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Performances and limitations of the HRMS method for dioxins, furans and dioxin-like PCBs analysis in animal feedingstuffs Part I: Results of an inter-laboratory study

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Abstract

The European strategy for dioxin monitoring of the food chain has defined high-resolution gas chromatography coupled to high-resolution mass spectrometry (HRGC/HRMS) method as the confirmatory method that can provide reliable and comparable results at sub-parts per trillion (ppt) level. This paper describes the first inter-laboratory study on dioxins, furans and dioxin-like PCBs by HRGC/HRMS method in animal feedingstuffs. Two different statistical approaches (ISO 5725 and Cofino's statistics) were used for the statistical evaluation. For this particular study, the performances of the HRGC/HRMS method seem to be congener-independent in repeatability and reproducibility conditions over a concentration range covering more than four orders of magnitude. Results clearly show the effect of precision loss below 0.1 ppt level per congener in repeatability conditions and below 0.2 ppt level per congener in reproducibility conditions. LODs reported by the laboratories give median values of 0.02 ng/kg for most of the toxic congeners. Relative standard deviation between the laboratories' mean values using upper-bound approach for TEQ calculation is 6.2%, more than twice the maximum level set at 0.75 ng TEQ/kg of product.

Keywords: Dioxins; Furans; Dioxin-like PCBs; HRGC/HRMS; Inter-laboratory study; Animal feedingstuffs

1. Introduction

The recent food contamination crises that have occurred in the past few years in Europe have led the European Union (EU) to develop and implement a strategy for the reduction of exposure to polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and dioxin-like polychlorinated biphenyls (D-L PCBs) [1,2]. The strategy is based on the reduction of the release of these undesirable substances into the environment and the reduction of their presence in the food chain. As human dioxin exposure is mainly the result of food consumption, the aim of the strategy is to decrease levels in food in order to bring the majority of the European population below the tolerable weekly intake (TWI).

In order to avoid exposure through the food chain, regulatory limits were set in foodstuffs and feedingstuffs and their levels are now monitored. The legislative measures consisted in establishing maximum action and target levels in different food and feed matrices. Maximum and action levels have already been set, while target levels will be established before the end of 2004 [3,4]. The target level would be the level to be achieved in order to bring human exposure below TWI. For the limits already established, only dioxins and furans are included. However, the approach is pro-active and is intended to incorporate the D-L PCBs when more reliable data on background levels is available [5].

Food contamination crises had led to the set-up of monitoring programs of the food chain inside the European market. To facilitate free trade of goods, harmonization of acceptance criteria for dioxin analysis is needed [6]. Measures have been taken and analytical requirements for dioxin analysis in food and feed have been adopted (directives 2002/69 and 2002/70) [7,8]. It is important to note that the

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analytical approach is particular for these contaminants. Specific requirements have to be met that comply with the confirmation method's objectives; each laboratory can use its own validated method once the specific requirements are fulfilled.

Quantification using isotopic dilution technique for HRMS should be carried out according to EPA method 1613 revision B [9].

Among the requirement criteria, one should underline the need for laboratories to participate in relevant inter-laboratory studies. Therefore the first inter-laboratory study on PCDDs, PCDFs and D-L PCBs by HRGC/HRMS in animal feed sample was organized. Thirteen selected European laboratories from eight different countries were invited to participate. Most of these laboratories are involved in the official food control program in their country, and some private laboratories participated as well. The aim of the present study was to assess the performances of the HRGC/HRMS method, and verify how close it came to meeting the requirements of the directive 2002/70/EC [8]. A second objective was to evaluate the method, to see if it produces reliable and comparable results across Europe.

2. Material and methods

The test material consisted of freeze-dried animal feed powder of plant origin, representing naturally contaminated feed samples that were collected during the 2000 monitoring program in Belgium. The concentration range of PCDDs and PCDFs in the test material spans more than four orders of magnitude.

The participants received 100 g of sample during November 2001 and were expected to deliver the results electronically by the end of January 2002. Due to technical problems encountered by some laboratories with their mass spectrometer, results were accepted until the end of March 2002. Laboratories were asked to perform the analysis in triplicate under repeatability conditions in order to estimate the within-laboratory variability. An Excel spreadsheet was designed for this purpose and participants were requested to report one value per congener and per replicate. For background levels, non-detected or 'lower than' limit of detection was indicated. Additionally, limits of detection (LODs) and limits of quantification (LOQs) of the method also had to be reported. Further spreadsheets describing the extraction, clean-up and analysis techniques were filled in to complete the report form. As already mentioned, no specific recommendations were given to laboratories regarding the extraction and clean-up techniques. It was only suggested to work with a minimum of 30 g of sample intakes to achieve low detection limits.

3. Homogeneity test

The material was tested for homogeneity before being distributed for proficiency testing. Several homogeneity tests were carried out at the Institute for Reference Materials and Measurements (IRMM, Geel, Belgium). During the bottling procedure, six representative samples were taken for Karl Fisher moisture determination (the percentage moisture mean value equal to 2.22% and the corresponding standard deviation 0.16%). The particle size measurements were carried out using a Sympatec particle size analyzer with a Helos measuring device. About 95% of the particles were smaller than 0.515 mm. The top particle size was smaller than 0.735 mm.

Dioxins and furans were also analyzed for 'sufficient homogeneity'. The test was based on a new method developed by Fearn and Thompson [10].

Practically, seven bottles out of the batch were randomly selected. Every bottle was homogenized and then two test portions of 30 g were weighed out. The 14 test portions were labelled and a sequence based on a random order of these labelled samples was constructed. The labels were related to the bottle number. The analytical procedure was carried out by respecting the previously determined sequence order. The analyses were conducted under repeatability conditions as much as possible. The test suggested that the analytical precision (in repeatability conditions) of the method used should not exceed the ratio $\sigma_{an}/\sigma_p < 0.5$ (where σ_{an} is defined as the standard deviation of the analytical method and σ_p the target standard deviation). The ratio values summarized in Table 1 complied with the criteria for all the congeners, except for 1,2,3,4,6,7,8-HpCDD, which was slightly higher than 0.5. As indicated in Table 1, some congeners at low levels in several different bottles were not detected. The test was not applied for those congeners for which some data were missing.

The detailed procedure is described elsewhere [10] and can be briefly summarized as follows: the analytical (s^2_{an}) and sampling (s^2_{sam}) variances are to be estimated. To this end, first the sum S_i and the difference D_i of each pair of duplicates is calculated; the sum of squares of the differences $\sum D_i^2$ is obtained, after which Cochran's test for outliers detection $C = D^2_{max}/\sum D_i^2$ is applied; the variance (ν_s) of the sum S_i is calculated, as well as $MS_w = (\sum D_i^2)/2m$ and $MS_B = \nu_s/2$; the analytical variance is estimated as $s^2_{an} = MS_w$ and the sampling variance as $s^2_{sam} = (MS_B - MS_w)/2$. Subsequently, the criterion applied to test for sufficient homogeneity is $s^2_{sam} \leq \sigma^2_{all}$ (where σ^2_{all} is the allowable sampling variance defined as $\sigma^2_{all} = 0.009\sigma_p^2$, σ_p being the target standard deviation of the inter-laboratory study). After little manipulation, this hypothesis test can be transformed to $s^2_{sam} \leq F_1\sigma^2_{all} + F_2 S^2_{an}$ where F_1 and F_2 are constants obtained from statistical tables ($F_1 = 2.1$ and $F_2 = 1.43$).

