New certified and candidate certified reference materials for the analysis of PCBs, PCDD/Fs, OCPs and BFRs in the environment and food

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Three new matrix-type certified reference materials (CRMs) have been produced for polychlorinated biphenyls (PCBs) in sterilized and wet (shell) fish matrices – BCR-682 (PCBs in mussels), BCR-718 (PCBs in herring) and BCR-719 (non-ortho PCBs in chub). Additional feasibility studies have been carried out to evaluate the conditions under which production and certification of CRMs are feasible for brominated flame retardants (BFRs), chlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs), PCBs and organochlorine pesticides (OCPs) in various food, animal feed and environmental matrices.

The results of these feasibility studies indicate that homogeneous and stable CRMs can be produced for all materials with the protocols developed during these studies. The user of these materials may need to apply simple rehomogenization of the material prior to use (e.g., for the milk material, slight phase separation resulting from sterilization was observed). The decreased precision or accuracy of applied analytical methods close to the method detection limit was found to affect the homogeneity and the stability for some low-concentration compounds studied. For example, as a result, the homogeneity and the stability of 1,2,3,4,7,8,9-HpCDF in the fish material could not be confirmed. However, based on analogy with compounds with the same degree of chlorination, we do not expect that 1,2,3,4,7,8,9-HpCDF would not be stable or homogeneously distributed over the sample matrix.

The BCR-682, −718 and −719 CRMs are strong quality-assurance tools to support laboratories analyzing (non-ortho) PCBs in fish and shellfish samples at the (low) levels determined in everyday routine samples. The materials from the feasibility studies can also support laboratories that analyze BFRs, OCPs, PCDD/Fs and dioxin-like PCBs (dl-PCBs) in the matrices investigated. However, these materials are currently not available for laboratories.

Keywords: BFR; Brominated flame retardant; Certified reference material; CRM; Dioxin; Environment; Feed; Fish; Food; PCB; Pesticide; Sediment

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1. Introduction

The analysis of persistent organic pollutants (POPs) in environmental, food or feed samples is complex and typically involves extraction, clean-up, further fractionation, and a final determination of the contaminants. Every stage of the analysis has critical parameters that should be optimized in order to reduce the uncertainty of the final result. Policy makers rely on data produced by various laboratories (e.g., when monitoring the compliance of products or carrying out risk assessments).

The ISO 17025 standard requires that accredited laboratories use validated methods, demonstrate traceability of calibrations, and apply an appropriate quality control programme. Proficiency testing (PT) schemes are important tools to compare a laboratory's performance with external laboratories. Nowadays, a number of international PT schemes are available for a wide range of contaminants in food and environmental matrices [www.quasimeme.org, www.fhi.no]. Certified reference materials (CRMs) are valuable tools to validate the trueness of analytical methods. Various CRMs have been produced for the analysis of organohalogen contaminants [1] but most of them show limitations, such as a limited number of certified contaminants, wide uncertainty ranges, concentrations (far) above the current values of interest or a physical state not matching routine samples (e.g., freeze-dried materials and oils). Consequently, there remains a clear need for additional CRMs to address the challenges that analytical laboratories currently face.

This article gives an overview of the developments in producing CRMs for the analysis of POPs that resulted from recent European projects. It describes the production and the certification of new CRMs for polychlorinated biphenyls (PCBs) in mussels and herring, and for non-ortho PCBs in chub. These CRMs can be obtained from the Institute for Reference Materials and Measurements (IRMM) for testing the accuracy of an analytical method. Furthermore, this article presents the outcome of feasibility studies on the production and certification of CRMs for brominated flame retardants (BFRs) in a freshwater sediment and flounder, organochlorine pesticides (OCPs) in flounder and polychlorinated dibenzo-*p*-dioxins and furans (PCDD/Fs) as well as PCBs (including alldioxin-like PCBs) in herring, pork, milk, fish oil and compound feed. Since this study evaluates whether production and certification of certain compound-matrix combinations is feasible, these materials are referred to as ''candidate CRMs''. Actual CRM production and certification should follow the feasibility study, although so far no initiatives for this have been taken.

2. Production of CRMs and candidate CRMs

The production of (candidate) CRMs can be sub-divided into four stages:

- production of the material;
- homogeneity study;
- stability study; and,
- certification study.

We give a general description of these phases below. The production of the materials was carried out according to ISO Guide 35 [2] and BCR (European Union, Community Bureau of Reference) guidelines [3–5].

2.1. CRMs

Information on the origin of the raw materials, production data and certified compounds of the BCR CRMs 682, 718 and 719 are shown in Table 1. Details on the production, homogeneity, stability and certification can be found in the certification reports [6–8].

Generally, production starts with collection of the tissue. The mussels (BCR-682) were cooked shortly before collection of the meat from the shells by vigorous shaking. The chub-muscle tissue (BCR-719) and the herring-muscle tissue (BCR-718) were collected by filleting the fish (removal of skin, intestines, head, tail, fins and bones). The fish muscle or mussels were subsequently minced to a final size of 3.5 mm^2. Subsequently, 10 batches of ca. 25-kg sample were homogenized for 3 min, after adding 0.02% butylhydroxytoluene (BHT), in a Stephan cutter (Stephan Machines, Almelo, The Netherlands, type UMM/SK25, made in 1979). After homogenization, each of the 10 batches of herring or chub mince (3.3 times 75 kg) was equally divided between 17 trays, resulting in 10 layers per tray. The trays were covered with aluminum foil, frozen in a blast freezer and subsequently stored in a freezer at −25°C. Before canning, the individual trays of herring or chub were homogenized again in a Stephan cutter for 3 min. For the mussels, one additional homogenization step was applied: in between mincing by the Stephan cutter and filling the trays, three batches of 25 kg were pooled in a stirring kettle and stirred for 5 min. Subsequently, 3 times 75 kg and a remaining amount of 25 kg were equally divided between 17 trays, which were homogenized again. After homogenization, coated tins (volume ca. 75 ml) were filled to the brim by hand with a stainless steel ice-spoon. The tins were sealed by a Lanico TVM 335 sealing machine and subsequently sterilized for 45 min at 122°C (pressure 1.4 bar).

