FIRST DATA CONCERNING PCDD/F AND PCB LEVELS IN FOOD SAMPLES FROM GREECE

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Introduction

Due to the new European Commission directive [1] which specifies maximum limits of dibenzo-p-dioxins (PCDDs) and dibenzo-furans (PCDFs) in food products, the Greek government decided the establishment of a laboratory specialized in the isolation and the determination of dioxins and related compounds.

The Mass Spectrometry and Dioxin Analysis Laboratory has been operating since 2002 in the National Center for Scientific Research "Demokritos" in Athens, Greece. It is equipped with specialized analytical instrumentation, suitable for the isolation and the determination of dioxins and PCBs in food samples and biological fluids and for trace analysis of POPs in general.

Collaboration has been established with the Greek official bureaus of food, environment and health control organizations.

The main objective of this study was to obtain representative data on levels of PCDDs, PCDFs and non-ortho polychlorinated biphenyls (non-ortho PCBs) in food consumed by the general population in Greece.

The study included the analyses of different kinds of milk products, pure virgin olive oil, meat and fish samples collected between August and December 2002.

Methods and Materials

Sampling

All food items were collected through the services of the Hellenic Food Authority and were properly transported to the laboratory between August and December 2002 for measurement of PCDDs/PCDFs and non-ortho-PCBs. The samples remained frozen until they were processed.

Extraction

Methods for the isolation of the lipid fraction from specific food products depended on the type of sample.

Fats and oils are generally assumed to be homogeneous, and normally do not require extensive extraction procedures. Aliquots of such samples were dissolved in dichloromethane to the desired fat concentration.

Meat and fish samples were initially blended and homogenised. Next, a representative test sample was ground with anhydrous sodium sulphate, until a free flowing powder was obtained. This mixture was then extracted using a Soxhlet extraction technique [2].

Milk was subjected to a liquid-liquid extraction procedure consisting of mixing with sodium oxalate and methanol, followed by extraction steps with a combination of diethyl ether- petroleum ether [3].

Clean Up

Samples' clean-up was performed according to the method described by Liem et al. [4]. A brief description follows.

Carbon Chromatography

A glass column (length 10 cm, 10 mm I.D.) equipped with mounting ends on both sides, was initially filled with glass wool, 2 g of Carbosphere and another plug of glass wool. The column was connected to a glass funnel. The sample residue was dissolved in 50 ml dichloromethane (~5 ml/g fat) and brought onto the top of the Carbosphere column. This volume of dichloromethane was sufficient to remove almost the complete fat amount (>99%) from the column. The Carbosphere column was placed in a reflux unit and refluxed for 2 h with 30 ml of dichloromethane. This fraction, including residual fat amounts was discarded. Next, the column was rinsed with 20 ml of toluene and refluxed with 30 ml of toluene for 60 min. This fraction, containing the non-ortho PCBs, was concentrated to a volume of about 2 ml in a rotary evaporator and then carefully evaporated to dryness under a gentle stream of nitrogen.

Then the Carbosphere column was inverted in the reflux unit and the PCDD/F fraction was eluted from the column by refluxing with 40 ml of toluene for 16 h. The PCDD/F fraction was concentrated to a volume of about 2 ml and then evaporated to dryness under a gentle stream of nitrogen.

Alumina Chromatography

The obtained residue containing the non-ortho PCBs, was dissolved in 5 ml of hexane and the mixture was brought onto a column (length 30 cm, 8 mm I.D.) pluged with glass wool and filled with 0.5 g of 44% H_2SO_4-silica gel and 5 g of alumina. The non-ortho PCBs were eluted with 50 ml of a hexane/dichloromethane mixture (1:1 v/v). Finally, the eluate was evaporated to dryness and redissolved in 50 µl of toluene containing 2 ng/ml of injection standard (^{13}C12 PCB 80).

The residue containing PCDD/Fs, was dissolved in 5 ml of hexane and the mixture was brought onto a new column prepared as above. PCDDs and PCDFs were eluted with 50 ml of a hexane/dichloromethane mixture (60:40 v/v). Finally, the eluate was evaporated to dryness and redissolved in 50 µl of toluene containing 2 ng/ml of injection standard (^{13}C6 1,2,3,4 TCDD).

Instrumental Analysis

The quantification of non-ortho PCBs and PCDD/Fs was performed by HRGC-HRMS (EI) in MID mode on a Trace GC gas chromatograph (ThermoFinnigan) coupled to a MAT-95 XP mass spectrometer (ThermoFinnigan) equipped with a CTC A 200S autosampler at 10000 resolving power (10% valley definition). Instrumental conditions and purity control criteria are according to the EPA 1613B method [5]. The quantification was carried out by the isotopic dilution method. For TEQ calculations the WHO-98 [6] toxicity equivalent factors (TEF) were used.

Results and discussion

This is the first study of this kind to be undertaken in Greece.

