European Commission

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REFERENCE MATERIALS

THE CERTIFICATION OF THE CONTENTS (MASS FRACTIONS) OF FIVE POLYCHLORODIBENZO-P-DIOXINS (D48, D54, D66, D67, D70) AND SEVEN POLYCHLORODIBENZO FURANS (F83, F94, F114, F118, F121, F124, F130) IN FLY ASH

CRM 490

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CONTENTS

GLOSSARY OF ABBREVIATIONS.

CI	: Confidence interval.
CV	: Coefficient of variation.
^{13}C	: Carbon 13 labelled isomers
D48	: 2,3,7,8 T4CDD
D54	: 1,2,3,7,8 P5CDD
D65	: 1,2,3,4,6,9 H6CDD
D66	: 1,2,3,4,7,8 H6CDD
D67	: 1,2,3,6,7,8 H6CDD
D70	: 1,2,3,7,8,9 H6CDD
F83	: 2,3,7,8 T4CDF
F89	: 1,2,3,4,8 P5CDF
F94	: 1,2,3,7,8 P5CDF
F114	: 2,3,4,7,8 P5CDF
F118	: 1,2,3,4,7,8 H6CDF
F119	: 1,2,3,4,7,9 H6CDF
F121	: 1,2,3,6,7,8 H6CDF
F124	: 1,2,3,7,8,9 H6CDF
F127	: 1,2,4,6,8,9 H6CDF
F130	: 2,3,4,6,7,8 H6CDF
GC	: Gas chromatography.
HRMS	: High resolution MS
LRMS	: Low resolution MS
H6CDD	: Hexachlorodibenzo-p-dioxin
H6CDF	: Hexachlorodibenzo furan
I-TEF	: International toxic equivalent factor
I-TEC	: International toxic equivalent concentration
MS	: Mass spectrometry.
P5CDD	: Pentachlorodibenzo-p-dioxin
P5CDF	: Pentachlorodibenzo furan
PCDD	: polychlorodibenzo-p dioxin
PCDF	: polychlorodibenzo furan
SB	: Between-laboratory standard deviation.
S.D.	: Standard deviation
SW	: Within-laboratory standard deviation.
T4CDD	: Tetrachlorodibenzo-p dioxin
T4CDF	: Tetrachlorodibenzo furan.

1. INTRODUCTION

1.1. Background and justification of the project

The group of chemicals which are commonly designated as 'dioxins' consists of two series of tricyclic aromatic compounds with similar chemical and physical properties: polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). The PCDDs and PCDFs can be substituted with one to eight chlorine atoms onto the dibenzo-p-dioxin and dibenzofuran nucleus (figure 1-1 and 1-2); this leads to 75 and 135 possible isomers and congeners of PCDDs and PCDFs, respectively.

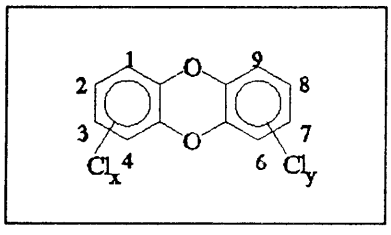

Figure 1-1:
structural formula of PCDDs

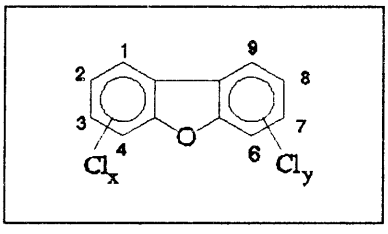

Figure 1-2:
structural formula of PCDFs

The compounds do not occur naturally, nor are they intentionally produced. Among their important physico-chemical properties are the high lipophilicity and the general resistance to chemical breakdown, which contribute to their bioaccumulation in the food chain and their environmental persistence.

The extreme toxicity of 2,3,7,8-T4CDD and related products in a variety of laboratory animal species has been extensively documented, since a number of accidental and occupational exposures drew the world's attention to the adverse effects of these unwanted by-products in many industrial processes. There is, however, still considerable controversy about the human toxicity of PCDDs and PCDFs and about their absolute degree of toxicity. The body of evidence clearly indicates that compounds showing 2,3,7,8-chlorine substitution, and particularly tetra-, penta- and hexa-chloro compounds, exhibit the highest toxicity. Emerged from several others, the international toxicity equivalency factor (I-TEF) scheme, tabulated in table 1-1, is now increasingly being accepted as a summary of the relative toxicities. The TEF for a specific congener is defined by the ratio of its toxicity relative to that of the most toxic congener 2,3,7,8-T4CDD. The current analytical approach, a congener-specific determination, closely responds to this knowledge about toxicity.