2.2. Candidate CRMs

A feasibility study aims to test the suitability of procedures for the production and the certification of a homogeneous, stable CRM, and, by that, to define the conditions under which a CRM can be produced and certified. Although in essence such study is similar to the production and the certification of a real CRM, slightly different approaches can be followed (e.g., testing novel production

Table 1. Production and certification details of BCR-682, 718 and 719

CRM	Sample type	Origin, year of production and number of lots produced	Certified CBs
BCR-682	Mussel (*Mytilus edulis*)	Wadden Sea, The Netherlands,1997, 2816 tins	28, 52, 118, 138, 149, 153, 170 and 180
BCR-718	Herring (*Clupea harengus*)	North Sea, The Netherlands,1998, 3600 tins	28, 52, 101, 105, 118, 128, 138, 149, 153, 156, 170 and 180
BCR-719	Chub (*Squalius cephalus*)	Moldau, Czech Republic,1998, 2700 tins	77, 81, 126 and 169
Candidate CRMs	**Sample type**	**Origin, year of production and number of lots produced**	**Target compounds**
BROC-01	Flounder (*Platichthys flesus*) muscle tissue	Western Scheldt, The Netherlands,2001, 305 tins	BFRs: BDEs[*] 28, 47, 49, 66, 99, 100, 153, 154, 183, HBCD[**] OCPs[***] : p,p′-DDT, p,p′-DDE, p,p′-DDD, o, p′-DDT, dieldrin, endrin, α-HCH, β-HCH, γ-HCH, β-HEPO, HCB, QCB, trans-nonachlor, cis-chlordane, trans-chlordane, oxy-chlordane
BROC-02	River sediment	Western Scheldt (Nauw van Bath), The Netherlands, 2001, ca. 300 bottles	BFRs: BDEs 28, 47, 49, 66, 85, 99, 100, 153, 154, 183, 209, HBCD
DIFF-01	Herring (*Clupea harengus*) muscle tissue	North Sea, The Netherlands, 2002, ca. 600 tins	PCDD/Fs[****]: 2,3,7,8-TCDD, 1,2,3,7,8-PCDD, 1,2,3,4,7,8-HxCDD, 1,2,3,6,7,8-HxCDD,
DIFF-02	Pork muscle tissue	The Netherlands, 2002, ca. 300 tins	1,2,3,7,8,9-HxCDD, 1,2,3,4,6,7,8-HpCDD, OCDD,
DIFF-03	Whole milk	The Netherlands, 2002, 388 tins	2,3,7,8-TCDF, 1,2,3,7,8-PCDF, 2,3,4,7,8-PCDF,
DIFF-04	Herring oil	North Sea, 2002, ca. 300 ampoules	1,2,3,4,7,8-HxCDF, 1,2,3,6,7,8- HxCDF,
DIFF-05	Compound feed for pigs	The Netherlands, 2002, ca. 1700 containers	1,2,3,7,8,9-HxCDF, 2,3,4,6,7,8- HxCDF, 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9- HpCDF, OCDF CBs[****]: CB 77, 81, 126, 169, 105, 114, 118, 123, 156, 157, 167, 189, 28, 52, 101, 138, 153, 180

[*]BDE, Bromodiphenylether.
[**]HBCD, Hexabromocyclododecane.
[***]β-HEPO, Heptachloroxide; HCH, Hexachlorocyclohexane; HCB, Hexachlorobenzene; QCB, Pentachlorobenzene.
[****]Target compounds for DIFF-01 to DIFF-05.

approaches). Below, we describe how candidate CRMs were produced according to applicable guidelines [2–5].

Basic information on the flounder and sediment material is provided in Table 1. The flounder-muscle tissue material (BROC-01) was prepared in a way similar to BCR-718 and BCR-719, the difference being that fewer tins were produced. The river sediment (BROC-02) was collected from the Western Scheldt. The material was dried in an oven at 40°C for 60 h and subsequently minimized by a breaker to <2 mm particles at the Wageningen Evaluating Programmes for Analytical Laboratories (Wageningen, The Netherlands). Afterwards, the dried sediment was transported to IRMM (Geel, Belgium) where it was ground by a Multi-Processing System (100 AFG Jet Mill/Ultrafine Classification System, Alpine, Augsburg, Denmark) to a <125 μm powder. The final homogenization was carried out in a multi-purpose cone mixer with semi-automatic filling equipment. Amber glass bottles of 100 ml were filled with 50 g of sediment and closed with a screw cap (with polyethylene insert). During storage, preparation and bottling, care was taken to avoid extended exposure to UV radiation to prevent degradation of BDE 209.

The materials DIFF-01 to DIFF-05 were produced to support EU policies on (analysis of) WHO PCDD/Fs and dioxin-like PCBs (dl-PCBs) as defined in EU Directives [9,10] (see Table 1). On top of that, the ICES-7 PCBs (CBs 28, 52, 101, 118, 138, 153 and 180) were included in this feasibility study. Basic information on the materials and analytes investigated is presented in Table 1.

The herring-tissue material (DIFF-01) was prepared in a way similar to BCR-718 and BCR-719, the difference again being that fewer tins were produced.

Contaminated pork meat (DIFF-02) was not available from stock, so a pig-feeding experiment was conducted (at the Animal Sciences Group, Lelystad, The Netherlands) using feed to which a spiked vegetable oil was added. To obtain adequate concentration levels, the contaminated pork meat was diluted with non-contaminated pork meat from a local butcher, minced and homogenized. The pork homogenate was tinned and sterilized according to the procedure described for fish tissue, the difference once more being that fewer tins were produced.

Whole milk (DIFF-03) was pasteurized for 10 s at 74°C. Suitable analyte concentrations were obtained by

spiking with PCDD/Fs and dl-PCBs, without changing the typical congener profile of naturally contaminated samples. After 20 min of stirring, the milk was again pasteurized, homogenized at 200 bar and 50°C, and transferred to 150-ml tins. Final sterilization of the batch of closed tins took place at 122°C for 20 min. The preparation of the milk sample was carried out at NIZO Food Research, Ede, The Netherlands.

The herring-oil material (DIFF-04) was produced by filtering the crude fish oil over a 0.45 μm paper filter and dispensing it into amber-colored ampoules. While filling the ampoules, the fish oil was heated to 40°C under constant stirring to homogenize the oil and to prevent crystallization of the larger triglycerides. The ampoules were closed directly after filling by flame sealing.

Production of a pig-feed material (DIFF-05) was carried out at Nutreco Maasweide Laboratory, Boxmeer, The Netherlands. A salmon oil spiked with additional PCDD/Fs and PCBs was used as lipid source (5.5%) for the compound feed. After mixing the 14 ingredients (barley (0.8%), wheat-bran sharps (3.0%), wheat-gluten feed meal (10.0%), soya-oil cake 48 (15.9%), palm-kernel flakes (6.0%), canola grist RE < 38 (12.0%), tapioca 66 (40%), beet pulp < 10 (4%), spiked salmon oil (5.5%), chalk 1 (0.6%), mixing salt 1 (0.2%), lysine 1 (0.3%), vitamin mix pig-37 (0.2%) and molasses (2.0%)), the feed homogenate was pelleted at 1.2 bar at a pellet size of 3.5 mm and packed in polyvinylchloride (PVC) containers.

3. Homogeneity of the materials

3.1. CRMs

The homogeneity of the materials was tested by determining the concentrations of (a selection of) the target PCBs. This method is preferred to the sometimes applied homogeneity testing by analysis of alternative constituents, such as metals or fat content because, in the latter case, possible inhomogeneity of the target compounds cannot be ruled out [11].

Homogeneity testing requires a very precise analytical method that is able to detect a possible inhomogeneity of the material at a certain sample intake. The homogeneity in this study was determined by the analysis of the target PCBs (CBs 52, 101, 118, 153 and 180 for BCR-682 and BCR-718 and CBs 77, 81, 126 and 169 for BCR-719) in 20 different tins (between-lot variance) and five times in one tin (within-lot variance).

