

Monitoring of Pcdd/Fs Levels in Selected Mediterranean Fish Species Collected from The Egyptian Coastal Harbors

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ABSTRACT

Alexandria and Port Said are two northern coastal Egyptian governorates located along the Mediterranean Sea. Both governorates were considered the most important harbors in Egypt. Many industrial activities located in it, especially fertilizers manufactories, recycling and waste incinerators which considered as a major source for food especially fish contamination with PCDD/Fs. This study highlighted the levels of PCDDs/Fs to assess the ecological and potential health risks derived from its intake and was performed to obtain information about the concentrations of PCDDs/Fs in fish that are high in production and commonly consumed. A total number of 26 fish samples that represent different types; Tilapia (*Tilapia Zilli*), grey mullet (*Mugil cephalus*), sardine (*Sardinella spp.*) and brush tooth lizard (*Saurida undosquamis*) samples were collected during 2006 from seven locations along the coastal Mediterranean borders for both governorates. The results demonstrated that the concentration of dioxins didnot exceeded the maximum level proposed by the Egyptian Organization for Standardization which relatively the same with the European Community which have set a limit of 4 pg WHO-TEQ/g of PCDD/Fs for fish and fish products on a wet weight basis. The results obtained from the analysis of seventeen 2,3,7,8-substituted congeners of PCCD/Fs in the four species of fish from seven areas over the Mediterranean Sea were determined and showed to be lower than the maximum permissible limits. Average concentration of total PCDD/Fs in tilapia, grey mullet, sardine and brush tooth lizard fish from the Mediterranean Sea was ranged from 0.22 to 2.9 pg WHO-TEQ/g fw, 0.38 to 1.2 pg WHO-TEQ/g fw, 0.21 to 0.47 pg WHO-TEQ/g fw, and 0.3 to 0.66 pg WHO-TEQ/g fw respectively.

Keywords; Egypt, Fish, PCDD/Fs

1. INTRODUCTION

Polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDDs and PCDFs) are two groups of chemicals within the persistent organic pollutants (POPs) found in the environment because of their persistent, toxic, and bioaccumulative properties. PCDD/Fs are fat-soluble and accumulate in animal tissues. They are among the more toxic

anthropogenic contaminants where PCDD/Fs are produced from combustion sources and manufacturing process. Such as combustion (*Baker and Hites, 2000*), incinerators (*Schuhmacher et al., 1997*) and vehicles exhausts (*Miyabara et al., 1999*), although they are 210 PCDD/F congeners, only 17 (those with a 2,3,7,8 substitution) have so far been found to be toxic. Among them, the most toxic compound is 2,3,7,8-tetrachlorodibenzo-p dioxin or TCDD. The toxicity of other PCDD/Fs is measured in relation to TCDD. PCDD/Fs have well described toxicities at extremely low concentrations, and a highly competent system, which allows inter-comparison of toxicities between different isomers on the basis of toxic equivalents (TEQ), has been developed for humans and wildlife (*Van den Berg et al., 1998*). These toxic substances tend to bioaccumulate further up the food chain, so PCDD/F levels in fish are often 100,000 times greater than those found in the surrounding environment (*Schechter et al., 2001*). Exposure to PCDD/Fs can cause several endocrine, reproductive and developmental problems in animals, including human beings (*Van den Berg et al., 1998*). In 1997, the International Agency For Research on Cancer (IARC) declared 2,3,7,8- TCDD carcinogenic for humans (*World Health Organization (WHO, 1997)*). Since 1990s food has been identified as a pathway of human exposure to dioxin compounds. Dietary intake contributes about 90-98% of the total daily dioxin intake of the general population. In which animal origin feed contribute about 90% of the daily intake in European country's (*Hoogenboom et. al.2004*). The Mediterranean Sea is no exception, as it is the recipient of extensive urban and industrial waste-water discharges from neighboring countries, while at the same time it is an attractive leisure region: there is thus an evident need for environmental risk assessment tools where it is the end point of various outlet that discharge large volumes of agriculture, municipal and industrial waste water, these contaminates are mainly ported to aquatic systems through the atmospheric deposition or directly via rivers (*Bruzuzuy and Hites, 1996*), All Wastes are originated from shipping activities, and maintenance works around. Marine ecosystem is the end point for a variety of pollutants that originate either from land or sea. Pollutants tend to accumulate in marine organisms reaching high concentration levels, posing potential threats to marine life and their consumers where fish is the primary protein source in diet of residents in some area along the Mediterranean Sea. In Egypt there is a lack of information to the PCDD/Fs evaluation studies (*Loutfy et al., 2006*) to estimate the PCDD/Fs exposure, the research work on dioxins in Egypt has been started recently and only

scarce information about PCDD/Fs contamination levels in food or the environment is available. The results from this work will provide an important data pool for setting the Egyptian National PCDD/Fs Standards in food and provide important information to decision makers in formulating policies to control sources and release of PCDD/Fs in the future.

