Hair mercury levels and dietary exposure of mercury in relation to fish consumption among coastal population in Negombo, Sri Lanka

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Abstract Consumption of contaminated fish is the major source of the human exposure to total mercury (T-Hg) and methyl mercury. This study reports the fish consumption habits of three groups based on the age category and their occupation; "Children" (age 9-20 years) and two groups of "Adults" (age above 21 years) based on their occupation; "Adults-Fishermen" (fishermen) and "Adults-Other". Hair T-Hg levels were measured from 30 individuals (age 9-48 years) from Negombo area in Sri Lanka. All individuals in this survey consumed fish at least six servings per week, which belongs to the high fish consumer category (> 3 servings/week) according to World Health Organization (WHO) guidelines. The main fish groups consumed by the respondents were mainly yellow-fin tuna (YFT), skipjack tuna (SKT), kawakawa and frigate tuna and small fish such as sardines (spotted sardines, goldstripe sardine, other sardines, scads and trevallies). The majority consumed an equal amount of big fish and small fish. The average weekly fish consumption per individual varied depending on the test group (Children; 1270 g/week per person, Adults-other; 1078 g/week per person and Adults-fishermen 1852 g/week per person) which exceeded United States Food & Drug Administration (USFDA) recommended level of 340 g/ week of seafood containing low concentration of Hg. The mean hair T-Hg of respondents of Negombo population was 4.89±3.23 μg/g (range 1.60-13.38 μg/g), which exceeded the United States Environmental Protection Agency (USEPA) reference dose (1 μg/g) for T-Hg level of hair. The total hair mercury in three respondent groups (Children 3.33±1.36 μg/g, Adults-other 2.89±1.26 μg/g and Adults-Fishermen 6.08±3.62 μg/g), were not significantly different (*p*>0.05) from each other. Real exposure value of three respondent groups were not significantly different (*p*>0.05). There was a low strength positive correlation (r=0.353, *p*>0.05) between hair T-Hg levels with the weekly large fish consumption.

Keywords: total mercury, methyl mercury, fish consumption, occupational groups

INTRODUCTION

Over the past few years, there has been an increasing evidence of mercury (Hg) pollution over the world and high Hg levels in human hair among coastal communities, for whom fish constitute the dietary mainstay (Barbosa et al. 2001). Mercury has caused a variety of adverse effects on human health and the environment throughout the world. Mercury is a neurotoxin, particularly to the developing nervous system. Mercury toxicity imposes on human and other organisms is dependent on the chemical form, amount of Hg in the exposure, the pathway of exposure, the life stage of the human/ organism, the time of exposure and health status of person or organism exposed. Human exposure to Hg may occur via a variety of pathways, including consumption of fish, occupational and household uses, dental amalgams and mercury-containing vaccines (Díez 2009).

Methyl mercury (M-Hg), which is known to be the most poisonous among the Hg compounds is created when inorganic Hg circulating in the general environment is dissolved into freshwater and seawater. Methyl Hg is a key status of Hg because of several reasons; irreversibility and severity of its effects (Tollefson and Cordle 1986), very long half-life in fish-approximately 2 years which is two to five times the half-life of inorganic mercury (Stopford 1975) and very slow process of discharge from the binding sites of animals tissue. These are major reasons that fish become a major source of M-Hg exposure. The steady increase of Hg in larger fish, older fish and predator organism at the top trophic levels have resulted in the highest Hg concentrations. The Minamata Bay (1953-1960) and Niigata (1965) poisoning episodes in Japan and Iraq grain contamination incident (1971-1972) are the most famous environmental contamination incidents by the foods by M-Hg (Bakir et al. 1973; Tsubaki 1979; Ministry of the Environment, Japan 2013). They showed the extreme effects of acute and extensive exposure to M-Hg on humans.

There are three epidemiological studies to evaluate the effects of low concentration or medium concentration of M-Hg exposure on human bodies via fish consumption without special contamination, the Faroe Islands, Seychelles and New Zealand. These studies showed that low and medium exposure of M-Hg and its negative health effects on different populations (Kjellstrom et al. 1989; Steuerwald et al. 2000; Myers et al. 2003).

