Dioxin and PCB levels in human samples from the Greek population

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Introduction

Polychlorinated biphenyls (PCBs) are commercial chemical substances produced in a large scale since 1930, with a wide range of applications in industry, such as for coolant fluids in transformers and dielectric fluids in capacitors. After their health effects became apparent, PCB production was banned in the late 1970s. However, humans are still exposed through PCB leakage of old capacitors and transformers and disposal of contaminated materials.

Dioxins (polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzo-furans (PCDFs)), are formed as undesirable by-products mainly during the production of chlorinated chemicals and during the combustion of municipal and hazardous waste.

Due to potential health hazard (dermal toxicity, immunotoxicity, reproductive effects, teratogenicity, endocrine disruption and carcinogenicity), their monitoring in humans is of high general concern [1,2]. Enough information on POP presence in human tissues from industrialized countries is available to suggest that the concentration of these compounds has decreased during the last 10 years [3,4].

Monitoring of human exposure to PCBs and dioxins, contaminants that accumulate in lipid tissue, is most conveniently performed by analysis of blood plasma or blood serum.

Monitoring of dioxins in human milk is of also great importance, since it is especially feared that lactational exposure to dioxins and related compounds may adversely affect brain development and the immune system of infants and children [5,6].

The present study includes the analyses of non-ortho, mono-ortho, indicator PCBs, and PCDD/Fs in human blood and human milk samples collected between November 2002 and February 2004 and is the first study of this kind to be undertaken in Greece.

Methods and Materials

Collection of samples

Blood samples were collected at the blood donor facilities of the Athens Regional General Hospital "G. Gennimatas" and the General Hospital of Kozani. Approximately 300 ml of blood were collected from each individual. Blood samples were collected in polyethylene recipients. Immediately after sampling, blood samples were processed for serum separation and were properly transported to the laboratory for measurement of PCDDs/PCDFs and PCBs. The blood serum samples, as well as the collected breast milk samples, remained frozen until they were analyzed.

Materials

All chemicals used were residue analysis picograde and were purchased from Promochem (Germany). The isomers for the preparation of the $^{13}C_{12}$ internal standard solutions were purchased from Wellington Laboratories (Canada). The sulfuric acid impregnated silica gel was prepared as follows: silica gel (100 g, 60–200 mesh), pre-washed with methanol and dichloromethane, was activated in an oven at 130 °C for at least two days and then mixed with concentrated sulfuric acid (37.5 ml).

Extraction

Breast milk and blood serum samples were 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 [7].

Clean Up

1. For the determination of PCDDs/PCDFs and non-ortho PCBs, the samples' clean-up was performed according to the method described by Liem et al. [8]. 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.) plugged 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}C 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}C 1,2,3,4 TCDD).

2. For the determination of mono-ortho PCBs and indicator PCBs, the clean-up was performed as follows: In breast milk samples, the residue obtained in the extraction step was dissolved in 5 mL of hexane and brought onto a column (length 30 cm, 8 mm I.D.) plugged with glass wool and filled with 10 g of 44% H_2SO_4-silica gel and 2 g sodium sulphate. The column was eluted with 100 ml of hexane. The eluate was concentrated to a volume of about 5 mL and brought onto an alumina chromatography column as described above and 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 20 ng/ml of injection standard (^{13}C PCB 80).
In blood serum samples, the residue obtained in the extraction step was dissolved in 5 mL of hexane and brought onto a column (length 30 cm, 8 mm I.D.) plugged with glass wool and filled with 2 g of 44% H_2SO_4-silica gel, 5 g alumina and 2 g sodium sulphate. The column was 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 20 ng/ml of injection standard (^{13}C PCB 80). This last procedure was also used for the simultameous determination of indicator, mono-ortho and non-ortho PCBs, in the case of the analysis of the blood samples from Kozani.

Instrumental Analysis
The quantification of non-ortho, mono-ortho and indicator 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 [9]. The quantification was carried out by the isotopic dilution method. For TEQ calculations the WHO-98 toxicity equivalent factors (TEF) were used [10].