2. MATERIALS AND METHODS

Chemicals and Reagents

All solvents used were from grade A and purity not less than 99%, Silica gel and basic Alumina were purchased from Aldrich (Brockmann I, Standard grade, Milwaukee, USA). Calibration standard solutions, labeled standard and injection solutions specified in EPA Method 1613B were obtained from Cambridge Isotopes Laboratories (Andover, USA).

Sampling and Sample processing

The procedures of sampling method were following the European commission regulation (*EC*) No 1883/2006. The collected samples during 2006 were represented from the most expected contaminated fishing area in Mediterranean Sea from Alexandria and Port Said harbors. The sampling strategy for the current survey targeted fish species that commonly eaten by the local people due to its cheaper prices in Egypt are one of the developments countries. Fish samples were analyzed in this survey include four different species of (Tilapia, grey mullet, sardine, and brush tooth lizard fish). All fish samples were mature for sale. The edible parts of the fish were removed, grounded, and then freeze-dried.

Extraction

N-hexane/ dichloromethane (1:1) were used for extraction for at least 18-24 h, an aliquot of freeze dried sample (equivalent to 20g wet samples) were spiked with known amounts of mixture of labeled PCDD/Fs, are extracted using Soxhlet after mixing the homogenized sample with anhydrous sodium sulfate, the extract solvent was evaporated using a rotary evaporator to concentrate the lipid, then lipid content were determined.

Clean-up

Steps were conducted according to EPA Method (U.S EPA 1613(B), 1994), using anthropogenic, multilayer silica gel, alumina and active carbon column.

HRGC/HRMS Instrument

Analyses were conducted using HP 6890 plus gas chromatograph coupled with Micromass/Autospec Ultima mass spectrometer operating in EI mode at 35 eV and with a resolution of 10,000 (5% valley). Sample injections were performed in the splitless mode on DB5 MS column (60m, 0.25 mm id, 0.1µm film thickness). The oven program was started from 90°C then takes 15min. to reach 220°C then held for 15 min, then from 220-290 in 8min then held for 17min. Helium (Ultra high purity) at a flow rate 0.8 ml/min. was used as a carrier gas. Injector temperature was 225 C; 1µl of the sample was injected using splitless mode.

Quantitative determination

Determinations of PCDD/Fs were performed by an isotope dilution method using relative response factors previously obtained from five standard solutions. The TEQ concentrations were calculated guided to World health Organization-toxic equivalent factor (WHO-TEFs, 1998), the result values are presented in pg WHO-TEQ/g fresh weight (fw) Multiplied by the associated WHO-TEF (Van den Berget al., 1998). It was assumed that non-detected isomer concentrations were equal to the limits of determination. As recommended by the European Regulation (Council Regulation EC No. 2375/2001, detection and quantification limits, as well as recoveries, for all PCDD/Fs congeners were in good agreement with requirements established in the European Commission Directives (2002/69/CE and 2004/44/EC), laying down the sampling methods and the methods of the analysis for the official control of PCDD/Fs. For each run the samples were prepared including a method blank and quality control samples are performed. All steps of analysis were conducted according to (U.S EPA 1613(B), 1994).

3. RESULTS AND DISCUSSION

3.1 PCDDs/Fs in fish samples

Seventeen 2,3,7,8-substituted congeners of PCDD/Fs profile in the edible parts of four species of fish from seven areas were determined. The levels of PCDD/Fs were shown in Table (1), figure (1) show the profile of the PCDD/Fs in Egypt, this study shows that the most dominant congeners was PCDDs where OCDD contribution was remarkable in higher percentage than any other congener in the four kinds of fish as shown in

figure (1), this congener has been reported to be dominant in emission from domestic combustion by (Weres et al., 2004).

