WORLDWIDE INTERLABORATORY STUDY ON PCDDs, PCDFs, DIOXIN-LIKE PCBs, MARKER PCBs AND PBDEs IN HUMAN PLASMA

Eppe G, De Pauw E, Focant J-F

CART Mass Spectrometry Laboratory, Chemistry Department, University of Liège, Allée de la Chimie 3, B-6c Sart-Tilman, B-4000 Liège, Belgium

Abstract

This paper describes a worldwide interlaboratory study on PCDDS, PCDFs, dioxin-like PCBs, indicator PCBs and PBDEs in no-artificially fortified human blood plasma. The study took place from January 2006 to July 2006. The test material was sent to 10 participants from 7 countries. The study design involved the analysis of one test sample in triplicate. The results reported for PCDDs were satisfactory with a range of relative standard deviation (RSD) of 9-25%, except OCDD (RSD 61%). Four congeners (2, 3, 4, 7, 8-PentaCDF, 1, 2, 3, 4, 7, 8-HexaCDF, 1, 2, 3, 6, 7, 8-HexaCDF, 1, 2, 3, 4, 6, 7, 8-HeptaCDF) out of the ten PCDFs were measured reliably (RSD 11-28%). Good results were achieved for PCBs except the CB 77 (RSD 48%), CB 28 (RSD 50%), CB 52 (RSD 63%), CB 101 (RSD 60%). Levels of PBDEs in the test material were very low (i.e. ng/L range). Results showed that a further improvement for BDE 47 (RSD 114%) and BDE 99 (RSD 81%) is needed. Too scarce results were reported for BDE 209 to assess its performances. The study also highlighted the issue of lipid determination. Enzymatic methods provide higher results compared to gravimetric methods. As scientific literature compares human exposure to these contaminants on a lipid weight basis in serum, the results presented here point out the necessity of standardizing lipid measurements.

Introduction

Human biomonitoring focused on the exposure to PCDDs, PCDFs, PCBs and PBDEs demands the availability of reliable data on the concentration of these contaminants in adipose tissues, blood and breast milk. The difficulties in obtaining human adipose tissues limit its use in epidemiological studies. Breast milk and blood collection are a much less invasive procedure but they present significant analytical challenges. Breast milk has the advantage to have high content of fat and high levels of target compounds compared to serum, making the extraction easier and the precision of the measurement on lipid weight basis easier. Since the mid-eighties, the World Health Organisation (WHO) has coordinated programme on possible health risks of those contaminants, especially in infants, due to exposure through contaminated breast milk. It has however the double disadvantage to be limited to a specific part of the general population and to require great care concerning the time point at which samples are collected in regards of toxicant depuration while breast feeding is taking place. Blood then appears as an interesting alternative as it can be easily obtained but it has the disadvantage to lower the target compound levels, as the lipid content is below 1% by weight. A previous international intercalibration study on PCDD/Fs in human milk and blood already pointed out that the RSDs of the data from blood tent to be systematically larger for a given PCDD/Fs congener than the data from milk[1]. This survey sums up the analytical performances of laboratories for PCDD/Fs, for some PCBs and also for PBDEs in blood more than fifteen years after the last exercise.

Materials and Methods

Test Material

The test material consisted of naturally contaminated human blood plasma not fortified with standards. It represents an aliquot of a pool of 5000 Belgian male and female donors aged from 18 to 65 years old. Participants received a sub-sample of 60-70 ml from a homogeneous batch of approximately one litre. The samples were shipped frozen (packed dry ice) and stored in a sealed amber glass vial. Most of the samples arrived frozen. For those damaged or

defrost during transport, they were immediately replaced. Thus, all the participants received the material in the requested conditions. The batch sample was tested for homogeneity before shipping. Six sub-samples of 20 ml were randomly sampled from six different bottles. Target analytes were analyzed under repeatability conditions and RSDs between 3% and 10% were achieved for the different congeners.