TEQ values of all compounds in pg/g are reported in Table 1, on a fat basis. Lower and upper bound TEQ are calculated assuming that non-detected congeners are equal to zero or to their corresponding limit of detection, respectively.

Milk and dairy products

Cow milk, powder milk, yogurt, butter, yellow cheese and feta cheese samples were analysed. For the milk and dairy products analysed, all concentration values were at low levels, far below the established maximum levels.

Table 1. Levels of non-ortho PCBs, PCDDs and PCDFs (pg/g fat) in the food samples analysed.

	TEQ: PCDD/Fs	**TEQ: *non-ortho* PCBs**	**total TEQ**
Breast Milk	Upperbound: 7.73 Lowerbound: 7.72	Upperbound: 3.52 Lowerbound: 3.52	Upperbound: 11.25 Lowerbound: 11.24
Powder Milk	Upperbound: 0.48 Lowerbound: 0.01	Upperbound: 0.01 Lowerbound: 0.00	Upperbound: 0.49 Lowerbound: 0.01
Cow Milk	Upperbound: 0.47 Lowerbound: 0.08	Upperbound: 0.34 Lowerbound: 0.34	Upperbound: 0.81 Lowerbound: 0.42
Yogurt	Upperbound: 0.51 Lowerbound: 0.04	Upperbound: 0.67 Lowerbound: 0.67	Upperbound: 1.18 Lowerbound: 0.71
Butter	Upperbound: 0.86 Lowerbound: 0.26	Upperbound: 0.66 Lowerbound: 0.66	Upperbound: 1.52 Lowerbound: 0.92
Cheese (yellow)	Upperbound: 0.48 Lowerbound: 0.09	Upperbound: 0.19 Lowerbound: 0.19	Upperbound: 0.67 Lowerbound: 0.28
Cheese (feta)	Upperbound: 1.29 Lowerbound: 1.26	Upperbound: 2.48 Lowerbound: 2.48	Upperbound: 3.77 Lowerbound: 3.74
Eggs (free range)	Upperbound: 0.45 Lowerbound: 0.02	Upperbound: 0.08 Lowerbound: 0.08	Upperbound: 0.53 Lowerbound: 0.10
Sea fish (aquaculture)	Upperbound: 2.38 Lowerbound: 2.30	Upperbound: 5.77 Lowerbound: 5.77	Upperbound: 8.15 Lowerbound: 8.07
Sea fish (wild)	Upperbound: 1.98 Lowerbound: 1.94	Upperbound: 6.65 Lowerbound: 6.65	Upperbound: 8.63 Lowerbound: 8.59
Olive oil	Upperbound: 0.32 Lowerbound: 0.00	Upperbound: 0.02 Lowerbound: 0.02	Upperbound: 0.34 Lowerbound: 0.02
Fish Oil	Upperbound: 1.75 Lowerbound: 1.70	Upperbound: 4.51 Lowerbound: 4.51	Upperbound: 6.26 Lowerbound: 6.21
Beef	Upperbound: 0.59 Lowerbound: 0.51	Upperbound: 0.69 Lowerbound: 0.69	Upperbound: 1.28 Lowerbound: 1.20
Sheep	Upperbound: 0.49 Lowerbound: 0.10	Upperbound: 0.31 Lowerbound: 0.31	Upperbound: 0.80 Lowerbound: 0.41
Pig	Upperbound: 0.41 Lowerbound: 0.13	Upperbound: 058 Lowerbound: 0.58	Upperbound: 0.99 Lowerbound: 0.71
Poultry	Upperbound: 0.32 Lowerbound: 0.01	Upperbound: 0.04 Lowerbound: 0.04	Upperbound: 0.36 Lowerbound: 0.05

Human milk
Concerning the analysis of breast milk from a secondipara mother, the total TEQ PCDD/PCDF level is in the lower end of the range (3.93 Brazil – 22.79 Egypt) measured in the 3rd WHO-monitored study for primiparae in 2001.

Meat and meat products
PCB and PCDD/Fs values of all meat samples (beef, sheep, poultry and pig) were lower than those typically monitored in other European and Mediterranean countries [7].

Fish
Aquaculture and wild fish samples presented similar non-ortho PCB and PCDD/F concentrations when expressed in pg/g fat.
Upperbound concentrations in fish, expressed in pg WHO-TEQ/g product, as established in the new European Regulations, were: 0.07 PCDD/F and 0.23 non-ortho PCB for the wild fish and 0.45 PCDD/F and 1.08 non-ortho PCB for the aquaculture fish.

Oil samples, Eggs
Concerning the olive oil sample, almost all PCDD, PCDF and non-ortho PCB congeners were below the detection limit. Similar was the situation of the free range egg samples, where only very few congeners were detected at low concentration levels. Fish oil exhibited the higher levels, the contribution of non-ortho PCBs representing approximately a 72% of the total TEQ value.

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