Annals of Agric. Sci., Moshtohor **ISSN 1110-0419**
Vol. 52(2) (2014), 205 – 210 **http://annagricmoshj.com**

HPLC MONITORING OF PCDD/Fs LEVELS IN FISH SPECIES COLLECTED FROM THE EGYPTIAN MARKET

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Abstract

Chemical pollutants released from various industries into water systems make fish a sources of various environmental toxicants to humans and classified fish consumption as one of the primary pathways of exposure to polychlorinated dibenzo-p-dioxins, dibenzofurans (PCDD/Fs) and dioxin like polychlorinated biphenyls (dl-PCBs). The current study was conducted on 31 randomly collected individual fish samples marketed in different governorates in Egypt, during 2011–2012 to evaluate tissue levels of the 17 laterally substituted polychlorinated dibenzo-p-dioxins, dibenzofurans (PCDD/Fs) and 12 dioxin like polychlorinated biphenyls (dl-PCBs). The mean total TEQ value found in fish was 0.625 and concentrations ranged from 0.063 to 3.981 pg WHO-TEQ/g product, such levels were lower than the EU regulations limits of 2006 (8pg WHO-TEQ/g product). The highest concentrations when the results were expressed in toxic equivalents corresponded to 2,3.4,7,8-PeCDF, PeCDD and TCDD; and PCB-126 being the most frequentof dl-PCBs. The average PCDD/Fs and dl-PCBs intakes based on adaily fish consumption of 14.5 g was estimated at 0.15pg WHO-TEQ kg^{-1}b.w. d^{-1} for adults, which was lower than range of TDI recommended by the WHO (1–4 pg WHO-TEQ kg^{-1}b.w. d^{-1}), and much lower than the TWI and PTMI adopted by SCF and JECFA, respectively.

Keywords: dioxin derivatives, determination, fish.

Introduction

Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) are three classes of chemically and structurally related polyhalogenated aromatic hydrocarbons and, Each of these groups consist of 75, 135 and 209 theoretical individual congeners,respectively, based on the number and position of chlorine atomsin the chemical structure**(Gilpin et al., 2003)**. They usually occur as a mixture of congeners, and their ubiquity, high chemical and metabolic persistence, and potent toxicity of some of the congeners make them a well recognized class of persistent organic pollutants (POPs) which are included in the Stockholm Convention **(UNEP, 2001).**

Food consumption is the main route for human exposure toPCDD/Fs and dioxin-like PCBs, following bioaccumulation of thesecompounds in the aquatic and terrestrial food chains. Fish and seafood,meat and meat products, and dairy products have been reportedas some of the major sources of exposure in adults and children **(Bordajandi et al., 2004)**. The strategy to reduce dioxin intakes has led to numerous surveystudies on the concentration of dioxins in particular food itemssuch as fish **(Gomara et al., 2005).** Several studies have performed assessments of the intake of dioxins from the diet **(Kroeset al., 2002)**, using different approaches such as market basket monitoring programmes **(Wang et al., 2009), (Bilau et al., 2008),** or total diet studies **(Windal et al., 2010).**

The present study is intended to assess the levels of the biologically active congeners in fish, and to evaluate whether the present levels of dioxins and dioxin-like PCBs exceed the maximum permitted levels according to EU Council regulation, to establish the contribution of dioxins, furans and coplanar PCBs to the toxic equivalent quantities (WHO-TEQ) in foodstuffs, and asses the dietary exposure of adults.

Materials and Methods

Sampling

A total of 31 fish samples were randomly collected from local markets in different governorates in Egypt during 2011–2012. Samples were analyzed in the central laboratory of residue

analysis and heavy metals in food, and the results grouped to evaluate average levels and ranges of PCDD/Fs and dioxin-like PCBs.

Chemicals and Reagents:

All solvents (Toluene, n-Hexane, Methanol,Methylene Chloride and Nonane) used were of pesticide grade and purity not less than 99%, Silica gel and basic Alumina were from Aldrich (Brockmann I, standard grade, Milwaukee, USA), anhydrous sodium sulphate and conc. H_2SO4 (96%) were from Riedel-deHaen, carbopack C- 80/100 (Supelco) and celite 545- (BDH or Aldrich). Calibration standard solutions, labeled standard and injection solutions specified in EPA Method-1613B were obtained from *Cambridge Isotopes Laboratories (Andover, USA).*

Extraction

Extraction of fat from fish samples (25 ±0.1g) was carried out by Soxhlet or Accelerated Solvent Extractor (ASE) using a solvent mixture of n-Hexane: Dichloromethane (1:1). The lipid was extracted from the samples using either soxhlet or ASE then transfered to cleanup steps.

