Investigation of PCDD/F, dioxin-like and markers PCBs in blood: results for french donors from pooled blood after validation of the method with 10 mL samples.

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Introduction

In the field of human exposure assessment to environmental contaminants, blood or plasma represent two matrices of choice for laboratory analysis. Indeed, these biological fluids are recognized to be good indicators of the organism exposure, they are available for all the population without age or sex restrictions, and the sample collection remains not so difficult in practice compared to other possible alternatives such as breast milk or adipose tissue. Nevertheless, the sample volumes (generally limited) required powerful analytical performances[1,2,3] that provides both sensitivity and specificity. In this context, the present study presents a new multi-residue analytical method dedicated to the identification of 17 PCDD/PCDF and 19 PCBs ("dioxin-like" and markers) from human serum or plasma using only 10 mL as sample volume. A complete validation of this method was performed according to current European criteria, demonstrating its suitability for routine analysis. Finally, the method was applied to the determination of the exposure level of two groups of French volunteers, one coming from a laboratory in charge of dioxin analysis, and one other coming from a university hospital service.

Materials and Methods

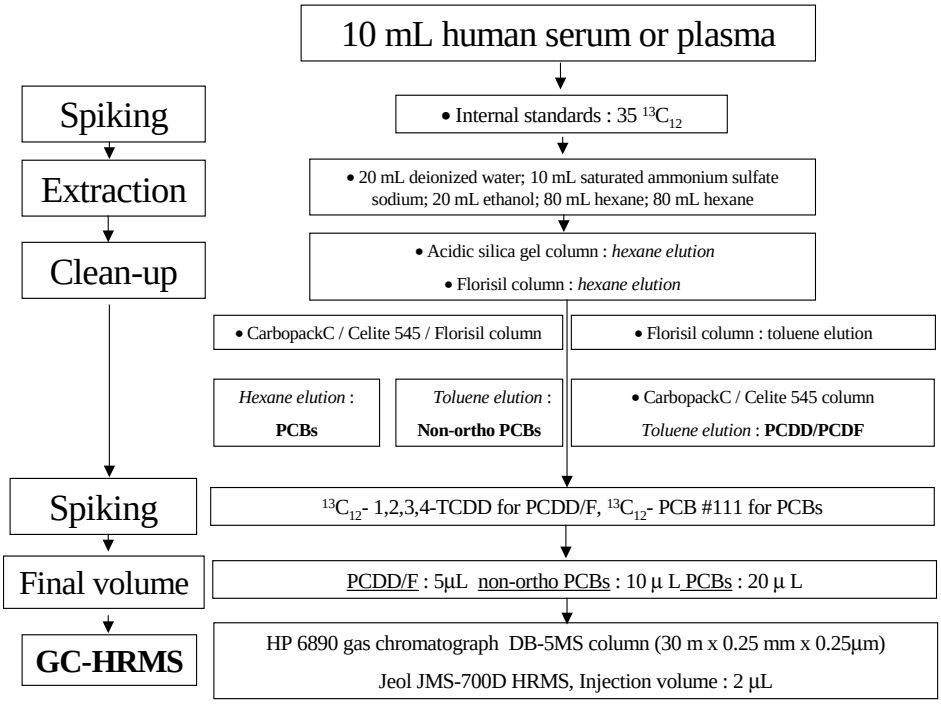
Samples

The first sample used in this study was a 250 mL pool of serum collected in 2003 from 22 healthy human volunteers working in the French National Reference Laboratory for dioxin. These donors included 12 female and 10 male subjects ranging from 22 to 60 years old (35 years old in average). This sample (S1) was then divided into nineteen 10 mL aliquots and two 20 mL aliquots. A second sample was a 250 mL pool of plasma collected in 2003 from 194 anonymous human volunteers hospitalized in a French university hospital service (Bichat-Claude Bernard, Paris). This sample (S2) was also divided into eighteen 10 mL aliquots and two 20 mL aliquots. The lipid content of these two samples was determined using classical biochemical assays (sample size was 100 µL).

GC/HRMS analysis

The identification and quantification of the analytes (dioxins and PCBs) were achieved by gas chromatography-high resolution mass spectrometry (GC-HRMS, R > 10000, EI ionisation), according to a previously described procedure[3] (Figure 1). Regarding the sample preparation, 10mL serum or plasma were diluted in 20 mL of deionised water and shaken for 1 min. Thus, 20 mL ethanol was added and the sample was shaken again for 1 min. The analytes of interest were then extracted with 2 x 80 mL of hexane. The analyses were performed in two separate batches on different weeks.

