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Dioxins and dioxin-like polychlorinated biphenyls in seafood: dietary exposure amongst Malaysian adult populations and its association with sociodemographic factors

*Leong, Y.H. and Majid, M.I.A.

National Poison Centre, Universiti Sains Malaysia, 11800 Penang, Malaysia

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Abstract

Seventeen polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans (dioxins) as well as twelve dioxin-like polychlorinated biphenyls (dl-PCBs) congeners were analysed in the seafood samples (prawn, squid, fish, processed seafood and dried anchovy). The average sum of dioxins and dl-PCBs in the seafood samples were 2.577 WHO_{2005}-TEQ (pg g^{-1} fw). Mean exposure to dioxins and PCBs (in pg TEQ kg^{-1} body weight day^{-1}) was assessed to be 0.333 and 0.643, respectively in the adult population (aged 18-59 years). Although the monthly dietary exposure of female was higher than male, but there is no significant difference found between the genders. Adults from rural area were reported to have the highest monthly exposure to dioxins and PCBs. Across the ethnicity, the highest exposure was found among the Malay, followed by Chinese and Indian. The daily, weekly and monthly exposure to all groups of the population were lower in comparison with the corresponding tolerable limits. However, the results obtained in this study indicated that there is a health risk to high consumer (those who consumes the amount of food corresponding to the 97.5th percentile of the consumption data) and caution of reducing the consumption of seafood should be taken.

Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are collectively designated as dioxins. Dioxins and dioxin-like polychlorinated biphenyls (dl-PCBs) are highly toxic and belong to a group of chemically and structurally related halogenated aromatic hydrocarbons. According to the International Agency for Research on Cancer (IARC), there are 210 theoretical possible congeners of PCDD/Fs, of which 17 show the same toxicology pathway with the most toxic 2,3,7,8-tetrachlorodibenzo-p-dioxin (2,3,7,8-TCDD). In contrast, of the 209 PCBs congeners, 12 show toxicological properties similar to that of dioxins (Safe, 1986) and therefore are often termed dl-PCBs. Dioxins and PCBs have been shown to pose severe adverse effects on human health, including carcinogenicity, immunotoxicity, and a range of endocrine effects related to reproductive function (Nakatani *et al*., 2011). These contaminants are persistent organic pollutants (POPs) and as a result, they accumulate in the body, especially in subcutaneous and visceral fat and liver, and to some extent are mobilised through the circulatory system (Hoogenboom *et al*., 2015).

Dioxins and PCBs are unwanted by-products formed via incomplete combustion, such as waste incineration, forest fires and some industrial processes (Srogi, 2008). In the past, PCBs have been produced and used in dielectric fluids in transformers, capacitors, and other electrical equipments (ATSDR, 2015). In the strategy to decrease human exposure to dioxins and PCBs, the European Commission (EC) has introduced maximum levels for dioxins and dl-PCBs in various food groups via Commission Regulation (EC) No. 1881/2006 (EC, 2006). WHO has set a tolerable daily intake (TDI) for the sum of PCDD/F and dl-PCB of 1–4 pg TEQ kg^{-1}body weight (WHO, 2000), which is comparable with a tolerable weekly intake (TWI) of 14 pg TEQ kg^{-1} body weight as fixed by the European Union Scientific Committee on Food (ECSCF, 2001). In 2002, a very similar health based guidance value which was extended to a monthly intake (provisional tolerable monthly intake, PTMI) of 70 pg TEQ kg^{-1} body weight was derived by the Joint FAO/WHO Expert Committee on Food Additives (JECFA, 2002). Recently, the US Environmental Protection Agency introduced a more stringent exposure limit (reference dose, RfD) of 0.7 pg TEQ kg^{-1} body weight day^{-1} based on effects on thyroid hormone levels and sperm production in children exposed in utero or postnatal in the Seveso incident (US EPA, 2012).