Target compounds

The content in all the seventeen 2,3,7,8 toxic PCDD/Fs, the twelve dioxin-like PCBs (77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, 189), the six indicator PCBs (28, 52, 101, 138, 153, 180), and eight PBDEs (28, 47, 99, 100, 153, 154, 183, 209) were measured by GC-HRMS.

Methods used by the participants

Participants applied their own extraction and clean-up procedures. Three laboratories performed an acidic pre-treatment of the sample prior to extraction; seven laboratories not. All laboratories spiked with the seventeen ^{13}C-labelled 2,3,7,8 substituted PCDD/Fs, the twelve ^{13}C-labelled dioxin-like PCBs, the six ^{13}C-labelled indicator PCBs and the seven or eight ^{13}C-labelled PBDEs. Extraction methods were mainly liquid/liquid (7x) and C_{18}-solid phase extraction (SPE) (3x). Classical multi-steps clean-up on columns with different adsorbents (silica, alumina, florisil and carbon) were carried, each laboratory has its own validated procedure. Different GC columns and different standard solutions were used but all used high-resolution sector instruments operating in electron impact at 10000 resolution in selected ion monitoring mode (except lab 7 for mono- and di-*ortho* PCBs). An overview of the GC-HRMS methods used is given in Table 1.

Lipid determination

Participants performed a separate lipid determination on the plasma. They used their own lipid determination procedure. Five laboratories used a gravimetric method and five laboratories reported data with an enzymatic method.

Study design

The statistical treatment of the data was performed on ‘results corrected for blanks’. For several reasons, some laboratories estimated not relevant or not necessary to remove the blank, others lost the blank. For those labs, the ‘results not corrected for blanks’ were used.

To determine the consensus value, we have selected the following approach. For all target analytes, median were calculated for all reported results. ‘ND’, ‘<LOD’ or ‘<LOQ’ were not used for assessment of the median due to the wide range of LOD/LOQ reported by participants. In addition, obvious outliers above ± 2 standard deviation (SD) were removed to assign values.

Results and discussion

Table 2 summarizes the performances. Due to low levels, high dispersion and few reported results, the following congeners (2,3,7,8-TetraCDF; 1,2,3,7,8-PentaCDF; 1,2,3,7,8,9-HexaCDF; 1,2,3,4,7,8,9-HepaCDF, OctaCDF, PCB 81, PBDE 28, PBDE 209) were not statistically treated. According to the provided data, only an indicative value ‘less than’ was reported. Basic statistics calculated for each analyte include the median, the mean and the relative standard deviation RSD (%). RSDs between 9% and 25% were obtained for PCDDs except for OCDD (61%) for which two laboratories reported much higher values. We already mentioned the difficulty to reliably measure five

Table 1 : (HR)GC-HRMS conditions for the participating laboratories.