A possible inhomogeneity is expressed as the uncertainty due to the between-unit inhomogeneity of the material (u_{hom}) and is quantified as:

$$u_{\text{hom}} = \sqrt{CV_b^2 - CV_w^2}$$

When CV_{within} (CV_w) is equal to or larger than $CV_{between}$ (CV_b), u_{hom} cannot be assessed by the above formula. In such a case, an estimate for a maximum between-unit variability that could be masked by method variations is given by establishing a kind of ''upper detection limit for inhomogeneity'', as follows [12]:

$$u^*_{\text{hom}} = CV_w \cdot \sqrt[4]{\frac{2}{v_w}}$$

where v_w is the number of degrees of freedom for determination of this coefficient of variation.

For BCR-682, 718 and 719, the performance of the analytical method was quantified by replicate GC determination of a standard (n = 10) and a cleaned extract (n = 5). The method performance was not taken into account in the u_{hom} calculation. However, the $CV_{m\ (clean\text{-}up)}$ does not contribute to a large extent to the inhomogeneity (u_{hom}). The CV of the method clean-up and GC determination was below 5% (see Table 2), except for CBs 81 and 169. The reason for CB 81 is not

Table 2. Homogeneity analysis of PCBs in three CRMs (BCR-682, 718 and 719), with CVs and u_{hom} presented as %

	BCR-682					BCR-718				
	$CV_{m\ (standard)}$	$CV_{m\ (cleanup)}$	CV_w	CV_b	u_{hom}	$CV_{m\ (standard)}$	$CV_{m\ (cleanup)}$	CV_w	CV_b	u_{hom}
CB 52	1.7	3.3	3.5	6.7	5.7	1.0	3.9	1.5	5.4	5.2
CB 101	2.1	2.9	3.7	5.3	3.8	1.1	3.1	1.8	2.7	2.0
CB 118	2.9	4.1	2.4	6.0	5.5	1.7	4.4	3.8	2.9	**3.2***
CB 153	2.3	2.5	2.2	6.0	5.6	1.3	4	2.2	3.0	1.9
CB 180	3.5	4.3	3.6	6.8	5.8	2.0	3.8	2.5	3.4	2.3
			BCR-719							
CB 77	4.0	4.8	2.2	4.2	3.6	–	–	–	–	–
CB 81	4.0	8.4	5.3	8.3	6.4	–	–	–	–	–
CB 126	3.4	3.0	6.9	4.8	**5.8**	–	–	–	–	–
CB 169	3.2	6.9	7.0	9.4	6.3	–	–	–	–	–

*Bold: u_{hom} based on u^*_{hom}.

known, but, for CB 169, the elevated CV can be explained by the low concentrations in the extract, which were close to the limit of quantification (LOQ). The inhomogeneity for BCR-682, 718 and 719 was in the range 1.9–6.4%, which was low. The materials are therefore considered suitable for the use in a certification study. The homogeneity was demonstrated at a sample intake of 18 g (BCR-682), 6 g (BCR-718) and 20 g (BCR-719).

3.2. Candidate CRMs

To investigate the homogeneity for flounder (BROC-01), sediment (BROC-02) and the DIFF-01 to DIFF-05 materials, the protocol followed was similar to that for BCR-682, 718 and 719, except that the between-unit homogeneity was tested on 15 lots instead of 20.

GC determination does not significantly contribute to the inhomogeneity of the flounder material, which is clear from the low $CV_{m\ (standard)}$ shown in Table 3. For the flounder, u_{hom} values were in the range 2.4–20% (see Table 3) for the BFRs and OCPs. The higher inhomogeneity (e.g., compared to BCR-718) indicates that flounder is more difficult to homogenize than other fish species. Despite a possible inhomogeneity, it was decided to continue the feasibility study with the flounder material.

The sediment showed an excellent homogeneity, with u_{hom} in the range 2.3–6.8%. For BDE 209, higher CVs of 14% were obtained for both CV_w and CV_b (resulting in a calculated u_{hom} of 0%). These elevated CVs are assumed to be caused by analytical difficulties [13] rather than inhomogeneous distribution in the sediment.

For the other materials (DIFF-01 to DIFF-05), the results are shown in Table 4. Generally, PCDD/Fs showed higher u_{hom} values than the dl-PCBs and ICES-7 PCBs. For example, for the compound feed (DIFF-05), u_{hom} values were 1.2–7.6% for the PCBs compared to 1.1–13% for the PCDD/Fs. This is not due to an inhomogeneity of the PCDD/Fs, but rather reflects analytical challenges in repeatable determination of these contaminants at very low concentrations in the sample matrix. This is confirmed by the $CV_{m\ (clean\text{-}up)}$ of compound feed (DIFF-05) and milk (DIFF-03), which showed that replicate GC determinations of PCDD/Fs in a cleaned extract resulted in higher CVs compared to those of the dl-PCBs and ICES-7 PCBs. Determination of the latter compounds is therefore the most suitable approach to demonstrate homogeneity, because of the lower method variance even at small sample-intake levels.

For whole milk (DIFF-03), long-term storage resulted in the deposit of a paste-like fatty substance on the lid of the tins, resulting in an inhomogeneity of the material as shown in Table 4 by the elevated u_{hom} values of 6–17% for the PCDD/Fs. Fat determination confirmed this inhomogeneity with a u_{hom} of 11%. However, a simple treatment, such as ultrasonication, enabled representative sub-samples to be taken. (After ultrasonication, a CV of the fat content of 0.8% was found based on six replicate samplings).

Concerning the herring tissue (DIFF-01), based on the good homogeneity results for the dl-PCBs, this material is considered homogeneous. The material is therefore suitable for the feasibility study.

The pork tissue (DIFF-02) showed somewhat higher u_{hom} values, also for the dl-PCBs (5.1–14%). A slight inhomogeneity could therefore not be excluded. Evaluation of the homogeneity through PCDD/F determination was particularly difficult due to the very low PCDD/F concentrations in the material.

The fish oil (DIFF-04) was found to be homogeneous, based on the dl-PCBs (u_{hom} range 1.7–6.1%). This

Table 3. Homogeneity analysis of BFRs and OCPs in two candidate reference materials, with CVs and u_{hom} presented as %

	Flounder (BROC-01)				Sediment (BROC-02)		
	$CV_{standard}$	CV_{within}	$CV_{between}$	u_{hom}	CV_{within}	$CV_{between}$	u_{hom}
BDE 28	2.0	19	18	**16***	4.8	8.3	6.8
BDE 47	1.8	5.1	10	8.6	2.0	6.3	6.0
BDE 99	1.5	2.2	12	12	1.1	4.6	4.5
BDE 100	1.7	2.8	10	9.6	1.2	5.0	4.9
BDE 153	0.7	1.0	10	9.9	4.2	4.8	2.3
BDE 154	1.5	1.4	11	11	2.5	4.4	3.6
BDE 209	2.1	<**	<	<	14	14	0.0
HBCD	5.3	8.6	16	13	5.8	5.6	**4.9**
HCB	1.4	4.2	9.3	8.3	–	–	–
γ-HCH	2.2	12	8.0	**10**	–	–	–
β-HEPO	1.3	13	24	20	–	–	–
transchlordane	1.1	9.6	9.9	2.4	–	–	–
p,p′-DDE	1.0	13	9.6	**11**	–	–	–
p,p′-DDD	2.1	7.5	9.9	6.5	–	–	–

*Bold: u_{hom} based on u^*_{hom}.
**Concentration <LOQ.