The main known primary sources of PCDDs and PCDFs into the environment are:

- chemical reactions in industrial processes which involve chlorine or chlorinated compounds (production and use of pesticides and technical products, chlorine bleaching of pulp,...);
- thermal reactions involving chlorine or chlorinated compounds (waste incineration, metallurgy,...).

Table 1-1: I-TEF values for PCDDs and PCDFs

PCDD CONGENER	I-TEF	PCDF CONGENER	I-TEF
2,3,7,8-T4CDD (D48)	1	2,3,7,8-T4CDF (F83)	0.1
1,2,3,7,8-P5CDD (D54)	0.5	1,2,3,7,8-P5CDF (F94)	0.05
1,2,3,4,7,8-H6CDD (D66)	0.1	2,3,4,7,8-P5CDF (F114)	0.5
1,2,3,6,7,8-H6CDD (D67)	0.1	1,2,3,4,7,8-H6CDF (F118)	0.1
1,2,3,7,8,9-H6CDD (D70)	0.1	1,2,3,6,7,8-H6CDF (F121)	0.1
1,2,3,4,6,7,8-H7CDD (D73)	0.01	1,2,3,7,8,9-H6CDF (F124)	0.1
OCDD (D75)	0.001	2,3,4,6,7,8-H6CDF (F130)	0.1
		1,2,3,4,6,7,8-H7CDF (F131)	0.01
		1,2,3,4,7,8,9-H7CDF (F134)	0.01
		OCDF (F135)	0.001

While the former gradually appears to become a historic source, due to the existing ban or strict regulation of production and use of the chemicals concerned, regulatory measures are now increasingly focusing on the formation of the compounds in combustion processes. The Commission of the European Union has set up Directive 94/67/CE, establishing a limit value for emissions of PCDDs and PCDFs from hazardous waste incineration plants of 0.1 ng m^{-3} (as I-TEC), to come into force in January 1997 provided that appropriate standards allow to verify the compliance.

Emission inventories from several industrialised countries indicate that municipal waste incineration is or has been a very important, if not the most important, source of environmental dioxin contamination, and thus a primary target for legislative and technical efforts aiming at the reduction of dioxin emission. Consequently, evaluation and monitoring of new as well as existing installations as to their dioxin release has become a major concern. For accurate quantitative measurement of the PCDDs and PCDFs a full validation of the highly complex analytical method, including its extraction efficiency, is essential. This requires the availability of reliably certified reference materials related to municipal waste incineration.

1.2. Choice of the material and the certification procedure

Following the preparation and certification of a crude fly ash extract for the twelve most toxic PCDDs and PCDFs in 1993 [1], which enables to check clean-up and instrumental procedures, the need for a certified fly ash was recognised. Such a certified fly ash would fulfil the objective of making available to analysts a reliable tool to validate their entire analytical procedure for congener-specific dioxin determination and/or verify its performance.

Fly ash has a PCDD and PCDF pattern that is representative for emission samples, and is an inexpensive and stable material. Furthermore the quantities of dioxins (in gram per year) emitted with residues of incineration processes are often several times larger than those emitted to air.

This report first describes the preparation of an appropriate and representative candidate CRM, starting from a regular fly ash which originates from an at the time relatively modern municipal waste incinerator. It then presents relevant details on the interlaboratory study set up to certify the dioxin content. The underlying principle on which the present certification was based is the agreement between a range of widely different methods, applied in laboratories working independently; many of the selected European expert laboratories already had been involved in an earlier step-by-step feasibility study supported by the Commission [2].

Taking into account the toxicity data actually available and the state-of-the-art in dioxin analysis, it was decided to aim at a certified value for the content of each of the twelve most toxic PCDDs and PCDFs (tetra-, penta- and hexachlorinated congeners with 2,3,7,8-chlorine substitution), and at an indicative value for each of the remaining 2,3,7,8-chlorine substituted congeners (hepta- and octachlorinated). Below the former will also be referred to as the target congeners, the latter as the supplementary congeners.

