Levels of contamination for various pollutants present in Belgian human plasma

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Introduction

During the last century, numerous compounds, such as polychlorinated biphenyls (PCBs) or organochlorine pesticides (OCPs), were banned because of their bioaccumulative and toxic properties, while other compounds, such as polybrominated diphenyl ethers (PBDEs), appeared on the market and consequently in the environment. The experiences gained from accidents involving PBBs, PCBs or PCDD/Fs are useful to conduct scientific investigations focused on preventing similar catastrophies with the newly introduced compounds. Several studies have reported potential increase in the concentration of PBDEs in food and wildlife. Monitoring the levels of toxic chemicals is therefore useful to understand the exposure pathways, sources and trends.

The aim of the paper is to present actual contamination's levels of various pollutants in human plasma from Belgium. Several classes of pollutants, such PCDD/Fs, PCBs and OCPs were determined in 20 human plasmas. In addition, perfluorooctanesulfonate (PFOS) and related fluorochemicals, which are of current concern, were measured. Although anticipated, concentrations of PBDEs in the same samples were not yet determined. Through this study, a good approximation of the contamination level in Belgian human is given, allowing thus comparison with concentrations observed in other countries.

Methods and Materials

Sample collection

Twenty individual plasma samples were collected in 1998 and 2000 by the Belgian Red Cross and frozen at -80°C after donation. In 2003, samples were thawed and divided in aliquots depending of the amount of plasma needed for the various analyses. All aliquots were stored in PE tubes and kept at -20°C until sending to the different laboratories.

Of the 20 human blood plasmas, only four samples were given by women. The donors were living all over Belgium. The age range of participants varied from 19 to 63 years, with a mean and median of 43.3 and 43.5 years, respectively. The body mass index (BMI) was calculated for 18 donors for whom information on weight and height were available. Mean and median BMI were 26.4 and 25.3 kg/m² (range: 19.4-39.9). Only one donor lived near a municipal solid waste incinerator. The number of current smokers, ex-smokers and non smokers were of 6, 2 and 12 respectively. Additional information on dietary habits and the use of herbicides by donors were also available.

Chemical analyses

Analyses of perfluorooctanesulfonate (PFOS), perfluorooctanoic acid (PFOA) and perfluorohexanesulfonate (PFHS) were performed by the Wadsworth Center (Albany, NY, USA). These compounds were extracted of the plasma samples using an ion-pairing extraction procedure and were determined using an HPLC interfaced with ES-MS/MS. Details on recoveries and limits of quantification were previously published[1].

Concentration of PCBs and OCPs were determined by the Toxicological Center (Antwerp, Belgium). The OCPs under investigation were α-, β-, γ-isomers of hexachlorocyclohexane (HCH), o,p´-DDE, p,p´-DDE, o,p´-DDD, p,p´-DDD, o,p´-DDT and p,p´-DDT, octachlorostyrene (OCS), hexachlorobenzene (HCB), oxychlordane (OxC), trans-nanochlor (TN), trans-(TC) and cis-chlordane (CC). The target PCB congeners were PCB 18, 28, 31, 44, 52, 66, 74, 95, 99, 101, 105, 110, 118, 128, 132, 149, 153, 156, 163, 167, 170, 177, 180, 183, 187, 194, 196 and 199. Details of the procedure were described elsewhere[2]. Briefly, after addition of internal standards and formic acid, 4 mL of plasma was extracted on a solid phase disk extraction cartridge. The extract was then cleaned on acidified silicagel and injected into a GC/MS operated in EI mode for PCBs and into a GC/MS operated in ECNI mode for OCPs.

The Center of Analysis of Residues in Traces (CART, Liège, Belgium) analyzed 17 polychlorinated dibenzo-dioxins and –furans (PCDDs and PCDFs) and 4 non-ortho PCBs. About 40 mL of sample were spiked with a mixture of ^{13}C-labelled standards and mixed with formic acid. This mixture was loaded on a Isolute C_{18} cartridge for extraction, followed by clean-up on a Power-prep system. The purified extracts were then injected in a GC-HRMS[3].

Bio-analyses

CALUX® analyses were performed by the Belgian Scientific Institute of Public Health (Brussels, Belgium). Ten mL of sample were extracted following a liquid-liquid extraction with a mixture of acetone/hexane, and purified on an acid silica column in series with an activated carbon column. After elution from the silica column, the extract was fractionated on the carbon column to yield dioxin and PCB fractions. Only the fraction containing PCDD/PCDFs was applied on mouse hepatoma H1L6.1 cells (XDS Inc., Durham, USA) for the determination of AhR activity. Results were expressed in pg TEQ/g lipids[4].