Accelerated Solvent Extractor - Dionex 350; condition of ASE: Oven temp.: 125 C˚, Static cycle time: 5 minute, Cycles: 4, Rinse volume: 80%, Purge time: 70 Sec, Cell pressure: 1500 psi (nitrogen gas) and Total extraction time: 30 min per sample.

Clean-*up*

Clean-up steps were conducted according to EPA Method **(USEPA 1994)**, using acidified silica gel, anthropogenic, multilayer silica gel, alumina and active carbon column. Finally PCDDs/Fs and dl-PCBs were determinated by using HRGC/HRMS.

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 injection was performed in the splitless mode on DB5 MS column (60m, 0.25 mm id, 0.1µm film thickness). The oven program was started from 90ºC then took 15min. to reach 220ºC then held for 15 min, then from 220-290 ºC 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; 2µL of the sample injected using splitless mode.

Quality Assurance/Quality control (QA/QC):

The QCAP lab. Followed the quality assurance system as shown in Table 1. Method blank, ongoing precision and recovery (OPR), certified reference material (CRM) and quality control samples were included with each batch of 20 samples to confirm the laboratory performance and method validation. Method of analysis of PCDDs/Fs and dioxin-like PCBs in fish were accredited by the Finnish Accreditation Service body (FINAS) according to the requirements of the International Standard ISO/IEC 17025.

Table 1. LOD and average recoveries of PCDDs/Fs tested with fish samples analyzed.

Labeled PCDD/Fs	LOD (ng/ml)	Expected Conc. (ng/ml)	Average Recovery %	Acceptance Av. Rec. Range %
2,3,7,8-TCDD	0.01	100	55.2	25-164
1,2,3,7,8-PeCDD	0.01	100	55.8	25-181
1,2,3,4,7,8-HxCDD	0.05	100	77.1	32-141
1,2,3,6,7,8-HxCDD	0.05	100	81.7	28-130
1,2,3,4,6,7,8-HpCDD	0.05	100	65.1	23-140
1,2,3,4,6,7,8,9-OCDD	0.1	200	52.8	17-157
2,3,7,8-TCDF	0.01	100	69.1	24-169
1,2,3,7,8-PeCDF	0.01	100	61.7	24-185
2,3,4,7,8-PeCDF	0.01	100	55.5	21-178
1,2,3,4,7,8-HxCDF	0.05	100	81.7	26-152
1,2,3,6,7,8-HxCDF	0.05	100	85.4	26-123
2,3,4,6,7,8-HxCDF	0.05	100	79.5	28-136
1,2,3,4,6,7,8-HpCDF	0.05	100	89.6	28-143
1,2,3,4,7,8,9-HpCDF	0.05	100	74.8	26-138
1,2,3,4,6,7,8,9-OCDF	0.1	200	57.6	17-157

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 by the World Health Organization-toxic equivalent factor **(WHO-TEFs, 1998)**, The tetra through octa PCDD/F results were identified, quantified and presented in pg WHO-TEQ/g fat

weight multiplied by the associated WHO-TEF **(Van den Berget et al., 1998)**. It assumed that non-detected isomer concentrations were equal to the limits of determination. As recommended by the European Regulation **(EC 2006)**, detection and quantification limits, as well as recoveries, for all PCDD/Fs congeners were in good agreement with requirements laying down the sampling methods and methods of analysis for the official control of PCDD/Fs. For each run, samples were prepared including a method blank and quality control samples. All steps of analysis were conducted according to the U.S adverse consequences of the observed effects **USEPA 1994.**

Results and Discussion

3.1 Toxic equivalency in fish

Concentrations of congeners for fish samples are expressed in pg WHO-TEQ/g product, in accordance with the European Council Regulation. The mean total TEQ value found in fish was 0.625 with a concentration range of 0.063 to 3.981 pg WHO-TEQ/g product; this level is lower than the EU limits 8 pg WHO-TEQ/g product. The mean concentration of PCDD/F was 0.592 pg WHO-TEQ/g products, this level was lower than the EU limits 4pg WHO-TEQ/g product with concentration range of 0.06 to 3.618 pg WHO-TEQ/g product. For dl-PCBs the mean concentration was 0.033 pg WHO-TEQ/g product with concentration ranged of 0.003 to 0.065 pg WHO-TEQ/g product see table 2.

Table 2. Minimum, maximum and mean range of PCDD/Fs and Dl-PCBs for fish samples in pg WHO-TEQ/g product.

