

CASE STUDY

Total Diet Study approach in estimating mercury and cadmium levels using selected fish: A case study from Sri Lanka

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Abstract: Total Diet Study (TDS) is a world-recognized approach in quantifying nutrients and contaminants in complete diets. In the present study, the TDS approach was used to determine the dietary exposure to mercury (Hg) and cadmium (Cd) by regular consumption of yellowfin tuna (YFT), swordfish (SWF) and sardinella (SAF) fish in Gampaha district, Sri Lanka. The study was based on the TDS method established by the World Health Organization (WHO). Hg and Cd were analyzed using Atomic Absorption Spectrophotometer after microwave digestion of samples. Survey results revealed that the weekly average fresh fish consumption was 301 g per person. Hg and Cd levels were calculated on the wet weight basis. The maximum allowable limits of Hg and Cd were compared with European Union/European Commission (EU/EC) regulation limits for each species. None of the samples exceeded the EU/EC maximum limit for Cd, but two samples of SWF exceeded the maximum limit for Hg, recommended by the EU/EC. The Provisional Tolerable Weekly Intake (PTWI) for Hg was calculated for risk assessment. The percentage contributions of SWF, YFT, and SAF for PTWI-Hg were 64.92 and 40.16 and 8.55 respectively. Accordingly, the SWF were close to exceed the PTWI value. YFT and SWF are considered as the top predators in the marine food web. Cooking did not significantly reduce the Hg and Cd contents in selected fish species. Further studies are recommended to evaluate the PTWI values for predatory fish species before consumption.

Keywords: Provisional Tolerable Weekly Intake (PTWI), Mercury, Cadmium, Risk, Seafood.


INTRODUCTION

Seafood refers to food including mainly fish, crustaceans, mollusks, and seaweeds derived from aquatic resources originating from marine and brackish water environments. Seafood provides a variety of nutrients thus beneficial in making healthy diets. Dietary guidelines state that the increase in consumption of fish and fishery products contain health benefits (Morales and Higuchi, 2018). The per capita fish consumption of a Sri Lankan is 15.3 kg/year, but according to Ministry of Health (2016) it should be increased up to 21 kg/year (Ministry of Health, 2016).

As larger fish species are in higher positions of food webs compared to smaller fish species, hazardous substance accumulation of the former might be 1,000 times higher than that of the latter (Johnson and Finley, 1980). Hazardous substances such as persistent organic pollutants (POP's), Polychlorinated biphenyl (PCB's), dioxins, furans and other environmental contaminants like mercury (Hg), cadmium (Cd) and lead (Pb) are accumulated in fish and eventually transfer to humans through consumption (Jinadasa *et al.*, 2016).

Mercury is a naturally occurring non-essential trace metal that exists in several forms such as metallic, organic and inorganic in the environment including water, air, and soil (Jinadasa *et al.*, 2015). Organic Hg, especially in the form of methylmercury (MeHg) is mainly found in seafood and it forms an extremely stable complex with sulfhydryl groups (Schmidt *et al.*, 2018). As Methyl Hg is the most toxic form it attracts more attention globally (Jinadasa *et al.*, 2015). Previous studies demonstrate that Hg contaminants can't be removed through trimming, cooking and skinning because MeHg binds to protein in muscle tissues of fish. Urine and the feces are the most common excretory pathways of inorganic Hg (Berger *et al.*, 2003) {B, 2003 #21;Berger, 2003 #285}. The accumulation of Hg especially MeHg in humans may damage the central nervous system, especially in the fetal and early growth stages. European Union/European Commission (EU/EC) and Government of Sri Lanka regulations (EU/EC-1881, 2006; GOSL,1528/7) indicate safety levels for Total Hg (T-Hg) in fish and fishery products. According to those safety regulations, the maximum Hg level in wet weight basis is 1 mg/kg for yellowfin tuna (YFT) and swordfish (SWF) and 0.5 mg/kg for sardinella (SAF). Based on the exposure risk assessment, Food and Agricultural Organization (FAO) and World Health Organization (WHO) Joint Expert Committee on Food Additives (JECFA) published the provisional tolerable weekly intake (PTWI) values (Popovic *et al.*, 2018). Food and Drug Administration of the United States of America (US-FDA) and EU also advise avoiding the consumption of fish which contain T-Hg higher than 1mg/

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kg (USFDA, 1994).