An overview of the results is presented in Table 2. Cochran's test indicated that no outliers were detected (C values are lower than their corresponding critical values). For some congeners, when the sampling variance estimation is negative, s^2_{sam} was equal to zero. All the PCDD and PCDF congeners passed the test, indicating that the material is sufficiently homogeneous for this inter-laboratory study.

Table 1
Ratio σ_{an}/σ_p

Compounds	Bottle number														σ_{an}	σ_p	Ratio σ_{an}/σ_p
	38-1 (ng/kg)	38-2 (ng/kg)	44-1 (ng/kg)	44-2 (ng/kg)	55-1 (ng/kg)	55-2 (ng/kg)	79-1 (ng/kg)	79-2 (ng/kg)	98-1 (ng/kg)	98-2 (ng/kg)	107-1 (ng/kg)	107-2 (ng/kg)	111-1 (ng/kg)	111-2 (ng/kg)			
2,3,7,8-TCDD	0.010	nd	0.009	nd	0.008	0.008	0.011	0.008	0.009	nd	nd	0.008	0.012	0.011	0.001	0.007	0.21
1,2,3,7,8-PeCDD	0.028	0.022	0.026	0.037	0.022	0.026	0.025	0.040	0.027	0.026	0.025	0.026	0.031	0.029	0.005	0.019	0.26
1,2,3,4,7,8-HxCDD	0.131	0.116	0.116	0.142	0.149	0.134	0.118	0.149	0.125	0.147	0.119	0.144	0.108	0.105	0.016	0.041	0.38
1,2,3,6,7,8-HxCDD	2.147	2.137	2.012	2.115	2.155	2.114	2.075	2.242	2.221	2.240	2.173	2.064	2.362	2.177	0.088	0.216	0.41
1,2,3,7,8,9-HxCDD	0.787	0.788	0.756	0.796	0.784	0.760	0.786	0.881	0.825	0.816	0.675	0.756	0.834	0.763	0.047	0.104	0.46
1,2,3,4,6,7,8-HpCDD	161.1	159.1	163.4	169.8	172.6	169.6	161.1	178.3	167.3	176.2	178.0	171.3	185.0	167.9	7.5	14.5	0.52
1,2,3,4,6,7,8,9-OCDD	789.6	817.7	784.9	828.7	815.6	819.0	792.2	879.4	827.6	873.0	903.6	800.6	905.4	899.1	44.4	109.1	0.41
2,3,7,8-TCDF	0.097	0.082	0.114	0.102	0.105	0.112	0.107	0.135	0.113	0.109	0.102	0.096	0.129	0.108	0.013	0.027	0.49
1,2,3,7,8-PeCDF	0.027	nd	0.020	0.029	0.020	0.026	0.025	0.029	0.023	0.028	0.023	0.027	0.036	0.028	0.004	0.015	0.29
2,3,4,7,8-PeCDF	0.053	0.042	nd	0.042	0.041	0.044	0.045	0.054	0.045	0.051	0.040	0.044	0.059	0.051	0.006	0.021	0.28
1,2,3,4,7,8-HxCDF	0.102	0.059	0.084	0.097	0.082	0.086	0.080	0.102	0.091	0.086	0.093	0.085	0.115	0.091	0.013	0.028	0.46
1,2,3,6,7,8-HxCDF	0.048	0.044	0.043	0.050	0.039	0.041	0.042	0.053	0.044	0.041	0.050	0.039	0.052	0.054	0.005	0.012	0.46
2,3,4,6,7,8-HxCDF	0.031	0.025	0.027	0.037	0.028	0.028	0.025	0.030	0.032	0.032	0.030	0.029	0.041	0.029	0.004	0.012	0.37
1,2,3,7,8,9-HxCDF	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd	nd			
1,2,3,4,6,7,8-HpCDF	1.423	1.280	1.505	1.333	1.344	1.321	1.226	1.522	1.352	1.423	1.507	1.390	1.593	1.436	0.103	0.333	0.31
1,2,3,4,7,8,9-HpCDF	0.203	0.172	0.216	0.215	0.201	0.198	0.217	0.198	0.174	0.208	0.244	0.190	0.248	0.205	0.022	0.056	0.39
1,2,3,4,6,7,8,9-OCDF	13.10	12.29	13.74	12.37	12.30	12.68	11.90	13.39	13.20	12.54	13.25	12.86	14.47	13.10	0.673	2.430	0.28

nd: non-detected.

Table 2
Homogeneity test

Compounds	D 38	D 44	D 55	D 79	D 98	D 107	D 111	S 38	S 44	S 55	S 79	S 98	S 107	S 111	$\sum D_i^2$	C	Critical value	s_{an}^2	ν_s	MS_B	s_{sam}^2	σ_{all}^2	$F_1\sigma_{all}^2 + F_2 s_{an}^2$
2,3,7,8-TCDD			0.001	0.003			0.001			0.016	0.019			0.023	0.00001	0.909	0.967	2.04E−06	1.21E−05	6.0E−06	1.99E−06	4.65E−06	1.27E−05
1,2,3,7,8-PeCDD	0.006	0.011	0.004	0.014	0.000		0.002	0.050	0.062	0.048	0.065	0.053		0.060	0.0004	0.535	0.781	3.2E−05	4.92E−05	2.5E−05	0	3.38E−05	0.000117
1,2,3,4,7,8-HxCDD	0.015	0.026	0.015	0.031	0.022	0.025	0.002	0.247	0.258	0.283	0.267	0.272	0.263	0.213	0.0032	0.300	0.727	0.000232	0.000523	0.00026	1.47E−05	0.000154	0.000655
1,2,3,6,7,8-HxCDD	0.009	0.104	0.041	0.168	0.020	0.109	0.184	4.284	4.127	4.270	4.317	4.461	4.238	4.539	0.0869	0.392	0.727	0.006207	0.019292	0.00965	0.00172	0.004217	0.017731
1,2,3,7,8,9-HxCDD	0.001	0.039	0.024	0.094	0.009	0.081	0.072	1.575	1.552	1.545	1.667	1.641	1.431	1.597	0.0227	0.392	0.727	0.001624	0.005899	0.00295	0.000663	0.000967	0.004353
1,2,3,4,6,7,8-HpCDD	2.0	6.4	3.0	17.2	8.9	6.7	17.1	320.3	333.2	342.2	339.3	343.6	349.3	352.9	767.74	0.387	0.727	54.839	117.5379	58.769	1.965071	18.94526	118.2046
1,2,3,4,6,7,8,9-OCDD	28.1	43.8	3.4	87.3	45.4	103.0	6.3	1607.3	1613.6	1634.7	1671.6	1700.6	1704.3	1804.6	23035.2	0.460	0.727	1645.37	4712.147	2356.07	355.3516	1070.343	4600.601
2,3,7,8-TCDF	0.014	0.012	0.007	0.029	0.004	0.006	0.021	0.179	0.216	0.217	0.242	0.221	0.198	0.237	0.0017	0.481	0.727	0.000123	0.000469	0.00024	5.58E−05	6.51E−05	0.000313
1,2,3,7,8-PeCDF		0.009	0.006	0.003	0.006	0.004	0.009		0.049	0.046	0.054	0.051	0.050	0.064	0.0003	0.325	0.781	2.2E−05	3.87E−05	1.9E−05	0	2.08E−05	7.46E−05
2,3,4,7,8-PeCDF	0.011		0.002	0.009	0.006	0.003	0.008	0.095		0.085	0.099	0.096	0.084	0.109	0.0003	0.410	0.781	2.65E−05	8.62E−05	4.3E−05	8.27E−06	3.91E−05	0.00012
1,2,3,4,7,8-HxCDF	0.043	0.013	0.003	0.022	0.006	0.008	0.024	0.161	0.181	0.168	0.183	0.177	0.178	0.206	0.0031	0.578	0.727	0.000225	0.000202	0.0001	0	7.19E−05	0.000473
1,2,3,6,7,8-HxCDF	0.004	0.008	0.002	0.012	0.003	0.011	0.002	0.092	0.093	0.081	0.095	0.085	0.089	0.107	0.0003	0.397	0.727	2.4E−05	6.71E−05	3.4E−05	4.72E−06	1.19E−05	5.96E−05
2,3,4,6,7,8-HxCDF	0.006	0.009	0.000	0.004	0.000		0.012	0.056	0.064	0.055	0.055	0.064		0.070	0.0003	0.485	0.781	2.3E−05	3.76E−05	1.9E−05	0	1.21E−05	5.82E−05
1,2,3,7,8,9-HxCDF																							
1,2,3,4,6,7,8-HpCDF	0.144	0.172	0.024	0.296	0.072	0.117	0.157	2.703	2.838	2.665	2.749	2.775	2.897	3.029	0.1820	0.481	0.727	0.013003	0.015656	0.00783	0	0.009998	0.03959
1,2,3,4,7,8,9-HpCDF	0.031	0.001	0.004	0.019	0.035	0.053	0.043	0.375	0.431	0.399	0.415	0.382	0.434	0.453	0.0072	0.392	0.727	0.000514	0.000827	0.00041	0	0.000278	0.001319
1,2,3,4,6,7,8,9-OCDF	0.81	1.37	0.38	1.49	0.66	0.40	1.37	25.39	26.12	24.98	25.28	25.74	26.11	27.57	7.3728	0.302	0.727	0.526626	0.731127	0.36556	0	0.531304	1.868815

