Feasibility of new CRMs for dioxins and PCBs in food and feed: DIFFERENCE leads to confidence

Rudy Van Cleuvenbergen[1], Stefan van Leeuwen[2], Jacob de Boer[2]

[1]Vito
[2]Netherlands Institute for Fisheries Research

Introduction

Certified reference materials (CRMs) are an important tool to validate analytical methods and to ensure the quality of monitoring data obtained. Though some materials for PCDD/Fs and dioxin-like PCBs in food and feed exist (e.g. milk powder BCR 607, chub tissue BCR 719, cod liver oil NIST 1588a, carp tissue NRC CARP-2, fish tissues CIL EDF-2524 to 2526), they together cover only few types of matrices; moreover each of these materials suffers from one or more of the following drawbacks:

- only a limited set of congeners was studied;
- certified uncertainties are relatively large, or only indicative values are given;
- the concentration level significantly exceeds the current EU maximum limit;
- the material does not enable a check of the complete analysis, incl. extraction.

One of the objectives of the EU-DIFFERENCE project was to assess the feasibility of the preparation and certification of new reference materials. Here we report on the results of an interlaboratory study on 5 food/feed materials conducted with 12 expert GC-HRMS laboratories, aimed at investigating whether sufficient agreement and fit-for-purpose confidence intervals can be reached for the whole range of PCDD/Fs, dioxin-like PCBs and indicator PCBs in a future certification project.

Materials and methods

Three food and two feed materials were selected and prepared for this feasibility study:
- a wet herring tissue, naturally contaminated (DIFF-01);
- a wet pork tissue, obtained by mixing regular meat with meat from a feeding experiment (DIFF-02);
- a whole milk, to which additional PCDD/Fs and dioxin-like PCBs were spiked (DIFF-03);
- a herring oil, naturally contaminated (DIFF-04);
- a compound feed for pigs, of which the lipid source was a salmon oil to which additional PCDD/Fs and dioxin-like PCBs were spiked (DIFF-05).

The materials were selected and prepared so that the levels in the final materials were close to the limits specified in the EC Directive 2001/102/EC and EC Regulation 2375/2001, except for the fish (with PCDD/F-TEQ level of about half the EC limit). Homogeneity and stability of the materials were demonstrated according to the BCR guidelines[1]. The PCDD/F-TEQ and total TEQ, calculated from the congener consensus values of the interlaboratory study using the WHO-TEFs, are given in Table 1. For the fish oil lower and upper bound TEQ were slightly different, so both are presented. The extractable fat content in Table 1 is the mean value obtained by the labs. Similarly, a moisture content in the compound feed of 9.3% was calculated.

The interlaboratory study was organised in two rounds between Dec 2003 and Nov 2004, according to a detailed protocol agreed upon between the participants in an introductory meeting. All laboratories used their in-house methods of extraction, clean-up and final determination. After each round a technical meeting between the participants was organised to evaluate the methods, QC data and results. After elimination of all technically explainable outlying values and a detailed statistical analysis of the remaining data set[1, 2], consensus values and their confidence intervals were calculated.

Material	PCDD/F-TEQ	total TEQ	extractable fat

	(pg/g product)	(pg/g product)	(%)
Herring muscle tissue (DIFF-01)	1.89	3.76	17.6
Pork muscle tissue (DIFF-02)	0.307	0.446	23.6
Whole milk (DIFF-03)	0.0945	0.220	3.68
eFish oil (DIFF-04)	5.46-5.56	11.0-11.2	-
Compound feed (DIFF-05)	0.463	1.23	6.79

Table 1: Material characteristics

Results

On an overall basis, for 65% of the parameters a half-width of the 95% confidence interval of less than 10% of the mass fraction was obtained; for 80% of the parameters this relative uncertainty was less than 15% of the mass fraction. Somewhat smaller relative uncertainties were obtained for milk and compound feed than for the other matrices. The worst comparability was observed for pork tissue, which also was the only material in which no consensus value could be reached for one of the important congeners with regard to TEQ contribution (outlying lab mean for PCB 126).

The relative uncertainties for PCDD/F and dioxin-like PCB congeners are shown in Figure 1 and 2. For the indicator PCBs relative uncertainties amounted to 2.9 - 10.7%.

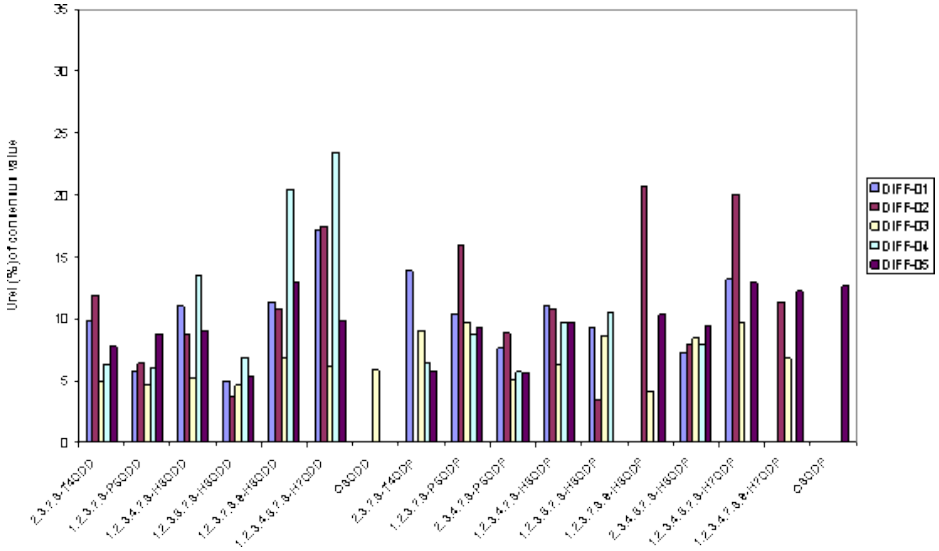

Figure 1: Relative uncertainties of the consensus values for PCDD/F congeners.