The estrogen activity of plasma samples was performed by XDS Inc.(Durham, USA) using the LUMI-CELL™ ER recombinant bioassay for estrogen screening. Samples were analyzed by mixing 5 µL of plasma with DMEM media then applying to the BG1Luc4E2 cells[5].

Results and Discussion

Perfluoroalkylated compounds

Concentrations of PFOS, PFOA and PFHS in Belgian plasma are shown in Table 1a). Among the three fluorochemicals, PFOS was present at the highest concentration, followed by PFOA. The observed range of PFOS concentrations is similar to those reported by the OECD for Belgium[1] (4.9 - 22 ng/mL). Mean PFOS concentration in the plasma of Belgian donors was two times lower than those reported for US donors and four times higher than those found in Italy[1]. Concentration of PFOA in Belgian donors was similar to those observed in US donors (4.7 ng/mL) and higher than those reported for Italian donors. This seems to indicate that the pattern of exposure to perfluorochemicals in Belgium is similar to the US.

No significant differences were found for the contamination with fluorochemicals between sexes. Moreover, those concentrations were not age-, BMI- and smoking habit-dependent. Concerning dietary habits, the PFOS concentration was significantly influenced by meat and milk consumption. These observations can be partially explained by the presence of some of these compounds in food. A US study has reported the presence of PFOS in a small number of milk and ground beef samples (data not published).

Polychlorinated biphenyls congeners

PCB concentrations found in the samples are summarized in Table 1a). The sum of the 7 marker PCBs (PCB 28, 52, 101, 118, 138, 153, 180), sum of mono-ortho PCBs (PCB 105, 118, 156 and 167), as well as the total sum of PCBs were calculated to make comparison easier. Since PCB 18, 44, 66, 110, 128, 132 and 149 were found below the limit of quantification in all samples, they were not taken into consideration in calculations. The mean value observed for the 7 marker PCBs is similar to the mean value previously reported in Belgium by Fierens et. al[6] and Koppen et. al[7] and higher than those obtained by Voorspoels et. al[8]. The difference with this latter can however be explained by the mean age of the sampled population, which is higher in the present study. As shown in Figure 1, the congener profile is dominated by the PCB 153, PCB 180 and PCB 138/163. These congeners represent more than 70% of the sum PCBs. Similar profiles were previously observed in Belgium and other countries, as well as in other human matrices (adipose tissue, breast milk or liver)[6-9].

The sum of the 7 marker PCBs, as well as the sum of mono-ortho PCBs and the sum PCBs, were positively correlated with the age of donors. No effect of BMI, smoke, gender or use of herbicides was detected. Following these results, the concentrations of PCBs were not significantly explained by any food consumption.

Organochlorine pesticides

From the 15 targeted OCPs, 8 compounds (α-HCH, o,p´-DDD, o,p´-DDE, o,p´-DDT, p,p´-DDD, TC, CC and OCS) were not quantifiable in any of the 20 plasma samples. In all samples, p,p´-DDE was the major pesticide, followed by HCB, OxC and β-HCH. The predominance of p,p´-DDE is in accordance with previous reports on OCPs in human samples[8,10]. The contamination level with p,p´-DDE is lower than the levels reported in Belgium by Koppen et al.[7], but higher than those reported by Voorspoels et al.[8]. However, the important standard deviation observed in this study must be taken into account. The age of donors was the only determinant observed for β-HCH, HCB and p,p´-DDE.

PCDD/F congeners

When expressed in pg/g lipids, the profile of dioxins congeners was dominated by higher chlorinated compounds such OCDD, 1,2,3,4,5,7,8-HpCDD and 1,2,3,6,7,8-HxCDD. For furans, 2,3,4,7,8-PeCDF was the most predominant congener, followed by 1,2,3,4,7,8-HxCDF and 1,2,3,4,6,7,8-HpCDF. Similar to Sweden and Spain, PCDDs account for 90% of the total PCDD/F concentration[10]. The total PCDD/F levels observed in Belgium is in the same range of concentrations than those previously reported in Sweden[11], Germany[12], United States[13] and lower than that reported in Spain[11] and Portugal[14]. The profile of toxic equivalents reported as pg TEQ/L of plasma is presented in Figure 2. This pattern is dominated by 2,3,4,7,8 PeCDF and 1,2,3,7,8-PeCDD, which represent together more than 70% of the total TEQ for PCDD/F. The mean TEQ-value of 26.1 pg TEQ/g lipids is similar to those observed on larger sampling studies in Belgium[15,16] and slightly higher than levels measured in Germany[12], United States[13] and Portugal[14].