4. Analytical procedures

Analyses were performed using each laboratory's own analytical method. Extraction techniques were mostly Soxhlet (nine times) with toluene as extracting solvent, but various mixtures of toluene/ethanol, toluene/acetone, hexane and cyclohexane were also used. Ultra-turrax (one time) with hexane/dichloromethane, hot extraction (one time) with toluene or toluene/ethanol and mechanical shaking (one time) with ethylacetate and ASE (one time) with hexane/acetone were also reported. A variety of different clean-up procedures were reported, but most of them were essentially based on silica, alumina, florisil and carbon columns. Some purification pre-treatments were also described: sulphuric acid, silica gel KOH/H_2SO_4 and gel permeation chromatography (GPC). All analyses were carried out by high-resolution gas chromatography coupled to high-resolution mass spectrometry (HRGC/HRMS) using the isotopic dilution technique.

5. Statistics

Two different statistical approaches were used and compared for the statistical evaluation of the study: the classical ISO 5725 [11] Standard and a new and novel concept developed by Cofino et al. [12,13]. The Standard ISO 5725 is based on the classical ANOVA technique that gives an estimation of the gross average, intra-laboratory and inter-laboratory variances, repeatability and reproducibility of the method [14]. Two tests were applied for outlier detection. The within-laboratory variability was examined using Cochran's test and the between-laboratory variability using Grubb's test.

The concept of the second approach based on Cofino's statistics replaces data points by the so-called laboratory measurement functions (LMFs). These functions are defined as the root of probability density functions (pdfs), i.e. each data point is replaced by the LMF that is considered proper. The LMFs form a basis set, in which inter-laboratory measurement functions (IMFs) are constructed. Knowledge of the IMFs enables the calculation of the sought after population characteristics. This approach derived from quantum chemistry is more robust than the conventional ISO Standard for outliers and asymmetrical distributions of data. The model requires an estimation of the uncertainties associated with the data.

In this study, each LMF was constructed as the square root of a normal distribution using the average of the three replicates as mean. Three different approaches were evaluated to estimate the standard deviations of the laboratories. First, the laboratory standard deviations were calculated straightforwardly from triplicates. Second, the average within-laboratory standard deviation of all laboratories was calculated; this value was then attributed as the standard deviation for all laboratories. Third, the normal distribution approximation of the model was employed. In this case, the model constructs a value that is used as laboratory standard deviation assuming that population of data is characterized by a normal distribution. The three approaches described generally gave very similar results. The straightforward calculation of the laboratory standard deviations proved to be less appropriate. Estimation of a standard deviation using only three data is fraught with a high uncertainty. In addition, some laboratories reported duplicate or even single measurements. This approach was therefore abandoned. Application of the normal distribution approximation was considered less appropriate, as many of the distributions encountered deviate significantly different from normality. Therefore, the second approach was adopted in this study, i.e. the within-laboratory standard deviation was calculated and each laboratory was given this value as standard deviation.

The inter-laboratory measurement functions (IMFs) are linear combinations of the laboratory measurement functions. The model renders for n LMFs and also n IMFs. For each IMF, the expectation value, the standard deviation and a percentage p, giving the degree to which the IMF describes the data set is calculated. The IMFs are ordered according to the value of p, IMF_1 having the highest percentage. IMF_1 provides the best estimate for the mean of the data set. Evaluation of the percentages of the other IMFs gives insight into the structure of the data set. For instance, the data set has a bimodal character when the difference between the percentages p of IMF_1 and IMF_2 is small. A poor comparability is reflected by, e.g. a low percentage of IMF_1 and several other IMF's with appreciable percentages.

6. Assessment of results

For several reasons, five laboratories were unable to analyze either the seventeen 2,3,7,8-PCDDs and PCDFs or the 12 D-L PCBs. Two laboratories did not report results for the D-L PCBs; one laboratory was unable to perform analysis for the non-*ortho*-PCBs and two laboratories were unable to produce results for the mono-*ortho*-PCBs. Moreover, one laboratory returned one replicate result for the D-L PCBs and another one reported dioxins and furans results only in duplicate.

Table 3 gives the raw data corresponding to the mean analyte levels (triplicate measurements expressed in ng/kg of product) for the participating laboratories. In the schematic processing, for both ISO 5725 and Cofino's model, the 'less than' limit of detection results were deleted from the data set and are indicated by a value in Table 3. Values indicated by a 'b' correspond to one replicate reported by the laboratory. Scarce results (less than 8) were reported for 1,2,3,7,8,9-HxCDF, PCB 114, 123, 156, 157, 167 and 189 and were therefore not statistically significant for ISO 5725 Standard procedure.