Cadmium (Cd) is a metal that can become toxic to all life forms including humans even at low concentrations. Cadmium is associated with cancer, birth defects and genetic mutations (Lundh *et al.*, 2016). Cadmium is released into the environment by industrial (coal power plant, mining *etc.*) or domestic effluents (electronic waste) or through natural activities (volcanic eruptions). Fish can accumulate Cd through contaminated food chains, finally exposing to humans who regularly consume shellfish and fish organs such as liver and kidney. As YFT and SWF are on the upper level of the food web, the bio-accumulation of contaminants including T-Hg is higher than in small pelagic fish species (Jinadasa *et al.*, 2014).

In Sri Lanka, different cooking methods are used to prepare fish and fishery products. The main cooking methods are milk/chilli curry, roasting and frying. Several researchers have evaluated Hg in different food types, but not much information is available on cooked fish. Mieirol *et al.* (2016) demonstrated that high-temperatures in cooking fish may reduce the volatile character of MeHg. Most countries practice the Total Diet Study (TDS) approach to quantify the nutrients and contaminants in complete diets. It is an universal approach to estimate and monitor dietary exposures to chemical substances such as additives, residues of phytosanitary products or veterinary medications, environmental contaminants, natural toxins, nutrients and micronutrient (USFDA, 2014). The TDS approach is mainly based on the food consumption data collected from a sample of a population (more than 1% of the total population). Development of the food list, food sampling and kitchen preparation of samples are also based on in the TDS survey. The main objective of the present study was to establish a methodology for TDS relevant to Sri Lanka and to analyze T-Hg and Cd contents in cooked samples of YFT, SWF and SAF.

MATERIALS AND METHODS

The methodology for TDS was established based on the WHO guidelines and previous TDS studies carried out in France, Tanzania, and Italy (Peltonen *et al.*, 2011, Gimou *et al.*, 2008). Food consumption data were collected from the Gampaha district which represents both urban and rural populations, during the period from January to August 2016 using a questionnaire (Sandaruwan *et al.*, 2017). Data were obtained through two non-consecutive 24-hour dietary questionnaires taken from 152 families. Negombo fish landing site and other popular fish markets (Peliyagoda and Chilaw) are located close by Gampaha district. The samples were analyzed from September to December 2016.

Sample collection

List of fish species was prepared using food consumption data. The individual approach was followed to analyze the trace metals, T-Hg and Cd. YFT and SWF are the most abundant larger pelagic species and SAF is the most abundant small pelagic fish species consumed by the people in the Gampaha district. YFT, SWF, and SAF samples were collected from mobile fish vendors

as well as from the Ceylon Fishery Corporation (CFC) fish stall, Peliyagoda fish market and super markets. The collected samples (11 samples each weighing 500 g) were immediately transported in insulated boxes containing ice to the laboratory at NARA.

Sample preparation and culinary process

Details of cooking methods, ingredients (varieties and quantity), containers, and energy sources were collected from the TDS survey. Accordingly, YFT, SWF, and SAF were prepared using the cooking methods obtained from the TDS survey. The T-Hg and Cd contents of cooked fish samples were analyzed. YFT and SWF fillets were taken (devoid of skin and bone) while SAF were cleaned by removing the gut, gills, and scales before cooking. The fish samples were cooked according to the culinary methods used by the local people using the same spices and other ingredients. The fish samples were prepared as a milk curry, chili curry and 'ambulthial' at the ratio of 6:3:2, and the cooking times were 15, 20 and 25 minutes in the ratio of 5:5:1. The energy sources used were gas: fire wood at the ratio of 5:6. Clay pots and aluminum containers were used in the ratio of 9:2.

