主論文

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Mercury contamination and exposure assessment of fishery products in Korea

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In this study, total (T-Hg) and methyl mercury (Me-Hg) contamination was investigated in fishery products including canned fish, fish sauces, dried bonito and frozen tuna *sashimi,* collected from retail markets in Korea, to assess dietary exposure. Direct mercury analyser and gas chromatography-electron captured detector were employed to measure T-Hg and Me-Hg, respectively. The highest T-Hg and Me-Hg contamination was present in tuna *sashimi,* followed by dried bonito, respectively. Canned tuna showed more frequent detection and higher content than other canned fishery products. The weekly exposure estimate indicates that exposure to mercury from fishery products is safe, showing 2.59% provisional tolerable weekly intake (PTWI) for T-Hg, 1.82% PTWI for Me-Hg and 4.16% reference dose for Me-Hg. However, it should be addressed to monitor the mercury contamination in fish and fishery products regularly, to safeguard vulnerable population such as children, to limit intake of these food products.

Keywords: total mercury; methyl mercury; fishery products; mercury intake; PTWI; RfD

Seafood products are important sources of nutrients such as high-quality proteins, omega-3 polyunsaturated fatty acids, vitamins and minerals, which are essential for human health. Meanwhile, seafood can also accumulate chemical contaminants such as mercury, cadmium and PCBs that can affect consumer's health when ingested. Mercury occurs naturally and is distributed throughout the environment by both natural process and human activities. The adverse health effect of mercury is well known for neurotoxicity. It affects the development and functioning of human central nervous system, especially during prenatal exposure. Mercury exists in various forms, such as metal mercury, inorganic and organic in nature. Organic mercury is mostly found as methyl mercury (Me-Hg), which is the most toxic form of Hg and accumulates in higher trophic animals throughout the food chain (Kidd et al. 2004). Fish and fish products are the main route of mercury exposure in the diet. Thus, mercury contamination in fish and fishery products is a serious public health concern, which contrasts with the health benefits of fish consumption (Agusa et al. 2005; Maulvault et al. 2013).

Fish consumption has increased over the past few years in Korea (Choi et al. 2011), and fishery products are also easily consumed at home and in catering systems for its convenience and preference. Consumers are well aware of mercury concern of fishes, but reporting on fishery products is not enough to provide information in Korea. Dietary intake of T-Hg and Me-Hg varies considerably between countries and between population groups within countries. Special subgroups of populations may have higher risks from dietary mercury intake than the general population. Therefore, in the present study, T-Hg and Me-Hg are measured in fishery products, including canned fish, fish sauces, frozen and dried fishes and the intake through fishery products is estimated for the general population and subgroups according to age in Korea. In addition, exposure assessment of T-Hg and Me-Hg for the Korean population was performed by comparison with tolerable levels suggested by international authorities.

Materials and methods

Sampling and chemicals

A total of 126 samples, including 87 canned fishes, 15 tuna sauces, 2 dried bonito (*Katsuobushi*) and 2 frozen tuna *sashimi* samples, were collected from markets in Seoul. Canned fish samples were homogenised and stored in a freezer at −20°C until analysis. For Me-Hg analysis, toluene (pesticide grade) for extraction was purchased from Fisher Scientific Korea (Seoul, Korea) and L-cysteine from Sigma-Aldrich (C-7880, St. Louis, MO, USA) and hydrochloric acid from Wako Chemicals (Tokyo, Japan). The single mercury standard solution (5% HNO_3) of Perkin Elmer Pure Plus (Shelton, CT, USA) was used for total mercury (T-Hg) measurement. The stock solution of T-Hg was dissolved with 5% HNO_3 containing 0.001% L-cysteine to 100 μg kg^{-1} of working solution. The Me-Hg standard was purchased from Sigma-Aldrich Chemical as Me-Hg (II) chloride (442534, St. Louis, MO, USA). The stock solution of Me-Hg standard was dissolved with toluene, and working solutions were

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prepared between concentrations of 10.31 and 103.1 μg kg^{-1} to construct calibration curves.