*Corresponding author.
Email: *yinhui_leong@yahoo.com*
Tel: +604 6534690; Fax: +604 6534688

The main pathways of human exposure to dioxins and dl-PCBs are dermal contact, inhalation and dietary intake. Over 90% of exposure to these contaminants for the general population is via food ingestion, in particular products of animal origin and fish (Hsu *et al*., 2007; Fernandes *et al*., 2010). In recent years, a number of studies on the occurrence of dioxins and PCBs in various food samples and the human exposure estimation have been reported in several countries over the world (Kilic *et al.,* 2011; Marin *et al*., 2011; Nakatani *et al.,* 2011; Tornkvist *et al*., 2011; Perello *et al.,* 2012; Sirot *et al*., 2012; Rauscher-Gabernig *et al*., 2013; Zhang *et al*., 2013; Husain *et al*., 2014). Some authors only investigated on particular food groups such as fish and seafood (Storelli *et al*., 2011; Wei *et al*., 2011; Wang *et al*., 2015), baby food (Lorán *et al*., 2010; Pandelova *et al*., 2010; Costopoulou *et al*., 2013; Jeong *et al*., 2014), fruit and vegetables (Grassi *et al*., 2010) as well as egg and egg products (Olanca *et al*., 2014). Fish as the main contributing food group to the exposure has been reported in certain studies (Zhang *et al*., 2008; Lorber *et al*., 2009). In this paper, we investigate the level of dioxins and dl-PCBs in seafood samples from Malaysia and estimate the dietary exposure amongst the adult population. The associations between the exposure with different genders, age groups and ethnics are also discussed in the present study.

Materials and Methods

Sampling

A total of 145 seafood samples, including fish, prawn, squid, dried anchovy, and other processed seafood products were collected in 2012 in Malaysia. In this study, a variety of fish species, such as tilapia, grouper, pomfret, barramundi, horse mackerel, and snapper, were investigated. Processed seafood products were referred to canned sardine, canned crab meat, fish ball, octopus ball, tempura seafood, and crab stick. Samples were treated by homogenising the edible part using a food blender and stored at -18^{o}C prior to analysis.

Standards solutions and chemical reagents

Solvents (dichloromethane, toluene, and hexane) were of pesticide grade purchased from Fisher Scientific (Leicester, UK). Hydromatrix was obtained from Agilent Technologies (Lake Forest, CA, USA). Dioxins calibration standards (EDF-9999), dl-PCB calibration standards (EC-4976), 13C12-labelled internal standard for dioxins (EDF-8999) and PCBs (EC-4937), and recovery standard for dioxins (EDF-5999) and PCBs (EC-4978) were supplied by Cambridge Isotope Laboratories, Inc. (Andover, MA, USA).

Extraction and instrumental analysis

Methodology for extraction and instrumental analysis has been described somewhere (Leong et al., 2014). Basically, extraction of dioxins and dl-PCBs from seafood samples was adopted from United States Environmental Protection Agency (USEPA) Method 3545, using a Dionex ASE 350 accelerated solvent extractor (ASE) device. A clean-up process through a series of multilayer silica, alumina, and carbon columns, was performed using Power-Prep Fluid Management System (Fluid Management System Inc., Waltham, MA, USA). The clean-up procedure was derived from the Smith–Stallings method outlined in USEPA Method 8290. High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry (HRGC/HRMS) was used for dioxins and dl-PCBs analysis following the USEPA Methods 23, 1613, and 8290.

Dietary intake calculation and statistical analysis

Dietary intakes of dioxins and dl-PCB was calculated by multiplying the TEQ of dioxins and dl-PCBs (pg TEQ g-1) by the food consumption data from Malaysian food consumption statistics (Ministry of Health Malaysia, 2006). An average body weight of 60 kg for adults was taken for the purpose of estimating monthly dietary exposure in pg TEQ kg^{-1} body weight month^{-1}.

Data were analysed using the Statistical Package for Social Science (SPSS, version 17.0 for Windows). One-way ANOVA was conducted to determine significant interactions of dietary exposure with several demographic factors. Significant findings were further analysed using Tukey's post hoc tests. Pearson correlation was used to correlate two quantitative variables. The probability value of 0.05 was used to determine statistical significance.