Total mercury (T-Hg) and cadmium analysis

The chemicals used in the analyses were of the analytical grade. Standard solutions of the metals (1,000 mg/L) were obtained from Fluka, Switzerland. The glassware was soaked overnight in a liquid detergent solution and thoroughly rinsed with tap water, before soaking them in 10% (v/v) HNO₃ overnight and rinsed in de-ionized water and oven dried before using. The plastic ware was air dried prior to use.

One gram of homogenized sample was weighed in a microwave digester tube and 10 mL of concentrated nitric acid (65%, AR-Sigma) was added and kept for 15 minutes in a fume hood for pre-digestion. After pre-digestion, the samples were digested using a microwave accelerated system (CEM-Mars-6). Each sample was analyzed in duplicate. Two blank tubes prepared in a similar manner (but without the muscle) were included in each batch of analysis. Microwave conditions were set at 800 PSI pressure, 200 °C temperature with a 10 min holding time.

The digests were allowed to cool to room temperature and the pressure was released carefully by opening the valve. Each digested fish sample was transferred into 50 mL volumetric flask and was diluted up to the mark with deionized water. The determination of T-Hg was performed using a Cold Vapor Atomic Absorption Spectrometry system (CV-AAS: Varian VGA 77) in a Varian 240 FS atomic absorption spectrophotometer (Varian 240 FS, Varian Inc., Mulgrave, Victoria, Australia) using SnCl₂ as a reduction solution throughout the analysis. The Cd was analyzed using a graphite tube atomizer (Varian GTA 120). A calibration curve for T-Hg (5-50 µg/L) and Cd (0.5-3.0 µg/L) were obtained. Using the standard curve, readings were obtained for blanks, samples and quality control sample (QC).

Quality control procedure

The accuracy of the analytical procedure was maintained throughout the analysis using certified quality control material (canned fish muscle, T/07194) from Food Analysis Performance Assessment Scheme (FAPAS, Sand Hutton, York, UK) for T- Hg and Cd. The average field blank, derived from sample field blanks, and three times its standard deviation were used to evaluate the limit of detection (LOD). The limit of quantification (LOQ) was $3 \times \text{LOD}$. The descriptive analyses were done using SPSS software version 17.0.

RESULTS AND DISCUSSION

This study provides information about T-Hg and Cd contents in cooked YFT, SWF and SAF species. The suitability of this method was evaluated by calculating the Limit of Quantification (LOQ) and the recovery of certified quality control measures for T-Hg and Cd. The LOQ for T-Hg and Cd was 0.07 and 0.006 mg/kg respectively. The assigned recovery value of quality control of samples ($n = 10$) for Hg (T-7194QC) was 141 $\mu\text{g}/\text{kg}$ and the recovery was 106.7 % (± 7.90) and the assigned value for Cd for the same QC sample was 4.99 $\mu\text{g}/\text{kg}$ and the recovery was 95.10 % (± 13.34). The expanded uncertainty ($k=2$) of Hg and Cd was 9.0 and 8.8%, respectively. The results indicate the validity of this method for determining the T-Hg and Cd concentrations in the samples investigated.

The food consumption data were collected from a population-based food consumption survey conducted in the Gampaha District in 2016 (Sandaruan *et al.*, 2017). Data were obtained through two non-consecutive 24-hour dietary questionnaires taken from 152 families. Based on the survey, weekly average fish consumption was 301 g per person. Eleven common fish species namely Indo-Pacific sailfish, shark, mackerel, yellowfin tuna, skipjack tuna, seer fish, trevally, spotted sardinella, sardinella, anchovy and mullets were identified. However, the

individual approaches were practiced on the first three species ranked in the survey. The T-Hg and Cd contents of cooked fish samples were shown in table 1 (given on wet basis).