Mercury measurement

The concentration of T-Hg in fishery products was measured using a direct mercury analyser (Hydra-C, Teledyne, USA). Briefly, 0.05–0.1 g sample was weighed in a sample boat and entered into the decomposition tube, where the sample is dried and then thermally decomposed by controlled heating of the Hydra-C system. The final decomposition products pass through a mercury amalgamator which collects Hg^0. The Hg amalgamator is heated to 700°C, and the Hg is released and detected at 254 nm by absorption spectrometry. The working solution was dissolved from 10 mg L^{-1} stock solution, in concentrations ranging 0.74–15.14 ng Hg.

For Me-Hg measurement, about 2 g homogenised sample was weighed into 50-mL conical tube. Dried bonito was weighed about 1 g into 50-mL conical tube, 10 mL of 25% sodium chloride solution was added and hand-shaken for 10 min, and 4 mL hydrochloric acid (60%) and 15 mL toluene were added and shaken vigorously for 10 min for extraction. After centrifugation at 4500 rpm for 10 min, the toluene layer was carefully transferred into a separate funnel and 10 mL 25% sodium chloride solution was added for washing; 5 mL L-cysteine solution was added to remove organic mercury compounds from the toluene. Finally, Me-Hg was extracted with toluene from sample.

Gas chromatography (Agilent GC-6890, USA) with (electron captured detector and HR-Thermon-Hg column (15m × 0.53 mm × 1.0 μm, Shimadzu, Kyoto, Japan) was employed for Me-Hg measurements. Instrument conditions were as follows: inlet temperature 160ºC, detector temperature 170°C, oven temperature 160°C, nitrogen gas flux 10 mL/min and 1 μl injection volume.

Quality assurance for analytical performance

Linearity, recoveries, limit of detection (LOD) and quantification (LOQ) and precision as repeatability were evaluated for analytical quality assurance. Results are shown in Table 1. Linearity of T-Hg was gained from calibration curves with concentrations between 0.74 and 15.14 μg kg^{-1} and those of Me-Hg with concentrations of 10.31–103.1 μg kg^{-1}. With regard to LOD and LOQ determination, the guideline of the International Conference on Harmonisation (ICH 1996) was used and the LOD was determined as the lowest acceptable concentration in the calibration curve for T-Hg and Me-Hg. The LOQ was calculated as 3x LOD. Certified reference material T 07153QC (FAPAS, UK, with assigned values of 244.0 μg kg^{-1} for T-Hg and 205.0 μg kg^{-1} for Me-Hg) was used to check the accuracy of the measurements, and the measured value was in good agreement with the certified value, since recoveries were 103.7% with RSD 2.8% and 103.8% with RSD12.5% for T-Hg and Me-Hg, respectively, as shown in Table 1.

Exposure assessment

For dietary exposure estimates, daily food consumption data for the Korean population were taken from the report of the Korean National Health and Nutrition Examination Survey (KNHANES 2008). The exposure assessment was performed by a point-estimate deterministic approach, which is an evaluation built by combining concentration of mercury and food consumption data and divided by the body weight. Therefore, the daily intake of mercury from each fishery product was calculated by multiplying the respective concentration in each fishery product by the average amount of the consumption by 8 years age groups and divided by the standard body weight of each age group to calculate the exposure level. The estimated weekly intake per body weight was compared with the provisional tolerable weekly intake (PTWI) values as established by the Joint FAO/WHO Expert Committee on Food Additives (JECFA) and reference dose (RfD) by United States Environmental Protection Agency (US EPA). When a result was below the LOD, this value was assumed to be half of the respective LOD.

