DATA QUALITY OF THE SERUM ANALYSIS OF PCDD, PCDF AND PCB IN THE FRENCH DIOXIN AND INCINERATORS STUDY

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Abstract

Serum analyses of PCDD, PCDF and PCB were carried out in 1030 adults to identify the determinants of the body-burden of these compounds in the population close to waste incinerators. During the study, several procedures were developed to insure the data quality of the serum analyses at low concentrations. Internal, external and blind quality controls were implemented to assess the performances at the various levels of concentrations found in the population. Two definitions of the limit of quantification (LOQ) were computed. One was based on the EU dioxin directive 2004/44/EC. The second took into account the levels of congeners found in the procedure blank, to define a more restrictive LOQ. The difference between these two definitions can be as large as one or two orders of magnitude for some congeners. However, the LOQ are still low enough to allow the quantification of the majority of the samples.

Introduction

The objective of French Dioxin and Incinerators Study was to determine whether the emissions of the waste incinerators contribute to the body-burden of PCDDs, PCDFs, and PCBs in the surrounding population. The body burden was estimated through an analysis of PCDDs, PCDFs and DL-PCBs levels in serum. The study involved 8 locations surrounding 8 incinerators and included 1030 adults (30-65 years old)[1].

For the purpose of the study, thousands of data were collected and data quality was a leading concern. Quality assurance was integrated from the beginning of the study, to define the data quality procedures and to set up the logistic and the analyses. The analyses of PCDDs, PCDFs and PCBs in serum at low levels were a focal point. An extensive work was done on data validation, quality control, blind control and limit of quantification, to ensure a high accuracy even at very low concentrations.

Material and Methods

General organisation

Analyses were carried out by a central laboratory. The traceability of the samples from the 8 sampling centres to the laboratory was carefully organised and checked.

The laboratory had to provide evidence of its capacities prior to and during the study, through repeated internal and external quality controls. Acceptable levels of detection, reproducibility, repeatability and uncertainty were defined between the laboratory and the National Institute for Health Surveillance. The performances of the analysis were to be consistent at very low level, with a special concern for results close to the limit of quantification.

Sample collection and Analytical methods

Sample collection of 200 ml of blood was undertaken by the local centres of the French national agency for blood, which are specialized in blood donation collections.
All serum samples were analyzed by the CART. A comprehensive description of the analytical methodology for PCDD/Fs and DL-PCBs by gas chromatography (GC) coupled to high resolution mass spectrometry (HRMS) is

given elsewhere[2]. The blood lipid determination was carried out using an enzymatic technique[3]. The WHO TEFs (1998) were used to compute the results in pgTEQ/g lipid.

Quality control

The target values of the internal quality controls (QC) allowed the assessment of the laboratory performances for the upper side of the concentration ranges encountered in the study. For the low concentration, a blind quality control was used. The CART also took part in an external QC.

Internal quality controls and laboratory blanks were analyzed every 20 samples. Quality controls were made of foetal beef serum, spiked with a known concentration of PCDD/F and PCB. The concentrations of the QC were of 157.3 pgTEQ/L for PCDD and PCDF, and of 122.04 pgTEQ/L for DLPCB.

Twenty samples of the blind control were prepared using a pool of blood available at the EFS of Normandie and hidden among the study samples. Four controls were randomly introduced in the samples set of the two first locations, and two controls were introduced in the samples set of the locations 3 to 8.

To estimate the target value of the control, an iterative procedure was used, for each congener and for the total TEQ.

- computation of the mean and standard deviations of the results obtained for the first 8 blind controls.
- all samples above or below the mean $\pm$ 2 standard deviations were removed, and a new mean and standard deviation were computed from this new dataset.
- each new result was then compared to the new mean $\pm$ 2 standard deviations. If the result fell in this acceptable range, they were then included in a new computation of the mean $\pm$ 2 standard deviations.

Limit of quantification

Two definitions were initially adopted for the computation of the limit of quantification (LOQ) and limit of detection (LOD). The first definition followed the EU dioxin directive 2004/44/EC recommandation[4]. The second definition was based on the mean blank level +3.28 SD at 95 % confidence level. Indeed, for congeners that are systematically present in procedural blanks, the calculation of the LOQs based on 2004/44/EC is no longer applicable, since the LOQ calculation approach has to rest on the estimation of the minimum amount of each congener that can reliably be distinguished from the average amount present in procedural blank.

Results and discussion

Blind quality control

The total TEQ target value computed on the acceptable blind controls was of 13.15 pgTEQ/g lipid. For each congener, the target value was close or below the mean concentrations obtained in our studies. The target value was below the limit of detection for five congeners (1, 2, 3, 7, 8 – PentaCDF, 1, 2, 3, 7, 8, 9 – HexaCDF, 1, 2, 3, 4, 7, 8, 9 – HeptaCDF, OctaCDF (OCDF), PCB 77 (non-ortho), PCB 81 (ortho), PCB 105 (ortho)). Target values for the TCDD and TCDF were close to the limit of quantification.

On total TEQ, all the blind control samples were within the acceptability limits, except for one sample. Further investigation of this sample revealed an external contamination. A coefficient of variation (CV) of 12.4% was computed from the 19 acceptable results. CV were below 16% for most of the congeners, below 25% for TCDF, PCB 114 and PCB 118. The highest CV were found for PCB 123 (43.6%) and PCB 126 (41.1%).

