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PCDDs, PCDFs, DIOXIN-LIKE PCBs, DI-ORTHO PCBs AND PBDEs ANALYSIS MADE POSSIBLE IN 20 ML HUMAN BLOOD SAMPLE

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Introduction

According to the literature, polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (PCDFs) analysis of human blood at a level of ≈ 10 pg-TEQ/g of fat requires at least 50 mL sample size. However, the collection of 50 mL blood sample is often at the root of anguish and pain for test subjects, especially for infants and aged persons. It is the reason why realistic methods should take into account the reduction of sample size[1]. Starting from 20 mL blood sample, the method presented hereafter is dedicated to the 17 PCDD/PCDF congeners as well as the 12 "dioxin-like" polychlorinated biphenyls (PCBs) in order to correctly assess TEQ concentration[2] in the samples. In addition, di-ortho PCBs and seven polybrominated diphenyl ethers (PBDEs) were determined.

Materials and Methods

All the organic solvents (Promochem) were Picograde® quality. Silica (Fluka), sodium sulfate and dipotassium oxalate (Merck), acetic acid and sulfuric acid (SDS) were of superior analytical quality. Native and ^{13}C-labeled standards were purchased from CIL and Wellington Laboratory. Standard solutions were prepared in toluene for PCDDs/PCDFs and PBDEs and in iso-octane for PCBs and stored in darkness at < 6 °C.

20 mL blood were collected from healthy human volunteers who worked in our dioxin laboratory for two years. In the same way, a blank (water) analysis was perfomed by carrying out the entire analytical procedure. Before extraction, 17 ^{13}C-labelled PCDDs/PCDFs, 18 ^{13}C-labelled PCBs and 7 ^{13}C-labelled PBDEs were added to the samples. After spiking, the samples were extracted with adequate solvents using a liquid/liquid extraction for blood[3]. Blood was diluted with deionized water (20 mL) and shaken for 30 min. Extraction procedure was performed as following: addition of 20 mL aqueous saturated ammonium sulfate sodium, shaking for 1 min, extraction with 20 mL of hexane then extraction with 40 mL of hexane. The hexane layer was dried with anhydrous sodium sulfate and evaporated at 40°C to dryness, permitting the estimation of the fat weight. The residue, which represents the fat content, was weighed and redissolved in hexane for sample clean-up. A three step purification was performed, using successively silica, florisil and celite/carbon columns. After removal of fat on a silica gel column loaded with sulfuric acid, PCBs were separated from PCDDs/PCDFs by means of a Florisil column. The PCDD/PCDF fraction was further cleaned up onto a column consisting of a mixture of Carbopack C/Celite 545. Separation of coplanar (non-ortho) PCBs from non-planar PCBs and PBDEs was achieved on a activated mixture of Florisil/ Carbopack C/Celite 545 (overnight at 130°C). After addition of external standards for the recovery calculation ($^{13}C_{12}$-1,2,3,4-TCDD for the PCDD/F, $^{13}C_{12}$-PCB #111 for the PCBs, $^{13}C_{12}$-PBDE #139 for the PBDEs), the final sample extract was evaporated

under a nitrogen stream to dryness and reconstituted by addition of 5 μL of toluene for the PCDD/Fs, 10 μL of toluene for coplanar PCBs and 50 μL of toluene for non-planar PCBs and PBDEs.
The GC-HR-MS detection was performed on a HP 6890 gas chromatograph, equipped with a DB-5MS column (30 m x 0.25 mm, 0.25 μm film thickness), and coupled to a Jeol JMS-700D high resolution mass spectrometer. The injection volume was 2 μL.

Results and Discussion

Average results through this work (≈ 12 pg-TEQ(PCDD/F)/g of fat) are comparable with data published recently regarding in German population[4,5] and demonstrate a low rate of exposure for people working in the laboratory for two years (aged under forty). As an example of results, all the values of one sample (10,41 pg-TEQ(PCDD/F)/g of fat) are shown in Table-1 and some corresponding chromatograms of low concentration compounds are illustrated in Figure 1.
All compounds are detected except for four of them (1,2,3,7,8-PeCDF, 2,3,4,6,7,8-HxCDF, 1,2,3,7,8,9-HxCDF and 1,2,3,4,7,8,9-HpCDF) and recoveries were in the 40-90 % range. The gas chromatographic separation of isomers was sufficient (<25 % peak to peak between 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF). Therefore, with 20 mL blood, this method is applicable with accuracy to dioxin analysis for human-blood at low pg-fat concentration level (≈ 10 pg-TEQ/g of fat) but also for PCBs "dioxin-like", di-ortho PCBs and PBDEs from an unique sample extract.
Moreover, we can observe that "dioxin-like" PCBs contribute approximately to 50 % of the total TEQ, which confirms the importance to determine these compounds with accuracy (all compounds are detected). In the samples, PCB#138, PCB#153, PCB#180, and PBDE#47 are the most abundant. This is a good representation of the human exposure of these compounds, which are very persistent in the environment.