2. PARTICIPANTS

2.1. Preparation of the material

Vlaamse Instelling voor Technologisch Onderzoek (VITO)	Mol	BE
Institute for Reference Materials and Measurements (IRMM)	Geel	BE

2.2. Homogeneity and stability testing

Vlaamse Instelling voor Technologisch Onderzoek (VITO)	Mol	BE

2.3. Certification measurements

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Bayer - Zentralforschung-DZA	Leverkusen	DE
Centre d'Analyse et de Rech. sur les Substances Organiques (CARSO)	Vernaison	FR
Centro de Investigación y Desarrollo (CID-CSIC)		
- Laboratori de Espectrometria de Masses	Barcelona	ES
Elf Atochem - Centre d'Application de Levallois	Levallois-Perret	FR
ENEL - Centro Ricerca Termica	Pisa	IT
The Finnish Pulp and Paper Research Institute Oy Keskuslaboratorio		
- Centrallaboratorium (KCL)	Espoo	FI
Istituto di Ricerche Farmacologiche "Mario Negri"	Milano	IT
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- CSL Food Science Laboratory	Norwich	GB
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TNO Milieu en Energie - Instituut voor Milieuwetenschappen (IMW)	Delft	NL
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- Dept. of Occupational Hygiene and Toxicology	Helsinki	FI
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3. PREPARATION OF THE MATERIAL

End February 1991, about 40 kg (3 batches of 14 kg) of fly ash were collected from an at the time relatively modern municipal waste incinerator. Three 1 g samples, one taken randomly from the bulk material of each batch, were analyzed, using a verified laboratory procedure, in order to assure that the levels of the seventeen target or supplementary congeners were adequate, i.e. at least five times the limit of detection for modern standard equipment. The collected material fulfilled this requirement.

After transfer to the Institute for Reference Materials and Measurements in Geel (BE), the three batches were homogenised for 4 h using a Turbula mixer. The whole amount was next sieved to less than 1 mm; about 500 g of the material was removed at this stage. Fine grinding of the fly ash was carried out using an Alpine multi-processing system. This comprised a jet mill, at the bottom section of which nozzles are mounted through which jets of air can be blown; the air accelerates the feed material particles, which impact on each other and generate size reduction, without any contamination and virtually without residue. The fine particles were extracted through a classifier wheel on top of the chamber; following a test series, the jet-milling was carried out with a classifier wheel speed of 3300 r.p.m.. This resulted in a total amount of ground fly ash powder $< 125\ \mu m$ of about 36 kg, three subsamples of which were again checked analytically for their dioxin content by the Vlaamse Instelling voor Technologisch Onderzoek (BE). After two hours homogenisation in a Turbula mixer, the powder was finally divided in 8 times 8 batches (64) using a laboratory sample divider. It was then bottled in 30 g amounts into 60 ml well cleaned brown glass bottles with aluminium inserts and plastic screw caps. A total of about 1200 bottles have been prepared.

Production control at the Institute for Reference Materials and Measurements (BE) included eight representative particle size analyses. The maximum particle size was found $< 125\ \mu m$; over 99 % of the material volume had a particle size smaller than 90 μm. Furthermore ten representative moisture analyses were carried out with the Karl-Fisher method during the bottling procedure. An average moisture content of 2.4 % mass fraction of water was detected. Microscopic examination showed a grey powder spiked with black particles.

The candidate CRM is stored at the Institute for Reference Materials and Measurements (BE) in a air conditioned room at 18 °C.

4. HOMOGENEITY AND STABILITY TESTING

4.1. Homogeneity testing

Due to the nature and origin of the material, crude fly ash must be suspected to be rather heterogeneous. The grinding and homogenisation at the preparation stage of the candidate CRM, however, aims at eliminating any inhomogeneity or at least reducing it to a level negligible as compared with other parts of the uncertainty of the certified properties. Earlier experience with jet-milling for sample preparation has indicated that homogeneity down to the 100 mg level or even lower can easily be reached.

A homogeneity study was set up in June 1992 using twenty units in their final packaged form, randomly selected during the bottling process. In addition to the properties to be certified, i.e. the twelve target PCDDs and PCDFs, also the five supplementary congeners were quantitatively determined. From each of two bottles, five sub-samples were analyzed to obtain an estimate of the within-bottle homogeneity. One sub-sample from each of the remaining eighteen bottles was analyzed to estimate the between-bottle homogeneity.
To achieve the best repeatability, a sample intake of 1 g was considered appropriate. This compares favourably with the sample intakes typically encountered for congener-specific dioxin analysis in fly ash, which vary from 1-5 g to 5-15 g, depending on whether high or low resolution mass spectrometric detection is applied.