Several studies showed a higher level of Hg exposure in the populations with high fish consumption levels where lives near lakes, rivers and oceans (Díez 2009). Hair-Hg concentrations in children from four riverine communities in the Brazilian Amazon showed high Hg levels; 3.80 (0.5-12.4), 11.9 (0.7-35.8), 25.4 (0.6-83.5), 17.7 (7.3-63.8) µg/g (Grandjean et al. 1999). Hong et al. (2012) analyzed the blood concentration of T-Hg and M-Hg in 400 residents in 30 areas of Busan, Ulsan and Gyeongsangnam-do province in Korea. They reported that the concentration of M-Hg was 4.05 µg/L, which was 78.53% of the T-Hg concentration (5.27 µg/L), that males showed a higher M-Hg concentration than females, that the blood M-Hg concentration increased significantly as the T-Hg concentration increased, and that the M-Hg concentration had a significant correlation with fish intake.

In Sri Lanka, Negombo (western part of the country) area is one of the main fishery areas in the country with consists of a major fishery harbour. Also, majority of the coastal population is mainly engaged in fishing and related activities as their livelihood. As the coastal communities have ready accessibility to fish, they are vulnerable to Hg exposure if any Hg contaminated fish is consumed (Hanidza et al. 2010; Yasutake et al. 2003). Hence, it is important to investigate the levels Hg exposure of coastal populations in relation to their fish consumption rate. The present study was therefore focused on the fish consumption rate and hair T-Hg levels in the coastal community, Negombo Sri Lanka.

MATERIALS AND METHODS

Sampling

Hair samples were collected according to guidelines of the standard operation procedure (SOP) published by the Ministry of Environment, Japan in 2004. A total of 30 hair samples were collected from the male population in Negombo harbour area, a western coastal region of Sri Lanka. The survey participants answered questions of potential relevance to the food pattern and lifestyle. Twenty strands of long hair and many numbers of shorter hair were cut from each person in the sample and stored in a sealed polyethylene bag on which the identification (ID) number of the participant is indicated. Samples were then transported to Analytical Chemistry Laboratory, National Aquatic Resources Research and Development Agency (NARA) for analysis.

Chemical and glassware

All chemicals were of analytical grade and certified for low trace metals content. De-ionised water was used throughout. Nitric acid, acetone and Hg standards were obtained from Sigma-Aldrich (Dorset, United Kingdom). Precautions were taken to avoid contamination of samples with trace metals. All glassware and plasticware used for the study were soaked in 10% HNO_3 overnight and rinsed three times with de-ionised water prior to use.

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Equipment

A MARS XP 1500+ microwave accelerated system (CEM, Matthews, USA) was used for sample digestion and Atomic Absorption Spectrophotometer (Varian 240FS; Varian Pvt. Ltd, Mulgrave, Victoria, Australia) was used for determination of trace metal. The T-Hg was analyzed by vapour generation accessory (Varian VGA 77).

Sample preparation

Hair sample was transferred into a clean beaker, washed with neutral detergent (diluted 100-fold) and distilled water by decantation, and washed again with a small amount of acetone to remove the water. Hair sample was dried under the ambient temperature. Dried hair sample was transferred into a new sealed polyethylene bag and cut into small pieces with dissection scissors to make a sample for analysis.

Approximately 0.2 g of hair sample was weighed in a microwave digestion tube. Then 10 mL of 65% HNO_3 acid was added to sample and samples were digested under pressure in a closed vessel heated by microwaves using a microwave-accelerated system. The digests were allowed to cool to room temperature and transferred into 50 mL volumetric flask and made up to 50 mL with de-ionized water as a diluent. Freshly prepared Hg standard solution (1 mg/L) was made by appropriate dilution and used for the prepared working standard solution. A $SnCl_2$ solution was used as the reductant and distilled water used as an acid solution to cold vapour VGA-AAS.

Quality control

Quality control for each analytical procedure consisted of certified reference material (CRM) from the National Institute for Environmental Studies (NIES), Japan (human hair, CRM No. 13) and the quality control (QC) sample from the Food Analysis Performance Assessment Scheme (FAPAS), United Kingdom (canned fish, T07194QC). The CRM was digested according to the procedure used in the preparation of hair samples for the metal analysis. The analytical chemistry laboratory at the NARA has participated proficiency testing program within the same time in FAPAS UK with satisfactory results (report no. 07215/2014, Z value for Hg: 0). Calibration curves were prepared with five points including zero. Optimum wavelength was used to determine the concentration of a particular metal and the calibration curve was optimized for the concentration of samples to be within the range of 0.2-0.8 in a linear graph. In every calibration curve, correlation coefficient (r value) was maintained more than 0.995. The average field blank, derived from sample field blanks, and three times of its standard deviation were used to evaluate the limit of detection (LOD). The limit of quantification (LOQ) was $3 \times$ LOD and below that value was reported as not detected (<0.07 μg/g).