Table 3
Raw data: mean levels (ng/kg of product) of PCDDs and PCDFs in animal feed material

Compounds	Laboratory 01	Laboratory 02	Laboratory 03	Laboratory 04	Laboratory 05	Laboratory 06	Laboratory 07	Laboratory 08	Laboratory 09	Laboratory 10	Laboratory 11	Laboratory 12	Laboratory 13	Number of labs
2,3,7,8-TCDD	[a]	[a]	0.010	[a]	0.020[b]	[a]	[a]	0.076[b]	0.012	0.011	0.067	0.011	0.013[b]	8
1,2,3,7,8-PeCDD	[a]	[a]	0.028	0.064	0.073	[a]	0.043	0.148[b]	0.024	0.037	0.037	0.026	0.025[b]	10
1,2,3,4,7,8-HxCDD	0.173	0.178	0.184	0.144	0.180	0.133	0.160	0.221	0.117	0.194	0.143	0.127	0.140	13
1,2,3,6,7,8-HxCDD	2.273	2.015	2.127	1.917	2.200	1.910	1.856	1.888	1.600	2.410	1.853	1.933	2.201	13
1,2,3,7,8,9-HxCDD	0.800	0.716	0.904	0.696	0.880	0.743	0.669	0.836	0.630	0.915	0.830	0.723	0.800	13
1,2,3,4,6,7,8-HpCDD	149.7	128.9	153.3	132.0	166.0	151.0	130.7	145.2	160.7	150.5	124.5	145.9	159.6	13
1,2,3,4,6,7,8,9-OCDD	799.0	723.8	931.3	627.3	723.0	616.7	633.9	637.5	771.3	744.5	671.5	709.8	909.2	13
2,3,7,8-TCDF	0.130[b]	0.085	0.145	0.092	0.097	0.087	0.063	0.083	0.058	0.079	0.103	0.094	0.106	13
1,2,3,7,8-PeCDF	[a]	[a]	0.043	0.061	0.030	0.020	0.028	0.123	0.014	0.021	0.027	0.030	0.013	11
2,3,4,7,8-PeCDF	0.030[b]	[a]	0.061	0.067	0.050	0.040[b]	0.050	[a]	0.037	0.036	0.040	0.068	0.059	11
1,2,3,4,7,8-HxCDF	0.093	0.070[b]	0.110	0.096	0.037	0.060	0.075	0.213	0.019	0.085	0.073	0.080	0.076	13
1,2,3,6,7,8-HxCDF	0.030[b]	0.050[b]	0.052	0.053	0.043	0.030	[a]	0.102	[a]	0.048	0.033	0.053	0.041	11
2,3,4,6,7,8-HxCDF	0.050	0.060[b]	0.051	0.059	0.050	0.047	0.165	0.103	0.042	0.067	0.040	0.049	0.043	13
1,2,3,7,8,9-HxCDF	[a]	[a]	0.005	[a]	[a]	[a]	[a]	0.138	[a]	[a]	[a]	0.002	[a]	3
1,2,3,4,6,7,8-HpCDF	1.167	1.286	1.423	1.400	2.000	1.427	1.278	1.500	1.867	1.773	1.617	2.172	1.625	13
1,2,3,4,7,8,9-HpCDF	0.200	0.153	0.187	0.180	0.270	0.170	0.198	0.307	0.163	0.262	0.143	0.146	0.211	13
1,2,3,4,6,7,8,9-OCDF	15.43	10.78	14.77	11.78	18.00	12.25	11.51	12.78	18.33	14.469	9.990	12.14	13.23	13
PCB 77	[a]	[a]	7.917	6.827	[a]	6.380	[a]	5.936	7.133	5.343	6.837	8.437	10.44	9
PCB 126	0.470[b]	[a]	0.476	0.588	[a]	0.467	[a]	0.323	0.437	0.350	0.587	0.534	0.494	10
PCB 169	0.070[b]	[a]	0.102	0.215	[a]	0.080[b]	[a]	0.062	0.071	0.065	0.113	0.091	0.070	10
PCB 81	[a]	[a]	0.457	0.356	[a]	0.370	[a]	0.111	0.370	0.345	0.607	0.545	0.607	9
PCB 105	32.00[b]	[a]	32.20	25.97	[a]	20.00	[a]	23.32	[a]	24.95	34.73	36.83	[a]	8
PCB 114	[a]	[a]	2.610	1.827	[a]	[a]	[a]	[a]	[a]	2.030	2.273	2.957	[a]	5
PCB 118	86.00[b]	70.00	113.3	89.93	[a]	90.00	[a]	81.38	[a]	78.07	103.8	120.1	[a]	9
PCB 123	[a]	[a]	3.397	1.803	[a]	[a]	[a]	[a]	[a]	1.610	4.250	2.147	[a]	5
PCB 156	15.00[b]	[a]	14.80	10.70	[a]	[a]	[a]	[a]	[a]	11.24	10.59	15.37	[a]	6
PCB 157	2.400[b]	[a]	2.587	1.750	[a]	[a]	[a]	[a]	[a]	1.963	2.043	2.522	[a]	6
PCB 167	6.800[b]	[a]	7.313	6.597	[a]	[a]	[a]	[a]	[a]	6.127	16.81	6.959	[a]	6
PCB 189	1.100[b]	[a]	1.680	1.567	[a]	[a]	[a]	[a]	[a]	1.165	1.250	1.492	[a]	6

[a] Values not reported by the participants (ND, <LOQ).
[b] One replicate analysis.

7. ISO 5725

By applying Cochran's test to all intra-laboratory variances, seven outliers and three stragglers were detected. The outliers were rejected and the stragglers were left in the set. By repeating the test a second time on the congeners for which outliers were removed, one outlier and two stragglers were detected. The outlier was removed and the straggler left in the set. No further outliers or stragglers were found after repeating Cochran's test on the remaining congener.

Afterwards, Grubb's test for single largest and single lowest mean value was applied to the remaining data set. No stragglers and six outliers were found. The outliers were rejected. By repeating single Grubb's test to these congeners, two outliers and no stragglers were detected. The test was applied a third time to those two congeners and one outlier was found. No more outliers or stragglers were found when applying the test a fourth time. Then, Grubb's test for two largest and two smallest mean values was applied to the set. The test was only applied to the congeners for which no outliers had been found during single Grubb's test. No stragglers or outliers were found when analyzing two extreme observations.

Thus, a total of 17 outlying observations were found by applying at the same time Cochran's test and single and double Grubb's test, corresponding to 6% of the overall data set.

Table 4 presents the statistical ISO 5725 characteristics of the results for PCDDs, PCDFs and D-L PCBs obtained by the participants for the animal feed test material. The ratio of eliminated laboratories to the total number of laboratories is below the ratio of 2/9 for most of the congeners, except for the 2,3,7,8-TCDD, where four outliers were detected. In that instance, the ISO procedure was not effective. It was, however, important to note that the level of 2,3,7,8-TCDD in the test material was below the detection limit of many laboratories.

The results were expressed in concentration (ng/kg of product) and on WHO-TEQ basis. The congeners' concentration varied from part per quadrillion (ppq) level to a hundred parts per trillion (ppt) level, hence covering more than four orders of magnitude. The total WHO-TEQ consensus mean value was 2.02 ng WHO-TEQ/kg of product (less than three times the maximum level set at 0.75 ng WHO-TEQ/kg of product). The pattern indicated that dioxin congeners contribute 93.1% of the total TEQ, with a high contribution of the 1,2,3,4,6,7,8-HpCDD (72.4%) and 1,2,3,6,7,8-HxCDD (9.8%) congeners. Furans contribute 3.6% and D-L PCBs, 3.3% to the total TEQ.