Compounds	Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7	Lab 8	Lab 9	Lab10
PCDD/Fs										
Column 1 (sat. phase, length, ID, Film thickness)	DB-5MS 60m x 0.25mm x 0.25µm	DB5-MS 60m x 0.25mm x 0.10µm	DB5 30m x 0.25mm x 0.10µm	DB5-MS 30m x 0.25mm x 0.25µm	DB5-MS 60m x 0.25mm x 0.25µm	DB5 60m x 0.25mm x 0.10µm	BPX-5 50m x 0,32mm x 0,17µm	VF5-MS 50m x 0.20mm x 0.33µm	DB5-MS 30m x 0.25mm x 0.25µm	Not measured
run time	53 min	50 min	40 min	30 min	45 min	45 min	40 min	50 min	47.5 min	
Column 2 (sat. phase, length, ID, Film thickness)		RTX-2330 60m x 0.25mm x 0.2µm								
run time		55 min								
Detector	HRMS	HRMS	HRMS	HRMS	HRMS	HRMS	HRMS	HRMS	HRMS	
Non-Ortho PCBs										
Column 1 (sat. phase, length, ID, Film thickness)	DB-5MS 60m x 0.25mm x 0.25µm	DB5 60m x 0.25mm x 0.10µm	HT8 50m x 0.22mm x 0.25µm	DB5-MS 30m x 0.25mm x 0.25µm	DB5-MS 60m x 0.25mm x 0.25µm	DB5 60m x 0.25mm x 0.10µm	BPX-5 50m x 0,32mm x 0,17µm	VF5-MS 50m x 0.20mm x 0.33µm	HT8 60m x 0.25mm x 0.20µm	Not measured
run time	53 min	45 min	40 min	30 min	45 min	45 min	40 min	50 min	55 min	
Detector	HRMS	HRMS	HRMS	HRMS	HRMS	HRMS	HRMS	HRMS	HRMS	
Mono-ortho PCBs										
Column 1 (sat. phase, length, ID, Film thickness)	DB-5MS 60m x 0.25mm x 0.25µm	HT8 60m x 0.25mm x 0.20µm	HT8 50m x 0.22mm x 0.25µm	DB5-MS 30m x 0.25mm x 0.25µm	DB5-MS 60m x 0.25mm x 0.25µm	DB5 60m x 0.25mm x 0.10µm	HT5 25m x 0,22mm x 0,25µm	HT8 25m x 0.22mm x 0.25µm	HT8 60m x 0.25mm x 0.20µm	DB5-MS 30m x 0.25mm x 0.25µm
run time	53 min	50 min	40 min	30 min	45 min	45 min	30 min	30 min	55 min	30 min
Detector	HRMS	HRMS	HRMS	HRMS	HRMS	HRMS	LRMS	HRMS	HRMS	HRMS
Di-ortho PCBs										
Column 1 (sat. phase, length, ID, Film thickness)	Not measured	HT8 60m x 0.25mm x 0.20µm	HT8 50m x 0.22mm x 0.25µm	DB5-MS 30m x 0.25mm x 0.25µm	DB5-MS 60m x 0.25mm x 0.25µm	DB5-MS 60m x 0.25mm x 0.20µm	HT5 25m x 0,22mm x 0,25µm	HT8 25m x 0.22mm x 0.25µm	HT8 60m x 0.25mm x 0.20µm	DB5-MS 30m x 0.25mm x 0.25µm
run time		50 min	40 min	30 min	45 min	45 min	30 min	30 min	55 min	30 min
Detector		HRMS	HRMS	HRMS	HRMS	HRMS	LRMS	HRMS	HRMS	HRMS
PBDEs										
Column 1 (sat. phase, length, ID, Film thickness)	DB-5MS 60m x 0.25mm x 0.25µm	DB-5HT 15m x 0.25mm x 0.10µm	Not measured	Not measured	Not measured	DB1 10m x 0.18mm x 0.18 µm	Not measured	HT8 25m x 0.22mm x 0.25µm	UB5 premium 15m x 0.25mm x 0.10µm	DB5-HT 15m x 0.25mm x 0.10µm
run time	53 min	30 min				30 min		30 min	30 min	20 min
Detector	HRMS	HRMS				HRMS		HRMS	HRMS	HRMS