Calculation of actual exposure of total mercury via fish consumption

Actual exposure of the respondents was analyzed based on the respondents' weekly dietary details. Published T-Hg levels of the fish species/ groups in Sri Lankan waters or Indian waters were used for the actual exposure calculations. For the calculation of actual exposure from big fish consumption, mean T-Hg levels from published data in Sri Lankan catch landings for yellowfin tuna and skipjack tuna were used and for the small fish consumption, mean T-Hg value for Indian oil sardine was used (Table 1).

Table 1 Reported values of mean total mercury (T-Hg) for mostly consumed fish species in Negombo area

Species	Mean T-Hg concentration (mg/kg wet weight basis)	Authority
Yellowfin tuna (*Thunnus albacares*)	0.39	Jinadasa et al. (2010)
Yellowfin tuna (*Thunnus albacares*)	0.16	Jinadasa et al. (2014)
Yellowfin tuna (*Thunnus albacares*)	0.30	Jinadasa et al. (2013b)
Skipjack tuna (*Katsuwonus pelamis*)	0.13	Jinadasa et al. (2015)
Indian oil sardine (*Sardinella longiceps*)	0.001	Rameshkumar et al. (2016)

Statistical analysis

Two groups were selected based on the age categories and their occupation i.e., "Children" (age 9-20 years) and "Adults" (age above 21 years). Further, adults group were divided into two groups based on their occupation i.e., "Adults-Fishermen" (fishermen) and "Adults-Other". The results were statistically analysed by the statistical package for social sciences (SPSS). A one-way Analysis of Variance (ANOVA) was performed, followed by Tukey's test for comparisons of significant differences. Pearson's correlation test was performed to test the relationship between hair mercury levels and fish consumption rates.

RESULTS

Fish consumption

All thirty participants in this survey consumed fish at least six servings per week. The majority of the participants consumed an equal amount of big fish and small fish. The small fish to large fish consumption ratio is approximately 1:1. The main fish groups of the correspondents consumed were tuna; mainly yellowfin tuna, skipjack tuna, kawakawa and frigate tuna and small fish such as

sardines (spotted sardinella, goldstripe sardine and other sardines). Apart from them, participants consumed sailfish, jacks, trevallies and scads. Survey results showed that Negombo male sample population could be listed into high fish consumers relevant to World Health Organization (WHO) guidelines. According WHO guidelines for identifying populations at risk from Hg exposure, it is necessary to conduct exposure assessment for all groups consuming a high number of fish servings; >3 servings/week (WHO 2008). Also responded participants exceeded the United States Food and Drug Administration (USFDA) recommended level of 12 ounces (340.2 g) per week of seafood containing low concentration of mercury (USFDA 2009).

Real exposure of total mercury via fish consumption

The real exposure values were calculated with variety of fish consumption of respondents. A one-way ANOVA results showed no significant difference ($p>0.05$) of real exposure values between groups. The real exposure of T-Hg gradually increased as follows; Adults-Other (1.34±0.63), Children (1.95±1.04) and Adults-Fishermen (3.15±1.73) µg/kg bw per week per person (Table 2).

Table 2 Real Exposure of mercury via fish consumption in the male population of Negombo area, Sri Lanka (µg/kg bw per week per person)

Group	Number (n)	Mean±SD µg/kg bw per week per person	Median µg/kg bw per week per person	Minimum µg/kg bw per week per person	Maximum µg/kg bw per week per person	Mean Weekly fish consumption g/week/ per person		
						Small fish	Large fish	Total
Children	7	1.95±1.04	2.31	0.05	2.94	814±894	461±260	1274
Adults-Other	7	1.34±0.63	1.21	0.42	2.37	654±609	424±215	1078
Adults-Fishermen	16	3.15±1.73	2.59	1.30	7.94	825±310	1027±555	1852

Study results clearly showed that detected hair Hg levels of sample groups corresponded to their real Hg exposure values with respect to fish consumption. The average real exposure value of Adults-Other group (1.34±0.63 µg/kg bw per week per person) is well below the WHO Provisional

Tolerable Weekly Intake (PTWI) level of Hg for adult; 3.2 µg/kg bw per week per person. Real exposure value of Adult-Fishermen group (3.15±1.73 µg/kg bw per week per person) was slightly lower than the WHO PTWI level of Hg for adult; 3.2 µg/kg bw per week per person. Children's

real exposure value (1.95±1.04 µg/kg bw per week per person) was well above the WHO PTWI level of Hg for children up to about 17 years of age category, 1.6 µg/kg bw per week per person.