The concentration of the less chlorinated PCDD and PCDF congeners are in the range of LOD/LOQ of the analytical method. The LODs and LOQs reported by the laboratories varied roughly by a factor of 10 for the dioxin and furan congeners. For the D-L PCBs, a factor of around 50 and up to 1000 between the lowest and the highest LODs were reported, indicating that for some laboratories the method's performance for those congeners needs to be improved. Their data might be the result of blank contamination. A thorough discussion on the different approaches used for LOD/LOQ is provided in the second part of these reports.

In general, after discarding outlying observations, the results summarized in Table 4 are characterized by a good repeatability and RSD_r are between 5 and 30% for PCDDs and PCDFs. For low PCDD/F levels, i.e. between 0.03 and 0.2 ppt, RSD_r vary from 17 to 30% and above 0.2 ppt level, RSD_r are much lower (from 5 to 10%). For D-L PCBs, the RSD_r vary from 5 to 14% in the range of 0.08–93 ppt.

The inter-laboratory reproducibility of the method is obviously more important, the range of RSD_R is between 10 and 53% for PCDDs and PCDFs and between 13 and 43% for D-L PCBs.

In this study, 6% of the overall data set were considered as outlying observations by applying ISO Standard procedure. For example, the 2,3,7,8-TCDD had four outliers detected. Fig. 1B represents the distribution of laboratories' mean value for this congener. The scattering of data is far from representing a Gaussian distribution. The classical approach, which is based on the normal distribution law assumption, therefore has difficulty coping with such a figure. The number of outliers entailed the rejection of the study for the 2,3,7,8-TCDD congener.

8. Cofino's statistics

A comparison of the two statistical approaches for all the dioxins, furans and D-L PCBs is summarized in Table 5. The results of IMF_1 function are presented. The expectation values calculated from IMF_1 are characterized by a high percentage of probability (P). On one hand, P-values between 53 and 83% were obtained for PCDDs and PCDFs, and on the other hand, P-values between 37 and 78% for D-L PCBs. That signifies values from IMF_1 can be compared with the means calculated by the conventional approach. In addition, the total standard deviation presented here for these statistics can be related to the ISO inter-laboratory reproducibility. Comparison of the results indicated very good agreement, not only on total TEQ basis, but also for all the individual congeners expressed as a concentration (ng/kg). The difference between the ISO mean and Cofino's model mean is below 10% for most of the congeners, except for 2,3,7,8-TCDD (13.7%), 1,2,3,7,8-PeCDD (16.6%) and PCB 123 (28.6%). It can also be observed that, in general, Cofino's model gives lower means than ISO. A thorough analysis inside the data set allows understanding specific cases. The situation seems to occur frequently when the distribution of laboratories' mean values diverts from normality. For instance, when a shoulder or a remaining tail appears in the data set distribution, this group of values is generally not discarded by Grubb's test as an outlier observation. A typical example is given by the IMF_1 function for OCDD

Table 4
Statistical characteristics of the results obtained by the participants laboratories (S_r, repeatability standard deviation; S_R, reproducibility standard deviation; r, repeatability; R, reproducibility)

Compounds	TEF	Eliminated laboratories	Consensus mean (ng/kg D.M.)	Consensus mean (ng WHO-TEQ/kg D.M.)	Contribution TEQ (%)	LOD range (ng/kg D.M.)	S_r	S_R	RSD_r (%)	RSD_R (%)	95% r	95% R
2,3,7,8-TCDD	1	4/8	0.011[a]	0.011	0.5	0.005–0.04						
1,2,3,7,8-PeCDD	1	1/10	0.040	0.040	2.0	0.005–0.04	0.008	0.019	20	49	0.022	0.055
1,2,3,4,7,8-HxCDD	0.1	0/13	0.160	0.016	0.8	0.008–0.12	0.035	0.041	22	26	0.098	0.117
1,2,3,6,7,8-HxCDD	0.1	1/13	1.985	0.198	9.8	0.008–0.12	0.122	0.216	6	11	0.346	0.613
1,2,3,7,8,9-HxCDD	0.1	0/13	0.779	0.078	3.9	0.008–0.12	0.066	0.104	8	13	0.185	0.293
1,2,3,4,6,7,8-HpCDD	0.01	0/13	146.286	1.463	72.4	0.013–0.35	7.2	14.5	5	10	20.4	41.1
1,2,3,4,6,7,8,9-OCDD	0.0001	1/13	742.237	0.074	3.7	0.025–0.40	46.2	109.1	6	15	130.7	308.6
2,3,7,8-TCDF	0.1	0/13	0.092	0.009	0.5	0.005–0.07	0.017	0.027	19	29	0.049	0.076
1,2,3,7,8-PeCDF	0.05	1/11	0.029	0.001	0.1	0.005–0.04	0.009	0.015	30	53	0.024	0.043
2,3,4,7,8-PeCDF	0.5	0/11	0.051	0.026	1.3	0.005–0.04	0.010	0.021	19	41	0.027	0.059
1,2,3,4,7,8-HxCDF	0.1	1/13	0.073	0.007	0.4	0.008–0.12	0.012	0.028	16	39	0.034	0.080
1,2,3,6,7,8-HxCDF	0.1	1/11	0.044	0.004	0.2	0.008–0.12	0.008	0.012	19	26	0.023	0.033
2,3,4,6,7,8-HxCDF	0.1	2/13	0.049	0.005	0.2	0.008–0.12	0.011	0.012	23	24	0.032	0.033
1,2,3,7,8,9-HxCDF	0.1	3				0.008–0.12						
1,2,3,4,6,7,8-HpCDF	0.01	0/13	1.582	0.016	0.8	0.013–0.25	0.173	0.333	11	21	0.490	0.943
1,2,3,4,7,8,9-HpCDF	0.01	0/13	0.195	0.002	0.1	0.013–0.18	0.034	0.056	17	29	0.096	0.157
1,2,3,4,6,7,8,9-OCDF	0.0001	1/13	13.129	0.001	0.1	0.025–0.24	0.78	2.43	6	19	2.21	6.88
TOTAL PCDD/Fs			906.74	1.95	96.7							
PCB 77	0.0001	0/9	7.327	0.001	0.0	0.008–0.50	0.539	1.552	7	21	1.53	4.39
PCB 126	0.1	2/10	0.446	0.045	2.2	0.008–0.50	0.027	0.077	6	17	0.08	0.22
PCB 169	0.01	1/10	0.082	0.001	0.0	0.008–0.08	0.007	0.020	9	25	0.02	0.06
PCB 81	0.0001	0/9	0.434	0.0000	0.0	0.008–0.50	0.045	0.152	10	35	0.13	0.43
PCB 105	0.0001	0/8	28.642	0.0029	0.1	0.02–20	1.897	6.443	7	22	5.37	18.23
PCB 114	0.0005	0/5	2.362[a]	0.0012	0.1	0.02–20	0.140	0.473	6	20	0.40	1.34
PCB 118	0.0001	0/9	93.677	0.0094	0.5	0.02–20	4.333	17.584	5	19	12.26	49.76
PCB 123	0.0001	0/5	2.715[a]	0.0003	0.0	0.02–20	0.350	1.170	13	43	0.99	3.31
PCB 156	0.0005	0/6	12.508[a]	0.0063	0.3	0.02–20	1.129	2.562	9	20	3.20	7.25
PCB 157	0.0005	0/6	2.157[a]	0.0011	0.1	0.02–20	0.125	0.392	6	18	0.35	1.11
PCB 167	0.00001	1/6	6.759[a]	0.0001	0.0	0.02–20	0.869	0.855	14	13	2.61	2.42
PCB 189	0.0001	0/6	1.422[a]	0.0001	0.0	0.02–20	0.197	0.272	14	19	0.56	0.77
TOTAL dioxin-like PCBs			158.53	0.07	3.3							
TOTAL			1065.27	2.02	100.0							

[a] Values with ISO 5725.

