

Relationship between Se concentrations in the toenails and Hg concentrations in the hair

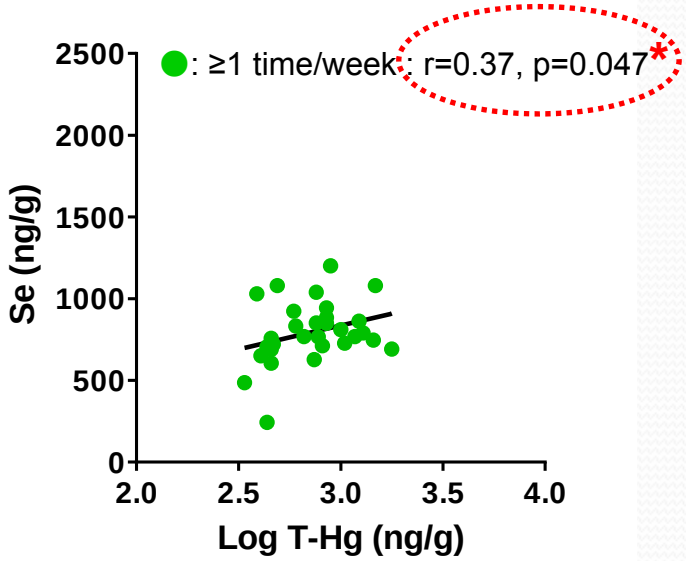
Freshwater fish



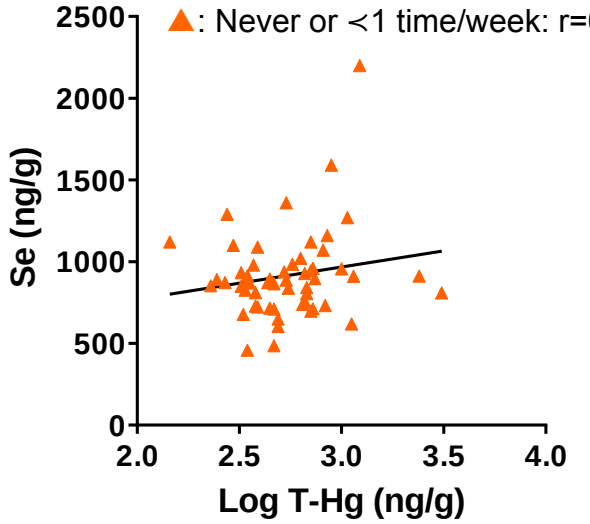
Freshwater fish



Marine fish



Marine fish



Fish species commonly consumed by males and females in Hanoi, Vietnam

Species		Scientific name	Male		Female		Hg levels
			N	%	N	%	(WHO, 2010)
	Total		**93**	**100**	**96**	**100**	
Freshwater fish	Tilapia	Oreochromis mosambicus	72	77.4	76	79.2	**<0.1 ppm**
	Common carp	Cyprinus carpio Linnaeus	71	76.3	70	72.9	**0.1-0.5 ppm**
	Roho labeo	Labeo rohita	51	54.8	53	55.2	**<0.1 ppm**
	Total		**56**	**100**	**63**	**100**	
Marine fish	Mackerel	Scomberomorus maculatus	38	67.9	39	61.9	**<0.1 ppm**
	Scad	Decapterus	22	39.3	25	39.7	**<0.1 ppm**
	Salmon	Oncorhynchus spp.	17	30.4	16	25.4	**<0.1 ppm**
	Tuna	Thunnini	13	23.2	8	12.7	**0.1-1.3 ppm[a]**
	Basa	Pangasius bocourti	9	16.1	20	31.7	**<0.1 ppm**

[a] *Ministry of Health, Labour and Welfare, 2003*

Summary

1. The geometric mean of Hg levels in the hair of males and females was 617 ng/g and 575 ng/g, respectively.
2. The Hg concentrations in hair of the Vietnamese participants tended to increase with age in both males and females.
   Fish consumption varies with age: older age groups of both males and females may eat more fish than the younger age groups.
3. A significant difference in the age-adjusted geometric mean of Hg levels in hair from females were related to the frequency of freshwater fish consumption.
4. Hg levels in hair and Se levels in toenails increased with increased frequency of marine fish consumption, indicating that the significant positive correlation between hair Hg levels and toenail Se levels may be related to the consumption of marine fish.

