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PCDDs and PCDFs in Pelagic Fish along the Straits of Malacca

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ABSTRACT

Fish and shellfish are rich sources of long chain fatty acids, especially DHA and EPA. High consumption of fish helps to elevate the level of these compounds in the body. However, fish also are easily exposed to chemical contaminants, such as dioxins (PCDDs) and furans (PCDFs). Exposure to PCDDs and PCDFs may lead to negative health effects, such as cancer, chloracne, hyperpigmentation and others. Level and type of PCDDs and PCDFs were determined in 20 pelagic fish samples of six different species collected from the Straits of Malacca using HRGC/HRMS. The most toxic congener (2,3,7,8-TCDD) was found in all the samples at a very low level of 0.04-0.05 pg/g sample, except in Spanish mackerel (south-T2) and Indian mackerel (middle-T1). Meanwhile, the level of the total PCDDs and PCDFs ranged from 0.13 pg/g to 0.38 pg/g of the wet weight of the samples. The value of the total PCDDs and PCDFs was in a descending order of Hardtail scad, Spanish mackerel, Indian mackerel, fourfinger threadfin, silver pomfret and dorab wolfherring. Generally, the results of this study indicate that fish and shellfish caught along the Straits of Malacca are safe as in terms of PCDDs and PCDFs levels and the data can serve as baseline information for future monitoring of these organochlorine compounds.

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INTRODUCTION

The fisheries along Straits of Malacca contribute approximately 70% of the total marine resources of Peninsular Malaysia (Annual Fisheries Statistics, 2004). These resources are made up of two major groups, which are demersal and pelagic. Pelagic fish live in the water column of coastal, ocean and lake waters, but not at the bottom of sea or lake. They can be compared with demersal fish which live near or at the bottom of the sea (Atmadja, Sadhotomo & Suwarso, 1995). The marine pelagic environment is the largest aquatic habitat on earth and it comprises 11% of the known fish species (Atmadja *et al.,* 1995). The families of *Pampus, Megalapsis, Epinephulus, Eleutheronema, Rasterlliger, Chirocentrus* and *Scomberomorus* are some examples of pelagic fish (Lui, 1992).

The concern of dietary recommendation to achieve an adequate intake of long chain (LC) *n*-3 PUFA is growing as knowledge about its beneficial effects has become a more concerning issue among people (Isabelle *et al*., 2008). A few studies reported that a modest increase in the consumption of LC *n*-3 PUFA would have important and beneficial health outcomes (Gebauer *et al*., 2006; Wang *et al*., 2006). Increased fish and shellfish consumption is suggested as a good approach and possible strategy to increase LC PUFA intakes in order to bridge the gap between the current intakes and dietary recommendations (Isabelle *et al*., 2008). At the same time, however, fish and other shellfish are also sources of persistent chemical contaminants that accumulate in the marine environment by bioaccumulation (Giesy *et al*., 1997). As a result, this strategy comes out with some conflict issues, whereby increasing fish intake to elevate omega-3 PUFA intake will also simultaneously increase the intake of contaminants to a level of toxicological concern (Giesy *et al*., 1997).

The Straits of Malacca, which occupies along the west coastal water of Peninsular Malaysia, is one of the world's busiest oil transport routes (Lui, 1992). The marine environment of the Straits may be affected by any accidental oil spills that has occurred. For example, the incidence of oil and grease content spill found on the Perak coast in 1990 had affected the coastal areas of Negeri Sembilan, Melaka and Selangor (Environmental Quality Report, 1990). Coastal marine environment pollution is also one of the major threats to the Malaysian fisheries industry. Besides marine pollution, land-based pollution, destruction of natural habitats and incomplete combustion from factories could also be the major treats to the marine environment (Lulofa, 1977; Sasekumar, 1980). All these may lead to high abundance of persistent organic pollutants (POPs), such as polychlorinated dibenzo-*p*-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) in fish and shellfish caught along the Straits of Malacca, thus increasing exposure to human. Therefore, it is important to investigate the presence of PCDDs and PCDFs in fish and shellfish in a way to minimize any health problem consequences.