The mean TEQ-value obtained by CALUX® bioassay is similar to the one given by GC-HRMS when using human WHO-TEF. However, the comparison of CALUX® and GC-HRMS results for each sample showed discrepancies ranging from -32 to 52.1 pg TEQ/g lipids. These differences are mainly explained by antagonistic and synergistic effects of other AhR ligands, such as PBDD/PBDFs, possibly present in the dioxin fraction analysed by CALUX®. Actually, those ligands are not completely identified.

None of the possible studied factors by statistical treatment has shown a good correlation with the TEQ-values obtained by GC-HRMS or CALUX®.

Estrogen activity

None of the sample demonstrated any Estrogen activity over the detection limit of 34.67 pg/µL β-Estradiol equivalent using the LUMI-CELL™ ER.

Correlation between chemical compounds

As previously observed[7,15,17,18], significantly high correlations were found between **Σ**7 PCB markers, **Σ**m-o PCBs, **Σ**PCBs, HCB, OxC, TN, PCDD/F TEQ-value, **Σ**PCDF and **Σ**cPCBs. β-HCH was also significantly correlated with **Σ**m-o PCB, HCB and p,p´-DDE.

The CALUX TEQ-value increases significantly as the PCDD/F TEQ value or the **Σ**PCDFs raises. This last observation indicates that the TEQ value is mainly dependent of the furans. This good correlation between CALUX and GC-HRMS TEQ-values were already several times reported for blood samples[4,7].

Concerning the fluorochemicals, no association was found with chlorinated compounds. However, the significant relationship between PFOS and PFOA suggest that the sources of exposures of those fluorochemicals are the same.

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Table 1: Mean and median concentrations for organohalogenated compounds observed in the 20 Belgian human plasma samples.

A)	Concentration (ng/mL)		Concentration (ng/g lipid)	
	Mean (SD)	Median [range]	Mean (SD)	Median [range]
PFOS	15.66 (5.85)	17.20 [4.52 - 27.04]	2268.6 (941.6)	2215.1 [885.3 - 4629.5]
PFOA	4.81 (2.99)	4.12 [1.08 - 12.84]	693.6 (450.3)	677.7 [239.5 - 2112.4]
PFHS	1.26 (0.10)	1.25 [1.14 - 1.44]	171.8 (21.9)	167.5 [144.5 - 197.2]
Σ 7 marker PCB	2.77 (1.48)	2.55 [0.65 - 5.71]	388.5 (226.9)	359.2 [72.6 - 938.7]
Σ m-o PCBs	0.28 (0.16)	0.28 [0.08 - 0.58]	38.6 (23.4)	32.9 [8.7 - 83.8]
Σ PCBs	3.61 (1.96)	3.54 [0.73 - 7.30]	504.8 (298.5)	468.6 [82.5 - 1197.4]
γ-HCH	0.04 (0.02)	0.03 [0.025 - 0.12]	5.4 (2.7)	4.5 [3.1 - 14.6]
β-HCH	0.17 (0.11)	0.15 [0.04 - 0.41]	23.1 (14.7)	16.7 [5.6 - 53.2]
HCB	0.22 (0.14)	0.20 [0.06 - 0.51]	32.0 (19.6)	27.2 [7.3 - 66.9]
OxC	0.16 (0.14)	0.11 [0.03 - 0.48]	22.3 (19.9)	15.8 [4.0 - 67.4]
TN	0.06 (0.03)	0.06 [0.02 - 0.13]	8.4 (4.7)	7.6 [2.5 - 16.7]
p,p´-DDE	2.74 (2.27)	2.48 [0.26 - 9.91]	365.0 (313.2)	344.2 [37.4 - 1390.3]
p,p´-DDT	0.06 (0.02)	0.06 [0.05 - 0.09]	9.1 (2.5)	8.3 [6.0 - 13.5]