Congeners	Min	Max	Mean
2,3,7,8-TCDD	0.006	0.261	0.058
1,2,3,7,8-PeCDD	0.010	0.658	0.105
1,2,3,4,7,8-HxCDD	0.001	0.011	0.003
1,2,3,6,7,8-HxCDD	0.001	0.041	0.008
1,2,3,7,8,9-HxCDD	0.001	0.008	0.003
1,2,3,4,6,7,8-HpCDD	0.0002	0.007	0.002
1,2,3,4,6,7,8,9-OCDD	0.000004	0.001	0.0003
2,3,7,8-TCDF	0.003	0.079	0.037
1,2,3,7,8-PeCDF	0.001	0.018	0.006
2,3,4,7,8-PeCDF	0.009	1.991	0.255
1,2,3,4,7,8-HxCDF	0.002	0.286	0.050
1,2,3,6,7,8-HxCDF	0.001	0.150	0.023
2,3,4,6,7,8-HxCDF	0.001	0.069	0.015
1,2,3,7,8,9-HxCDF	0.0005	0.018	0.005
1,2,3,4,6,7,8-HpCDF	0.0002	0.091	0.019
1,2,3,4,7,8,9-HpCDF	0.00008	0.003	0.001
1,2,3,4,6,7,8,9-OCDF	0.000002	0.001	0.0001
PCB 77	0.000003	0.001	0.0002
PCB 81	0.000002	0.00004	0.00001
PCB 126	0.003	0.052	0.026
PCB 169	0.0002	0.001	0.0004
PCB 105	0.00001	0.002	0.001
PCB 114	0.00002	0.001	0.0003
PCB 118	0.00002	0.005	0.003
PCB 123	0.000003	0.001	0.0002
PCB 156	0.0002	0.003	0.002
PCB 157	0.0001	0.001	0.0003
PCB 167	0.000002	0.00003	0.00002
PCB 189	0.00001	0.00005	0.00003
Total PCDD/F	0.060	3.618	0.592
Total PCBs	0.003	0.065	0.033
Total TEQ	0.063	3.981	0.625

This trend was in agreement with that derived from studies by **Scott et al., (2009)** who reported that the total TEQ concentration ranged from 0.1 to 4.3 pg WHO-TEQ/g product for wild-caught fish. Also, the obtained results were in agreement with that reported by **Wei et al., (2011)** who reported that the total PCDD/Fs TEQ levels in fish meat was 0.27 to 3.8 pg WHO-TEQ/g product and dl-PCBs ranged from 0.065 to 5.25 pg WHO-TEQ/g product.

The percentage of each PCDD/F congener in fish samples, as concentrations (pg/g product) and toxic equivalents (pg WHO-TEQ/g product) are shown in Figure 1.The profile of concentrations was dominated by OCDD,1,2,3,4,6,7,8 HpCDF and OCDF congeners whose combined concentrations account for 67.50% of all PCDD/Fs. When the percentage is expressed in toxic equivalents, the most abundant congeners were 2,3.4,7,8-PeCDF, PeCDDand the TCDD, that together account for the 68.14% of total dioxins.This could be explained because TCDD, PeCDD and 2,3.4,7,8-PeCDF congeners present the highest WHO-TEF for TCDD , PeCDD (TEF = 1) and for PCDFs (TEF = 0.5).