Table; (1) Average concentration of PCDD/Fs in fish pg/g in Alexandria and Port Said

Governorate	Alexandria				Port Said				
	Kind Of fish	Tilapia	Grey	Sardine	Brush Tooth	Tilapia	Grey	Sardine	Brush Tooth
No of Samples	5	3	3	3	3	3	3	3	3
Σ PCDDs	4.6	3.9	3.3	3.3	21.6	19.2	11.9	10	
Σ PCDFs	2.3	2.3	1.2	1.6	2.7	5	2.3	2.8	
Σ PCDDs/Fs	6.9	6.2	4.5	4.9	24.3	24.2	14.2	12.8	

This study showed that Tilapia fish contained the highest concentration found in Alexandria 6.9 pg/g fw ranged from 3.8 to 13.1 pg/g fw where the most abundant congener was OCDD 3.8 pg/g fw which contributes about 54% from Σ PCDD/Fs ranged from 2.1 to 5.4 pg /g fw, where the OCDD was the highest congener, While OCDF 0.52 pg/g fw which contributes about 8% from Σ PCDD/Fs ranged from 0.28 to 0.63 pg/g fw. (Bacher et al., 1992), reported that OCDD was the most abundant in open fire of garden waste where the dominance PCDFs has been reported in the chimney soot, Samples arising from wood burning was reported in the United Kingdom ambient air Samples from an area where the domestic burning of wood and coal is wide spread (Lohmann et al., 2000).

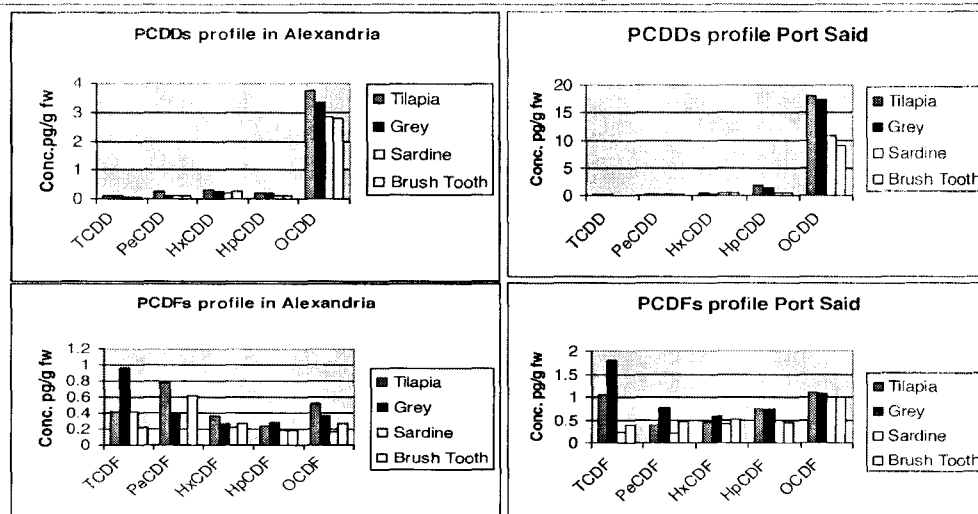


Figure (1) Average concentration of profile PCDDs/Fs of fish pg/g for Alexandria and Portsaid harbors.

Port Said tilapia fish average concentration was 24.2 pg /g fw ranged from 11.9to 48.3 pg/g fw where the most abundant congener was also OCDD 18 pg/g fw which contributes about 74% from \sum PCDD/Fs ranged from 7.6 to 37.4pg /g fw as shown in figure(1),The profile of dioxin when calculated in Port Said showed that tilapia was the highest concentration (pg/g) but in (pg WHO-TEQ/g) Grey Mullet was the highest concentration this difference is according to sum of PCCD/Fs multiplied by Toxicity equivalency(TEQ), OCDF was detected in all kinds of fish but with small amounts compared with OCDD which reflects that Egypt nature is not industrial as the source of OCDF is caused mainly by chemical manufacturing especially polyvinyl chloride (PVC), manufacturing process (Bakoglu et al., 2005).