PCDDs and PCDFs are commonly known as dioxins and furans, respectively, and they are also considered as persistent, bioaccumulative and toxic environmental contaminants (Geyer *et al*., 2002). PCDDs and PCDFs compounds have been shown to accumulate in fish and wildlife (Kadokami *et al.,* 2002; Brunstrom *et al.,* 2001; Huang *et al.,* 1999; Woodford *et al.,* 1998). Therefore, they are likely to be present in oily fish. Their presence in the marine environment leads to accumulation in the food chain of fish, with levels being highest in large predatory species (Jacobs *et al.,* 1998).

More than 90% of the intakes of PCDDs and PCDFs by general population are derived from meat, dairy products, and fish (Schecter, 1997; Bocio & Domingo, 2005; Charnley & Doull, 2005; Huwe & Larsen, 2005). In Tokyo, Japan, 40% of the daily intakes of PCDDs and PCDFs are derived from fish and shellfish (Sasamoto *et al*., 2006). Meanwhile, a study in Spain showed that around 31% of PCDDs and PCDFs were found in fish and other seafood intakes (Llobet *et al*., 2003).

As PCDDs and PCDFs have now become worldwide concerns, studies on these contaminants are therefore needed to monitor fish and shellfish intakes and achieve the nutritional requirement without exceeding toxicological thresholds. The determination of the type and level of these contaminants in food is very important for dietary exposure assessment, protection of public health, and increasing availability of safe marine fish to consumers (Elliot *et al*., 1996). Therefore, the aim of the study was to determine the levels of PCDDs and PCDFs in fresh pelagic fish species available at fish landing areas along the Straits of Malacca. This included viewing the trend of these contaminants in the fresh marine products. The results obtained are useful as baseline information for the control of PCDDs and PCDFs release into the marine environment.

MATERIALS AND METHODS

Reagents

All reagents used were of analytical grade. Meanwhile, the chemicals used are such as hydromatrix (Framton Ave, Harbour City), dichloromethane (DCM) (Fisher Scientific, Fair Lawn, New Jersey) and hexane (Fisher Scientific, Leicestershire, UK). These chemicals were used for extraction, pre-treatment and clean-up of the samples before PCDDs and PCDFs determination using high-resolution gas chromatography/ high-resolution mass spectrometry (HRGC/ HRMS).

Fish Samples and Preparation

The stratified sampling method was used in the collection of the samples. Fresh samples consisted of 20 available fish samples from six different species which had been collected from three regions along the Straits of Malacca: North (Kuala Perlis, Kuala Kedah, Teluk Bahang, Pulau Betong), Middle (Melaka, Port Dickson, Muar) and South (Kuala Selangor, Manjung Utara, Matang). All the samples were collected at two different times in August,

2008 (T1) and November, 2008 (T2). The collection of samples was carried out with the help of some officers from Lembaga Kemajuan Ikan Malaysia (LKIM). The pelagic fish consisted of the following species: *Rastrelliger kanagurta* (Indian mackerel), *Scomberomorus guttatus* (Spanish mackarel), *Pampus argenteus* (silver pomfret), *Megalapsis cordyla* (Hardtail scad), *Eleutheronema tradactylum* (Fourfinger threadfin), and *Chirocentrus dorab* (Dorab wolfherring).

The collected samples were brought to the laboratory on the same day. The samples were delivered to the laboratory in a sealed polystyrene box and stored in a freezer (-20°C) at Dietetic laboratory, UPM. For the analysis, the muscle tissues of the fish were taken and weighed in gram (g). The composite sample of the same species from the same region was prepared after the samples had been gutted, washed and filleted. The prepared samples were transferred into a polyester covered cup and stored in a freezer (-20°C) before further analyses. The samples were sent to Doping Control Centre (DCC), Penang, in a cold box to prevent from any damage. The determination of PCDDs and PCDFs in fish lipid samples was carried out at DCC, USM Penang, which is one of the Accredited Laboratories for doping control analyses and recognized by World Anti-Doping Agency, (WADA).

Analysis of the PCDDs and PCDFs Concentrations in the Samples

The procedure utilized for the determination of dioxins and furans was adopted based on the principle laid by US EPA method 8290 for PCDDs and PCDFs by HRGC/HRMS. The method was slightly modified by DCC which provides procedures for the detection and quantitative measurements of PCDDs and PCDFs in variety matrices at part per trillion (ppt) concentrations. The modified procedure uses matrix specific extraction, analyte, specific clean-up and HRGC/HRMS analysis techniques.