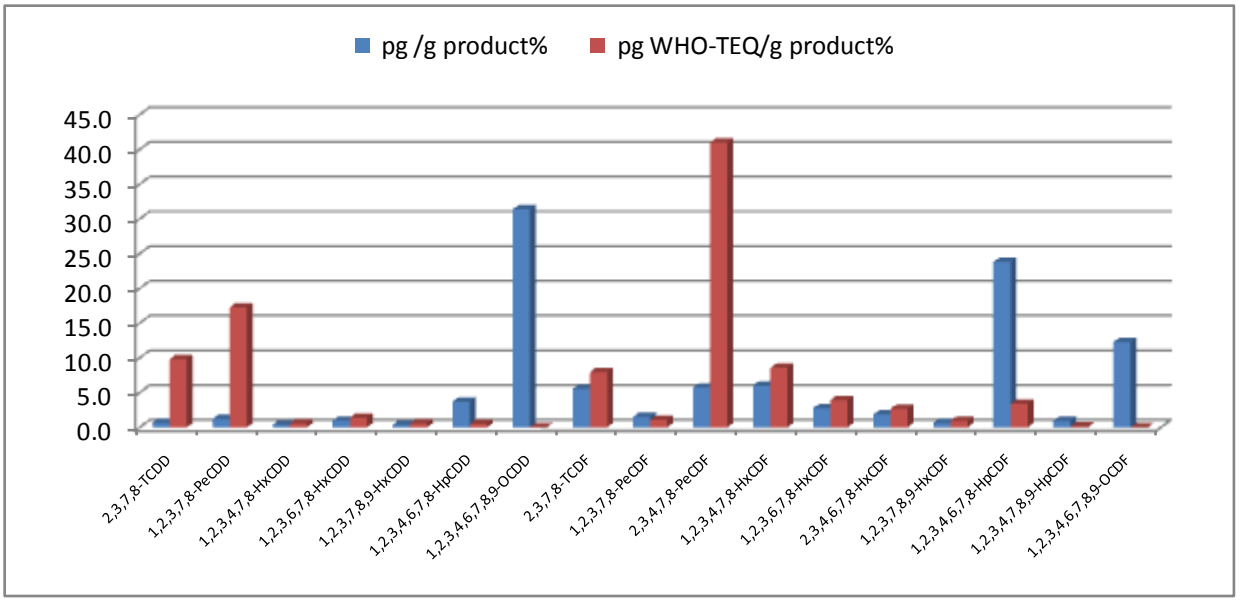

Figure 1: The percentage of individual PCDD/Fs congeners in fish samples as toxic equivalents.

For dl-PCBs, the mono-ortho PCB-118 represents 55.48% of the total concentration, however, in terms of percentage from toxic equivalents, it is the most toxic PCB-126 (WHO-TEF = 0.1) that accounts for the highest contribution (79.6%), as shown in figure 2.

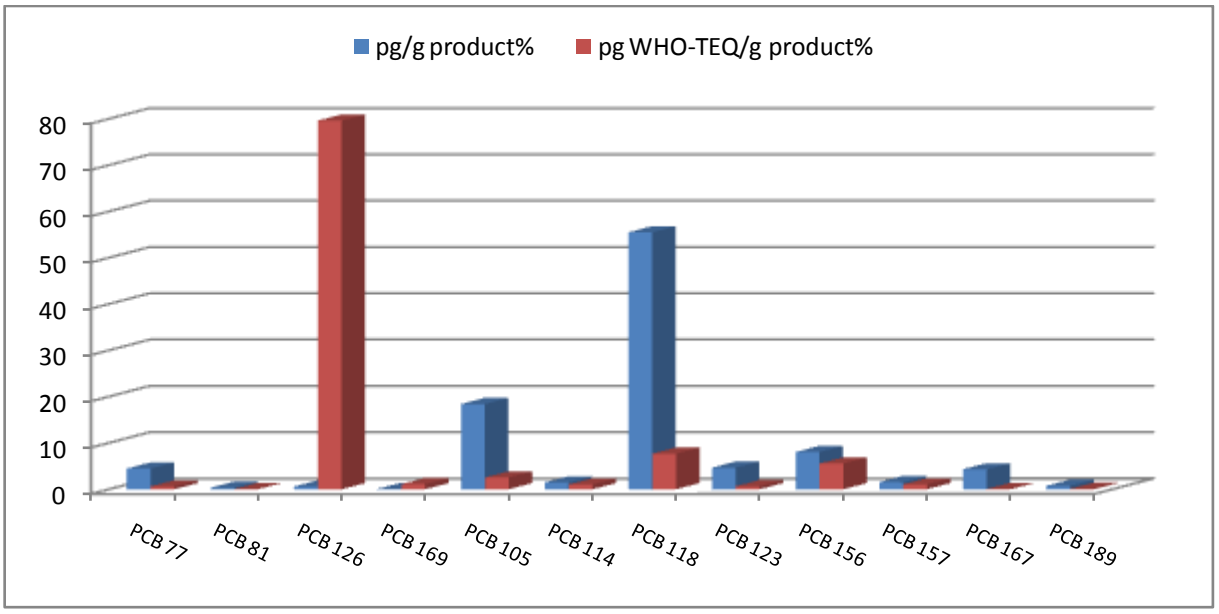

Figure 2. The percentage of individual DL-PCBs congeners in fish samples as toxic equivalents.

For PCDD/Fs samples of the highest content were found in Al Qalyobia governorate; the mean result was 2.575 pg WHO-TEQ/g product. The lowest samples were found in Giga governorate, the mean result was 0.094 pg WHO-TEQ/g products, as shown in Figure 3.