4.1.1. Analytical method

Prior to analysis, the bottles were rehomogenized by shaking manually during 30 s. For each analysis an amount of 1.0 g of the fly ash was weighed in an Erlenmeyer flask and 20 ml of a 4 % hydrochloric acid solution was added. The suspension was swirled for two hours on a magnetic stirrer. Subsequently it was filtered on a double filter paper and the precipitate was rinsed with ultrapure water until a filtrate of neutral Ph was obtained. The residue was air-dried at room temperature.
The residue, filter paper included, was then transferred into a precleaned extraction thimble and 25 μl of an internal standard solution was added; this spike contained, at a concentration of ca. 50 $\mu g\ l^{-1}$ in nonane, one ^{13}C-labelled isomer for each product class and each chlorination degree, i.e. a mixture of the ^{13}C-labelled isomers of 2,3,7,8-T4CDD, 1,2,3,7,8-P5CDD, 1,2,3,7,8,9-H6CDD, 1,2,3,4,6,7,8-H7CDD, OCDD, 2,3,7,8-T4CDF, 2,3,4,7,8-P5CDF, 1,2,3,7,8,9-H6CDF, 1,2,3,4,6,7,8-H7CDF and OCDF respectively. The sample was extracted in a Soxhlet apparatus with 80 ml of toluene during 42 h.

After concentration to about 2 ml under a dry nitrogen stream, the extract was loaded on a column (10 mm I.D.) packed with 2.5 g of basic alumina (Super I activity) and a top layer of 2 g of anhydrous Na_2SO_4. Elution was carried out consecutively with first 15 ml of benzene, next 20 ml of a 98/2 (vol/vol) n-hexane/dichloromethane mixture and finally 30 ml of a 50/50 (vol/vol) n-hexane/dichloromethane mixture. Fractions 1 and 2 were discarded, whereas fraction 3 was concentrated under a dry nitrogen stream and transferred to a closed vial with insert for the automatic injector. At the end of the clean-up 30 μl of a solution containing 33 $\mu g\ l^{-1}$ of ^{13}C-labelled 1,2,3,4-T4CDD was added as a recovery standard. The final volume was about 50 μl.

The quantitative determination was performed by gas chromatography with high resolution mass spectrometric detection (HRGC-HRMS). A summary of the gas chromatographic and mass spectrometric conditions is given in table 4-1.

Table 2. Results of the homogeneity studies, expressed as CV% [a]

Compound	Within-bottle 1 $n = 10$	Within-bottle 2 $n = 10$	Between bottle $n = 20$	Standard solution $n = 10$
Pyr	7.5	7.1	8.2	5.8
BaA	9.8	12.1	11.1	11.5
BaP	13.1	11.9	14.3	8.5
BeP	10.8	11.7	14.5	8.1
BbF	12.1	12.0	13.1	9.0
BkF	13.1	12.1	11.8	9.2
INP	10.5	9.8	11.8	7.5
BNT	n.d.	n.d.	10.1	n.d.
fluorene	(9.8)	(11.2)	(10.8)	(8.3)
phenanthrene	14.1	10.9	13.1	9.1
anthracene	12.8	13.8	14.0	8.2
fluoranthene	(16.8)	(15.8)	(16.0)	(12.0)
PCP	17.0 [b]	[b]	19.0	n.d.

[a] Values between brackets, indicative values (cf. section 4.1); n.d., not determined

[b] five replicates in each of two bottles.