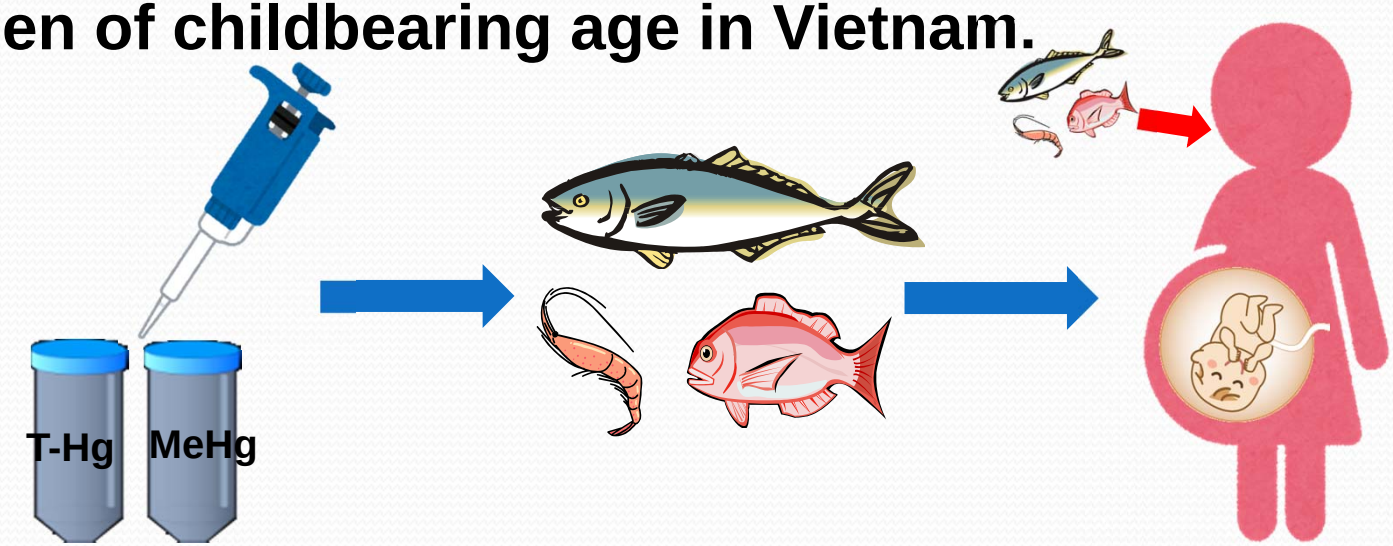
This is the first cross-sectional study to investigate the association between hair Hg levels and fish consumption in Vietnam. These findings provide valuable information for future assessments of the health risk of MeHg exposure through fish consumption in Vietnam.

Future studies

1. Encouraging the distribution of this analytical method to determine Hg concentration in food and human samples in Vietnam.

2. An epidemiological study on MeHg exposure through seafood consumption, especially in susceptible population groups such as unborn children and young children in Vietnam.

➢ **Contributing to providing guidance on fish and seafood consumption for assessing the risk of MeHg exposure to women of childbearing age in Vietnam.**



Mercury concentrations 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)	7.1	6.8	95.8
Sardines in tomato sauce	(b)	5.1	5.1	100
Mackerel in tomato sauce	(c)	8.1	8.1	100
Tuna in oil	(d)	76.5	76.1	99.5
Tuna in oil	(e)	54.4	52.9	97.2
Yellowfin tuna chunks in vegetable oil	(f)	622.9	589.5	94.6
Light tuna chunks in oil	(g)	63.2	62.5	98.9
Tuna in vegetable oil	(h)	29.7	29.6	99.7
Tuna chunks in brine	(i)	78.3	76.1	97.2

n=1 for each type of canned fish examined