Proficiency test for dioxins and dioxin-like PCBs in fats intended for the production of animal feed

Elbers I.J.W [1], , Egmond van H.[1], Hoogenboom L.A.P.[1],Traag W.A.[1]

[1]RIKILT Institute of Food Safety, Wageningen UR, Akkermaalsbos 2, 6708 WB Wageningen, The Netherlands

Introduction

The aim of this proficiency test was to give laboratories the possibility to evaluate or demonstrate their competence for the analysis of dioxins and dioxin-like PCBs in fat of plant origin intended for use in animal feed. The analysis must be carried out according to Commission Regulation (EC) No 152/2009. The preparation of the materials, including the suitability testing of the materials and the evaluation of the quantitative results were carried out according to ISO 17043 [1]. Fifteen European laboratories subscribed for participation in the proficiency test and reported within the deadline.

Materials and methods

Four samples were prepared for this test. They were prepared by either adding standard solutions of PCDD/Fs and/or dioxin-line PCBs (dl) or non-dioxin-like PCBs (ndl) or by adding contaminated fat, either fish or chicken fat to a sunflower oil basis (table 1):

-sunflower oil spiked with dioxins and PCBs (material 1)
-sunflower oil mixed with contaminated fish oil (material 2)
-sunflower oil mixed with contaminated chicken fat and spiked with 2,3,7,8-PCDF (material 3)
-sunflower oil mixed with contaminated chicken fat and spiked with six ndl PCBs (material 4)

Table 1. Aimed spiking levels.

	Material 1	Material 2	Material 3	Material 4
dioxins in ng TEQ/kg	1.01	0.48	1.15	1.15
dl-PCBs in ng TEQ/kg	0.89	0.85	2.36	2.36
dioxins and dl-PCBs in ng TEQ/kg	1.90	1.33	3.40	3.40
ndl-PCBs in µg/kg	15.0	7.76	-	36.51

After homogenization, the sample materials were divided into sub-portions and stored in glass jars. Each contained 10 ml of sample. The samples for the participants were randomly selected and coded from 001 through 120. For each laboratory a sample set was prepared consisting of one randomly selected sample of material 1, 2 and 3a ór 3b. The homogeneity of the materials was tested according to The International Harmonized Protocol for Proficiency Testing of Analytical Laboratories [2] and ISO 13528 [3]. For the four materials PCB 138 and PCB 153 were selected to determine homogeneity. Ten randomly selected containers of materials 1 and 2 were analysed in duplicate for PCB 138 to determine the homogeneity of the materials. Materials 3a and 3b were analysed in duplicate for PCB 153.
All four materials demonstrated to be sufficiently homogeneous for use in the proficiency test. Each of the participating laboratories received a randomly assigned laboratory code. The laboratories were asked to store the samples until analysis according to their own laboratory procedure. A single analysis according to EC/152/2009 of each sample was requested. Whether the dioxin and/or dioxin-like PCBs or the ndl-PCB levels in the samples were compliant or non-compliant according to regulations was an additional request.
For the evaluation of the quantitative results the consensus value, the uncertainty of the consensus value, a target standard deviation and z-scores were calculated according to the International Harmonized Protocol for the Proficiency Testing of Analytical Laboratories [2], elaborated by ISO, IUPAC and AOAC and ISO 13528 [3] in combination with the insights published by the Analytical Methods Committee [4,5] regarding robust statistics.

The uncertainty of the consensus value is calculated to determine the influence of this uncertainty on the evaluation of the laboratories. In case the uncertainty of the consensus value is higher than 0.3*target standard deviation for proficiency testing, the uncertainty of the assigned value was taken into account when evaluating the performance of the participants regarding the accuracy.

For illustrating the performance of the participating laboratories with regard to the accuracy a z_a-score is calculated. For the evaluation of the performance of the laboratories, ISO 13528 [3] is applied. According to these guidelines z_a-scores are classified as presented in table 2.

Table 2. Classification of z_a-scores.

$\|z_a\| \leq 2$	Satisfactory
$2 < \|z_a\| < 3$	Questionable
$\|z_a\| \geq 3$	Unsatisfactory

Results and discussion

Fifteen laboratories participated in the proficiency test for dioxins and dioxin-like PCBs in fat. For statistical evaluation, the results which were reported with smaller than values ('<') were changed to the corresponding detection limits. Participants were asked to report according to the Commission Regulation 225/2012 [6].

For the detection of PCBs, ten labs applied GC-HRMS, one lab applied GC-MS, one lab applied GC-MS/MS, one lab applied a combination of GC-HRMS and LRMS and one lab applied the CALUX assay. The amounts of fat equivalents injected on to the GC-column varied from 0.004 to 2.4 grams. The lowest and highest limits of detection for PCDD/Fs varied from a factor 1.25 for 2,3,7,8-TCDD to a factor 40 for 1,2,3,4,6,7,8,9-OCDF (lowest LOD was <0.05 ng/kg, highest was <2 ng/kg). The lowest and highest limits of detection for PCBs varied from a factor 2 for PCB 77 to a factor 500 for PCBs 28 and 180 (lowest LOD was <2 ng/kg, highest was 1000 ng/kg).