Before extraction, the samples were thawed at room temperature and this was followed by spiking 10 g of wet sample of fish into 50 µl C_{13}-labelled internal standard (8999) (Cambridge Isotope Laboratories Inc., USA). Later, 10 g of hydromatrix was mixed into the sample before homogenization with a mortar. The homogenized sample was then dried in an oven (Petaling Jaya, Selangor, Malaysia) at 50°C for two minutes to hydrate the moisture content until powder was formed. The powder was placed in a cell (size 33) that was closed with Ottawa sand (Fisher Scientific, Leicestershire, UK) before entering into Accelerated Solvent Extraction [ASE 200) (DIONEX Corporation U.S Patents, Sunnyvale, USA)] machine for 20 minutes for fat extraction. The fat was extracted using DCM solvent. The mixture of the extracted fat and DCM was dried using a rotary evaporator (BUCHI Labortechnik, Flawil, Switzerland) for 20 minutes and filtered to get crude fat extract. The fat content was determined gravimetrically, and later, hexane was mixed into the extract to form an aliquot.

The aliquot was placed in a fully automated device, Power-Prep Fluid

Management System (FMS) (Fluid Management System, Inc. Waltham, USA) for a clean-up process that involved three types of column (Fluid Management System, Inc. Waltham, USA), silica (CLDS-ABN-STD), alumina (CLDA-BAS-011), and carbon (CLDC-CCE-034). The clean-up process involved 23 steps to remove the bulk matrix components and enrichment of the target analysis. The involvement of the three column chromatographic clean-up procedure was derived from Smith Stalling method outlined in the US EPA Method 8290. After completing the FMS procedure, the collected PCDDs and PCDFs (mixed with solvent) were dried using a rotary evaporator before spiking them with 50 µl external standard (5999). Later, the solvent was dried using heating block (Dri Block DB-20, Staffordshire, OSA, UK) and nitrogen gas (TESCOM Corporation, ELK River, USA) at 60°C. The collected crude PCDDs and PCDFs mixture was placed in a small covered aluminium foil vial before the analysis.

HRGC/HRMS (GC: Thermo scientific, Trace GC Ultra Rodano, Milan, Italy; MS: Hannah-Kunathstr, Bremen, Germany) were used for the analysis of PCDDs and PCDFs. Each analysis included the determination of seventeen dioxin and furan congeners with 2, 3, 7, 8-chloro-substitution (ten PCDFs and seven PCDDs). Meanwhile, calibration standard was used to construct the calibration curve using the QUAN programme in the XCalibur software (Thermo scientific, Milan, Italy). EDF-4141 window-defining standard (Thermo scientific, Milan, Italy) was used to ensure the first and the last eluting analytes in each sample. Concentrations in the fish samples were calculated on a wet weight (w.w.) basis. Recoveries for internal standards were more than 50% for all the congeners.

Toxicity equivalency (TEQ) was calculated using the procedures developed by World Health Organizational (2005). In this study, PCDDs and PCDFs toxicity were expressed as Toxicity Equivalents (i.e., total toxicity) of the seventeen 2, 3, 7, 8-substituted PCDDs and PCDFs congeners. TEQ was calculated by multiplying the absolute concentration of each congener by a numeric factor that expresses the concentration in terms of the most toxic dioxin molecule, 2, 3, 7, 8-TCDD (tetrachlorodibenzodioxin), which is given a value of one. In cases where congeners were reported as non-detects, limit of quantification (LOQ) would be used as result.

RESULTS AND DISCUSSION

This study was undertaken to investigate the levels of PCDDs and PCDFs in selected pelagic fish. Table 1 shows the fat content (%) and the level of PCDDs/PCDFs (WHO I-TEQ) from the analyses of the fish fillet for each sample. The Indian mackerel species from the middle region the and Spanish mackerel from the south region contained high percentages of fat content, with 5% and 4.35%, respectively. The lowest percentage of fat content was observed in the hardtail scad and Indian mackerel from the north region at 0.8% and 2.05%, respectively.