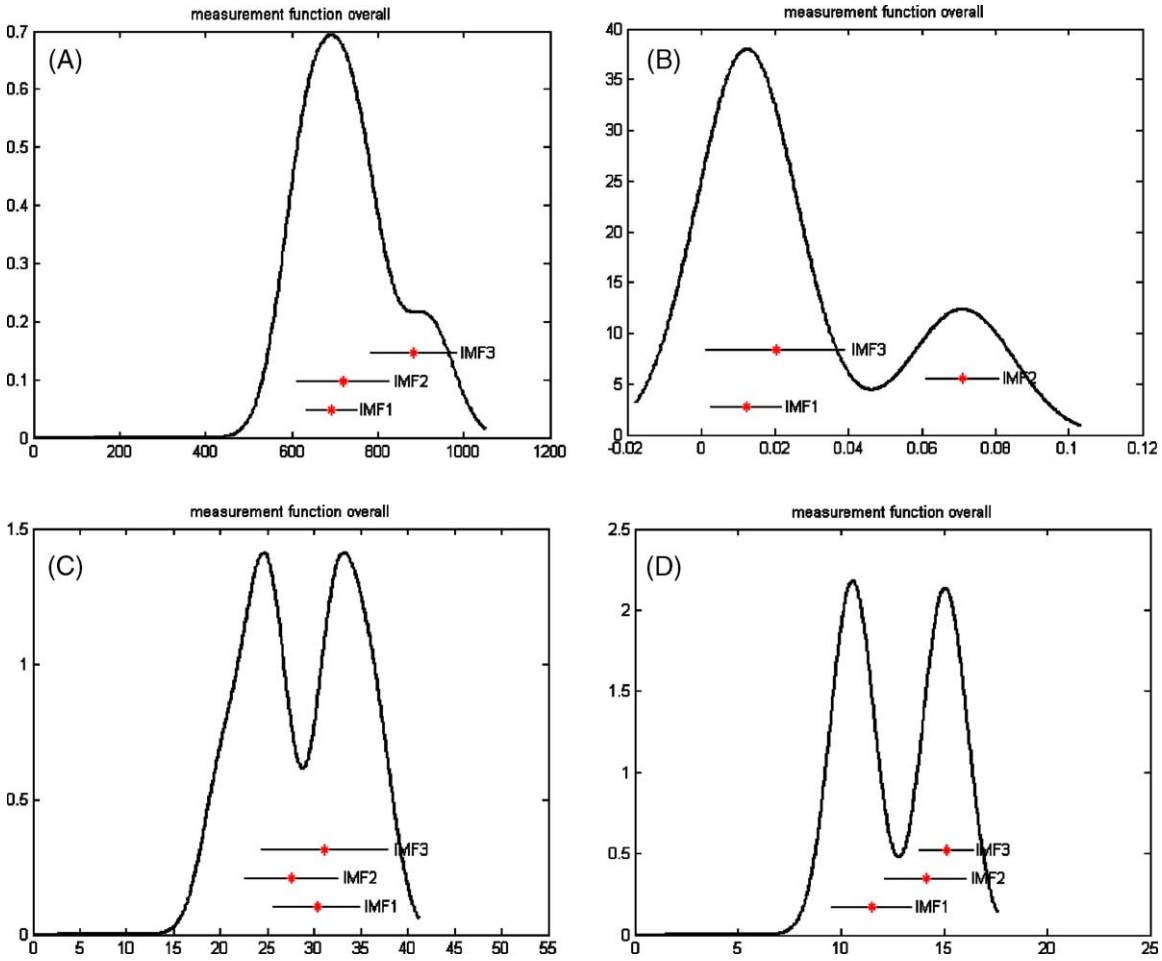

Fig. 1. Distribution of laboratories' mean values for OCDD (A), TCDD (B), PCB 105 (C) and PCB 156 (D).

(see Fig. 1A). The outlier detected by ISO procedure was an outlier identified by Cochran's test and has, in this case, no influence on the distribution. Two laboratories reported higher values (not discarded by Grubb's test) characterizing a normality discrepancy in the distribution of means. Thus, the mean value calculated by the conventional approach is influenced more by the weight of these higher values than the new model. Alternatively, when an extreme situation characterized by obvious outliers and a distribution of data representing a 'two-humped backed camel' (see Fig. 1B), then ISO can correct the situation by discarding one or several outliers and the tendency is then not observed.

The special case of 2,3,7,8-TCDD, already mentioned above, is presented in Fig. 1B. The IMF_1 provided an assigned value of 0.012 ng/kg of product with a P factor of 73%, indicating that it is probably the best estimate of the true value. The mean value obtained by the classical approach is strongly influenced by the highest results and after discarding them (four outliers), an indicative mean of 0.011 ng/kg of product was assessed. The new model is not affected by these higher results. Instead, IMF_2 has a reasonable probability (24%) and points to a second cluster of results with a high mean value of 0.07 ng/kg of product.

PCB 105 and PCB 156 also presented some interesting features, which are characterized by similar probability factors (P) for IMF_1 and IMF_2, signifying a possible bimodal distribution (see Fig. 1C and D). Unfortunately, the combination of the very similar intensity of the two peaks and the difference in concentration of the two modes compared to the uncertainty of the data renders it impossible for the model to resolve the two peaks. For PCB 156 ($P = 50\%$), the mean value has the same probability of being 11.49 ng/kg (IMF_1) or 14.12 ng/kg (IMF_2), while the corresponding mean value estimated by the classical approach (12.51 ng/kg) is situated at the minima between the two peaks.

9. Performances of the HRMS method

In order to evaluate the performance of the confirmatory HRMS method and interpret the results, the repeatability S_r and the reproducibility S_R standard deviations of all congeners were plotted against their corresponding mean level (Fig. 2). Two nice linear relationships were obtained. Their corresponding coefficients of correlation were respectively $r^2 = 0.978$ and $r^2 = 0.985$ and data points, i.e. the PCDD/F