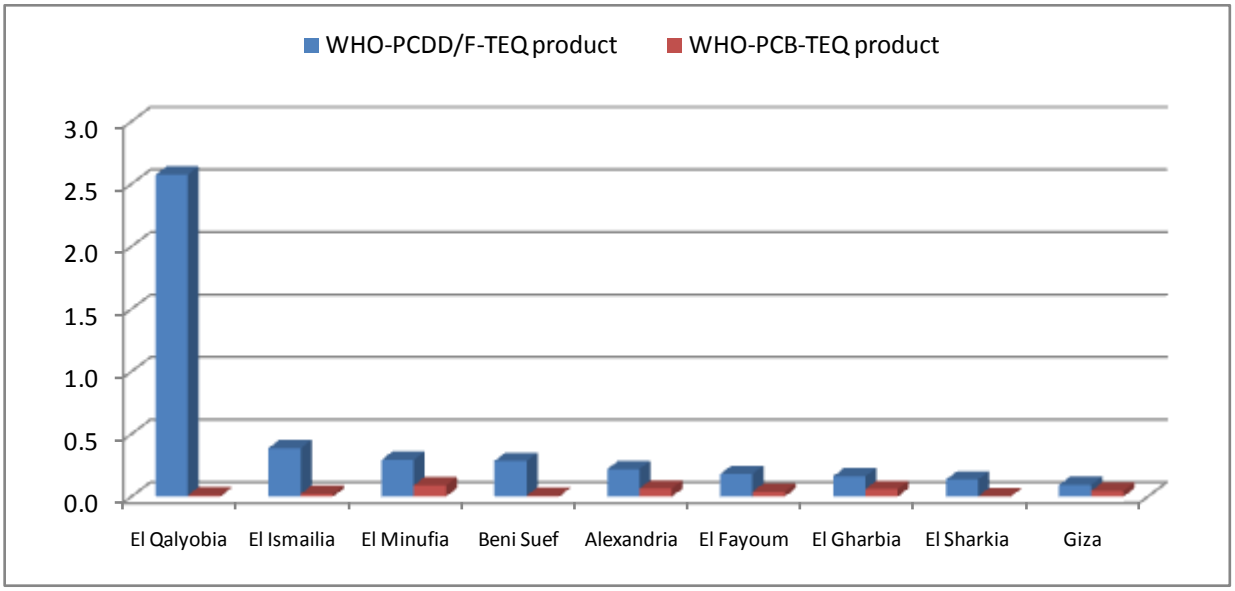

Figure 3. Average concentration of profile PCDDs/Fs of fish (pg WHO-TEQ/g) from different Egyptian governorates in fish samples.

3.2 Estimate of dietary exposure to PCDD/Fs and dl-PCBs

This monitoring study has been designed mainly to assess contamination levels of PCDD/Fs and dl-PCBs in fish and to evaluate if the levels are in accordance with those established by the EU legislation. The results could also be used to perform an approximate estimation of the average daily intake of the population of Egypt. In the current survey, food consumption data were obtained from **GEMS 2006**. The average PCDD/Fs and dl-PCBs intakes based on adaily fish consumption of 14.5 g was estimated at 0.15pg WHO-TEQ kg^{-1}b.w. d^{-1} for adults, which was lower than the range of TDI recommended by the WHO (1–4 pg WHO-TEQ kg^{-1}b.w. d^{-1}), and much lower than the TWI and PTMI adopted by SCF and JECFA, respectively

The percentage ofPCDD/Fs daily intake and dL-PCBs daily intake are shown in Figure 4.

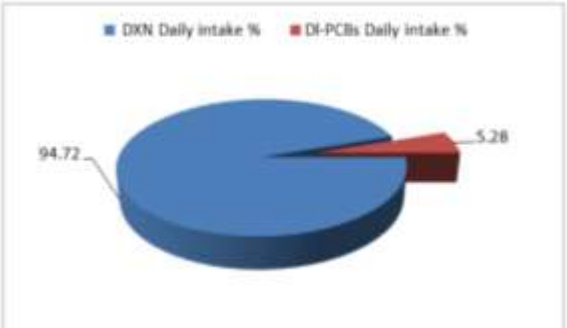

Figure 4. Distribution of dioxin and dl-PCBs daily intake from fish markets.

Conclusion

In all analyzed samples, the most found and the highly detected PCDD/Fsand dl-PCBs congeners were OCDDthen OCDF; and PCBs 118, 105 and 156, respectively.This means that there are ongoing sources for these pollutants. Therefore there must be a strict supervision on all sources that may cause contamination of Egyptian food. It is recommended to continue the monitoring programs in the Egyptian markets to trace, investigate and estimate these pollutants in food to help decision makers to take steps that protect the health of Egyptian Citizens.

Acknowledgements

For Central Lab of Residue Analysis of Pesticides and Heavy Metal in Food-(QCAP), Agricultural Research Center, Egypt.

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