Table 4. Homogeneity analysis of PCDD/Fs and PCBs in five candidate reference materials, with CVs and u_{hom} presented as %

	Herring tissue DIFF-01			Pork tissue DIFF-02			Milk DIFF-03				Fish oil DIFF-04			Compound feed DIFF-05			
	CV_{within}	$CV_{between}$	u_{hom}	CV_{within}	$CV_{between}$	u_{hom}	$CV_{m\ (cleanup)}$	CV_{within}	$CV_{between}$	u_{hom}	CV_{within}	$CV_{between}$	u_{hom}	$CV_{m\ (cleanup)}$	CV_{within}	$CV_{between}$	u_{hom}
PCDD/F																	
2,3,7,8-TCDF	4.2	10	9.2	10	16	(12)[*]	5.4	17	12	**14**[**]	0.9	7.3	7.2	4.4	2.6	5.1	4.4
1,2,3,7,8-PCDF	3.7	10	9.7	9.8	19	(16)	8.4	13	17	11	5.6	4.4	**4.7**	9.3	12	7.8	**9.8**
2,3,4,7,8-PCDF	5.2	7.4	5.2	2.5	13	13	8.0	10	13	8.4	2.3	2.9	1.8	6.2	6.5	6.8	1.9
1,2,3,4,7,8-HxCDF	5.7	6.9	3.9	3.7	16	15	5.8	8.0	11	7.1	5.9	12	10	9.7	5.1	10	8.7
1,2,3,6,7,8-HxCDF	12	6.6	**10**	10	13	7.5	8.4	3.4	14	14	10	13	8.4	11	7.9	5.4	**6.6**
2,3,4,6,7,8-HxCDF	6.3	8.8	6.3	8.5	17	15	10.2	6.2	11	8.9	7.3	8.9	5.2	2.9	7.6	6.1	**6.4**
1,2,3,7,8,9-HxCDF	<[***]	<	<	29	27	**(25)**	2.4	7.9	11	7.8	<	<	<	8.6	3.3	10	9.4
1,2,3,4,6,7,8-HpCDF	7.6	14.9	13	9.2	14	11	7.8	6.1	12	10	20	29	(21)	8.6	6.8	6.9	0.8
1,2,3,4,7,8,9-HpCDF	<	<	<	9.3	23	(21)	4.6	5.4	14	12	<	<	<	1.1	3.8	12	11
OCDF	<	<	<	26	52	(45)	2.2	8.8	15	12	<	<	<	8.1	5.9	6.9	3.5
2,3,7,8-TCDD	9.6	10.5	4.3	10	11	4.7	10.3	17	18	13	8.4	6.7	**7.0**	12	16	10	**13**
1,2,3,7,8-PCDD	3.4	7.0	6.1	6.2	16	14	8.8	10	14	9.5	6.1	6.0	**5.1**	11	6.7	10	8.0
1,2,3,4,7,8-HxCDD	12	12	**10**	5.8	9.0	7.0	11	13	14	6.0	17	19	8.6	11	9.8	13	9.2
1,2,3,6,7,8-HxCDD	3.2	8.6	8.0	7.2	15	13	15.6	9.5	14	11	6.9	7.5	3.1	8.3	13	13	1.1
1,2,3,7,8,9-HxCDD	8.4	12	8.5	18	17	**(15)**	7.1	7.2	18	17	24	29	(15)	13	6.3	8.0	4.9
1,2,3,4,6,7,8-HpCDD	3.1	13	13	9.0	22	21	4.0	4.4	10	9.5	11	21	(18)	7.0	5.6	7.0	4.2
OCDD	6.9	14	12	20	28	20	2.5	6.3	11	9.4	19	45	(41)	4.0	4.3	5.5	3.4
Non-ortho PCB																	
CB 81	5.0	9.5	8.1	2.0	7.4	7.1	2.9	6.4	10	7.9	5.0	5.3	1.7	1.6	4.3	3.2	**3.6**
CB 77	3.5	6.4	5.4	2.2	8.8	8.5	1.1	17	14	**14**	2.5	3.1	1.8	0.8	6.8	4.9	**5.7**
CB 126	4.1	5.0	2.8	10	11	5.1	1.2	4.5	8.4	7.1	7.2	4.9	**6.1**	1.1	1.7	1.8	0.6
CB 169	1.8	4.6	4.3	4.8	9.1	7.8	2.1	6.4	9.0	6.4	4.2	2.4	**3.5**	4.6	3.5	2.5	**2.9**
Mono-ortho PCB																	
CB 105	5.4	6.3	3.2	16	13	**13**	5.5	10	5.1	**8.4**	2.1	3.7	3.1	4.5	4.7	3.6	**3.9**
CB 114	7.5	7.0	**6.3**	17	14	**14**	<	<	<	<	6.0	9.2	7.1	<	<	<	<
CB 118	6	5.3	**5.0**	17	12	**14**	1.4	1.8	3.3	2.7	2.1	2.0	**1.7**	3.2	1.7	2.2	1.4
CB 123	6.9	10	7.7	15	14	**13**	<	<	<	<	6.9	7.5	3.1	<	<	<	<
CB 156	5.2	5.2	**4.4**	3.6	10	8.4	4.2	3.4	6.2	5.2	2.0	2.0	**1.7**	9.2	6.5	5.6	**5.5**
CB 157	6.7	5.5	**5.6**	5.2	10	9.6	<	<	<	<	1.6	2.2	1.6	<	<	<	<
CB 167	2.1	4.1	3.6	5.2	9.9	8.8	8.6	7.1	7.3	1.6	1.8	6.2	5.9	6.8	5.6	4.0	**4.7**
CB 189	6.7	6.2	**5.6**	7.4	9.7	6.4	<	<	<	<	1.7	2.8	2.2	<	<	<	<
Indicator PCB																	
CB 52	–	–	–	–	–	–	<	<	<	<	–	–	–	3.7	2.3	4.8	4.2
CB 101	–	–	–	–	–	–	<	<	<	<	–	–	–	3.1	1.9	2.3	1.2
CB 138	–	–	–	–	–	–	4.5	3.0	6.7	6.0	–	–	–	5.8	3.4	3.9	1.7
CB 153	–	–	–	–	–	–	2.4	5.3	8.8	7.1	–	–	–	3.0	1.6	3.2	2.7
CB 180	–	–	–	–	–	–	7.1	7.3	9.5	6.0	–	–	–	4.8	3.7	8.4	7.6
Extracted fat	–	–	–	–	–	–	–	13	10	**11**	–	–	–	–	2.3	2.5	1.0

[*]Between brackets: concentration close to LOQ.
[**]**Bold:** u_{hom} based on u^*_{hom}.
[***]Concentration <LOQ.

material also contained PCDD/Fs at low concentrations, which hampered the homogeneity evaluation through the PCDD/Fs.