Table 5
Comparison of the two statistical approaches

Compounds	ISO 5725				Cofino model					Difference (%)
	n	Mean value (ng/kg D.M.)	RSD_r (%)	RSD_R (%)	Unc S.D.	Mean value (IMF_1) (ng/kg D.M.)	S.D. total	RSD total	*P* (%)	
2,3,7,8-TCDD	8 (4)	0.011[a]			0.009	0.012	0.010	77	73	13.7
1,2,3,7,8-PeCDD	10 (1)	0.040	20	49	0.012	0.033	0.014	43	68	16.6
1,2,3,4,7,8-HxCDD	13	0.160	22	26	0.030	0.159	0.036	23	83	0.6
1,2,3,6,7,8-HxCDD	13 (1)	1.985	6	11	0.120	1.986	0.172	9	66	0.1
1,2,3,7,8,9-HxCDD	13	0.779	8	13	0.050	0.780	0.081	10	63	0.1
1,2,3,4,6,7,8-HpCDD	13	146.286	5	10	6.680	149.347	10.233	7	61	2.0
1,2,3,4,6,7,8,9-OCDD	13 (1)	742.237	6	15	39.973	693.714	60.337	9	58	6.5
2,3,7,8-TCDF	13	0.092	19	29	0.014	0.090	0.018	20	71	2.9
1,2,3,7,8-PeCDF	11 (1)	0.029	30	53	0.014	0.026	0.016	60	77	9.3
2,3,4,7,8-PeCDF	11	0.051	19	41	0.013	0.048	0.016	34	83	5.9
1,2,3,4,7,8-HxCDF	13 (1)	0.073	16	39	0.014	0.078	0.018	23	64	7.3
1,2,3,6,7,8-HxCDF	11 (1)	0.044	19	26	0.013	0.044	0.015	33	82	0.5
2,3,4,6,7,8-HxCDF	13 (2)	0.049	23	24	0.017	0.051	0.018	36	81	3.9
1,2,3,7,8,9-HxCDF	3				0.046	0.038	0.058	154	77	
1,2,3,4,6,7,8-HpCDF	13	1.582	11	21	0.141	1.477	0.209	14	61	6.6
1,2,3,4,7,8,9-HpCDF	13	0.195	17	29	0.034	0.184	0.043	24	74	5.6
1,2,3,4,6,7,8,9-OCDF	13 (1)	13.129	6	19	0.858	12.339	1.315	11	52	6.0
TOTAL-TEQ PCDD/Fs		1.95				1.98				1.2
PCB 77	9	7.327	7	21	0.365	6.733	0.580	9	46	8.1
PCB 126	10 (2)	0.446	6	17	0.041	0.486	0.059	12	61	8.3
PCB 169	10 (1)	0.082	9	25	0.017	0.077	0.020	26	75	5.8
PCB 81	9	0.434	10	35	0.051	0.396	0.081	20	52	8.6
PCB 105	8	28.642	7	22	1.459	30.291	4.658	15	37	5.4
PCB 114	5	2.362	6	20	0.219	2.239	0.355	16	61	5.2
PCB 118	9	93.677	5	19	3.434	85.474	5.361	6	40	8.8
PCB 123	5	2.715	13	43	0.422	1.939	0.558	29	58	28.6
PCB 156	6	12.508	9	20	0.746	11.490	1.957	17	50	8.1
PCB 157	6	2.157	6	18	0.235	2.212	0.356	16	69	2.5
PCB 167	6 (1)	6.759	14	13	0.854	6.736	0.913	14	78	0.3
PCB 189	6	1.422	14	19	0.234	1.372	0.284	21	83	3.5
TOTAL-TEQ dioxin-like PCBs	0.07					0.07				
TOTAL WHO-TEQ		2.02				2.05				1.2

[a] Value with ISO 5725.

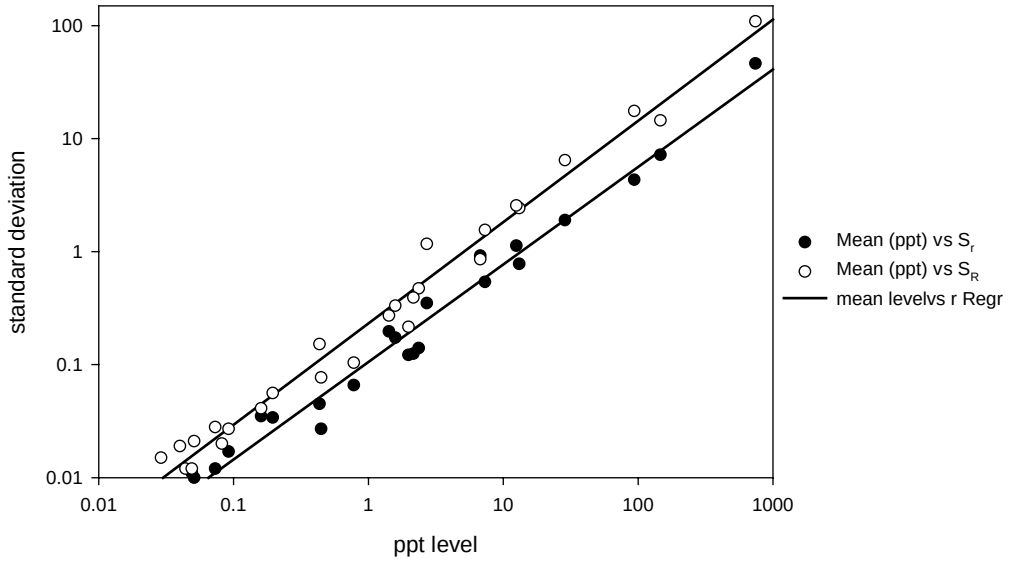

Fig. 2. Mean level (ppt) vs. repeatability (S_r) and reproducibility (S_R).

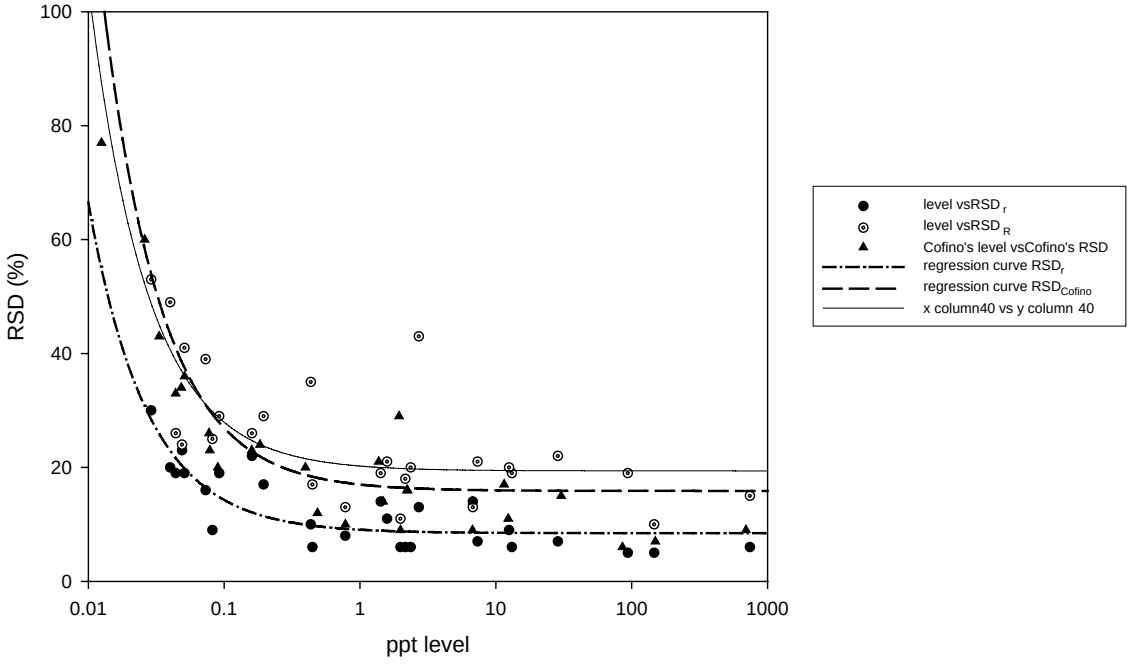

Fig. 3. RSDs vs. mean level (ppt).

and the D-L PCB congeners are distributed on both sides of the expected relationship. Consequently, the precision of the confirmatory HRMS method, for this particular study, seems to be congener-independent in terms of repeatability and reproducibility performances over the concentration range. Moreover, the two lines are parallel and thus the interval between the repeatability and the reproducibility of the method is constant from 0.1 to 1000 ppt. Expressed in RSD (%), the difference between reproducibility and repeatability is roughly 10% in the linear range.