Results and discussion
i) <u>Blood serum samples</u>
A total of 62 blood samples from the Athens area (general population, average age 40.9 years, range 27-55 years) were analysed for mono-ortho and indicator PCBs. Ten of these samples were also analysed for non-ortho PCB, PCDD/F contamination.
22 blood samples were collected in Kozani, a rural area (general population, average age 43.5 years, range 28-65 years) and analysed for mono-ortho, non-ortho and indicator PCBs.
The average WHO-TEQ values for individual congeners (except for indicator PCBs, expressed in pg/g fat concentrations) as well as the sums for each category of congeners, along with minimum and the maximum total WHO-TEQ values (total concentrations in the case of indicator PCBs) are given in Tables 1- 3. The contaminating levels were calculated as the TEQ values by multiplying with the corresponding WHO-TEFs for each congener (WHO-ECHS, IPCS, 1998). Concentration and TEQ values of all compounds are reported on a fat basis (pg/g fat). Upperbound concentration TEQ values are calculated, assuming that non-detected individual congener concentrations are equal to their corresponding limit of detection.

Table 1. Levels of indicator-PCBs (ng/g fat) and mono-ortho PCBs (pg/g fat WHO-TEQ) in blood sera analysed. *p** value for regional comparison of the data expressed per g of serum fat.

Indicator PCB (ng/g fat)	**Athens (N=40)**	**Kozani (N=22)**	***p**** **values**
PCB 28	1.80	0.39	0.1133
PCB 52	0.03	0.31	0.0959
PCB 101	0.53	1.51	0.2170
PCB 138	32.17	11.75	<0.0001
PCB 153	58.61	22.59	<0.0001
PCB 180	58.25	20.43	<0.0001
Total	151.40 (54.57-660.24)	56.99 (24.06-122.55)	<0.0001
Mono-ortho PCB (pg/g fat WHO-TEQ)	**Athens (N=40)**	**Kozani (N=22)**	***p**** **values**
PCB 105	0.17	0.10	<0.0001
PCB 114	0.12	0.08	<0.0001
PCB 118	0.69	0.41	<0.0001
PCB 123	0.02	0.01	0.2883
PCB 156	2.04	0.99	0.2122
PCB 157	0.50	0.21	0.0363
PCB 167	0.02	0.01	0.4473
PCB 189	0.06	0.03	0.0002
Total	3.62 (0.98-16.96)	1.84 (0.59-4.64)	0.0003

Indicator PCBs and mono-ortho PCBs in the Athens and Kozani serum samples were analysed using the same method. The regional differences are examined. Statistical analysis was performed with SAS Institute software (SAS Inc.). Means were compared across the two areas by t-test. Significance probabilities (p* values) were calculated for the respective number of samples analysed, based on the formula for comparison of two groups of normally distributed values.

There was regional difference observed. Concerning indicator PCB values, the population mean in Athens (151.40 ng/g fat) was significantly different compared to the population mean in Kozani (56.99 ng /g fat), (*p**<0,0001). Means for PCB138, PCB153, PCB180, and total indicator PCBs values were statistically "significantly different" between the two populations, for a confidence level of 95%.

Concerning mono-ortho PCB values, the population mean in Athens (3.62 pg TEQ/g fat) was significantly different compared to the population mean in Kozani (1.84 pg TEQ/g fat), (*p**<0,0001). Means for PCB105, PCB114, PCB118, PCB157, PCB189 and total mono-ortho PCBs values were statistically "significantly different" between the two populations, for a confidence level of 95%.

Table 2. Levels of non-ortho PCBs, PCDDs and PCDFs (pg/g fat WHO-TEQ) in blood serum from Athens population (N=10) analysed.