4. Stability of target compounds in the materials

4.1. CRMs

The stability of the target PCBs in BCR-682, 718 and 719 was tested in tins stored at −25°C (reference temperature) and elevated temperatures (+20°C and +37°C) for a period of maximum 24 or 26 months (see Table 5). In addition, tins were stored at +50°C for 3 months (BCR-718 and 719) or 6 months (BCR-682), to provide information about the short-term stability at more extreme conditions. After each storage period, all target PCBs were analyzed in five replicate tins (per temperature). Stability monitoring was performed by a state-of-the art analytical method [14] to ensure that a possible instability of the target compounds could be detected. As the analysis were spread over a long time (partial results after 3, 12 and 24 months), good within-lab reproducibility was a major prerequisite. Relative CB concentration ratios (R_T) were calculated for each storage period by dividing the mean of the replicates at T = +20°C, +37°C or +50°C (X_T) by the mean of the replicates of samples stored at the reference temperature T = − 25°C ($X_{-25°C}$): $R_T = X_T/X_{-25°C}$. A stable compound should in principle yield R_T values of 1 at every time-temperature combination. Stability can thus be monitored by assessing the change of R_T values at the different testing times. A possible consistent degradation can be determined by regression analysis of the R_T values. In the case of no degradation, regression analyses shows no slope <1. However, a significant slope (at 95% or 99% confidence level (CL)) indicates a change of the target compound concentration over time. The results of the regression analysis are shown in Table 5.

For nearly all PCBs in BCR-682, 718 and 719, regression analysis did not show a consistent decrease or increase of concentration over time. For BCR-682, CBs 138 and 153 show increasing concentrations. An analytical artifact is the likely cause for the increasing concentration over time, rather than instability of these compounds. In addition, the stability results of BCR-718 do not confirm increasing concentrations of CBs 138 and 153 over time.

Concerning BCR-718, CB 101 shows a decreasing $R_T = X_T/X_{-25°C}$ ratio of 1.00 (0 months) to 2.32/2.44 μg/g ww = 0.95 (24 months). Although this is only a slight decrease, the trend is consistent and significant at 95% CL. However, it is not believed to be caused by degradation but rather to be an analytical issue, as the concentrations are very close (2.32 μg/g ww at 37°C and 2.44 μg/g ww at reference temperature −25°C). Suggestions of instability, which in fact are due to analytical issues, have been recognized by Lamberty et al. [15], who designed the isochronous approach for stability studies to overcome this type of chemo-analytical problems.

For CB 126 in BCR-719, a possible degradation cannot be ruled out, but, for CB 169, the cause of decrease is an inaccuracy in the analytical method, as the concentration is very close to the method LOQ, so the stability of CB 169 could not be confirmed.

4.2. Candidate CRMs

For the BFR and OCP stability study, an isochronous approach was applied [15]; the samples of all temperature/time combinations were analyzed at the end of the study, except those for the short-term stability at elevated temperature (3 months at +45°C).

The slopes of the linear regression for all compounds at each temperature in the flounder did not differ significantly throughout the study period (12 months), suggesting that instability of such materials is unlikely. For some BDEs (e.g., 66, 153 and 154) the concentrations were close to the LOQ. The higher analytical variance at these low concentrations hampered the determination of the stability. Although BDEs 66, 153 and 154 can be assumed to have the same behavior as the other BDEs,

Table 5. Stability evaluation by regression analysis R_T/R_{Tref} over the test period at 95% and 99% confidence levels (CL), with upward trend (+) indicating a possible increase of R_T/R_{Tref} over the test period, and (−) indicating a possible decrease

Material	No trend (95% or 99% CL)	Significant trend (95% or 99% CL)
BCR-682 (tested at 20°C and 37°C at 0, 6, 12 and 26 months)	CBs 28, 52, 101, 105, 118, 128, 138, 149, 153, 156, 170, 180	CB 138 (20°C, +) at 95 and 99%, CB 153 (37°C, +) at 95%
BCR-718 (tested at 20°C and 37°C at 0, 3, 12 and 24 months)	CBs 28, 52, 101, 105, 118, 128, 138 (+163), 149, 153, 156, 170, 180	CB 101 (37°C, −) at 95%
BCR-719 (tested at 20°C and 37°C at 0, 3, 12 and 24 months)	CBs 77, 81, 126, 169	CB 126 (37°C, −) at 95%, CB 169 (37°C, −) at 95%
Flounder (tested at 5°C, 20°C and 45°C at 0, 3 and 12 months)	α - HCH, γ - HC H, β-HEPO, dieldrin, p,p′-DDE, p,p′-DDD, HCB, cis-chlordane, trans-chlordane, oxy-chlordane, trans-nonachlor, BDEs 28, 47, 66, 99, 100, 119, 153, 154, HBCD	–
Sediment (tested at 5°C, 20°C and 45°C at 0, 3 and 12 months)	BDEs 28, 47, 66, 85, 99, 100, 153, 154, 183, 209, HBCD	BDE 100 (45°C) at 95%

stability of these compounds in this material could not be confirmed. Also the determination of hexabromocyclododecane (HBCD) using GC-MS proved to be difficult due to instability of this compound at high temperatures (>180°C) [16]; for a future certification, it is recommended to use LC-MS to determine the stability of the HBCD isomers [17].

For the OCPs, no significant degradation was observed throughout the study period (12 months), and a separate three-month 45°C study did not show significant degradation. The results of this stability study showed that the majority of the BFRs and OCPs were stable in the matrices investigated. However, it should be mentioned that the concentrations for some OCPs in this material were close to or below the LOQ, which made determination of (in)stability difficult (e.g., dieldrin), if not impossible (e.g., QCB).

Concerning materials DIFF-01 to DIFF-05, long-term stability tests were performed at −20°C (reference), 5°C and +20°C after 0, 6, 12 and 18 months. A short-term test was performed at 45°C (0 and 3 months). Stability has not been evaluated by regression analysis on the resulting data set.

However, stability can also be assessed by judging individual R_T values at different temperature-time combinations. Arbitrarily, R_T limits for stable compounds can be set at 0.9–1.1. For the herring material (DIFF-01), the R_T values are between those limits at nearly all t, T combinations. Unexpectedly, a considerable increase of R_T after 18 months at +20°C was obtained for the majority of PCDD/Fs and dl-PCBs. This phenomenon will need further attention in a future certification of a fish-tissue material; some compounds do not follow the trend, so an analytical error cannot completely explain the problem. On the other hand, it is obvious that the target compounds are not formed at a temperature of +20°C.