Figure 4.1: Particle size distribution of CRM 524

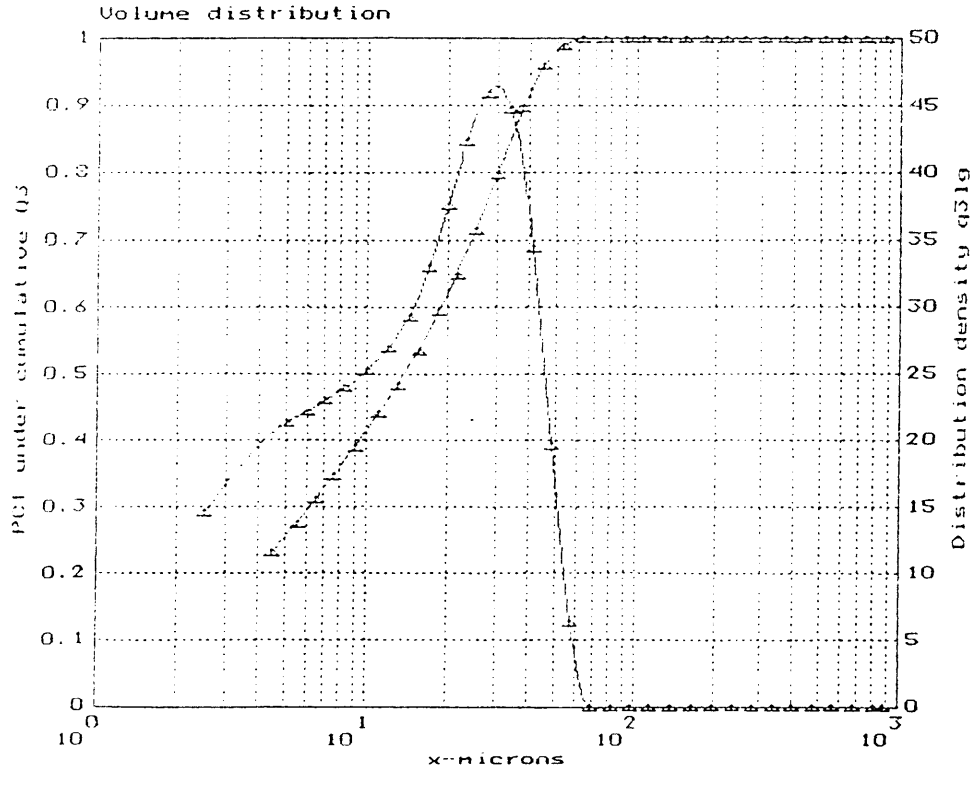


Table 4-1: Gas chromatographic and mass spectrometric conditions

GC COLUMN		
- type:	DB-Dioxin	CP-Sil 88 [a]
- dimensions:	60 m x 0.25 mm	50 m x 0.25 mm
- film thickness:	0.25 μm	0.25 μm
- program:	160 °C, 1 min; 20 K min^{-1} to 240 °C, 35 min; 15 K min^{-1} to 270 °C, 58 min	110 °C, 1 min; 30 K min^{-1} to 170 °C; 3 K min^{-1} to 230 °C, 22 min; 5 K min^{-1} to 240 °C, 43 min
INJECTION		
- type:	splitless (1 min), autosampler	splitless (1 min), autosampler
- volume:	1 μl	1 μl
- temperature:	270 °C	270 °C
- carrier gas:	He (200 kPa)	He (180 kPa)
MS DETECTION		
- type:	HRMS (SIM mode)	HRMS (SIM mode)
- interface temp.:	270 °C	240 °C
- source type:	EI+	EI+
- source temp.:	250 °C	250 °C
- electron energy:	34 eV	34 eV
- ion masses:		
T4CDF	303.9016, 305.8987 315.9419, 317.9389	303.9016, 305.8987 315.9419, 317.9389
T4CDD	319.8965, 321.8936 331.9368, 333.9339	319.8965, 321.8936 331.9368, 333.9339
P5CDF	339.8597, 341.8567 351.9000, 353.8970	339.8597, 341.8567 351.9000, 353.8970
P5CDD	355.8546, 357.8516 367.8949, 369.8919	355.8546, 357.8516 367.8949, 369.8919
H6CDF	373.8208, 375.8178 385.8610, 387.8580	373.8208, 375.8178 385.8610, 387.8580
H6CDD	389.8156, 391.8127 401.8559, 403.8529	389.8156, 391.8127 401.8559, 403.8529
H7CDF	407.7818, 409.7788 419.8220, 421.8191	407.7818, 409.7788 419.8220, 421.8191
H7CDD	423.7767, 425.7737 435.8169, 437.8140	423.7767, 425.7737 435.8169, 437.8140
OCDF	441.7428, 443.7399 453.7831, 455.7802	441.7428, 443.7399 453.7831, 455.7802
OCDD	457.7377, 459.7348 469.7780, 471.7750	457.7377, 459.7348 469.7780, 471.7750
lock masses	330.9792, 342.9792, 380.9760, 430.9728, 454.9728	330.9792, 380.9760, 404.9760, 430.9728,