TABLE 1
WHO I-TEQ (pg/g), fat content (%) and percentage of variation of PCDDs and PCDFs in pelagic fish by region along Straits of Malacca

Region	Species	Common name	Fat content (%)		Mean	Percentage of variation (%)	WHO I-TEQ (pg/g)		Mean	Percentage of variation (%)
			T1	T2			T1	T2		
North	*Pampus argentus*	Silver pomfret	3.6	3.7	3.65	1.94	0.19	0.12	0.16	30.94
	Megalapsis cordyla	Hardtail scad	0.6	1.0	0.80	35.36	0.17	0.16	0.13	5.44
	Eleutheronema tradactylum	Fourfinger threadfin	2.0	2.7	2.35	21.06	0.17	0.13	0.15	18.85
	Rastrelliger kanagurta	Indian mackarel	1.0	3.1	2.05	72.44	0.21	0.73	0.14	262.64
Middle	*Megalapsis cordyla*	Hardtail scad	2.8	3.5	3.15	15.71	0.34	0.37	0.21	10.10
	Eleutheronema tradactylum	Fourfinger threadfin	1.8	3.3	2.55	41.59	0.13	0.12	0.17	4.16
	Rastrelliger kanagurta	Indian mackarel	3.3	6.7	5.00	48.08	0.12	0.14	0.18	7.86
South	*Megalapsis cordyla*	Hardtail scad	0.4	3.1	1.75	109.09	0.90	1.57	0.38	124.67
	Chirocentrus dorab	Dorab wolfhering	3.8	3.5	3.65	5.81	0.12	0.14	0.16	8.84
	Scomberomorus guttatus	Spanish mackarel	3.2	5.5	4.35	37.79	0.25	0.30	0.30	11.79

(T1 = trip 1; T2 = trip 2)

On the contrary, high fat contents were found in the species from the middle and south regions compared to the north region samples of the Straits of Malacca. Based on the results obtained, the percentage of fat by trip was higher in the samples taken from trip 1 (T1) as compared to the sample from trip 2 (T2), except for dorab wolfherring species (T1: 3.8%; T2: 3.5%). The differences in the fat content between T1 and T2 could be related to the differences in the maturity of the fish caught as the analysed samples were those available at the point(s) of collection. Previously, Osman *et al*. (2001) reported lower fat contents of Spanish mackerel (1.46%), fourfinger threadfin (2.24%), hardtail scad (3.08%) and silver pomfret (2.91%) species compared to the data of the present study. The higher level of fat in the samples of this study was found to be related to the different extraction methods used, in which ASE operated at higher temperature (125°C) and pressure (1500 psi), and thus allowed an optimum extraction of fat and provided good recovery and precision for determination of organochlorine compounds, as shown in Table 1 (Ezzell *et al*., 1996).

In this study, the levels of PCDDs and PCDFs were reported as WHO I-TEQ in pg/g wet weight of sample. The hardtail scad and Spanish mackerel species taken from the south region showed the highest values of the total PCDDs/PCDFs at 0.38 pg/g and 0.30 pg/g, respectively. Conversely, both hardtail scad and Indian mackerel species from the north region showed the lowest values of PCDDs/PCDFs at 0.13 pg/g and 0.14 pg/g, respectively. Generally, the levels of PCDDs and PCDFs were higher in the samples from the south region compared to the ones taken from the middle and north regions. Besides, the levels of PCDDs and PCDFs were also higher in T2 as compared to T1, especially for the sample from the middle and south regions. It has been well established that biotic and abiotic factors affect the degree and exposure of chlorinated compounds in fish including the proximity of fish to contaminated sediment, magnitude of contamination in their habitats, fish movement patterns, the tropic status, growth rates, fish age and bioavailability of the contaminants (Stow *et al*., 1994; Bentzen *et al*., 1996). Besides, species' specific metabolism and detoxification of contaminants, reproductive and maturational, as well as the level of body fat can affect the accumulation of contaminant in fish tissues (Bentzen *et al*., 1996; Larsson *et al*. 1996). Therefore, some of the above mentioned factors may explain the differences observed.