According to the EU regulation (EU/EC 1881/2006 GOSL 1528/7) for T-Hg, the maximum allowable level was 1 mg/kg for SWF and YFT, while 0.5 mg/kg for SAF in wet weight basis. However, the EU/EC 488/2014, the maximum level of Cd was 0.1 mg/kg for YFT while 0.25 mg/kg for SWF and SAF. Sample numbers 6 (chili curry cooked for 15 minutes) and 7 (milk curry, 25 minutes) of SWF exceeded the maximum allowable level recommended by EU and GOSL. The YFT and SWF are considered as the top predators in the marine food chain and commercially important fish species in the Sri Lankan fish market. The results of the present study also suggest that the T-Hg content in the fish muscle is comparatively higher in larger fish species than in small pelagic fish species such as SAF. However, none of the samples exceeded the EU maximum allowable limits for Cd.

Chemical constituents in fish may also vary between individuals, due to age, gender, the habitat of the fish and also sampling site (Vollenweider *et al.*, 2011). SWF and YFT are carnivorous species present in the upper part of the food web compared to that of YFT. SWF feed on fish, while YFT feeds on fish, crustaceans, and squids. Crustaceans and squids are in the lower part of the food web, and they also feed on zooplankton. The SAF mainly feed on phytoplankton and zooplankton (Gushchin and Corten, 2015). As a result, the bio-accumulation and bio-magnification of T-Hg is reduced in large fish than in small fish. This is perhaps the reason that the highest T-Hg content recorded in SWF than in YFT.

Risk assessment of fresh and cooked fish

The exposure for Hg was estimated and calculated using the consumption data received from the TDS survey. The

Table 1: T-Hg and Cd contents (mg/kg) in cooked fish samples of SWF (swordfish), YFT (yellowfin tuna) and SAF (sardinella).

Fish No	Hg, mg/kg			Cd, mg/kg		
	SWF	YFT	SAF	SWF	YFT	SAF
1	0.16	0.11	#ND	ND	0.007	0.021
2	0.45	0.25	ND	0.006	0.012	0.019
3	0.06	0.27	0.07	ND	0.012	0.016
4	ND	0.51	ND	0.006	0.049	0.014
5	0.44	ND	ND	0.027	0.015	0.014
6	1.47	ND	0.07	0.029	0.015	0.007
7	1.07	0.11	0.07	0.014	0.010	0.014
8	0.42	0.95	0.09	ND	0.021	0.014
9	0.19	0.44	0.07	ND	0.007	0.016
10	0.11	0.19	ND	ND	0.009	0.016
11	0.34	ND	0.08	0.021	0.016	0.021

#ND-Not Detected

PTWI values for Hg and Cd are 4 µg/kg and 7 µg/kg of body weight, respectively. However, the European Food Safety Authority has given the Tolerable Weekly Intake (TWI) value of 2.5 µg/kg bodyweight for Cd. The PTWI value was calculated assuming that an average body weight of a person is 50 kg. Accordingly, the PTWI values for an average person were 200 µg for T-Hg and 350 µg for Cd (Subasinghe *et al.*, 2012).

Weekly average fresh fish consumption per person was 301 g. Percentage contributions of SWF, YFT, and SAF for PTWI-Hg were 64.9, 40.2 and 8.6, respectively. The results suggest that SWF species was closer to exceed the PTWI value, indicating that an average person (50 kg body weight) should not eat more than 425 g of SWF per week. The EU & USFDA regulations have clearly mentioned that children, pregnant women, lactating women, and women who are planning to get pregnant should refrain from eating SWF and YFT. The study confirmed that the contribution of SWF and SAF for PTWI-Cd was negligible, but YFT recorded 2.37% for PTWI-Cd. The study recommends further studies to find out the efficiency levels of different cooking method to reduce T-Hg in fish.

CONCLUSION

The percentage contribution of SWF, YFT & SAF species for Hg, PTWI was 75.4, 40.5 and 7.6, respectively. As SWF was closer to exceed the PTWI value, people should be advised not to consume SWF more than 425 g per week. However, the levels of Cd in studied fish species were significantly lower than established PTWI values.

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