Similar to what was observed in the homogeneity study, the low concentrations of PCDD/Fs can hamper the accurate determination of stability (e.g., for 1,2,3,7,8,9-HxCDF). For the PCDD/Fs, more R_T values are found outside the 0.9–1.1 range as compared to the dl-PCBs.

Concerning the pork material (DIFF-02), more compounds were outside the R_T range 0.9–1.1 than can be explained only by the compound concentrations close to the LOQ. This may relate to a possible inhomogeneity, which was discussed earlier (see Table 4) so the stability of the majority of the PCDD/Fs cannot be confirmed based on the R_T criteria (0.9–1.1). However, it is not expected that these persistent compounds degrade or are generated under these stability conditions.

Nearly all dl-PCBs met the R_T criteria, showing that these compounds are stable in this matrix.

Due to an inhomogeneity problem for the milk sample (DIFF-03), as discussed earlier, the results are expressed on a fat-weight basis. The compounds then show excellent stability. The compounds in the fish oil (DIFF-04) showed excellent stability (R_T values within 0.9–1.1), except for some compounds with concentrations close to the method LOQ (see Table 4: 1,2,3,6,7,8-HxCDD; 1,2,3,7,8,9-HxCDD; OCDD; 1,2,3,7,8,9-HxCDF; 1,2,3,4,6,7,8-HpCDF; 1,2,3,4,7,8,9-HpCDF; and, OCDF). For these compounds, stability cannot be confirmed. The compound feed (DIFF-05) suffered from moisture losses due to the relatively high surface area of the feed pellets. The moisture losses added variability to the stability results. However, the compounds in the matrix showed excellent stability when results were expressed on a fat weight basis. In a real certification, this can be solved only by grinding and homogenizing (but not pelleting the feed mixture) in combination with a low headspace volume and an air-tight container.

For a future certification, concentrations close to the LOQ may pose a challenge on laboratories:

- on the one hand, the target-compound concentrations in the CRM should match the low concentrations, as found in routine samples; but,
- on the other hand, the current state-of-the-art analytical methods may not be sufficiently sensitive and precise at these very low concentrations to enable accurate stability determination.

A solution to this issue is to lower the method LOQs (e.g., by increasing the sample intake).

5. Technical and statistical evaluation

5.1. CRMs

Various laboratories with long-standing experience had recently demonstrated their ability to carry out accurate PCB determinations and were invited to participate in the certification study. Each laboratory was requested to perform six independent, replicate determinations of the target PCBs in the samples using its own method. To approach reproducibility conditions, the measurements had to be spread over at least two series, performed on at least two different days and using a new calibration curve for each series. A PCB standard solution, containing target compounds as well as possibly interfering PCBs with concentrations undisclosed to the participants, was made available to test the calibration performance. The final extracts were injected on at least two different apolar capillary GC columns using either electron capture (EC) or mass selective (MS) detection. Each participant verified its method by carrying out recovery experiments, procedure blanks and detector linearity tests. The entire analytical work was carried out according to a protocol discussed with the participants prior to the exercise. All relevant data, such as method description, method-performance characteristics, and final measurement results, had to be submitted by the participants using a standardized report. This detailed

information for BCR-682, 718 and 719 can be found elsewhere [6–8].

The collated method information and test results were discussed in detail in multiple-day technical meetings. Only technically sound data were accepted; reasons for rejecting data of individual PCBs from a participating laboratory were given (e.g., interfering peaks in the chromatogram, a high blank contribution, or absence of method-recovery information). The remaining data were statistically tested, using Soft-CRM software [http://www.eie.gr/iopc/softcrm/] specifically developed for statistical treatment of certification-study data. The statistical evaluation included:

- compatibility of data sets two by two (Scheffe's multiple t-test);
- outlying data sets (Dixon and Nalimov tests);
- outlying variances (Cochran test);
- calculation of the mean of means of the data sets;
- calculation of within-data-set standard deviation;
- calculation of between-data-set standard deviation;
- homogeneity of variances (Bartlett test);
- calculation of the data-set means;
- normality of the distribution of the data set of means (Kolmogorov-Smirnov-Lilliefors test);
- calculation of the half-width confidence interval.

Finally, the report of the study was discussed with external experts in a certification committee meeting, resulting in established certified values and uncertainties for BCR-682, 718 and 719.

Fig. 1 shows the results obtained for CB 81 in BCR-719. The overall performance exceeded the expectations, taking into account the low concentration of CB 81 in this lean fish material. The high quality of the data resulted in the first ever certification of CB 81 in fish. Furthermore, this CRM is unique for its certified value of CB 169 at the very low concentration of 1.8 ng/kg ww.

Table 6 shows the certified values and corresponding uncertainties of the CRMs BCR-682, 718 and 719. These CRMs are stored at, and distributed through, IRMM. On

Table 6. Certified concentrations and corresponding uncertainties (in brackets) for CRMs BCR-682, 718 and 719

	BCR-682		BCR-718	
	Mussel tissue (µg/kg ww)	**n***	**Herring muscle tissue (µg/kg ww)**	**n**
CB 28	0.30** (0.07)***	6/35	0.41 (0.04)****	6/36
CB 52	0.78 (0.09)	8/47	1.00 (0.04)	8/48
CB 101	–	–	2.12 (0.06)	8/48
CB 105	–	–	0.63 (0.06)	6/36
CB 118	2.6 (0.3)	6/36	1.78 (0.07)	9/54
CB 128	–	–	0.62 (0.11)	6/36
CB 138	4.6 (0.8)	6/36	2.97 (0.11)	6/36
CB 149	5.7 (0.9)	6/36	2.58 (0.11)	8/48
CB 153	9.2 (0.8)	10/60	4.62 (0.11)	11/66
CB 156	–	–	0.19 (0.09)	6/36
CB 170	0.17 (0.05)	6/36	0.35 (0.03)	7/42
CB 180	0.77 (0.07)	10/60	0.80 (0.03)	7/42
	BCR-719			
	Chub muscle tissue (ng/kg ww)	**n**		
CB 77	196 (7)****	10/59	–	–
CB 81	13.6 (0.5)	10/59	–	–
CB 126	20 (0.8)	10/59	–	–
CB 169	1.8 (0.23)	9/54	–	–

*Number of data sets/individual data.
**Unweighted mean of the means of n accepted data sets, each set being obtained in a different laboratory and/or with a different method of determination.
***Uncertainty calculated as half-width of the 95% confidence interval of the mean of means.
****Uncertainty calculated as combined uncertainty of the characterization by the interlaboratory study, the homogeneity and the stability of the sample, with k = 2.