Results and Discussion

In this study, all the results of the individual WHO-TEQs dioxins and dl-PCBs were calculated according to the WHO Toxic Equivalency Factors (WHO2005-TEFs) (Van den Berg *et al*., 2006) and reported on a fresh-weight (fw) basis. The concentration of dioxins ranged from 0.13 to 1.03 pg TEQ g^{-1} fw (mean 0.16) while dl-PCBs ranged from 0.34 to 0.44 pg TEQ g^{-1} fw (mean 0.36) (Table 1). The highest concentration of dioxins was detected in fish (average 0.201 pg TEQ g^{-1} fw), while processed food and dried anchovy were found to contain the highest

Table 1. WHO_{2005}-TEQ (pg g^{-1} fw) for PCDD/Fs and dl-PCBs analysis in seafood samples

Samples	PCDD/Fs (pg TEQ g-1 fw)*	dl-PCBs (pg TEQ g-1 fw)*
Prawn	0.153 (0.13-0.26) (n=20)	0.353 (0.35-0.39) (n=34)
Squid	0.156 (0.13-0.24) (n=9)	0.348 (0.34-0.35) (n=11)
Fish#	0.201 (0.13-1.03) (n=19)	0.354 (0.35-0.36) (n=13)
Processed Seafood	0.147 (0.13-0.31) (n=11)	0.362 (0.35-0.44) (n=17)
Dried anchovy	0.141 (0.13-0.16) (n=4)	0.362 (0.35-0.39) (n=7)
Total	0.798	1.779
Sum of PCDD/F and PCBs2.577		

*Results presented as mean in upper bound value and range in parentheses

#Total daily consumption of freshwater and marine fish

dl-PCBs (average 0.362 pg TEQ g^{-1} fw) among the samples. The average sum of dioxins and dl-PCBs in the seafood samples was 2.577 pg TEQ g^{-1} fw. All the 17 and 12 congeners of dioxins and dl-PCbs were detected in seafood samples except the octachlorinated dibenzofurans (OCDF) and PCB 167. Among the 17 congeners, 1,2,3,7,8-pentachlorodibenzo-p-dioxin (1,2,3,7,8-PeCDD) was the most abundant congener (average 40%) in the analysed samples. This finding was consistent with the reports from Matthews et al. (2008) and Nasir *et al*. (2011). For the dl-PCBs, PCB 126 contributed the highest values, accounting around 84% on average followed by PCB 169. Similar pattern in seafood species from Australia has also been reported by Matthews *et al.* (2008).

Mean exposure to dioxins and PCBs (in pg TEQ kg^{-1} body weight day^{-1}) was assessed to be 0.333 and 0.643, respectively in the adult population. However, the dietary exposure estimations were much lower than the tolerable daily intake (TDI) of 1-4 pg TEQ kg^{-1} body weight per day as recommended by the WHO. Estimated dietary intake of dioxins and dl-PCBs for average and high consumer in Malaysia, according to different socio-demographic factors are shown in Table 2. High consumer is defined as those who consumes the amount of food corresponding to the 97.5th percentile of the consumption data from the Malaysian Adult Nutrition Survey 2003. Dietary intakes (pg TEQ kg-1 body weight $month^{-1}$) for different category of socio-demographic factors ranged from 7.43 to 12.60 and 14.98 to 24.37 respectively, for dioxins and PCBs for the average consumers. For high consumers, the dietary intakes were from 34.90 to 59.22 pg TEQ kg^{-1} body weight $month^{-1}$ for dioxins and from 71.09 to 118.88 pg TEQ kg^{-1} body weight $month^{-1}$ for PCBs. The monthly dietary intakes of total dioxins and PCBs for average population were much lower than the PTMI of 70 pg TEQ kg^{-1} body weight established by JECFA (22.41-36.97 pg TEQ kg^{-1} body weight $month^{-1}$). The level of dietary exposure in this study was also lower than study conducted by Zhang *et al.* (2008) and Wang *et al.* (2015) in China. However, dietary intakes of total dioxins and PCBs for the high consumers in all categories were found exceeding the PTMI, with the highest intake reported in adults from 18-19 age group (178.10 pg TEQ kg^{-1} body weight $month^{-1}$). Although PTMI is derived for lifetime exposure, however, caution should be taken among the high consumers to reduce the seafood consumption in their daily diet. Domingo and Bocio (2007) reported that some population who frequently consuming fish and seafood could be significantly increasing their health risk due to the dioxins and PCBs exposure.