The findings of this study showed that the PCDDs and PCDFs levels in pelagic fish were not influenced by the fat content of the samples that much since the fat content in the fish sample was not directly proportional to the levels of PCDDs and PCDFs. The concentration of lipid found in the fish samples ranged from 0.4% to 6.7%. A wide variation (1.94% - 109.09%) in terms of the fat content exist between the samples collected in T1 and T2 was due to the differences in the fish's maturity and sizes as the samples were those available

during the sampling time. According to Jacobs *et al.* (2002), younger fish consume lower levels of feed and thus tend to store lower lipid in their adipose tissue compared to mature fish. This situation may reflect the lipid content of the samples (Jacobs *et al*., 2002). Besides the differences in maturity, the variation in the fat contents could also be due to certain individual fish that utilize high-energy diets but deposit little lipid in their flesh, but tend to have greater adiposity in some others (Bell, 1998).

In an Italian study, the reported mean PCDDs/PCDFs in mackerel species was relatively low at 0.22 pg/g WHO I-TEQ (Taioli *et al*., 2005). Meanwhile, in the samples from Japan, Sasamoto *et al*. (2006) found that the total concentration of PCDD/PCDFs and dioxin-like PCBs in fish and shellfish ranged from 0.98 to 0.91 pg/g WHO I-TEQ between 1999 and 2004, respectively. Moreover, the concentrations of PCDD/PCDFs in 40 species of marine organisms from the Korean coastal waters were shown to vary from 0.02 to 4.39 pg/g WHO I-TEQ (Moon & Ok, 2006). A study done by Li *et al*. (2007) in China found that aquatic foods which had been obtained from a local market contained high concentrations of PCDDs and PCDFs at 0.18 pg/g WHO I-TEQ, compared to the other food groups. The findings from various studies revealed that the levels of PCDDs and PCDFs were relatively low, i.e. below the permitted level (< 4pg/g) underlined by World Health Organization (WHO, 2005). The data obtained in the present study also showed low levels of PCDDs and PCDFs in the pelagic fish samples along the Straits of Malacca, ranging from 0.13 to 0.38 pg/g WHO I-TEQ.

There are many fish landing areas along the Straits of Malacca, and they serve as fish and shellfish collection sites (Annual Fisheries Statistics, 2004). Based on the brief observation during the sample collection, there were quite a number of industrial spots near the collection sites. Human activities, such as land development, agriculture and high population density, have been well established as the main causes of marine water pollution (UNEP/GPA, 2006). Previously, Choo *et al*. (1994) reported that the activities of agro-based and pesticide industries along the West Cost of Peninsular Malaysia might have important contributions to PCDDs/PCDFs in the marine environment. The existence of PCDDs and PCDFs in the fish samples also could be due to the strategic location of the strait as a major international shipping lane (Annual Fisheries Statistic, 2004; Choo *et al*., 1994). In fact, the growth of agriculture and industrial sectors, as well as urbanization which predominates the west coast of Peninsular Malaysia, is among the sources of these persistent organic pollutants (Annual Fisheries Statistic, 2004). Additionally, the north, middle and south regions of the Malacca Straits have different industrialization stages and types (Chua *et al*., 1989), and these may have contributed to the release of PCDDs/PCDFs into the marine environment.

The by-products formation resulted from industrial processes such as

smelting, bleaching and processing of paper pulp (Abbot & Hinton, 1996), manufacturing of some herbicides or pesticides, polychlorinated biphenyls (PCBs), pentachlorophenol, and polyvinyl chlorides (PVCs), burning of plastics and toxic waste at high temperatures with waste incinerators or kilns, as well as motor vehicle exhaust, charcoal grills and cigarette smoke are the major sources of PCDDs and PCDFs (Harte *et al*., 1991; Hoffman *et al.,* 1995; Schettler *et al*., 1999; Im *et al*., 2002). The industry which operates within the nearest coastal vicinity is probably one of the factors contributing to the increasing level of persistent organic pollutants in the marine ecosystem which simultaneously increase the levels of dioxins and furans in pelagic fish.