Figure 1. Schematic diagram of the method



Results and Discussion

The results for lipid content were 0.458 % and 0.444 % for S1 and S2, respectively.
The results obtained in the two analyzed samples for dioxin + "dioxin like" PCBs and indicator PCBs are presented in Tables 1 and 2, respectively. According to the actual regulation, dioxin and "dioxin like" PCBs results are expressed in pg/g of fat (ppt) and a toxic equivalent amount (TEQ) is calculated, while indicator PCBs results are expressed in ng/g of fat (ppb). Except three of them (1,2,3,7,8-PeCDF, 1,2,3,7,8,9-HxCDF, 1,2,3,4,7,8,9-HpCDF), all the target congeners were detected with recoveries in the 50-90 % range. The GC separation of isomers was found to fulfil the actual requirements (resolution better than 25 % peak to peak between 1,2,3,4,7,8-HxCDF and 1,2,3,6,7,8-HxCDF). The intra-day repeatability was also satisfying, with RSD lower than 5 % for WHO-TEQ (dioxin and "dioxin like" PCBs) and in the 2.7 to 12.0 % range for each indicator PCBs congener. Graphical displays of these contamination profiles are shown in Figure 2, which appeared very similar for the two analyzed samples.

Table 1. PCDDs, PCDFs and dioxin-like PCBs values (pg/g of lipid). ND = not detected.

	S1			*S2*		
	10 mL (n=19)		*20 mL (n=2)*	*10 mL (n=18)*		*20 mL(n=2)*
	Mean	SD %	Mean	Mean	SD %	Mean
2,3,7,8 - TeCDD	3.01	16.0	2.54	1.85	28.4	2.00
1,2,3,7,8 - PeCDD	10.28	9.4	9.91	7.16	18.1	7.94
1,2,3,4,7,8 - HxCDD	5.34	13.5	5.49	3.35	13.9	2.93
1,2,3,6,7,8 - HxCDD	36.56	8.4	37.75	26.2	9.3	29.14
1,2,3,7,8,9 - HxCDD	7.04	13.4	6.96	4.70	22.4	4.19
1,2,3,4,6,7,8- HpCDD	61.09	9.5	54.91	54.56	14.3	51.46
OCDD	314.26	13.8	305.98	293.20	12.7	309.49
2,3,7,8 - TeCDF	0.82	35.3	0.63	0.56	39.5	0.56
1,2,3,7,8 - PeCDF	ND	-	ND	0.63	45.5	0.51
2,3,4,7,8 - PeCDF	21.26	4.9	20.40	15.87	9.1	15.77
1,2,3,4,7,8 - HxCDF	6.10	12.5	6.50	5.47	14.1	5.66
1,2,3,6,7,8 - HxCDF	7.12	12.5	6.74	5.88	12.4	5.69
2,3,4,6,7,8 - HxCDF	2.44	21.6	2.54	2.31	23.3	2.34
1,2,3,7,8,9 - HxCDF	ND	-	ND	ND	-	ND
1,2,3,4,6,7,8 -HpCDF	13.96	13.1	12.47	13.24	9.9	11.94
1,2,3,4,7,8,9 -HpCDF	ND	-	ND	ND	-	ND
OCDF	4.01	39.6	1.99	3.90	25.1	1.86
3,3',4,4'-TeCB (#77)	15.8	19.1	8.8	42.0	9.3	24.8
3,4,4',5-TeCB (#81)	4.8	15.5	4.1	4.1	20.3	4.3
3,3',4,4',5-PeCB (#126)	95.0	7.5	90.0	69.2	5.1	70.7
3,3',4,4',5,5'-HxCB (#169)	114.6	7.6	123.1	80.2	7.3	80.0
2,3,3',4,4'-PeCB (#105)	6809	3.2	7136	4905	2.6	5093
2,3,4,4',5-PeCB (#114)	2306	4.9	2386	1604	3.1	1710
2,3',4,4',5-PeCB (#118)	32348	4.4	32660	25853	3.1	28073
2',3,4,4',5-PeCB (#123)	251	25.2	280	241	27.1	201
2,3,3',4,4',5-HxCB (#156)	21661	4.2	22338	15252	5.2	16554
2,3,3',4,4',5'-HxCB (#157)	4372	3.9	4494	2968	3.9	3166
2,3',4,4',5,5'-HxCB (#167)	5832	5.1	5901	4127	6.7	4623
2,3,3',4,4',5,5'-HpCB (#189)	2376	14.0	2506	1811	10.3	2172
WHO-TEQ (PCDDs/PCDFs)	**31.77**	**5.1**	**30.64**	**23.05**	**8.4**	**24.08**
WHO-TEQ (Dioxin-like PCBs)	**28.81**	**4.1**	**28.91**	**20.78**	**3.9**	**21.97**
Total WHO-TEQ	**60.58**	**4.5**	**59.55**	**43.83**	**6.1**	**46.05**