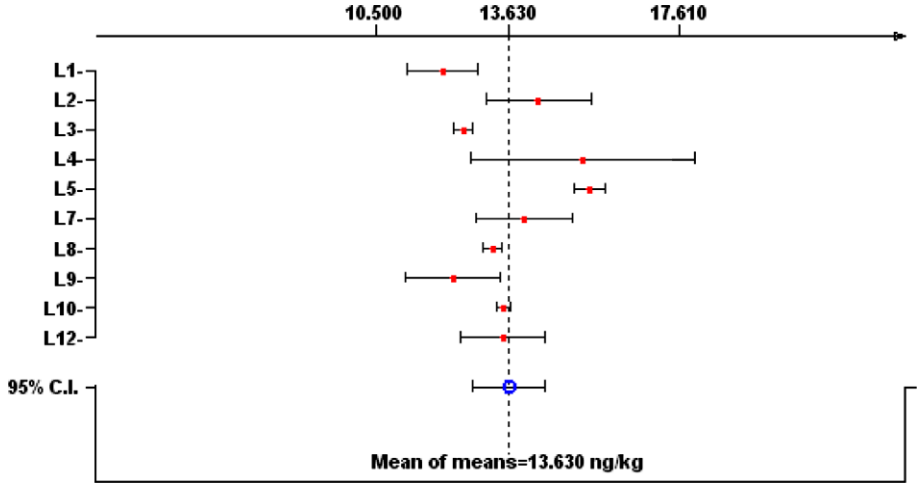

Figure 1. Distribution of laboratory means (and confidence intervals) of CB 81 in BCR-719. X-axis: Concentration of determinand. Y-axis: Laboratory code. The lowest data point in each graph represents the mean of all laboratory means with the 95% confidence interval.

a wet (total) weight basis, BCR-682 has a PCB content comparable to the NIST (National Institute for Standards and Technology, USA) standard reference material (SRM) 1974b (frozen mussel-tissue homogenate) [18]. The uncertainties of BCR-682 are slightly higher than those of SRM 1974b.

NIST also certified freeze-dried mussel materials (NIST SRMs 2977 and 2978). PCB concentrations in Cambridge Isotope Laboratories (CIL) materials EDF-2524 (herring) and EDF-2525 (lake trout) are one to two orders of magnitude higher, and the uncertainties (half-width of the 95% confidence interval) considerably larger, compared to those in BCR-682 and BCR-718 [19,1]. An advantage of the NIST materials over the BCR or CIL materials is that they have been certified for a broader range of contaminants (e.g., PCBs, OCPs and PAHs) rather than for a limited number of PCBs only. However, BCR-682 and BCR-718 offer the advantage of a certified concentration for CB 138 without interference of CBs 163 and 164.

As regards the non-ortho CBs, EDF-2524, EDF-2525 and EDF-2526 (salmon) have consensus values for the CBs 77, 126 and 169. The congener profiles of these materials differ from those of BCR-719, and they show wide 95% confidence intervals, for which the cause is unknown [19].

5.2. Candidate CRMs

The experimental set-up and technical and statistical evaluation of the data presented below was carried out with a CRM procedure similar to that described above, including a thorough statistical assessment of the data and a multiple-day technical discussion meeting. However, no certification committee meeting and no certification took place.

As is apparent in Table 7, the most abundant BFRs in the flounder material (BDE 47, 99, 100) were analyzed successfully by many laboratories; problems were observed for less common and/or low-concentration compounds, such as BDEs 49, 66 and 183. However, BDE 153 could be successfully analyzed by seven laboratories, although the concentrations were lower than 0.1 μg/kg ww. BDE 154 showed no overlap due to one outlying data set.

Concerning the sediment material, surprisingly only for three out of 12 compounds was there overlap between the individual data sets, although the concentrations were sufficiently high for accurate analysis. In some of these cases, the reason for no overlap was the low variance in the data sets of the individual laboratories, which in fact meant that their data were very precise (individual data not shown). Although for BDEs 47, 99, 100 and 153 the data sets showed no overlap, they had half-width 95% confidence intervals (of the concentration) similar to the flounder material (ca. 10%), which was good.

The data set for BDE 209 seemed to be bimodal (data not shown) although there was overlap caused by a wide variance of one laboratory.

For both sediment and flounder, results for HBCD appeared to be method dependent, as discussed earlier [16,17].

For BDE 49 at the time of the study, only some laboratories had standards available, and that was reflected

Table 7. Statistical evaluation of the candidate CRMs for BFRs and OCPs in flounder and BFRs in sediment

	Flounder (BROC-01)							Sediment (BROC-02)		
Compound	**No labs**	**Mean of means (μg/kg ww)**	**Overlap data set***	**Compound**	**No labs**	**Mean of means (μg/kg ww)**	**Overlap data set**	**No labs**	**Mean of means (μg/kg ww)**	**Overlap data set**
p,p′-DDT	3	0.03 (0.02)**	Y	BDE 28	7	0.09 (0.01)	Y	6	0.63 (0.13)	Y
p,p′-DDE	9	1.86 (0.21)	Y	BDE 47	10	3.33 (0.29)	Y	7	10 (1.4)	N
p,p′-DDD	10	0.72 (0.10)	Y	BDE 49	3	0.28 (0.10)	N	4	2.75 (0.77)	N
o,p′-DDT	3	0.08 (0.17)	N	BDE 66	6	0.06 (0.01)	N	5	0.29 (0.04)	N
Dieldrin	6	0.77 (0.25)	N	BDE 85	–	–	–	7	0.66 (0.12)	N
Endrin	2	0.03 (0.01)	Y	BDE 99	8	0.27 (0.03)	Y	7	14 (1.4)	N
α-HCH	3	0.05 (0.17)	N	BDE 100	8	0.60 (0.06)	Y	9	3.04 (0.29)	N
β-HCH	4	0.09 (0.13)	N	BDE 153	7	0.09 (0.01)	Y	8	1.93 (0.16)	N
γ-HCH	7	0.16 (0.04)	N	BDE 154	7	0.14 (0.01)	N	8	1.71 (0.16)	Y
β-HEPO	7	0.21 (0.03)	N	BDE 183	2	0.02 (0.12)	N	7	0.45 (0.14)	N
HCB	9	0.22 (0.03)	Y	BDE 209	–	–	–	6	1164 (120)	Y
QCB	3	0.04 (0.02)	Y	HBCD	4	0.9 (0.40)	N	5	96 (56)	N
Trans-nonachlor	6	0.09 (0.03)	N	–	–	–	–	–	–	–
Cis-chlordane	5	0.06 (0.01)	N	–	–	–	–	–	–	–
Trans-chlordane	5	0.05 (0.01)	Y	–	–	–	–	–	–	–
Oxychlordane	6	0.03 (0.004)	Y	–	–	–	–	–	–	–

*Overlap of confidence intervals for individual data sets.
**Uncertainty calculated as half-width of the 95% confidence interval of the mean of means.

in the low number of laboratories that have analyzed this compound. In a future certification, this will become less problematic, as an increasing number of high quality standards have become commercially available over the last few years.

For OCPs in flounder, overlap of data sets was found for half the compounds. Some compounds (e.g., dieldrin and *p,p′*-DDT) were difficult to analyze as they were easily degraded due to treatment of the extract with concentrated sulphuric acid or dirty liners in the GC injector. This negatively affected the quality of the data and the coherence of the individual data sets. Overlap of data sets was found for half the compounds included in this study.

