Certification of the contents of PCDDs and PCDFs in milkpowder

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1 INTRODUCTION

The reliable determination of PCDDs and PCDFs in foods is of considerable importance. The use of Certified Reference Materials (CRMs) based on appropriate matrices is increasingly a requirement of good laboratory quality systems, and is necessary for interlaboratory harmonisation and recognition.
Following problems in the trade of milk powder the Bureau Communeautaire de Reference (BCR) conducted a preliminary intercomparison on the determination of PCDDs and PCDFs in natural and spiked milk powder (1) which indicated that the preparation and certification of a reference material was feasible.
We now report on the preparation and homogeneity testing of a candidate milk powder reference material and on the results of the certification exercise.

2 MATERIALS AND METHODS

2.1 Preparation of milkpowder

A quantity (1650 liter) of cows milk, known to contain dioxins, was transported to NIZO (Netherlands Institute for Dairy Research) in Ede and heated in 10 sec. to 74°C and homogenised at 55°C at 200 bar; whereafter inline cooling to 5°C took place. The homogenized product was concentrated to 46% dry matter in four temperature steps in a falling stream evaporator from 74° to 46°C. The concentrate was sprayed using a nozzle atomiser at a temperature of 72°C to give a final water content of about 2%. After mixing in a Nauta mixer the powder was filled into 6 sacks (25 kg each) and transported to IRMM (Institute for Reference Materials and Measurements) Geel, Belgium for bottling and labelling into 1470 bottles, each containing 100 g of milkpowder.

2.2 Method of analysis

Measurements of PCDD and PCDF concentrations to determine homogeneity and stability of the powder were completed at RIKILT using methods described in the literature (2,3,4)

and in detailed internal standard operating procedures. In outline, ^{13}C labelled PCDD and PCDF internal standards are added to a known amount of milk powder, and fat extracted quantitatively. The native and labelled PCDDs and PCDFs are separated from the fat by gel permeation chromatography, and purified by cleanup using columns of basic aluminina and of porous, graphitised carbon. Final determination was carried out with capillary gas chromatography and high resolution mass spectrometry.
Internal control samples (blanks and PCDD and PCDF containing fat samples) and chemical blanks were analysed with the milk powder samples.

3 RESULTS

3.1 Homogeneity

To assess within-sample homogeneity five replicate analyses were completed on subsamples from each of two bottles of milk powder. The mean TEQ content of the milk powder was 1.94 pg TEQ/g (sd = 0.03; CV = 1.4%).
To assess homogeneity between samples analysis was performed on single subsamples from each of 18 bottles. The mean TEQ value was 1.83 pg TEQ/g milk powder, (sd = 0.09; CV = 4.8%).
The homogeneity studies were being acceptable, it was decided to proceed with the stability studies.

3.2 Stability

Bottles samples were stored at -20°C, +20°C and +35°C and, for each temperature one analysis each made from each of five bottles was made after periods of one, six and twelve months. Samples stored at -20°C and in the dark were assumed to be stable in respect of their PCDD and PCDF concentrations and matrix and were used as a reference for comparison for the samples stored at +20°C and +35°C.
The uncertainty U_t was obtained from the CVs of each set of five determinations according to the general formula:

$$U_{(t,T)} = (CV^2_{(t,T)} + CV^2_{(t,-20°)})^{1/2} \cdot \frac{R(t,T)}{100} \quad \text{in which} \quad R_{(t,T)} = x(t,T) / x(t,-20°)$$

In the ideal situation R_t should be 1.00.
Generally the expected value (= 1.00) was between $R_t + U_t$ and $R_t - U_t$.
It was concluded that the PCDD and PCDF content in the milk powder was stable after six and twelve month's storage at 20 and 35°C and certification was possible for this material.

3.3 Certification exercise

The detailed protocol for analysis and reporting of results was issued following discussion between potential participants at a preliminary meeting.
Each laboratory was required to determine a total of twelve target tetra-, penta- and hexa-CDD/DF congeners. Five independent replicate analyses were required and laboratories were instructed to measure material from at least two different bottles of milk powder and to carry out determinations on at least two separate days.
Each laboratory had a free choice of extraction and cleanup methodologies, and of conditions for the final determination steps provided that gas chromatography and high resolution mass spectrometry were used. This latter (gas chromatography with high resolution mass spectrometry) included choice of injection, capillary columns, mass ions monitored etc.

A series of quality control parameters had to be checked and reported by each participant to demonstrate the traceability and reliability of its analytical procedure. They include the following principal aspects.
Each laboratory received a set of twelve ampoules containing quantitative reference standards for the target compounds supplied by BCR (RM 432-443). An ampoule containing a solution of all 17 congeners at undeclared concentrations was also distributed and laboratories were instructed to analyse this as an unknown.
Laboratories were also requested, if possible, to report data for five supplementary hepta- and octa-CDD/DF congeners. BCR reference standards for these congeners were not available and laboratories were asked to calibrate their measurements using in-house standard solutions.