Table 2. Marker PCBs values (ng/g of lipid)

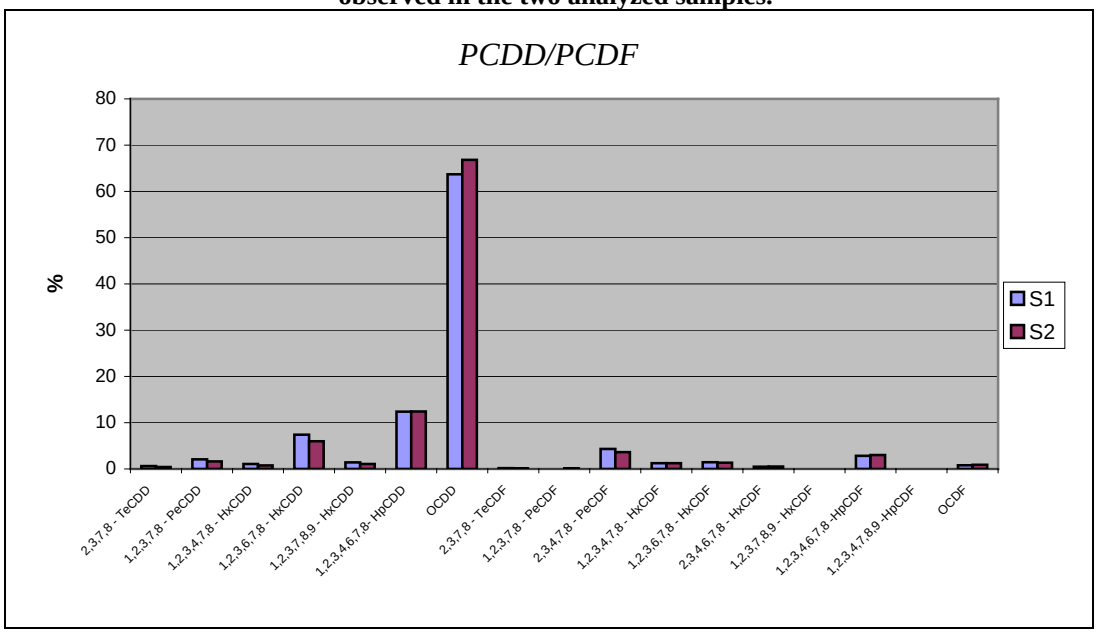
	S1			S2		
	10 mL (n=19)		*20 mL (n=2)*	*10 mL (n=18)*		*20 mL(n=2)*
	Mean	SD %	Mean	Mean	SD %	Mean
2,4,4'-TriCB (#28)	3.1	4.6	2.9	2.7	2.7	2.7
2,2',5,5'-TeCB (#52)	1.2	12.0	1.1	1.1	6.8	1.1
2,2',4,5,5'-PeCB (#101)	1.4	8.5	1.3	1.5	5.0	1.5
2,3',4,4',5-PeCB (#118)	32.3	4.4	32.6	25.9	3.1	28.1
2,2',3,4,4',5'-HxCB (#138)	89.2	4.8	91.4	79.4	4.5	80.4
2,2',4,4',5,5'-HxCB (#153)	184.9	4.6	195.5	155.4	6.0	159.1
2,2',3,4,4',5,5'-HpCB (#180)	162.2	8.1	187.9	119.3	7.9	127.2