Table 2: Overview of major statistical parameters

Congeners	n	Median	mean	RSD	Outliers
Dioxins (pg/L)		(pg/L)	(pg/L)	(%)	
2, 3, 7, 8 - TetraCDD	22	9,5	9,9	22	1
1, 2, 3, 7, 8 - PentaCDD	22	32,0	32,3	9	0
1, 2, 3, 4, 7, 8 - HexaCDD	22	17,3	17,2	21	1
1, 2, 3, 6, 7, 8 - HexaCDD	24	122,0	119,6	9	1
1, 2, 3, 7, 8, 9 - HexaCDD	22	17,4	18,5	22	1
1, 2, 3, 4, 6, 7, 8 - HeptaCDD	24	145,4	152,8	25	2
OctaCDD	24	1390,0	1635,4	61	4
Furans (pg/L)					
2, 3, 7, 8 - TetraCDF	10	<5,0	<5,0	-	-
1, 2, 3, 7, 8 - PentaCDF	7	<5,0	<5,0	-	-
2, 3, 4, 7, 8 - PentaCDF	24	82,5	78,6	14	0
1, 2, 3, 4, 7, 8 - HexaCDF	22	24,0	23,2	14	1
1, 2, 3, 6, 7, 8 - HexaCDF	22	28,0	28,0	11	1
1, 2, 3, 7, 8, 9 - HexaCDF	2	<5,0	<5,0	-	-
2, 3, 4, 6, 7, 8 - HexaCDF	18	7,9	9,8	57	1
1, 2, 3, 4, 6, 7, 8 - HeptaCDF	23	35,0	34,9	28	
1, 2, 3, 4, 7, 8, 9 - HeptaCDF	3	<5,0	<5,0	-	-
OctaCDF	5	<40,0	<40,0	-	-
dioxin like PCBs (pg/L)					
PCB 77 (non-ortho)	16	186,1	171,1	48	0
PCB 81 (non-ortho)	7	<100,0	<100,0	-	-
PCB 126 (non-ortho)	22	247,9	249,4	14	3
PCB 169 (non-ortho)	24	334,0	349,7	19	1
PCB 105 (ortho)	26	11740,0	12131,1	10	2
PCB 114 (ortho)	20	3873,0	3982,9	16	1
PCB 118 (ortho)	26	74702,4	72948,0	13	1
PCB 123 (ortho)	19	830,0	776,9	26	2
PCB 156 (ortho)	26	56128,6	55926,7	9	2
PCB 157 (ortho)	26	10412,2	10600,6	8	2
PCB 167 (ortho)	26	16354,4	16706,4	9	1
PCB 189 (ortho)	26	8606,9	8604,0	12	2
Indicator PCBs (ng/L)					
PCB 28	16	11,1	11,48	50	0
PCB 52	16	7,9	7,7	63	0
PCB 101	19	5,7	5,9	60	1
PCB 138	25	290,7	289,5	17	3
PCB 153	25	507,0	511,3	10	2
PCB 180	24	443,7	447,4	7	3
PBDEs (ng/L)					
PBDE 28	10	<3,0	<3,0	-	-
PBDE 47	16	6,4	9,7	114	1
PBDE 99	16	2,5	3,4	84	2
PBDE 100	13	1,5	1,7	51	0
PBDE 153	16	6,3	6,0	22	0
PBDE 154	11	0,4	0,5	68	2
PBDE 183	14	1,6	1,7	40	1
PBDE 209	5	<100	<100	-	-
Total Lipids (g/L)					
gravimetric	15	4,6	4,6	13	0
enzymatic	18	5,4	5,6	6	2

out of the ten PCDFs. RSDs between 11% and 57%. No major difficulties were observed for dioxin-like PCBs (RSDs between 8% and 26%) except CB 77 and 81. The large RSD for CB 77 is due to the conjugated effect of its high ubiquity in procedural blank samples and its weak level in the human plasma. The same reasons can also explain the high variability observed for CB 28, 52, 101 (RSDs > 50%). Less data were reported for PBDEs and, compared with the results of chlorinated compounds, the RSDs for most of the PBDEs were higher. This indicates an immature QA/QC approach for the analysis of PBDEs in serum samples at background European levels.
Finally, Table 2 also shows the results of the lipid determination performed on the test material. Ten laboratories submitted results. These were evenly divided between gravimetric and enzymatic methods (both methods reported by laboratory 2). Enzymatic methods tend to yield higher results (median of 5,4 g/L compared to the median of 4,6 g/L). Dispersion of gravimetric methods is greater than for enzymatic (SD of 0,3 g/L compared 0,6 g/L). This is probably due to the fact that enzymatic methods are generally performed by automated systems using commercial kits whereas gravimetric methods are manual and more dependent on the skill of the operator. As scientific literature compares human exposure to these contaminants on a lipid weight basis in serum, the results presented here point out the necessity of standardizing lipid measurements.

References

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