Participant were additional requested to provide information whether a sample is regarded as being compliant or non-compliant. A variety of results was reported for the compliancy/non-compliancy of the samples and some questions were asked. Material 1 (considered non-compliant in the ideal case) was assigned as compliant by three labs, non-compliant by ten labs and compliant (for dioxins and sum dioxins and PCBs) ánd non-compliant (for sum non dioxin-like (ndl) PCBs) by one lab. Material 2 (considered compliant in the ideal case) was assigned as compliant by ten labs and non-compliant by four labs. For material 3a (considered non-compliant in the ideal case) one lab reported this material as compliant, six as non-compliant and one as non-compliant (for dioxins and sum dioxins and PCBs) ánd compliant (for ndl-PCBs). Material 3b (considered non-compliant in the ideal case) was assigned compliant by one lab and non-compliant by six labs. Labs 2, 6, 8, 11, 12, 13 and 16 assigned all materials correctly as compliant or non-compliant.

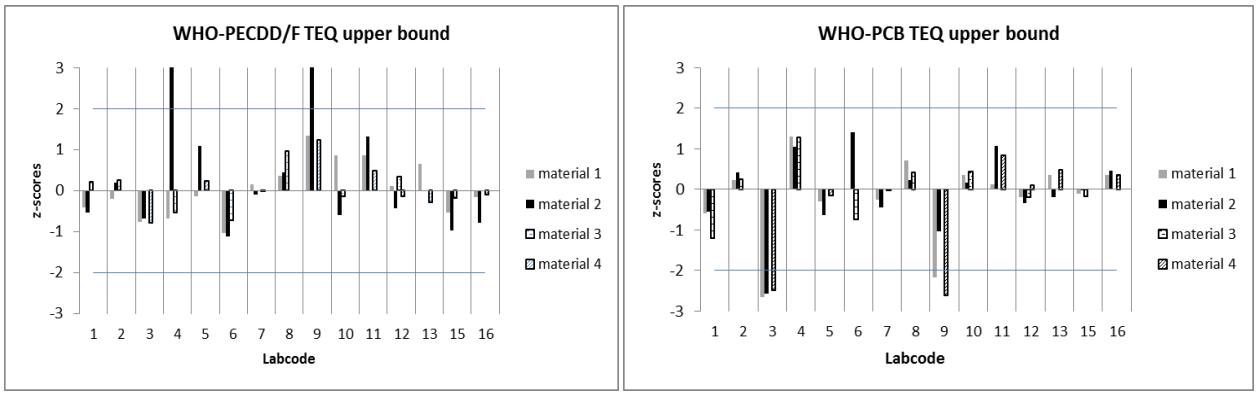

Figure 1. Overall z-scores for upper bound results of the participating laboratories.

Not all labs corrected for the moisture content as laid down in Commission Regulation no 152/2009 [7]. Also, in case CALUX assay, GC-MS or GC-MS/MS (screening techniques) were used in combination with non-compliant results, no lab suggested re-analysis with high-resolution mass spectrometry. In table 3 overall results of the PT are given.

Table 3: Overall results of all participating laboratories

Parameters	# reported results	# uncertainty > 0.3	# of questionable/ unsatisfactory z-scores
PCDD/F TEQ (3)	126	3	4
PCBs-TEQ (3)	126	0	11
PCDD/PCDF (17)	731	32	83
Dioxin like PCBs (12)	516	4	20
Non dioxin like PCBs (6)	240	4	18
Total	1739	43	136

Overall 7.8% of the total z-scores were questionable/unsatisfactory results. There is a difference of a factor 2.5 between the questionable/unsatisfactory z-scores for dl-PCBs (8.7%) and dioxins (3.2%). Also remarkable is the high percentage of questionable/unsatisfactory z-scores of 7.5% for the ndl-PCBs

Conclusions and recommendations

Fifteen laboratories participated and reported results for the proficiency test of dioxins and dioxin-like PCBs in fat. Out of these, no laboratories showed optimal performance by quantifying all congeners and sum-TEQs correctly. However, twelve labs (labs 1, 2, 5, 6, 7, 8, 10, 11, 12, 14, 15, 16) reported correct sum-TEQs for both PCDD/Fs and PCBs. Lab 3 reported |z-scores|>2 for the ub, mb and lb PCB-TEQ in all three materials, lab 4 for the ub, mb and lb PCDD/Fs-TEQ in material 2 and lab 9 for the ub PCDD/F-TEQ in material 2 and for the ub PCB-TEQ in materials 1 and 3.

Wide ranges of results were reported in this PT: for more than 25% of the compound-material combinations the uncertainty of the assigned value exceeded $0.3\sigma_H$, indicating large variability in results. Of 1739 possible z-scores, 138 results were questionable/unsatisfactory (7.9%).

Whether the samples were compliant or non-compliant resulted in a fair amount of questions and a variety in results. However, labs 2, 6, 8, 11, 12, 13 and 16 assigned all materials correctly as compliant or non-compliant.

Based on the results of this study it is concluded that:

- The quantification of PCBs leads to better results than the quantification of the PCDD/Fs, which may be related to the higher concentrations of PCBs present in the samples;
- The LODs reported by some participants are high and need additional attention.
- The assignment of the samples as compliant or non-compliant needs additional attention. Only seven out of 15 labs assigned all materials correctly as compliant or non-compliant.
- Not every lab applied EC/152/2009 for correction for moisture content and for re-analysis in case of a non-compliant screening result.
- More effort is needed for correct quantification of the individual congeners.

Acknowledgements
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