For γ-HCH and trans-chlordane, there was no overlap, due to one outlying data set that could not be removed from the complete data set, as there was no technical explanation for the deviation.

Only a few laboratories could determine the very low concentrations (e.g. of α-HCH and QCB). For production and certification of a CRM for OCPs, the material selected should preferably have higher concentrations of some OCPs.

To avoid increasing the size of this article considerably, we discuss the results for candidate CRMs for PCDD/Fs, dl- and ICES-7 PCBs more generally. Based on the consensus values obtained in the evaluation, toxic equivalents (TEQs) can be calculated.

The results in Table 8 show that all materials were below or in the concentration range defined in EU legislation on PCDD/Fs in food and feed [20,21]. A half-width of the 95% confidence interval of less than 10% of the concentration was obtained for 65% of the PCDD/Fs, dl-PCBs and ICES-7 PCBs investigated in five candidate CRMs for food or feed. For 80% of the parameters, this relative uncertainty was less than 15% of the concentration. Somewhat smaller relative uncertainties were obtained for milk and compound feed than for the other matrices. Pork-muscle tissue showed the worst comparability and was the only material without a consensus value for one of the important congeners with regard to TEQ contribution (CB 126 had an outlying laboratory

Table 8. Concentrations of PCDD/Fs and dl-PCBs (TEQ ng/g ww), obtained from consensus values. Lower bound values calculated without congeners with consensus values <LOQ

Calculated concentration (ng/g ww)	Herring tissue DIFF-01	Pork tissue DIFF-02	Milk DIFF-03	Fish oil DIFF-04	Compound feed DIFF-05
PCDD/F-TEQ lower bound	1.89	0.31	0.09	5.5	0.46
PCDD/F-TEQ upper bound	1.89	0.31	0.09	5.6	0.46
Total-TEQ lower bound	3.76	0.45	0.22	11.0	1.23
Total-TEQ upper bound	3.76	0.45	0.22	11.2	1.23

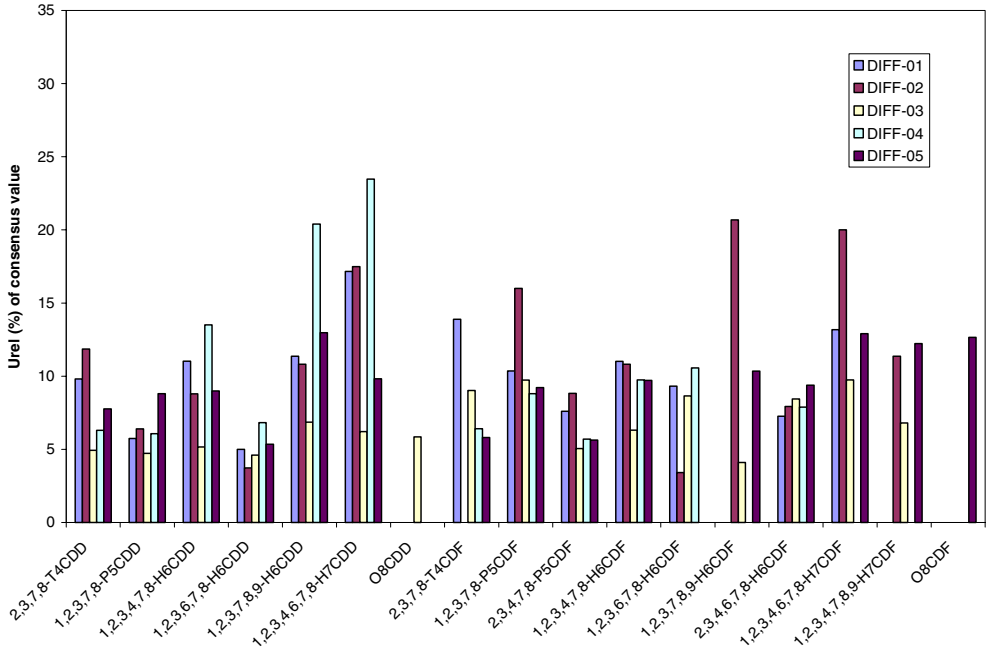

Figure 2. Relative uncertainties of the consensus values for PCDD/Fs. DIFF-01, Herring-muscle tissue; DIFF-02, Pork-muscle tissue; DIFF-03, Whole milk; DIFF-04, Herring oil; and, DIFF-05, Compound feed.

mean at $p = 0.01$ that could not be explained technically). 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):

- OCDD and OCDF;
- 1,2,3,4,6,7,8-HpCDD, 1,2,3,7,8,9-HxCDF, CB 114 and CB 123; and,
- 1,2,3,4,6,7,8-HpCDF, 1,2,3,4,7,8,9-HpCDF and CB 28.

Consequently, these congeners may be regarded as the most demanding for future certification, which is not unexpected because of the very low concentrations of some of them (especially when compared to others from the same compound group). As an example, a bar graph with the relative uncertainties for the PCDD/Fs is shown in Fig. 2.

In addition to stringent precautions with regard to blanks, extraction efficiency, recoveries and GC separation, the independence of replicate analyses within each participating laboratory is a critical factor to make a certification succeed. A compromise to reduce the workload and the cost of the study (e.g., analysis of two series of three replicates rather than six fully independent replicates) may result in data sets with very small relative standard deviations within-laboratory, probably not reflecting the typical random and systematic errors of the analytical process. This may cause poor overlap between data sets and increase the risk of outlying laboratory variances or means.

6. Conclusions

Three new matrix-type CRMs have been prepared and successfully certified for a range of PCBs (including non-ortho PCBs) in mussel, and herring-muscle and chub-muscle tissue. The certified concentrations, which reflect today's environmental concentrations, were established by replicate analysis and thorough technical and statistical evaluation of the results. The uncertainties for nearly all PCBs are <10% of the certified value (BCR-718 and 719) or just above 10% (BCR-682). Sterilization has resulted in stable CRMs that can be easily stored and transported. These CRMs are currently available through IRMM, and are very suitable for quality assurance in food and environmental laboratories that analyze fish and shellfish at (low) concentrations encountered in routine samples.

Feasibility studies show that CRMs for PCDD/Fs, dl-PCBs and ICES-7 PCBs, BFRs and OCPs in a variety of food and feed matrices as well as sediment can be prepared and certified successfully. Nevertheless, for future production and certification of these CRMs, some experimental factors have to be taken into account as they affect stability, homogeneity and certification results. Close to the LOQ of an analytical method, precision and accuracy decrease, and, in this study, this resulted in increased variability of results for target compounds with concentrations close to the method LOQ (e.g., 1,2,3,7,8,9-HxCDF). This hampered confirmation of the homogeneity and the stability of some of these compounds. Improving the method performance at these very low concentrations will certainly challenge analytical chemists in future CRM certification studies. New CRMs for BFRs, OCPs, PCDD/Fs and (dl-)PCBs with the concentrations found in routine samples are essential quality-assurance tools to support monitoring programmes and EU policies on (reduction) strategies for these POPs.

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