Statistical analysis

T- and Me-Hg data were statistically analysed by SPSS statistics software (IBM, SPSS 20, USA). Mean, standard deviation and ranges were presented for descriptive statistics. Coefficient of determination (R^2) was determined by correlation analysis in T- and Me-Hg concentration. ANOVA with Duncan's *post hoc* test was used to determine the significant difference of contamination between fishery products at $p < 0.05$.

Table 1. Quality assurance parameters (n = 6).

Analyte	Linearity R2	LOD (μg kg^{-1})	LOQ (μg kg^{-1})	Recovery (%)	RSD (%)	Repeatability RSD (%)
T-Hg	0.99986	0.74	2.22	105.2	2.8	4.4
Me-Hg	0.99944	3.21	9.62	103.8	12.5	7.6

Results and discussion

T-Hg and Me-Hg concentration in fishery products

The concentrations of T-Hg and Me-Hg in fishery products are summarised in Table 2. From one hundred six samples, the T-Hg and Me-Hg were detected in 100 and in 63 samples showing 94.3% and 59.4% of detection rate, respectively. T-Hg and Me-Hg contamination ranged from 0.001 to 0.771 (mean value: 0.059) mg kg^{-1} wet wt and from 0.003 to 0.205 (mean value: 0.019) mg kg^{-1} wet wt, respectively. The highest value was found in frozen tuna *sashimi:* 0.395 and 0.103 mg kg^{-1} wet wt for T-Hg and Me-Hg, respectively. Among canned fish, canned tuna was the highest ($p < 0.05$) with 0.075 (range: 0.013–0.386) for T-Hg and 0.029 (range: 0.003–0.168) for Me-Hg, respectively. Canned sardine, mackerel pike, mackerel and whelk exhibited similar levels from 0.032 to 0.42 for T-Hg and from 0.007 to 0.010 for Me-Hg. Meanwhile, tuna sauce showed very low T-Hg ($p < 0.05$) with only 0.003 mg kg^{-1} wet wt and Me-Hg was not detected at all. No sample exceeded the maximum permissible limit as set by the Korean Food and Drug Administration (KFDA 2013) and CODEX (2011) at 0.5 mg kg^{-1} for fish except predatory fish and 1.0 mg kg^{-1} for predatory fish for Me-Hg.

With regard to the Me-Hg to T-Hg ratio by fishery product group, canned fish ranged from 31.8% in canned sardine to 48.6% in canned whelk. Meanwhile, this ratio was below 30% in frozen tuna *sashimi* and dried bonito. The relationship between T-Hg and Me-Hg in all samples showed a high correlation ($R^2 = 0.867$, $p < 0.001$). Similar findings have been reported for fish and fishery products (Moon et al. 2011; Park et al. 2011). Mean values were above 40%, except for canned sardine that showed the lowest ratio, with 31.8%.

Compared with other studies, T-Hg level in canned fish in this study was lower than found in canned tuna (0.329 mg kg^{-1} wet wt) and canned mackerel (0.074 mg kg^{-1} wet wt) in France (Crépet et al. 2005), yellow fin tuna (0.258–0.362 mg kg^{-1} wet wt) in Mexico (Ruelas-Inzunza et al. 2011), canned fish that mainly originated from Spain, Thailand, France and Croatia, (0.017–0.384 mg kg^{-1} wet wt in canned tuna, 0.046–0.063 mg kg^{-1} wet wt in canned mackerel) sold in Slovenia (Miklavčić et al. 2011), even showing a wide variable species range. It has been reported that mercury levels can vary with the length of fishes (Storelli et al. 2010; Bonsignore et al. 2013). Because canning industry in Korea processes mainly small tuna species such as skipjack (*Katsuwonus pelamis*) rather than yellowfin or bluefin tuna, it can be argued that the mercury levels in this study showed lower values than other studies from different counties. This study shows also lower levels than a domestic study by Park et al. (2011) who found 0.007–2.581 mg kg^{-1} for T-Hg and 0.003–1.307 mg kg^{-1} for Me-Hg in processed marine products. They also revealed that fresh fish contained higher mercury levels than fish products like canned fishes (Rasmussen & Morrissey 2007), which could explain the lower mercury content found in the present study.