B)	Concentration (pg/L)		Concentration (pg/g lipid)	
	Mean (SD)	Median [range]	Mean (SD)	Median [range]
Σ n-o PCBs	1.22 (0.91)	0.81 [0.28 - 3.27]	176.0 (128.0)	132.6 [31.9 - 442.7]
Σ PCDD	3.71 (4.49)	2.57 [0.24 - 21.29]	520.1 (606.4)	375.2 [34.9 - 2885.6]
Σ PCDF	0.38 (0.24)	0.33 [0.10 - 0.86]	56.2 (37.4)	47.0 [13.2 - 144.3]
Σ PCDD/F	4.09 (4.62)	3.27 [0.35 - 22.15]	576.2 (624.5)	454.3 [51.9 - 3002.1]

C)	Concentration (pg TEQ/L)		Concentration (pg TEQ/g lipid)	
	Mean (SD)	Median [range]	Mean (SD)	Median [range]
CALUX TEQ	187.8 (144.8)	151.0 [41.7-597.4]	26.9 (23.2)	19.9 [4.9 - 102.5]
PCDD/F TEQ	176.2 (119.8)	122.5 [30.0-431.5]	26.1 (18.0)	18.6 [4.4 - 60.1]

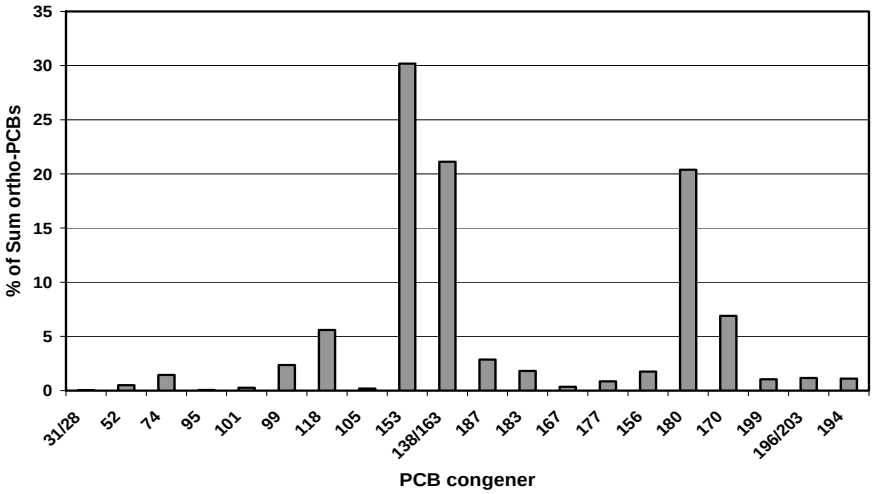

Figure 1: PCB profiles in Belgian human plasma

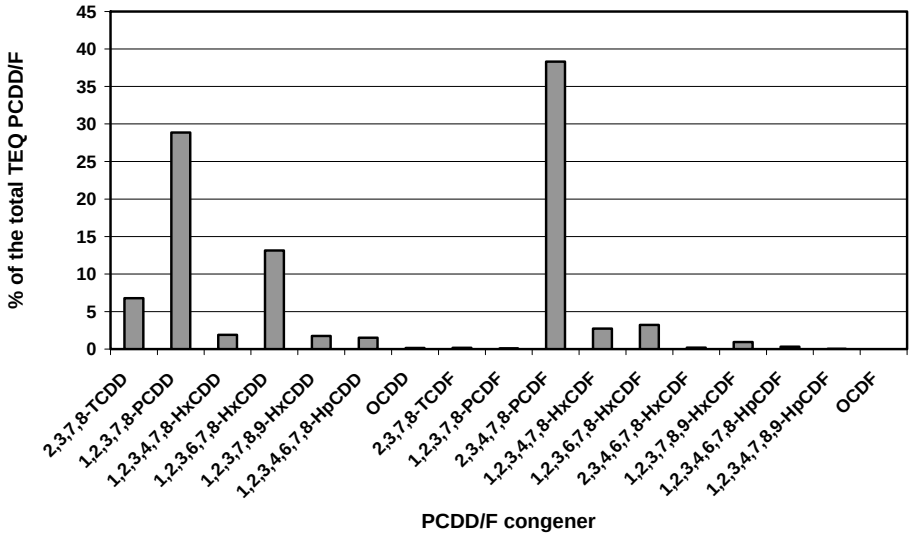

Figure 2: PCDD/F profile in Belgian human plasma