Fig.1, Fig.2 and Fig.3 depict the congeners of PCDDs/PCDFs in three species, with the highest PCDDs/PCDFs in T1 and T2. According to Gene *et al*. (2008), the congeners that contributed to the highest toxicity were 1,2,3,7,8-PeCDD, 2,3,7,8-TCDD, 1,2,3,4,7,8-HxCDD and 2,3,4,7,8-PeCDF. In all the studied samples, 1,2,3,7,8-PeCDD congener was present at the highest concentration compared to other congeners. Seventy-eight percent of the total toxicity was derived from the four congeners averaged toxicities (Schecter *et al.,* 1994). The most toxic congener of PCDDs and PCDFs was 2,3,7,8-TCDD, and it was classified as a Group 1 carcinogen (i.e. a known human carcinogen) by WHO's International Agency for Research on Cancer in 1997 (WHO, 1999). This particular congener was found in all the samples except in Spanish mackerel (south-T2) and Indian mackerel (middle-T1). The concentration of this congener, however, was very low, i.e. at 0.04 – 0.05 pg/g sample. The highest 2,3,7,8-TCDD congener was found in Indian mackerel (middle-T2) at 0.05 pg/g sample.

A summary of the total PCDDs and PCDFs contents is shown in Fig.4, whereby the different levels of these contaminants are clearly found in the different species. Overall, hardtail scad exhibited the highest levels of the total PCDDs and PCDFs contaminations as compared to the other species. Other species of pelagic fish

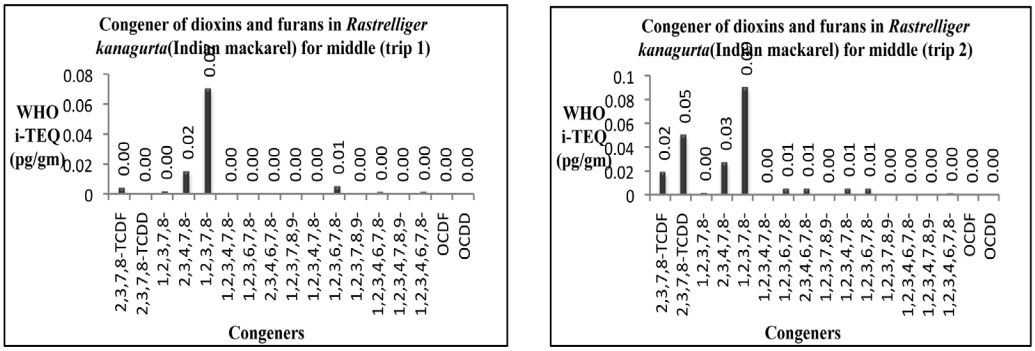

Fig.1: Dioxin and furan congeners concentration in *Rastrelliger kanagurta* (Indian mackerel)

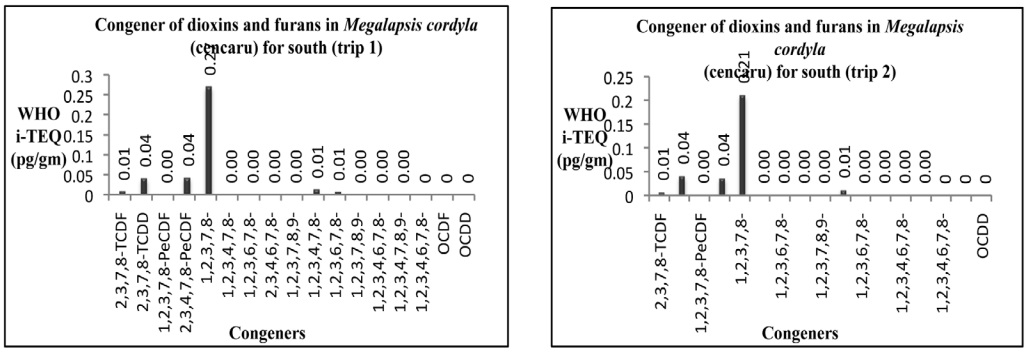

Fig.2: Dioxin and furan congeners concentration in *Megalapsis cordyla* (hardtail scad)