References

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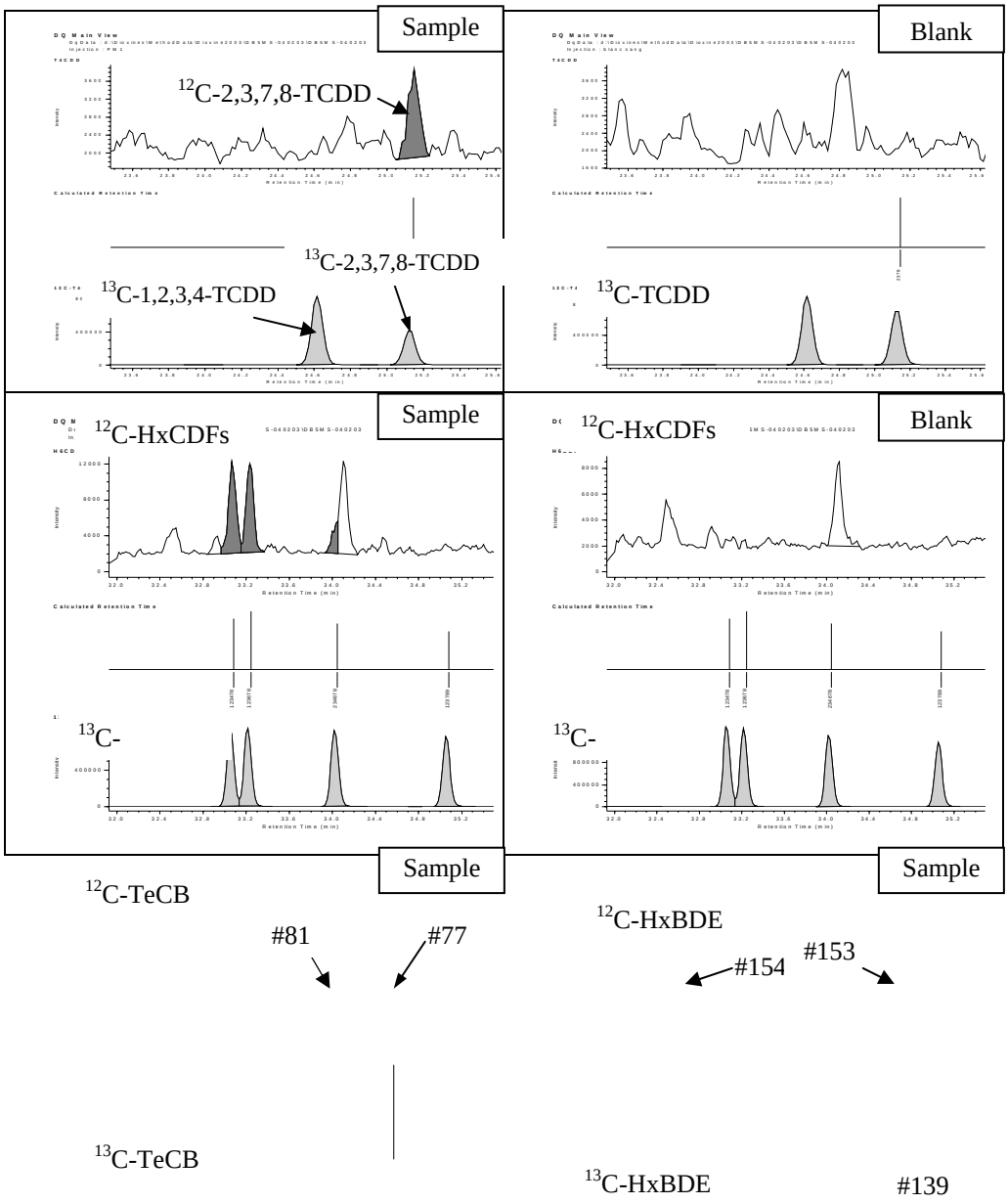
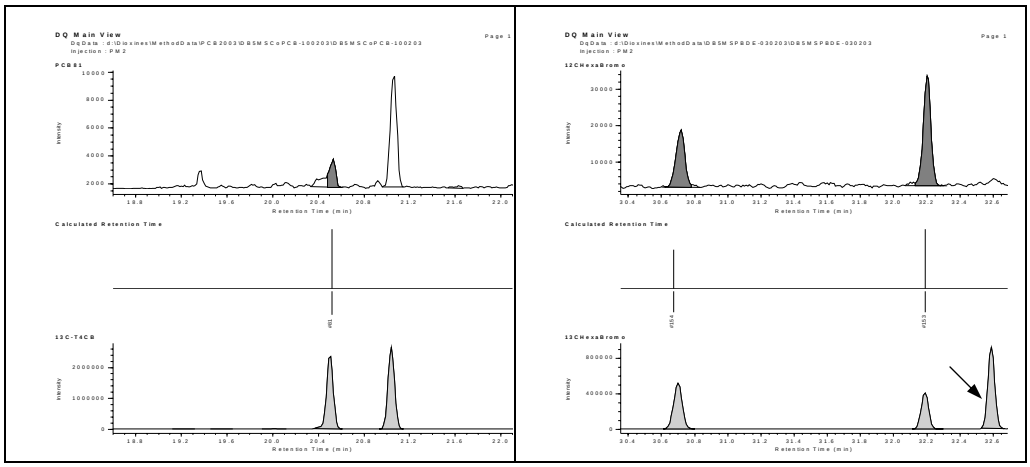
Table 1. Monitoring of 42 analytes in 20 mL blood sample from a 36 year old volunteer (1m75, 70 kg, Caucasian)