Table 2. T-Hg and Me-Hg concentrations in fish products (mg kg^{-1} wet wt). Values of the same column, followed by the same letter (a–d), are not significantly different at $p < 0.05$ by Duncan's test.

Sample type	N	T-Hg Mean ± SD (min–max)	Me-Hg Mean ± SD (min–max)	Me-Hg/T-Hg ratio (%)
Canned fish	87	0.055 ± 0.054 (<0.001–0.386)	0.019 ± 0.023 (<0.003–0.168)	43.2 ± 14.2
Tuna	44	0.075 ± 0.070[a] (0.013–0.386)	0.029 ± 0.028[a] (<0.003–0.168)	44.1 ± 9.06
Mackerel	13	0.034 ± 0.014[b] (0.015–0.065)	0.009 ± 0.008[b] (<0.003–0.018)	46.6 ± 20.9
Mackerel pike	14	0.036 ± 0.011[b] (0.017–0.055)	0.010 ± 0.009[b] (<0.003–0.023)	44.0 ± 10.7
Sardine	4	0.042 ± 0.011[b] (0.030–0.054)	0.007 ± 0.008[b] (<0.003–0.015)	31.8 ± 5.12
Whelk	12	0.032 ± 0.015[b] (0.019–0.067)	0.008 ± 0.010[b] (<0.003–0.024)	48.6 ± 21.2
Tuna sauce	15	0.003 ± 0.005[c] (<0.001–0.016)	<0.003	–
Frozen tuna sashimi	2	0.395 ± 0.532[d] (<0.001–0.771)	0.103 ± 0.145[c] (<0.003–0.205)	26.6
Dried bonito	2	0.309 ± 0.071[d] (0.259–0.359)	0.077 ± 0.015[c] (0.066–0.087)	26.0 ± 10.7
Total	106	0.059 ± 0.095 (<0.001–0.771)	0.019 ± 0.030 (<0.003–0.205)	43.7 ± 12.4 (16.9–78.4)

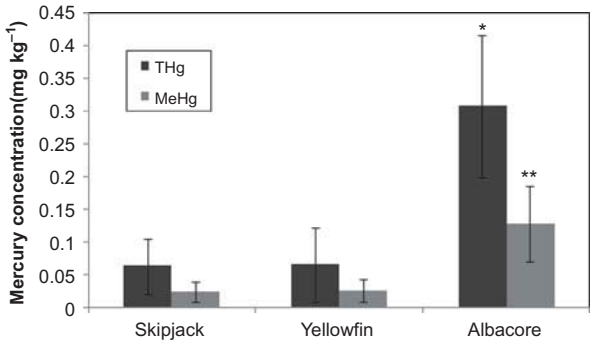

Figure 1. Mercury concentration (mg kg^{-1}) by canned tuna type: Skipjack (n = 32), Yellowfin (n = 10) and Albacore (n = 2). * and ** are significantly different at $p < 0.05$ (one-way ANOVA) for other types.

As seen in Figure 1, the mercury concentration in canned tuna varied with species. T-Hg and Me-Hg in albacore was significantly higher than in other types ($p < 0.05$). In particular, T-Hg concentration of canned albacore tuna produced in Thailand in our study was 0.309 mg kg^{-1}, which was higher than canned albacore (*Thunnus alalunga*) caught at the US Pacific Coast presenting 0.21 ± 0.05 mg kg^{-1}. A recent USA study (Mercury Policy Project 2012) reported mercury levels of canned albacore tuna of 0.560 mg kg^{-1} and 0.118 mg kg^{-1} in canned light tuna. It was also recommended that children should not eat albacore tuna because it contains roughly triple the mercury content found in light tuna.