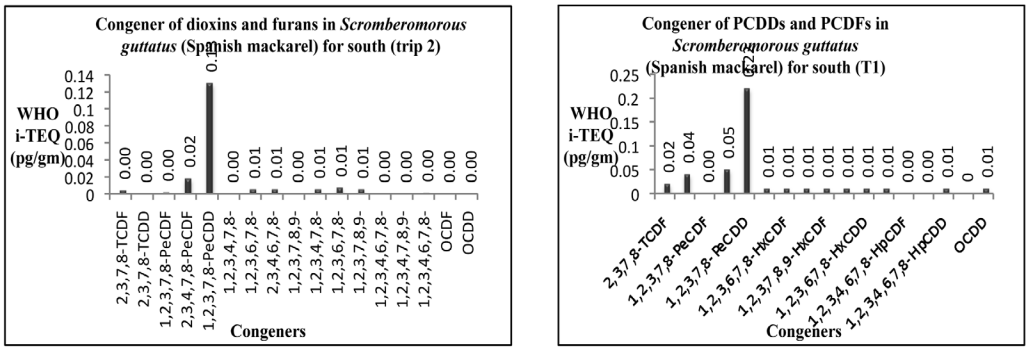

Fig.3: Dioxin and furan congeners concentration in *Scromberomorous guttatus* (Spanish Mackarel)

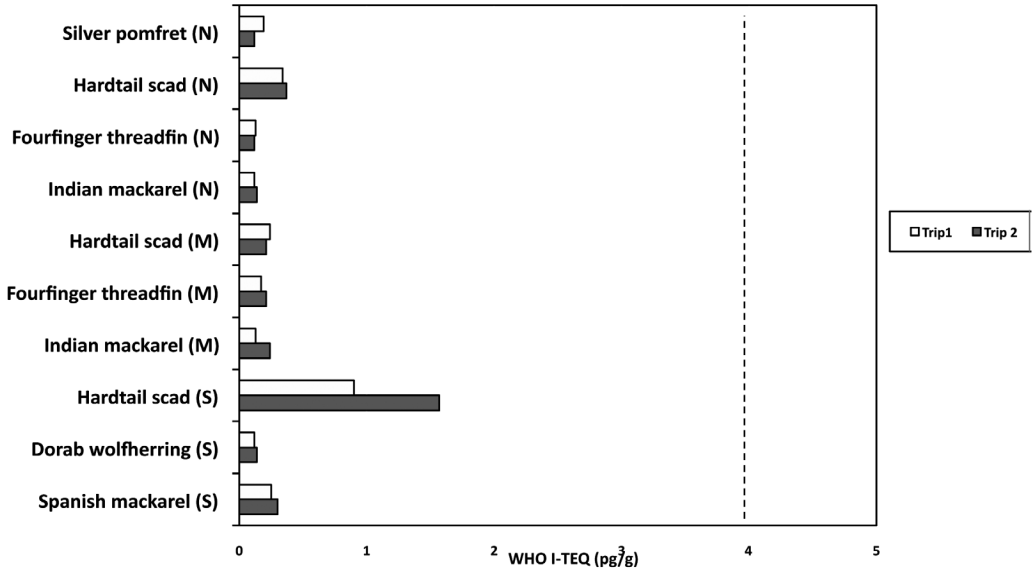

Fig.4: Total WHO i-TEQ of PCDDS and PCDFs distribution in selected pelagic fish by region and trip

had the total PCDDs/PCDFs of less than 0.5 pg/g WHO I-TEQ. Generally, the level of PCDDs/PCDFs in all the samples determined in this study was well below the permitted level of 4 pg/g as prescribed by WHO (2007) for total PCDDs/PCDFs. Although the data are limited, the findings of this study can serve as baseline information to increase public awareness of the PCDDs and PCDFs compounds in the fish samples from the Straits of Malacca.

CONCLUSION

This study has demonstrated the levels of PCDDs and PCDFs in the pelagic fish from Straits of Malacca. In particular, the levels of PCDDs and PCDFs ranged from 0.13 pg/g to 0.38 pg/g of the wet weight of the samples. The values of the total PCDDs and PCDFs were in a descending order of *Megalapsis cordyla* (hardtail scad), *Scomberomorus* (Spanish mackarel), *Rastrelliger kanagurta* (Indian mackarel), *Eleutheronema tradactylum* (fourfinger threadfin), *Pampus argentus* (silver pomfret) and *Chirocentrus dorab* (dorab wolfherring). In general, the detected levels of both PCDDs and PCDF in the studied samples were low compared to the international permitted level, and thus indicated the safety of fish species caught along the Straits of Malacca. These data are important as these serve as baseline information for future studies. However, continuous monitoring of these contaminants is strongly recommended to ensure fish safety.

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