Total hair mercury levels

Thirty male hair samples of Negombo area were tested for T-Hg. Hair Hg concentrations ranged from 1.60 - 13.38 µg/g (Table 3). The mean hair Hg

level of the respondents was 4.10±3.23 µg/g. The National Academy of Science and USEPA reference dose (RfD) for total hair Hg equals to 1 µg/g in hair (USEPA 1997). Almost 95% of Hg in hair is mainly in the organic Hg form. This RfD indicates the M-Hg level in the human body that is reasonably expected to cause no harm and is protective of all people. According to the present study, the mean hair Hg content of hair sample of males tested exceeded the USEPA RfD for Hg limit.

Table 3 Hair Hg levels (µg/g) in the male population of Negombo area, Sri Lanka

Group	Number (n)	Mean±SD µg/g	Median µg/g	Minimum µg/g	Maximum µg/g
Children	7	3.33±1.36	2.97	1.76	4.89
Adults-Other	7	2.89±1.26	2.18	2.10	5.04
Adults-Fishermen	16	6.08±3.62	5.46	1.60	13.38

Table 3 shows hair Hg level of three groups based on their age category and occupation, i.e., Children, Adults-Other and Adults-Fishermen. Results of the one-way ANOVA of hair Hg showed no significant differences ($p>0.05$) between groups. The mean hair Hg level gradually increased from lowest to highest as follows; Adults-Other (2.89±1.26 µg/g), Children (3.33±1.36 µg/g) and Adults-Fishermen (6.08±3.62 µg/g). Mean hair Hg level of Adults-Other group was more than 2.9 times higher, Children group was more than 3.3 times higher and Adults-Fishermen group was almost 6.1 times higher than USEPA RfD.

Influence of amount and type of fish eaten on hair mercury levels

There was no significant correlation between hair Hg levels of male population with their weekly consumption of small fish, large fish and total fish consumption. However there was a low strength positive correlation ($r=0.353$, $p>0.05$) between hair Hg levels with their weekly consumption of large fish (Fig. 1), indicating that hair Hg level in the studied population have influenced by consumption of large fishes (tuna, sailfish, marlin and swordfish species) which was reported to contain high Hg levels (Jinadasa et al. 2013a; Jinadasa et al. 2013b; Jinadasa et al. 2014; Rodrigues et al. 2016).
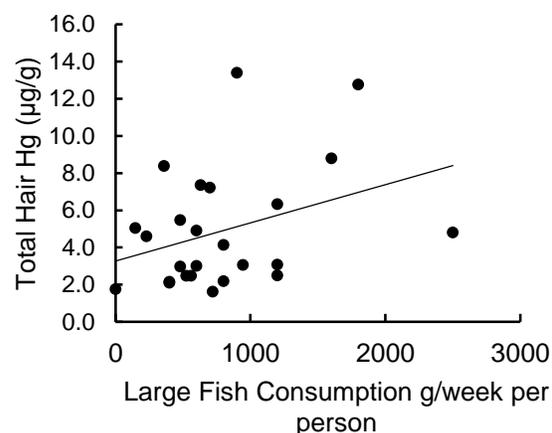

Fig. 1 Relationship between large fish consumption vs. total hair Hg

DISCUSSION

To the authors' knowledge, this is the first attempt to assess hair Hg concentrations related to fish consumption of coastal residents in Sri Lanka. All participants of the study consumed fish at least six servings per week, which fall into high fish consumption group according to WHO guidelines (WHO, 2008). Also, higher levels of individual and groups' mean weekly fish consumption (USFDA 2009) alert the potential risk of fish consumption of the study population. This high fish consumption

nature is especially inherited with the nature of the area, as Negombo is one of the major coastal fishery districts with major fishery harbor and major fish and fishery product trade centre. This offers the population to access quality seafood at a lower price compared to other areas. The high accessibility to fish and cultural background of the population in Negombo area collectively leads them into high Hg exposure. WHO guidelines for identifying populations at risk from Hg exposure stated that it is necessary to conduct exposure assessment for all groups consuming a high number of fish meals, i.e., >3 meals per week (WHO, 2008).