Exposure assessment

Estimated T-Hg and Me-Hg intake by average fishery product consumption for the Korean population is summarised in Table 3 and were estimated to be 6.217 and 1.746 per week, respectively. Frozen tuna *sashimi* accounted for the highest contributor accounting for 63.5% and 58.6% of the total intake of T-Hg and Me-Hg, due to the remarkably high concentration. Meanwhile, very low contribution was observed as 0.35% and 0.29% of total intake of T-Hg and Me-Hg in dried bonito because of the low consumption of 0.01 g day^{-1} as shown in Table 3. Canned sardine also showed very low contribution for mercury intakes due to the low consumption. Canned tuna and mackerel made similar contributions, collectively accounting for 29.8% and 34.5% of T-Hg and Me-Hg intake, respectively. The domestic study (Kim et al. 2012) that studied mercury level of Korean diet revealed that fishes including shark meat and tuna were the highest contributed food in mercury intake. They also calculated the PTWI (%) of T-Hg through fish consumption as 0.98% of Korean population. Another Korean study (Lee et al. 2006) listed up the top 10 major contributors accounting for 71% of T-Hg intake and reported that foods/dishes with fish such as broiled yellow croaker, broiled mackerel and canned tuna contained much higher levels of mercury than other foods, indicating almost 50% of T-Hg intake. This tendency was also documented in Cambodian study (Cheng et al. 2013), which showed fish had the highest contribution to the total daily intakes of T-Hg and Me-Hg.

T-Hg and Me-Hg estimated intakes by age groups ranged from 1.279 to 8.472 µg week^{-1} and from 0.521 to 2.797 µg week^{-1}, respectively. The 19–29 years age group had the highest and the 1–2 years age group the lowest intake among all age groups as presented in Figure 2(a). Generally, exposure levels of persistent toxic chemicals tend to increase over age due to the higher consumption. In the present study, this pattern reversed from the 30–49 years age group. Adjusting the intake by

Table 3. Estimated total and methyl mercury intake of the Korean population and evaluation by international exposure limits.

Fish product	Intake[1] (g/day)	Mean concentration[2] (mg kg^{-1})		Estimated weekly intake (µg/week)		Estimated intake adjusted for body weight (µg/kg bw/week)		% of PTWI[3]		% of RfD[4]
		T-Hg	Me-Hg	T-Hg	Me-Hg	T-Hg	Me-Hg	T-Hg	Me-Hg	Me-Hg
Canned fish										
Tuna	1.77	0.075	0.029	0.929	0.359	0.015	0.006	0.39	0.37	0.86
Mackerel	3.96	0.034	0.009	0.942	0.249	0.016	0.004	0.39	0.26	0.59
Mackerel pike	1.19	0.036	0.010	0.300	0.083	0.005	0.001	0.12	0.09	0.20
Sardine	0.01	0.042	0.007	0.003	<0.001	<0.001	<0.001	0.00	0.00	0.00
Whelk	0.3	0.032	0.008	0.067	0.017	0.001	0.0003	0.03	0.02	0.04
Frozen tuna sashimi	1.43	0.395	0.103	3.954	1.031	0.066	0.017	1.65	1.07	2.45
Dried bonito	0.01	0.309	0.077	0.022	0.005	<0.001	<0.001	0.01	0.01	0.01
Total	8.67	0.923	0.243	6.217	1.746	0.104	0.029	2.59	1.82	4.16

Note: [1] Average intake of each food group based on KNHANS (2008) consumption data
[2] Samples <LOD were calculated with LOD/2 values.
[3] FAO/WHO JECFA Provisional Maximum Weekly Intake data for T-Hg: 4 µg/kg bw/week and for Me-Hg: 1.6 µg/kg body weight/week.
[4] US EPA RfD for Me-Hg: 0.7 µg/kg body weight/week.