[a] only 1,2,3,7,8-PCDD is quantitatively determined using this column

As an interference-free determination of 1,2,3,7,8-P5CDD appeared to be impossible on the DB-Dioxin gas chromatographic column used, a supplementary injection on a CP-Sil 88 column was performed for this purpose. Fine-tuning of the mass spectrometer was carried out daily using perfluorokerosene as reference gas; the resolution was tuned to a value of 10000 at 10 % valley. Identification relied on the characteristic masses, on isotope ratios within 15% of the theoretical values, and on the retention times. Calculation of the concentration was based on the relative response factors (RRF's) of each analyte vs. the corresponding internal standard (see 5.5). The required RRF's were obtained by daily injection of calibration solutions at regular interval with the samples; three solutions of different concentration (1-500 $\mu g\ l^{-1}$) were used. Instrument blanks were included in the measurement series to verify the absence of cross-contamination.

As additional quality control, recoveries of the internal standards added before extraction were calculated vs. the recovery standard; for the target congeners average recoveries ranged between 88% (T4CDD) and 60% (H6CDD). Furthermore a procedure blank was treated in exactly the same way as the samples; none of the seventeen PCDD/PCDF congeners could be detected, with detection limits varying between 1.0 pg (T4CDF) and 25 pg (OCDF) per sample. One of the fly ash samples was extracted a second time; for all congeners the amount found in the second extract was negligible.

4.1.2. Results and conclusion

The averages and coefficients of variation resulting from the homogeneity tests are listed in table 4-2.

A two-tailed F-test at the 95 % confidence level indicates that there is no significant difference between the variability observed in both within-bottle homogeneity tests. The CV values are in the same range as the values obtained under similar circumstances for the crude fly ash extract CRM 429, i.e., between 2 and 10 % for the target congeners.

Generally the between-bottle CV values appear slightly higher, ranging between 4 and 12 % for the target congeners and up to 14 % for one of the supplementary congeners. As to the individual compounds, the apparent increase in variability is most striking for 1,2,3,4,7,8,9-H7CDF, 1,2,3,4,6,7,8-H7CDD, 1,2,3,4,7,8-H6CDD, 1,2,3,7,8-P5CDF and OCDF. A detailed analysis of variance (one-way ANOVA) confirms that the effect is statistically significant at the 95 % confidence level for the five congeners mentioned. There is, however, no physical or chemical reason to suspect any differentiation between those and the other similar congeners during the preparation of the candidate CRM.

It was concluded that the homogeneity of fly ash candidate CRM 490 at a 1 g sample intake level is satisfactory for certification of PCDDs and PCDFs.

Table 4-2: coefficients of variation from the within-bottle and between-bottle homogeneity study of PCDDs and PCDFs in fly ash candidate CRM 490, and from measurements of fly ash extract CRM 429 under similar circumstances.

CONGENER	FLY ASH (CANDIDATE CRM 490)			FLY ASH EXTRACT (CRM 429)
	WITHIN-BOTTLE CV (%)		BETWEEN-BOTTLE CV (%)	BETWEEN-AMPOULE CV (%)
	n = 5	n = 5	n = 18	n = 5
2,3,7,8-T4CDD	6.4	9.4	6.3	5.6
1,2,3,7,8-P5CDD	6.6	4.0	4.9	3.0
1,2,3,4,7,8-H6CDD	5.3	4.1	10.3	8.2
1,2,3,6,7,8-H6CDD	5.8	8.1	10.5	4.6
1,2,3,7,8,9-H6CDD	3.8	5.4	6.5	4.7
1,2,3,4,6,7,8-H7CDD	5.9	3.3	9.2	n.a. [a]
OCDD	4.6	5.8	6.7	n.a.
2,3,7,8-T4CDF	7.3	7.1	6.4	6.1
1,2,3,7,8-P5CDF	3.1	7.7	11.4	8.6
2,3,4,7,8-P5CDF	3.8	1.7	4.6	7.4
1,2,3,4,7,8-H6CDF	7.8	10.2	11.5	4.6
1,2,3,6,7,8-H6CDF	8.8	7.3	11.0	5.3
1,2,3,7,8,9-H6CDF	9.7	10.1	11.1	10.4
2,3,4,6,7,8-H6CDF	9.2	5.0	9.9	4.3
1,2,3,4,6,7,8-H7CDF	4.4	1.6	4.3	n.a.
1,2,3,4,7,8,9-H7CDF	5.7	7.0	13.6	n.a.
OCDF	4.8	5.7	7.7	n.a.

