Investigation on the Level of Furans and Diozins in Five Commonly Consumed Fish Species

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ABSTRACT: Five commonly consumed marine fish from Straits of Malacca were studied for their level of dioxins (PCDDs) and furans (PCDFs). The fish fillets were found to contain low to moderate level of fats. Among the moderate fat content species, *Plotosus spp.* (Sembilang) was found to contain the highest level of total PCDDs/Fs (1.23 $\pm$ 0.48 pg/g of wet weight) due to the high fat content of the species. Other species however, contain low level of PCDDs/Fs that range between 0.10-0.18 pg/g of wet weight. Generally the levels of total PCDDs/Fs in fish species determined in this preliminary study were well below the European limits and are therefore safe for human consumption.

Keywords: furans, PCDD, dioxins, PCDF, fish

Introduction

Polychlorinated dibenzo-para-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are two clusters of chemical compounds that comprise very similar properties and structures. These chemicals are insoluble in water, lipophilic and very persistent. They are by-products resulting from the production of other chemicals, incineration reactions, and the use of a variety of chemical products (UNEP, 2002). PCDDs are contaminants produced in the manufacture of polychlorinated biphenyls (PCBs) and organochlorine pesticides. PCDDs could also be produced by inefficient burning of organic carbon in the presence of chlorine (Meharg and Osborn, 1995). PCDFs are the key contaminant of PCBs. The toxicity of PCDDs and PCDFs to human have been well documented which include dermal toxicity (chloracne), immunotoxicity, carcinotoxicity, reproductive toxicity and possible neurobehavioral (cognitive) effects (SCF, 2000).

Potential public health risks from environmental exposures to chlorinated dioxins and related compounds continue to be the subject of much research, regulation and debate. Human exposure to PCDDs and PCDFs occurs mainly from foods that contain these chemicals. Among the foods are meat, dairy products and fish which could make up to more than 90% of the intake of PCDDs and PCDFs in diet (Huwe and Larsen, 2005). In Europe, it was reported that fish and fish products contribute to 2-63% of the dietary intake while other animal source such as meat and meat products as well as milk and dairy products contributed to 6-32% and 16-39%, respectively (EC, 2000).

Over the past few decades, nutritional research has identified a number of foods that are recommended for human diet on the basis of their potential beneficial effect on chronic diseases. Among the foods, fish have been recognised as a food group containing high protein with good biological value, low content of saturated fat and rich in certain minerals and vitamins (Sidhu, 2003). Marine foods including fish represent a very important source of vitamin D (Bender, 2002), unique source of long chain polyunsaturated fatty acids (PUFA) of the n-3 family mainly the EPA and DHA (Sidhu, 2003) and source of protein with high biological value (Brown, 2008). Several studies also showed that fish is perceived as a healthy food by consumers (Gross, 2003). However, fish can also be a major source of human exposure to contaminants such as methyl mercury, polychlorinated biphenyls (PCBs), dioxins, organochlorine pesticides and other environmental contaminants (Kris- Etherton *et al.*, 2002).

Due to the health benefits and scientific facts offered in fish consumption and the conflicting toxicological food safety, this study was carried out to obtain new data on the presence of PCDDs and PCDFs in local marine fish. As preliminary, this paper aims to determine the level of PCDDs and PCDFs in 5 species of marine fish collected from

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Straits of Malacca. The 5 species were among 10 species most commonly used in everyday diet of Malaysian (Osman *et al.*, 2001).

Materials and Method

Chemicals and reagents

All reagents used were of analytical grade. Chemicals used include dichloromethane, toluene, and pesticides. The chemicals were used for extraction, pre-treatment, and clean-up of samples before PCDDs and PCDFs determination using high-resolution gas chromatography/high-resolution mass spectrometry (HRGC/HRMS).

Sampling

Stratified sampling method was used to collect fish and shellfish samples. Fresh fish and shellfish samples were collected from several regions of identified fish landing areas along 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 samples were collected fresh from the various sites in November-December 2008.

Samples

Samples consists of 12 species of fish [(*Rastrelliger kanagurta* (kembung), *Scomberomorus guttatus* (tenggiri papan), *Pampus argenteus* (bawal putih), *Megalapsis cordyla* (cencaru), *Gymnura spp*. (pari), *Plotosus spp*. (sembilang), *Eleutheronema tradactylum* (senangin), *Nemipterus janonicus* (kerisi), *Epinephulus sexfasciatus* (kerapu), *Psettodes erumei* (sebelah), *Chirocentrus dorab* (parang) and Lutianus argentimaculatus (merah)] and 3 species of shellfish [*Anadara granosa* (kerang), *Macrobrachium rosenbergi* (udang) and *Sepia officinalis* (cuttle fish)]. However, as preliminary, only 5 species of fish were analyzed in this report.

Sample preparation

Composite sample of each species from the same region of a fish was prepared by mixing and grinding the respective fish species before storage in the freezer. Upon arrival at Dietetics laboratory, Univeristi Putra Malaysia, fish were individually measured for total body weight and length. Vicera were removed before being beheaded, washed, filleted, packed in polyethelene covered cup. All preparation and cleaning procedures were carried out using contamination-free tools. Samples for organochlorinated pollutants determination were kept frozen at -25°C without any prior treatment.