Compounds	Injection concentration pg/µL	Concentration pg/g fat
Dioxins		
2,3,7,8 - TeCDD	0.02	0.7
1,2,3,7,8 - PeCDD	0.09	4.0
1,2,3,4,7,8 - HxCDD	0.04	1.7
1,2,3,6,7,8 - HxCDD	0.30	13.1
1,2,3,7,8,9 - HxCDD	0.05	2.2
1,2,3,4,6,7,8- HpCDD	0.36	16.0
OCDD	2.32	102.1
2,3,7,8 - TeCDF	0.01	0.2
1,2,3,7,8 - PeCDF	<0.01	<0.1
2,3,4,7,8 - PeCDF	0.15	6.4
1,2,3,4,7,8 - HxCDF	0.01	2.5
1,2,3,6,7,8 - HxCDF	0.05	2.2
2,3,4,6,7,8 - HxCDF	<0.01	<0.2
1,2,3,7,8,9 - HxCDF	<0.01	<0.1
1,2,3,4,6,7,8 -HpCDF	0.08	3.4
1,2,3,4,7,8,9 -HpCDF	<0.01	<0.2
OCDF	0.03	1.4
Dioxin-like PCBs		
3,3',4,4'-TeCB (#77)	0.06	10.3
3,4,4',5-TeCB (#81)	0.01	2.5
3,3',4,4',5-PeCB (#126)	0.16	28.3
3,3',4,4',5,5'-HxCB (#169)	0.19	34.6
2,3,3',4,4'-PeCB (#105)	4.03	1831.4
2,3,4,4',5-PeCB (#114)	1.22	554.6
2,3',4,4',5-PeCB (#118)	19.45	8842.7
2',3,4,4',5-PeCB (#123)	0.73	331.8
2,3,3',4,4',5-HxCB (#156)	12.43	5650.0
2,3,3',4,4',5'-HxCB (#157)	2.51	1140.0
2,3',4,4',5,5'-HxCB (#167)	2.85	1297.3
2,3,3',4,4',5,5'-HpCB (#189)	1.58	720.0
Di-ortho PCBs		
2,4,4'-TriCB (#28)	1.48	671.8
2,2',5,5'-TeCB (#52)	1.06	479.6
2,2',4,5,5'-PeCB (#101)	1.56	707.3
2,2',3,4,4',5'-HxCB (#138)	68.50	31134.6
2,2',4,4',5,5'-HxCB (#153)	138.65	63020.5
2,2',3,4,4',5,5'-HpCB (#180)	103.18	46900.0
PBDEs		
2,4,4'-TriBDE (#28)	0.41	179.4
2,2',4,4'-TeBDE (#47)	24.64	10807.0
2,2',4,4',5-PeBDE (#99)	7.59	3327.6
2,2',4,4',6-PeBDE (#100)	1.38	603.5
2,2',4,4',5,5'-HxBDE (#153)	1.10	482.9
2,2',4,4',5,6'-HxBDE (#154)	0.24	104.8
2,2',3,4,4',5',6-HpBDE (#183)	0.46	202.6
WHO-TEQ (PCDDs/PCDFs)		**10.41**
WHO-TEQ (Dioxin-like PCBs)		**8.03**
Total WHO-TEQ		**18.44**

Figure 1. GC-HRMS ion chromatograms of low concentrated PCDD/F, PCBs and PBDE. For the dioxins, left and right column corresponds to sample and blank signals, respectively. In each windows, upper signal represents ^{12}C congeners and lower signal ^{13}C congeners.




DQ Main View
Page 1
PCB81
Intensity
Retention Time (min)
Calculated Retention Time
13C-T4CB
DQ Main View
Page 1
12CHexaBromo
Intensity
Retention Time (min)
Calculated Retention Time
13CHexaBromo