3.2 PCDDs/Fs pg/g(TEQ) Toxic equivalency in fish samples.

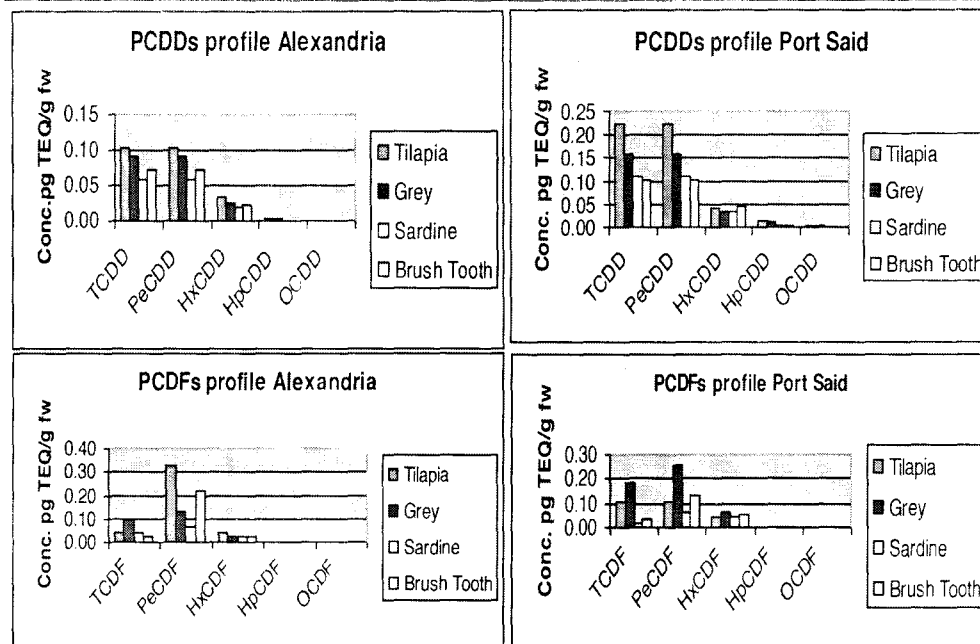
These results obtained from the analysis of seventeen 2,3,7,8-substituted congeners of PCCD/Fs in the four species of fish Multiplied by Toxic equivalency factor (TEF).

Table; (2) Average concentration of fish (pg WHO-TEQ/g) in Alexandria and Port Said

Governorate	Alexandria				Port Said				
	Kind Of fish	Tilapia	Grey	Sardine	Brush Tooth	Tilapia	Grey	Sardine	Brush Tooth
No of Samples	5	3	3	3	3	3	3	3	3
Fat (g)	0.7	1.4	0.8	1.1	1	1.7	0.83	0.97	
Σ PCDDs	0.41	0.22	0.16	0.22	0.42	0.33	0.27	0.27	
Σ PCDFs	0.41	0.26	0.13	0.27	0.27	0.51	0.13	0.22	
Σ PCDDs/Fs	0.82	0.48	0.29	0.49	0.69	0.84	0.4	0.49	

Average concentration of total PCDD/Fs in Tilapia, grey ,sardine and brush tooth lizard fish from Mediterranean Sea was 0.82 pg WHO-TEQ/g fw ranged from 0.22 to 2.93pg WHO-TEQ/g fw, 0.48 pg WHO-TEQ/g fw ranged from 0.38 to 1.2 pg WHO-TEQ/g fw, 0.29 pg WHO-TEQ/g fw ranged from 0.21 to 0.47 pg WHO-TEQ/g fw, 0.49 pg WHO-TEQ/g fw ranged from 0.3 TO 0.66 pg WHO-TEQ/g fw.

The Sum of PCDD and PCDF are presented in table (2), Mostly all analyzed samples are characterized generally by the dominance of PCDDs. These congeners have been reported to be dominant in emission from domestic combustion (Alcock et al., 2001), PCDFs usually dominate the typical finger prints from combustion sources (Huang and Buekens, 1995), The study shows that tilapia fish contained the highest concentration found in Alexandria (0.8 pg WHO-TEQ/g fw) ranged from 0.22 to 2.9 pg WHO-TEQ/g fw where it was the minimum fat content 0.7g, the most abundant congeners were PeCDFs 0.33pg WHO-TEQ/g fw which contributes about 40% from Σ PCDD/Fs , PeCDDs 0.27pg WHO-TEQ/g fw which contributes about 33% from Σ PCDD/Fs and TCDD 0.10 pg WHO-TEQ/g fw which contributes about 13% from Σ PCDD/Fs.



Figure; (2) Average concentration of profile PCDDs/Fs of fish (pg WHO-TEQ/g) for Alexandria and Portsaid harbors.