2,3,7,8 - TCDD	0.15
1,2,3,7,8 - PeCDD	0.58
1,2,3,4,7,8 - HxCDD	0.27
1,2,3,6,7,8 - HxCDD	1.07
1,2,3,7,8,9 - HxCDD	0.48
1,2,3,4,6,7,8 - HpCDD	0.49
OCDD	0.03
2,3,7,8 - TCDF	0.01
1,2,3,7,8 - PeCDF	0.01
2,3,4,7,8 - PeCDF	2.13
1,2,3,4,7,8 - HxCDF	0.43
1,2,3,6,7,8 - HxCDF	0.32
2,3,4,6,7,8 - HxCDF	0.41
1,2,3,7,8,9 - HxCDF	0.13
1,2,3,4,6,7,8 - HpCDF	0.30
1,2,3,4,7,8,9 - HpCDF	0.02
OCDF	0.00
Total PCDD/F	6.82 (1.86-11.01)
PCB-77	0.00
PCB-81	0.00
PCB-126	2.56
PCB-169	0.64
Total non-ortho PCB	3.20 (1.46-5.50)

Table 3. Levels of non-ortho PCBs (pg/g fat WHO-TEQ) in blood serum from Kozani population (N=22) analysed.

PCB 77	0.03
PCB 81	0.00
PCB 126	1.01
PCB 169	0.21
Total non-ortho PCB	1.24 (0.22-7.22)

The present study showed a relatively wide range of concentrations of PCBs and PCDD/Fs in the blood samples of people belonging to same population.
Non-ortho PCB WHO-TEQ concentrations seem to present regional differences similar to those observed for indicator and mono-ortho PCBs. Values in Athens (3.20 pg/g fat WHO-TEQ) are higher compared to those in Kozani (1.24 pg/g fat WHO-TEQ).
PCB and PCDD/F values of all blood samples were lower than those typically monitored in other European and Mediterranean countries [4,11].

ii) Human milk samples
Eight mother milk samples from the Athens area (general population, average age 33.5 years, range 28-44 years) were analysed for non-ortho, mono-ortho and indicator PCBs, dioxin and dibenzofuran contamination. Results are reported on Table 4.

Table 4. Levels of indicator-PCBs , mono-ortho PCBs non-ortho PCBs and PCDD/Fs in human milk analysed in the Athens area.

Indicator PCB (ng/g fat)	
PCB 28	1.12
PCB 52	0.73
PCB 101	0.86
PCB 138	24.0
PCB 153	43.9
PCB 180	23.8
Total	94.4 (50.6-231.4)
mono-ortho PCB (pg/g fat WHO-TEQ)	
PCB 105	0.25
PCB 114	0.17
PCB 118	0.69
PCB 123	0.11
9CB 156	1.86
PCB 157	0.34
PCB 157	0.01
PCB 189	0.05
Total	3.48 (1.76-8.47)

PCDD/F (pg/g fat WHO-TEQ)	
2,3,7,8 - TCDD	0.73
1,2,3,7,8 - PeCDD	2.14
1,2,3,4,7,8 - HxCDD	0.11
1,2,3,6,7,8 - HxCDD	0.53
1,2,3,7,8,9 - HxCDD	0.10
1,2,3,4,6,7,8 - HpCDD	0.05
OCDD	0.00
2,3,7,8 - TCDF	0.05
1,2,3,7,8 - PeCDF	0.01
2,3,4,7,8 - PeCDF	3.13
1,2,3,4,7,8 - HxCDF	0.16
1,2,3,6,7,8 - HxCDF	0.16
2,3,4,6,7,8 - HxCDF	0.07
1,2,3,7,8,9 - HxCDF	0.02
1,2,3,4,6,7,8 - HpCDF	0.01
1,2,3,4,7,8,9 - HpCDF	0.00
OCDF	0.00
Total	7.27 (3.43-11.28)
non-ortho PCB (pg/g fat WHO-TEQ)	
PCB-77	0.00
PCB-81	0.00
PCB-126	2.84
PCB-169	0.24
Total	3.08 (1.29-5.92)

Concerning the analysis of breast milk, the total TEQ PCDD/PCDF level is in the lowest end of the range measured in Europe [4,12].

In conclusion, contamination levels in blood and human milk from Greece reported here are low compared to the previously reported dioxin data from other European countries and give no indication of particular health risk.
Moreover, significantly higher values were found in Athens compared to the rural region of Kozani.

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