Obviously, if the RSD_r and RSD_R of all the congeners were plotted against their corresponding mean level, a constant range of RSD could be delimited. Fig. 3 represents the precision of the HRMS method for dioxins, furans and D-L PCBs in animal feed at ultra-trace level. The graph clearly showed the effect of precision lost below 0.1 ppt level for repeatability and below 0.2 ppt level for reproducibility of the method. On the other hand, the RSD_r of the HRMS method tends to an asymptotic value of 8%, while the RSD_R tends to 20%. The inter-laboratory reproducibility precision at ppt and sub-ppt level provides here RSD_R that is lower than the prediction of the Horwitz functions [15,16]. The same trends for precision at ppb levels were already reported by Thompson [17], who suggested applying different functions for reproducibility standard deviation according to the level of concentration. The HRMS method can be modeled here by an inverse first-order equation providing the best regression curve fitting.

$$\text{RSD}\,(\%) = ac^{-1} + b \quad (1)$$

where c is the level stated in parts per trillion, and a and b are constants.

The equation parameters a and b are summarized in Table 6 for inter-laboratory repeatability, reproducibility as well as for Cofino's statistics. A distinction was made between all the 29 congeners and the dioxin and furan congeners only. The repeatability precision (r) obtained for the D-L PCB congeners is comparable to the one obtained for PCDD and PCDF congeners, which is confirmed by similar constants. However, two D-L PCBs (81 and 123) are characterized by poor reproducibility precision (R), which leads the regression curve to tend to a higher asymptotic value when D-L PCBs are taken into account. On the other hand, the robust Cofino's statistics show less influence of the D-L PCBs' precision performances. The equation parameters also pointed out the good agreement of precision between both statistical approaches (RSD_R and total Cofino's RSD) when only dioxin and furan congeners are taken into account.

When the results are expressed on WHO-TEQ for PCDD/Fs only, a mean value of 1.95 ng WHO-TEQ/kg of product was assigned with a corresponding coefficient of variation (CV) of 8.4% if the lower bound is used for reporting, whereas a CV of 6.2% is obtained for upper-bound

Table 6
a and b equation parameters for repeatability, reproducibility and Cofino's statistics for PCDD/Fs only and PCDD/Fs and dioxin-like PCBs

	PCDD/Fs		PCDD/Fs and d-l PCBs	
	a	b	a	b
r	0.58	8.2	0.58	8.5
R	1.69	11.9	0.85	19.4
Cofino	1.69	10.9	1.58	11.6

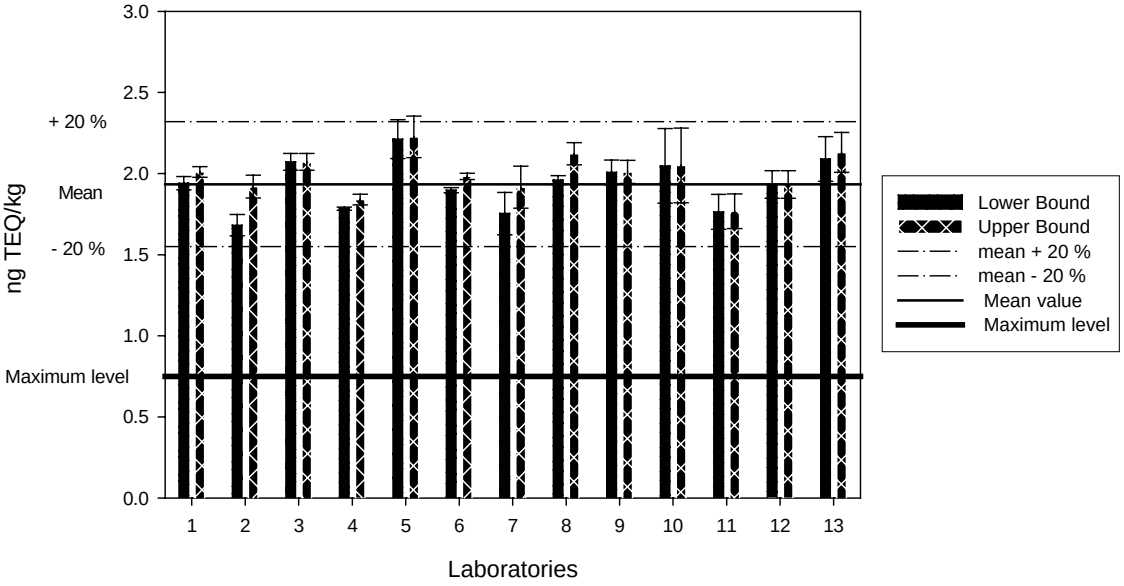

Fig. 4. Laboratories' mean value expressed on WHO-TEQ for PCDDs and PCDFs only.

value. Fig. 4 shows the participants' mean value on ng WHO-TEQ/kg, reported on lower-bound and upper-bound values. At more than twice the maximum level, the results comply with EU directive requirements, i.e. that the CV be lower than 15%. All the laboratories' mean values are within the ±20% range of the assigned value for trueness and the difference between upper-bound levels and lower-bound levels does not exceed 20%.

10. Conclusions

The laboratories taking part in the test included skilled participants and only a few with less experience in dioxin analysis in feedingstuffs. No significant differences in performances were observed. On the contrary, the study has shown a good agreement (CV of 6.2% on TEQ calculation in upper-bound value) between the laboratories at more than twice the level of interest (i.e. 0.75 ng TEQ/kg of product). Thus it should probably be possible to comply with the required criteria of CV < 15% at the level of interest. The performances obtained here at ppt levels are comparable with those achieved during certification exercise with selected laboratories [18]. However, one should also notice that for worldwide inter-laboratory studies in the field [19,20], grouping more than 50 laboratories, higher CVs are generally observed.

Laboratories have shown sufficient sensitivity performances to carry out dioxin analysis at background levels. Median LOD value was 0.02 ng/kg for tetra-penta-hexa-CDD/F congeners, whereas higher LOD values were reported for the hepta-octa-CDD/Fs, probably due to their presence in blank samples. It does seem to be sensitive enough to comply with current legislation. Nonetheless, important differences between detection limits reported by participants were pointed out. As the maximum residue limits will probably decrease in a near future, some laboratories should focus their efforts to improve the sensitivity of their analytical method.

In this particular study, the performances of the HRMS method seem to be congener-independent for repeatability and reproducibility over a range of concentration spanning more than four orders of magnitude. The method loses repeatability precision below an individual concentration per congener of 0.1 ppt. Moreover, promising results were also obtained for D-L PCBs. Except for two congeners (PCB 81 and PCB 123), repeatability and reproducibility were satisfactory, and performances were comparable to those obtained for the dioxin and furan congeners. Unfortunately, scarce data were reported and laboratories are encouraged to validate their method for these congeners.

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