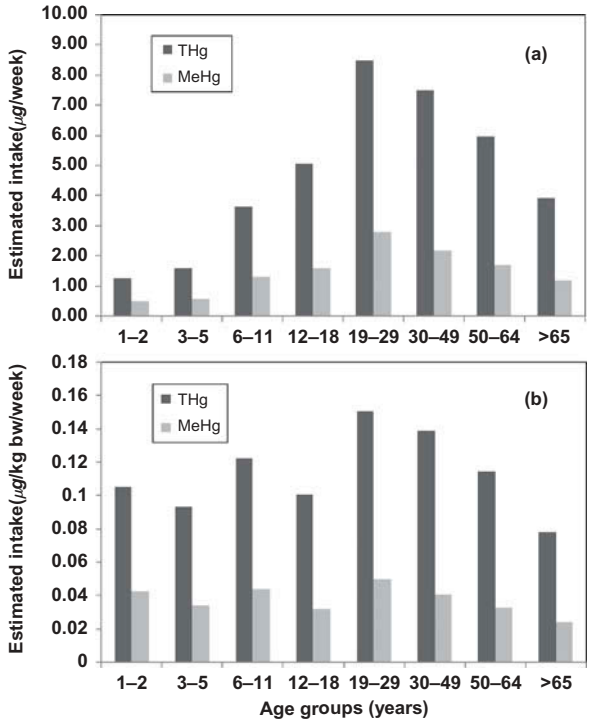

Figure 2. Estimated T-Hg and Me-Hg intakes from fishery products among age groups (a) Estimated weekly intake (μg per week) of T-Hg and Me-Hg (b) Estimated intake (μg kg^{-1} bw per week) adjusted by body weight per age group.

body weight of corresponding age groups, the estimated T-Hg and Me-Hg intake per body weight ranged from 0.078 to 0.150 μg kg^{-1} bw per week and from 0.024 to 0.049 μg kg^{-1} bw per week, respectively. Even though the 19–29 years age group showed the highest absolute estimated intake, the estimated intake adjusted by body weight showed a different view, as can be seen in Figure 2(b), which shows that the exposure level of children can be as high as adult groups. This suggests that children are relatively more vulnerable to mercury exposure and so a more careful intake guideline should be needed. Other previous researches (Crepet et al. 2005; Karjalainen et al. 2013) support that special age groups like children and childbearing women are the main target group for mercury intake control through fish consumption.

To estimate human health risk of mercury from fishery product consumption in Korea, the exposure level (as intake) was compared with tolerable maximum intake levels from other countries and international authorities. The JECFA established PTWI values of 1.6 μg kg^{-1} bw per week for Me-Hg and 4 μg kg^{-1} bw per week for inorganic mercury (JECFA 2010). The United States US EPA proposed 0.7 μg kg^{-1} bw per week as a RfD for Me-Hg (US EPA 2005). In the present study, as shown in Table 3, Me-Hg intake for the general population (0.104 μg kg^{-1} bw per week) and 8 years age groups were less than the levels suggested by JECFA and US EPA. This indicates that Me-Hg intake from fish product consumption in Korea is relatively safe.

Conclusion

T-Hg and Me-Hg were measured in commonly consumed fishery products in Korea. The levels were relatively lower than in fresh fishes found in domestic and other countries. Frozen tuna *sashimi* showed the highest mercury level, followed by dried bonito. Canned tuna exhibited the highest among canned fishes. With regard to tuna species, the albacore tuna showed the highest mercury level. Taking into account dietary exposure, T-Hg and Me-Hg intake by the Korean population via fish products was less than the allowable maximum levels as suggested by JECFA and US EPA. Within age groups, intakes increased with age to the 19–29 years age group; however, when the intake was adjusted for body weight, the under 12–18 years age groups revealed that these consumed similar T-Hg and Me-Hg intakes as did adult groups. This indicates that children should consume balanced food including fish, but it is very important which species of fish and how often fish is consumed. Because fish and fishery products are important food due to food quality and convenience, regular monitoring for T-Hg and Me-Hg should be accomplished.

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