External quality control

The lipids determination was assessed through the interlaboratory control organized by the Arctic Monitoring and Assessment Program (AMAP). Our results were systematically 13% higher than the target value calculated as the mean value of all data (i.e. both enzymatic and gravimetric methods). This discrepancy is explained by the

differences introduced by the measurement method: there is generally a 20% discrepancy between the gravimetric method and the enzymatic method, the last one being more reliable. For PCDD/PCDF concentrations, the CART participated in the interlaboratory control it organized, based on pooled plasma[5]. Satisfactory results were obtained for all congeners (z-scores below 2) except PCB 77 and 114.

Limit of quantification

The difference between LOQ values computed with the two definitions can be as large as one or two orders of magnitude for some congeners, depending on the blank level and its variability. The difference in LOQ depending on the chosen definition is striking on DL-PCBs and PCBs. However, the impact on the number of non quantified value remains acceptable (Table 1). Methods used to take into account these censored values are described elsewhere[6].
In the final statistical analysis, LOD computed by the mean blank level + 1.64 SD of the blank and LOQ computed by the mean blank level + 3.28 SD of the blank was preferred.

Conclusion

Feedback from this study shows that a close collaboration between the laboratory and health professionals is required to successfully manage the quality assurance of a large study. Traceability of the samples is one of the main issues.

Assessment of the data quality at low levels is another issue, for which the use of blind external was efficient. However, a blind control remains to be organised when sensitive biological material like blood is used. Similarly, internal QC should be set at levels close to the concentrations expected in the samples of the study. It is regrettable that reference materials do not always exist at such levels. External quality controls and inter-laboratory comparisons at low levels should also be encouraged. They provide precious information for the assessment of the quality of biomonitoring studies, and for their comparison.

Among the various methods available to compute a limit of quantification, the one based on the levels present in the blank was preferred. This definition gives in higher LOQ but provides more reliable results, since it allows a clear distinction between, the minimum amount of each congener and the average amount present in procedural blank. This choice does not compromise the study by increasing the number of non quantified values. Even with a restrictive definition, the LOQ were low enough to quantify most of the samples for the majority of the congeners, and were low compared to LOQ found in the literature.

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Table 1 – Comparison of LOQ definition and its impact on the number of non quantified values

	LOQ defined by the EU dioxin directive 2004/44/EC			LOQ defined by the mean blank level + 3.28 SD		
	LOQ pg/L	%<LOD	LOD<%<LOQ	LOQ pg/L	%<LOD	LOD<%<LOQ
2, 3, 7, 8 - TetraCDD	2	5,5	0,8	2	5,6	0,8
1, 2, 3, 7, 8 - PentaCDD	2	0,1	0,0	2	0,1	0,0
1, 2, 3, 4, 7, 8 - HexaCDD	5	0,7	1,1	5	0,7	1,0
1, 2, 3, 6, 7, 8 - HexaCDD	5	0,0	0,0	5	0,0	0,0
1, 2, 3, 7, 8, 9 - HexaCDD	5	0,5	0,5	5	0,5	0,4
1, 2, 3, 4, 6, 7, 8 - HeptaCDD	15	0,0	0,5	32	0,0	2,4
OctaCDD	80	0,0	0,2	89	0,0	0,2
2, 3, 7, 8 - TetraCDF	2	15,9	46,3	2	15,9	46,3
1, 2, 3, 7, 8 - PentaCDF	2	61,7	10,8	2	61,7	10,8
2, 3, 4, 7, 8 - PentaCDF	2	0,0	0,0	2	0,0	0,0
1, 2, 3, 4, 7, 8 - HexaCDF	5	0,0	0,7	11	0,0	11,6
1, 2, 3, 6, 7, 8 - HexaCDF	5	0,0	0,0	5	0,0	0,0
1, 2, 3, 7, 8, 9 - HexaCDF	5	69,6	12,2	5	69,6	12,2
2, 3, 4, 6, 7, 8 - HexaCDF	5	5,0	8,7	5	5,0	8,7
1, 2, 3, 4, 6, 7, 8 - HeptaCDF	10	0,0	8,1	20	0,0	29,4
1, 2, 3, 4, 7, 8, 9 - HeptaCDF	10	86,3	13,5	10	86,3	13,5
OctaCDF	10	0,1	90,4	48	0,1	99,0
PCB 77 (non-ortho)	200	0,0	88,7	1600	0,0	99,2
PCB 81 (ortho)	200	0,0	93,0	400	0,0	96,3
PCB 126 (non-ortho)	20	0,0	1,3	20	0,0	1,3
PCB 169 (non-ortho)	20	0,0	0,0	20	0,0	0,0
PCB 105 (ortho)	200	0,0	7,9	8000	0,0	37,3
PCB 114 (ortho)	20	0,0	0,1	900	0,0	2,0
PCB 118 (ortho)	200	0,0	1,0	24000	0,0	10,6
PCB 123 (ortho)	20	8,7	4,1	20	8,7	4,3
PCB 156 (ortho)	20	0,0	0,0	900	0,0	0,0
PCB 157 (ortho)	20	0,0	0,0	250	0,0	0,0
PCB 167 (ortho)	20	0,0	0,0	600	0,0	0,0
PCB 189 (ortho)	20	0,0	0,0	20	0,0	0,0
PCB 138	200	0,0	0,0	5000	0,0	0,0
PCB 153	200	0,0	0,0	5000	0,0	0,0
PCB 180	200	0,0	0,0	5000	0,0	0,0