Toxicity equivalency (TEQ) was calculated based on the WHO-TEFs for humans/mammals (Van den berg et al., 1998), Alexandria and Port Said have the most contributed amount of PCDDs/Fs but was in the permissible limits of the ESO. Agriculture drainage, Electricity Plant, factories for fertilizer, Metal, and petrochemical companies settled at Abu kier make it the highest concentration area in Alexandria and Porfouad in Port Said was the highest concentration area which have recycling rubbish factory, Abu Kir concentration was 2.9pg WHO-TEQ/g fw Where the most abundant congeners were PeCDFs 1.4 pg WHO-TEQ/g fw which contributes about 48% from \sum PCDD/Fs, PeCDDs 1pg WHO-TEQ/g fw which contributes about 35% from \sum PCDD/Fs, and TCDD 0.28 pg WHO-TEQ/g fw which contributes about 10% from \sum PCDD/Fs, and the lowest concentration was found in Sardine 0.3pg I-TEQ/g fresh weight were its fat percent 0.8g higher than Tilapia as shown in table(2),Port Said Grey Mullet was the highest fat content 1.7g also it was the highest concentration Found in Port Said 0.84pg WHO-TEQ/g fresh weight ranged from 0.56 to 1.2 pg WHO-TEQ/g fw where the most abundant congeners were PeCDFs 0.26pg WHO-TEQ/g fw which contributes about 31% from \sum PCDD/Fs, TCDF 0.18 pg WHO-TEQ/g fw which contributes about 21% from \sum PCDD/Fs, and

TCDD 0.16 pg WHO-TEQ/g fw which contributes about 20% from Σ PCDD/Fs. PorFouad contained the highest concentration 1.2 pg I-TEQ/g fresh weight Where the most abundant congeners found was PeCDFs 0.36pg WHO-TEQ/g fw which contributes about 31% from Σ PCDD/Fs, TCDF (0.27 pg WHO-TEQ/g fw) which contributes about 23% from Σ PCDD/Fs, and TCDD 0.23 pg WHO-TEQ/g fw which contributes about 20% from Σ PCDD/Fs, and the lowest concentration was found in Sardine 0.39pg I-TEQ/g fresh weight. PCDD/F concentrations in food samples ranged between 0.12 and 3.35 pg WHO-TEQ/g wet w Egypt. (*Tundo et al., 2004, 2005*).

4. CONCLUSION

The levels of PCDD/Fs were studied in four fish species collected from the Northern Egyptian harbors where Tilapia eight samples, Grey Mullet six samples, Sardine six samples and Brush tooth Lizard fish six samples from two governorate (Alexandria, Portsaid) along the Mediterranean Sea. The study showed that the levels of PCDD/Fs were fairly lower than the maximum permissible limits of European Community. Average concentration of total PCDD/Fs in Tilapia, grey mullet, sardine and brush tooth lizard fish from Mediterranean Sea was Ranged from 0.22 to 2.9 pg WHO-TEQ/g fw, 0.38 to 1.2 pg WHO-TEQ/g fw, 0.21 to 0.47 pg WHO-TEQ/g fw and 0.3 to 0.66 pg WHO-TEQ/g fw respectively.

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الملخص العربي

نقصى مستويات الداىوكسينات و الفيورانات فى بعض أسماك البحر المتوسط التى تم تجميعها من جمهورية مصر العربية

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مركز البحوث الزراعية

تم جمع (٢٦) عينة سمك سنة ٢٠٠٦ فى محافظتى الاسكندرية و بورسعيد فى جمهورية مصر العربية من البحر المتوسط و ذلك لتقدير مستويات الداىوكسين و الفيوران فى العينات ممثلة فى أربع أنواع مختلفة من السمك (بلطى - بورى - سردين - مكرونة) ، و قد أوضحت النتائج ان البلطى يحتوى على أعلى نسبة من الداىوكسين ثم يليه البورى ثم السردين ثم المكرونة ، و قد وجد ان التركيزات الوسطية لمجموع الداىوكسين و الفيوران (بالنسبة للوزن الصافى) كانت تتراوح بين ٢,٩ - ٠,٢٢ نانو جرام تيك / كيلو جرام و ذلك للبلطى و ١,٢ - ٠,٣٨ نانو جرام تيك / كيلو جرام للبورى و ٠,٤٧ - ٠,٢١ نانو جرام تيك / كيلو جرام للسردين و ٠,٦٦ - ٠,٣ نانو جرام تيك / كيلو جرام للمكرونة و هذه المستويات أقل من الحدود المصرية التى تتشابه مع حدود الاتحاد الأوروبى و النتائج المتحل عليها تعطى مدلول عن مدى التعرض العام للشعب المصرى بالنسبة للداىوكسين عن طريق الأسماك .

الكلمات الدالة : الأسماك - الداىوكسين و الفيوران