3.3.1 Internal standards
Laboratories were required to use at least one ^{13}C internal standard for each isomeric group of congeners.
All but one laboratories added the internal standards before starting the extraction procedure, to either the milk powder or to milk reconstituted from the milk powder as appropriate. Most laboratories reconstituted the milk.

3.3.2 Cleanup procedures
A wide variety of combinations of adsorbents were used, depending on each laboratories' preferences and experiences. Most procedures used only open, gravity-fed columns, but a few involved also flow controlled columns. One laboratory used the dialysis technique to separate PCDDs and PCDFs from the bulk of the fat.

3.3.3 GC-MS determination
It was required that final determination should be carried out on a capillary gas chromatograph with mass spectrometric detection, and that two isotope peaks should be measured for each analyte. Identification was based on comparison of retention times in sample and calibration solutions and agreement of the isotope ratios for PCDDs and PCDFs in the sample extracts with theoretical values. Quantification used relative peak areas of the two isotope peaks.
All participants checked the linearity of the system for each of the congeners to be determined, injecting five standard solutions, each of a different concentration.
All laboratories used fused silica capillary columns, in most cases with chemically bound stationary phases. Helium was the carrier gas in all cases. All but one lab used 60 m column lengths. Six labs used two different columns (with different polarity), in 2 cases to determine all compounds and otherwise to determine specific congeners on the most appropriate column. A wide variety of temperature programmes were employed. Two labs used on column injection, three used a temperature programmable injector and the remainder the splitless injection technique.
In all cases electron impact ionization was used working at resolutions above 6000. Two masses of the molecular ion cluster were used for identification and quantification, for each native and corresponding labelled congener.

3.3.4 Quality control
Participants were required to provide information from the analysis of laboratory blanks and were also required to analyse a solution containing the seventeen congeners at unknown concentrations.

In any case when the laboratory blank value was greater than 25% of the reported concentration then the data was withdrawn. Measureable blank values, if present but corresponding to less than 25% of the measured sample concentration, were subtracted from the final result. Most labs reported that blanks were negligible for most of the compounds considered.
In general the overall mean values for different congeners in the standard solution corresponded well with the target values, especially when the individual congener was available as an RM standard. See table 1.

Table 1. Concentration in ng/g for standard solution

	Mean	Standard deviation	Target		Mean	Standard deviation	Target
D48	10,10	0,66	10,32	F114	34,47	2,60	36,76
D54	20,37	1,73	20,83	F118	20,72	1,72	21,29
D66	5,91	0,86	6,37	F121	20,81	2,36	21,36
D67	19,32	1,53	21,08	F124	4,95	0,82	5,26
D70	10,93	1,15	10,86	F130	18,80	2,19	20,06
D73	28,53	4,13	33,91	F131	26,87	2,85	29,56
D75	45,54	4,80	47,17	F134	19,08	2,69	20,11
F83	10,15	0,92	10,88	F135	46,16	6,76	44,44
F94	10,05	1,18	10,87				

3.3.5 Certified values
After discussion of provided results and their statistical treatment, certified contents for five dioxins (D48, D54, D66, D67 and D70) and six furans (F83, F94, F114, F118, F121 and F130) are reported in table 2. For one furan (F124) no value could be reported as the concentration in milk was close to detection limits.
For the remaining PCDD/Fs (five hepta and octa congeners) only indicative values will be reported as for these compounds no standard reference material was available.

Table 2.

Contents of polychlorodibenzo-p-dioxins and polychlorodibenzo furans in natural milk powder (CRM 607)				
Mass fraction based on dry mass				Number of accepted sets of results p
Compound		Certified value µg/kg (1)	Uncertainty µg/kg (2)	
2,3,7,8-TCDD	D 48	0.25	0.03	9
1,2,3,7,8-PCDD	D 54	0.79	0.04	9
1,2,3,4,7,8-HxCDD	D 66	0.42	0.07	9
1,2,3,6,7,8-HxCDD	D 67	0.98	0.11	9
1,2,3,7,8,9-HxCDD	D 70	0.34	0.05	8
2,3,7,8-TCDF	F 83	0.05	0.03	5
1,2,3,7,8-PCDF	F 94	0.054	0.013	7
2,3,4,7,8-PCDF	F 114	1.81	0.13	9
1,2,3,4,7,8-HxCDF	F 118	0.94	0.04	9
1,2,3,6,7,8-HxCDF	F 121	1.01	0.09	8
2,3,4,6,7,8-HxCDF	F 130	1.07	0.05	8

(1) This value is the unweighted mean of the means of p accepted sets of results.
(2) The uncertainty is taken as the half-width of the 95% confidence interval of the mean given in (1).

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5 LITERATURE

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