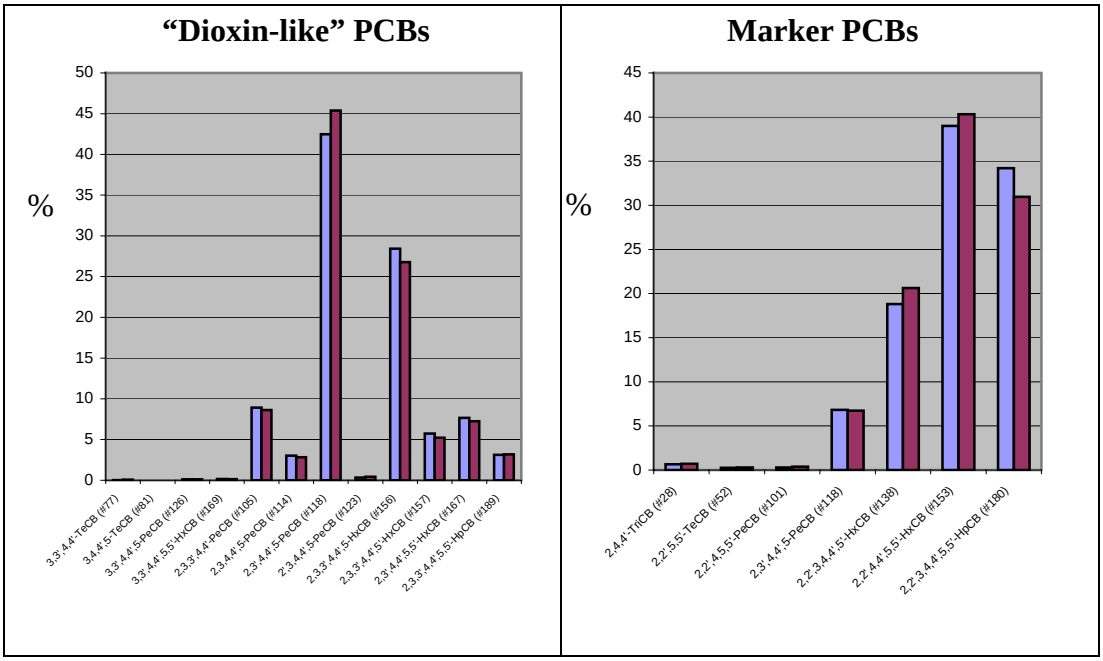
Regarding the dioxin and "dioxin like" PCBs, the three congeners 1,2,3,7,8-PeCDD, 2,3,4,7,8-PeCDF and 3,3',4,4',5-PeCB (#126) were the most abundant in the two analyzed samples, accounting for exactly 50.2 % of the total WHO-TEQ. Moreover, "dioxin like" PCBs contribute to around 50 % of the total TEQ (47.6 % and 47.4 % for S1 and S2, respectively). These results confirmed the importance to determine all the congeners involved in the TEQ determination with high accuracy. Regarding the marker PCBs, the congeners #138, #153 and #180 were found to be the most abundant, in accordance with the observations commonly reported in environmental compartments. No toxic equivalent factors (TEF) have been defined for these compounds, despite a recognized endocrine disruptive activity[4]. Therefore, a systematic measurement of these compounds in exposure assessment programs could be of particular interest in determining their overall interference on hormonal homeostasis in living organisms. The very similar results obtained for 10 mL and 20 mL samples demonstrated the absence of analytical troubles for reduced sample size and the suitability of the method for the 10 mL samples.

Conclusion

The present study presents an innovative analytical method dedicated to the analysis of dioxin and PCBs in serum or plasma samples. The advantages of the proposed approach were to authorize a large multi-residue determination (dioxin, "dioxin like" PCBs and marker PCBs) using a unique sample with a reduced volume of 10 mL only. The performances of the method were found to be compliant with the present European requirements. Its application to the determination of the contamination in two serum and plasma sample pools collected in France shown an exposure level comparable with data recently published regarding other European[5,6,7], USA[8] and Japanese[9] sub-population groups.

Figure 2. Dioxin, "dioxin-like" PCBs and Marker PCBs contamination profiles observed in the two analyzed samples.





Acknowledgements

We want to address our thanks to all the volunteers who participated to the study through a blood gift. Our special thanks to Nathalie Seta, for the coordination of the plasma samples collection in the Bichat-Claude Bernard Hospital, Paris.

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