It was noticed that the amount of dietary intake for both the dioxins and PCBs was higher in male than female, but the monthly dietary exposure of female was higher than male. However, there is no significant difference of dietary exposure found between the genders. Similar results were reported by Zhang *et al.* (2013). Perelló *et al.* (2012) also reported that female showed higher dietary intake of dioxins and PCBs than male in Spain. A significantly higher dietary exposure in female than that of male has been found in Hong Kong (FEHD, 2011). In this study, no correlation was observed between the amount of dietary intake and age of adults. This is probably the food consumed among adults from age 18 to 59 are almost similar. Undoubtedly, limited choice of food and relatively lower body weight in children would lead to higher exposure compared to adults. Literature reviews showed significantly higher exposure in children than that of adults (Bergkvist *et al.*, 2008; Perelló *et al.,* 2012; Zhang *et al*., 2013). The same situation might reflect for Malaysian children, although the estimation of daily intake for children is not performed in this study because of the lack of detailed food consumption data.

Adults from rural area were reported to have the highest intake of dioxins and PCBs among all the categories. The exposure was up to 12.60 and 24.37 pg TEQ kg^{-1} body weight $month^{-1}$ for dioxins and PCBs respectively and were significantly higher than those from urban. This may be due to the high consumption of fish (89.67 g day^{-1}) by rural adults whereby fish carried a highest level of contamination among the seafood samples. According to Norimah *et al.* (2008), fish ranked second among the top 10 daily consumed foods in Malaysia, especially in rural areas. In addition, this phenomena also reported by Arisawa *et al.* (2008), in which the dietary intake was highest

Table 2. Estimated dietary intake of dioxins and dl-PCBs for Malaysian population according to different socio-demographic factors

Socio-demographic factor	Amount of dietary intake (pg TEQ day^{-1})		Dietary exposure (pg TEQ kg^{-1} body weight month^{-1})			
	Dioxins	dl-PCBs	Dioxins		dl-PCBs	
			Mean*	P97.5#	Mean	P97.5
Gender						
Men	22.18	43.62	9.93	47.03	19.53	93.45
Female	21.11	41.30	10.92	54.19	21.39	106.63
Age group						
18-19	23.37	46.43	11.68	59.22	23.22	118.88
20-29	21.21	41.78	10.61	50.59	20.89	100.08
30-39	21.91	42.86	10.96	50.60	21.43	100.49
40-49	21.81	42.40	10.90	52.07	21.20	102.41
50-59	21.70	41.98	10.85	52.90	20.99	104.39
Area						
Urban	19.23	38.06	9.62	48.86	19.03	96.72
Rural	25.19	48.74	12.60	52.50	24.37	103.53
Ethnic						
Malay	24.49	47.67	12.25	53.63	23.83	106.27
Chinese	16.29	32.34	8.15	34.90	16.17	71.09
Indian	14.85	29.97	7.43	35.82	14.98	72.29

Average body weight of 60 was used for the calculation, except average of 67 kg (men) and 58 kg (women) were used for the calculation that involved gender

*Refer to mean of food consumption

#Refer to 97.5th percentile of food consumption

in fishing areas, followed by farming and urban areas in Japan. In the study of Tsuchiya *et al.* (2003), the mean dioxin concentrations in the fishermen, the farmers and the controls were 161,369, 79,079 and 100,500 pg g^{-1} fat, respectively. The elevated blood dioxin concentration with polychlorinated dibenzo-p-dioxins, polychlorinated dibenzofurans and PCBs found in the fishermen may be due to the frequent consumption of fish. There was a significant difference in dietary exposure of dioxins and PCBs across the ethnicity. The highest intake was found among the Malay, followed by Chinese and Indian for both the dioxins and PCBs. Different living style or cultural background leads to a very different food habits among the ethnic groups. Socioeconomic may also play a role and affect the dietary pattern. Most of the fishermen are Malays, therefore Malays are also consuming the fish the most among the three ethnics. According to Malaysian food consumption data, Malay consumes the highest fish amount (84.42 g day^{-1}), followed by Chinese (51.33 g day^{-1}) and Indian (42.84 g day^{-1}).

Conclusion

The occurrence of dioxins and dl-PCBs in seafood samples in Malaysia is considered low compared to other industrialised countries. The daily, weekly and monthly exposure to all groups of the population were lower than the corresponding tolerable limits. However, these results may present a health risk to high consumer. The chronic exposure will increase the human body burden and should be avoided by reducing the intake of certain high risk seafood. Therefore, routine monitoring of human food for these contaminants is of major importance and should be enforced and regulated by local authorities.

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