Legend: DIFF-01: herring muscle tissue; DIFF-02: pork muscle tissue;

DIFF-03: whole milk; DIFF-04: fish oil and DIFF-05: compound feed.

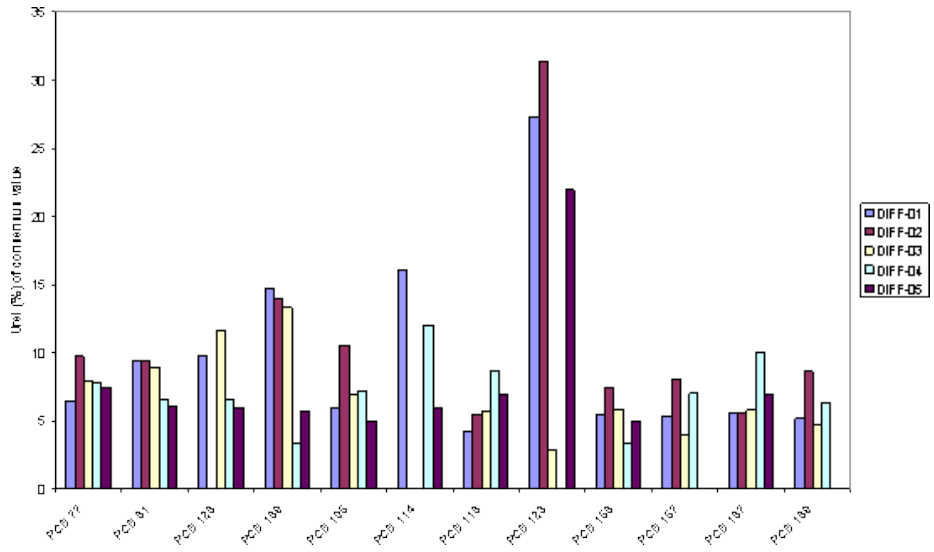

Figure 2: Relative uncertainties of the consensus values for dioxin-like PCB congeners. Legend: DIFF-01: herring muscle tissue; DIFF-02: pork muscle tissue;

DIFF-03: whole milk; DIFF-04: fish oil and DIFF-05: compound feed.

Indicative or "less than" values, or relatively large uncertainties (U_{rel} >15%), were obtained more than once for the following congeners (ranked according to decreasing occurrence of such values):

· O_8CDD and O_8CDF;

· 1,2,3,4,6,7,8-H_7CDD, 1,2,3,7,8,9-H_6CDF, PCB 114 and PCB 123;

· 1,2,3,4,6,7,8-H_7CDF, 1,2,3,4,7,8,9-H_7CDF and PCB 28.

These congeners consequently may be regarded as the most demanding for certification, which is not unexpected because of the very low concentration of some of them (especially when compared to others from the same compound group) as well as higher sample contamination risks.

Discussion

This feasibility study has demonstrated that the participating group of expert laboratories should be able to reach fit-for-purpose uncertainties in a future interlaboratory certification project, and this for most - if not all - of the 35 compounds of interest. It is hoped that European funds will now be made available through the Institute for Reference Materials and Measurements to prepare and certify the planned CRMs.A comprehensive protocol agreed upon prior to the measurements, and a thorough technical discussion of the methods and results afterwards, must be considered critical factors to make such a certification succeed. In the protocol, it is recommended to pay particular attention to the following points:

· the independency of the replicate analyses: depending on the compromise taken, part of the random errors of a typical determination may not be reflected by the within-lab RSDs (resulting in an increased risk of outlying variances), and also lab means may become affected by additional small systematic errors (resulting in an increased risk of outlying lab means at the 0.05 significance level);

· the extraction efficiency from wet tissues (fish, pork, ...): this feasibility study indicated that apolar solvents may not always completely extract the analytes from the matrix, even when recoveries for internal standards and the amount of fat extracted do not suggest any problem;

· the procedure blanks: because of the ultratrace level at which some of the analytes are present in food and feed, the procedure blank is nonnegligible in many labs; it should be carefully quantified to enable a reliable correction, and a limit on the contribution of the procedure blank to the result is recommended;

· the chromatographic separation : on the currently used GC columns, it is likely that some analytes cannot be sufficiently resolved from other analytes or coeuting compounds to enable an accurate peak integration; some critical pairs are the 1,2,3,4,7,8/1,2,3,6,7,8-H_6CDD and H_6CDF isomers, PCB 118/123, PCB 167/128, PCB 28/31, PCB 138/163; however, also for other congeners (e.g. PCB 114) possible coelution has been noticed in this feasibility study;

· the recovery of internal standards: for GC-HRMS, the Directives 2002/69/EC and 2002/70/EC have set 60-120% limits to the recovery (with an exception for PCDD/F congeners which together contribute less than 10% to the PCDD/F-TEQ); the results of this feasibility study indicate that particularly the lower limit cannot easily be met when multi-step cleanup schemes are applied, but recoveries somewhat below 60% generally did not affect the reliability of results; the amount of internal standards added and the spiking method (conditioning, ...) may be more important factors.

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