Fat extraction and clean-up

About 50 µl internal standard were spiked into 10 g of wet sample of fish. Then, 10 g of hydromatrix were mixed into the sample before being homogenized with a mortar. The homogenized sample was then dried in an oven for a few minutes to dehydrate the moisture content until it formed a powder. The powder was then placed into the cell (size 33) and the cell's surface was closed with otawa sand before placing into Accelerated Solvent Extraction (ASE 200) machine for 20 minutes (Method 11). The mixture of extracted fat and solvent was dried using a rotary evaporator for 20 minutes and then filtered to get crude fat extract. Hexane was mixed into the extract to form eluent. The eluent was placed in the Power-Prep Fluid Management System (FMS) for clean-up process involving three types of column: silica, alumina and carbon. After 23 steps of FMS have been completed, the collected PCDDs and PCDFs (mixed with solvent) mixture was dried using a rotary evaporator before being spiked with 50 µl external standard. The solvent was dried using a heating block (with nitrogen gas) at 60^{o}C. The crude dioxins and furans collected were placed in a small covered aluminium vial before analysis.

Analysis of dioxins and furans

High resolution gas chromatography/high resolution mass spectrometry (HRGC/HRMS) was used for analysis of dioxins and furans. Each analysis included the determination of 17 dioxin and furan congeners with 2,3,7,8-chloro-substitution. Congeners of dioxins and furans determined are as listed below. Selection of congeners was based on the most toxic to human.

a. Dioxins – 7 congeners
b. Furans – 10 congeners

Determination of PCDDs/Fs compounds in fish lipid samples was carried out at Doping Centre, Universiti Sains Malaysia, Penang.

Results and Discussion

TABLE 1 summarizes the results of the 5 fish species analyzed showing the percentage of lipid and the concentrations of PCDDs/Fs in fish samples. The highest level of total PCDFs/Ds was in *Plotosus spp.* while in other 4 species the levels were in the range of 0.10-0.18 pg/g of wet weight. The highest level of PCDDs and PCDFs in *Plotosus spp* was related to higher fat content in the fish sample (5.7% $\pm$ 0.4 fat). However, for other species that contain > 5% fat such as *Rastrelliger*

kanagurta (Kembung) and *Scomberomorus guttatus* (Tenggiri) the levels of these contaminants were relatively low.

TABLE 1- Level of total PCDDs and PCDFs in 5 species of marine fish

Species	Percent lipid	Total PCDDs/Fs WHO-TEQ (pg/g of wet weight)
Rastrelliger kanagurta (Kembung)	5.0 ± 2.4	0.10 ± 0.01
Pampus argenteus (Bawal Putih)	3.6 ± 0.1	0.13 ± 0.02
Plotosus spp. (Sembilang)	5.7 ± 0.4	1.23 ± 0.48
Megalapsis cordyla (Cencaru)	3.5 ± 0.1	0.12 ± 0.01
Scomberomorus guttatus (Tenggiri)	5.5 ± 0.2	0.18 ± 0.01

It is well established that the quality of seafood products is dependent on the genetic basis, size, reproductive period of fish as well as characteristics of the environment (pH, salinity, temperature of water, composition of phyto- and zooplankton during the year and the presence of other fish species) (Orban *et al.*, 2003). For wild fish, the main exposure to PCDD/Fs is associated with chronic contamination due to leaching of agricultural or industrial chemicals into surface waters.

In general, the preliminary data on the level of PCDDs/Fs in the studies samples showed low level of these contaminants in the muscle tissue of local marine fish. The maximum limit suggested by EC Regulation No 119/2006 is 4 pg/g of wet weight for total PCDDs/Fs and 8 pg/g of wet weight for PCDDs/PCDFs-PCBs (including the dioxin-like PCBs), respectively (EC, 2006). Our data indicates PCDDs/Fs in the five local marine fishes are of safe levels for consumption. In comparison, Ministry of Agriculture, Fisheries and Food (MAFF) in United Kingdom has observed a mean concentration of PCDDs, PCDFs and PCBs (of 12 samples) to be 25 pg/g fat in marine salmon (Ministry of Agriculture, Fisheries and Foods. 1999). Earlier reports have also reported significant levels of PCDDs, PCDFs and PCBs in fatty tissue of herring from Baltic sea (Rappe *et al.* 1989). A more recent study in Ireland on fish and fishery products available in Irish market, the levels of PCDD/Fs including dl-PCBs were, however, generally below 8.0 pg/g of wet weight (Tlustos *et al.* 2006). Similarly, in another study carried out in Spain on 14 species of fish and shellfish (Bocio *et al.* 2007), the concentration of PCDD/Fs marked a significant decrease in comparison to an earlier findings in 2003 (Llobet *et al.* 2003).

Conclusion

The local marine fish is safe for consumption in terms of PCDDs/Fs level based on our samples. Data on PCDDs/Fs exposure to human is important following concerns about the carcinogenicity and other negative health effects. Therefore determination of estimate intake of PCDDs/Fs via other sources should also be carried out. Specific control measures in controlling the emission of these chemicals into the marine environment should be monitored.

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