Majority of respondents in three groups' showed that they mainly consumed small fish and big fish at 1:1 ratio. Big fish includes mainly tuna species (yellowfin tuna, skipjack tuna, kawakawa and frigate tuna) and other species such as sailfish, marlin, Spanish mackerel and Wahoo. Small fish includes with sardine species (spotted sardines, goldstripe sardine and other sardine species), scads and trevallies. Estimated real exposure of three respondent groups with respect to their weekly fish consumption values were not significantly different ($p>0.05$). From three groups of respondent's categories, Adults-Other real exposure (1.34 ± 0.63 μg/kg bw per week per person) stayed well below the WHO, PTWI level for adults (3.2 μg/kg bw per week per person). This shows Adults-Other group stays in safe region of Hg exposure in relation to fish consumption. Childrens' real exposure value (1.95 ± 1.04 μg/kg bw per week per person) was higher than the WHO, PTWI level for children up to about 17 years of age (1.6 μg/kg bw per week per person). Adults-Fishermen groups' real exposure value (3.15 ± 1.73 μg/kg bw per week per person) was almost close to the WHO, PTWI level for an adult (3.2 μg/kg bw per week per person), which clearly showed a high risk of Hg exposure of studied population via fish consumption. These alarms need for necessary actions to remedy the situation. It is necessary to carry out further detailed studies heavy metal accumulation in coastal communities in relation to fish consumption pattern.

Thirty hair samples of respondents showed a higher level of T-Hg which ranged from 1.60 to 13.38 μg/g. Two of 30 respondents had hair Hg higher than 12 (12.76 and 13.38 μg/g). All respondents in the study exceeded the National Academy of Science and USEPA reference dose (RfD) for total hair Hg (1 μg/g in hair). Also, the mean hair Hg level of respondents was 4.89 ± 3.23 μg/g which more than 4.9-times higher than standard RfD. The mean hair Hg level of this study was higher than the values reported in similar studies for high fish consumption populations by ISACI (2013) reported 2.739 μg/g in Tokyo, Japan and 3.290 μg/g in Rarotonga, Cook Islands; Yasutake et al. (2003), who found 2.55 μg/g and 1.43 μg/g in Japanese males and females respectively. Similar higher concentrations of hair Hg (4.4 μg/g with range 0.5–8.9 μg/g) were observed from women of reproductive age in a Caribbean Island of Vieques (Ortiz-Roque and Lopez-Rivera. 2004).

Airey (1983) reviewed studies focused on the fish eaters and accumulation of heavy metals in some marine fisheries resources collected from Gulf of Mannar Marine Biosphere Reserve, Southeast Coast of India. According to Rameshkumar et al. (2012), "*Arithmetic mean mercury concentrations for people who ate fish 1–4 times each month were: Australia, 2.5 ppm; Canada, 1.2 ppm; China, 0.9 ppm; West Germany, 0.5 ppm; Hong Kong, 3.0 ppm; Italy, 1.5 ppm; Japan, 3.9 ppm; Monaco, 1.7 ppm; New Zealand, 1.3 ppm; Papua New Guinea, 1.8 ppm; South Africa, 1.9 ppm; U.K., 1.6 ppm and USA, 2.4 ppm. The differences are believed to be due to diet and environment. Mean hair mercury concentrations were significantly different for the group that ate fish once or less a month (1.4 ppm) once a fortnight (1.9 ppm) once a week (2.5 ppm) and once or more a day (11.6 ppm).*" Mean Hair Hg concentrations in this study were lower than Airey (1983) suggested for people eating fish once or more a day.

Hair Hg concentrations among the three groups of respondents in the study were directly proportional to their weekly fish consumption. Similar results showed in the Hair Hg concentrations and fish consumption patterns in the residents of Florida (Schaefer et al. 2014), where high fish consumption groups with higher hair Hg level. Differences weekly fish consumption among 3 groups in the study can be due to many reasons such as accessibility to fish, occupation and age variations.

There is a low positive correlation ($r=0.353$, $p<0.05$) between hair Hg levels with their weekly big fish consumption, which clearly illustrates the greater risk of higher weekly consumption of big fish in the studied population. There was no clear

relationship between the amount of small fish consumption rate and hair Hg levels in respondents. As such, it is possible to maintain high fish consumption rate to meet nutrients requirements of different populations by choosing low Hg contain fish groups.

CONCLUSION

The present study provides information about hair Hg levels in Negombo male population. In conclusion, study revealed that hair Hg level in the studied population exceeded USEPA RfD of 1 µg/g. Also weak positive correlation between hair Hg levels with their big fish consumption rate and lack of correlation with small fish consumption rate draw attention on further detailed studies on hair Hg levels of coastal populations in relation to fish consumption patterns. Initiation of awareness about fish consumption and Hg exposure is essential to address health problems while meeting nutrient requirements via fish consumption.

ACKNOWLEDGEMENTS

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