[a] n.a. = not analyzed

4.2. Stability testing

In general, PCDDs and PCDFs show a high chemical and thermodynamic stability. Taking elementary precautions (e.g. protection from light, avoidance of extremely high temperatures), there are no reasons to suspect that the dioxin content of a fly ash would change with time.

A stability monitoring program for the candidate CRM was set up using thirty units in their final packaged form, randomly selected during the bottling process. Immediately upon receipt of the samples in June 1992, ten bottles were stored in the dark at each of the following temperatures -20, 20 and 40 °C. After six months a set of five bottles at each temperature was taken for analysis. The remaining set of five bottles at each temperature was analyzed after twelve months. Each time the content after storage at -20 °C was used as the reference level. In addition to the twelve target PCDDs and PCDFs, also the five supplementary congeners were quantitatively determined.

4.2.1. Analytical method

Basically the same analytical method as for the homogeneity study (4.1.1.) was applied, but meanwhile additional ^{13}C-labelled congeners had become available, and therefore the number of internal standards added prior to extraction was increased from ten to seventeen, i.e. each analyte as ^{13}C-labelled compound.

The recoveries of the internal standards added before extraction, calculated vs. the recovery standard, averaged at 65% or better for all congeners.

4.2.2. Results and conclusion

To verify the stability, both after 6 and 12 months the ratio R of the mean value (X) of the measurements after storage at 20 or 40 °C, respectively, was calculated versus the reference level, i.e. the mean value after storage at -20 °C:

$$R_{(6\ m,\ 20\ °C)} = X_{(6\ m,\ 20\ °C)}/X_{(6\ m,\ -20\ °C)};\qquad R_{(6\ m,\ 40\ °C)} = X_{(6\ m,\ 40\ °C)}/X_{(6\ m,\ -20\ °C)}$$
$$R_{(12\ m,\ 20\ °C)} = X_{(12\ m,\ 20\ °C)}/X_{(12\ m,\ -20\ °C)};\qquad R_{(12\ m,\ 40\ °C)} = X_{(12\ m,\ 40\ °C)}/X_{(12\ m,\ -20\ °C)}$$

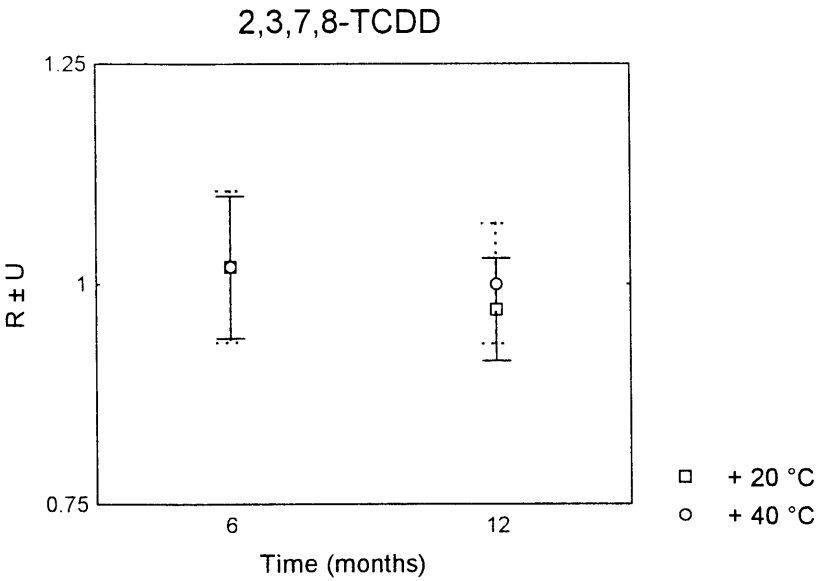
The uncertainty U was derived from the coefficient of variation (CV) of each set of measurements, according to the following example:

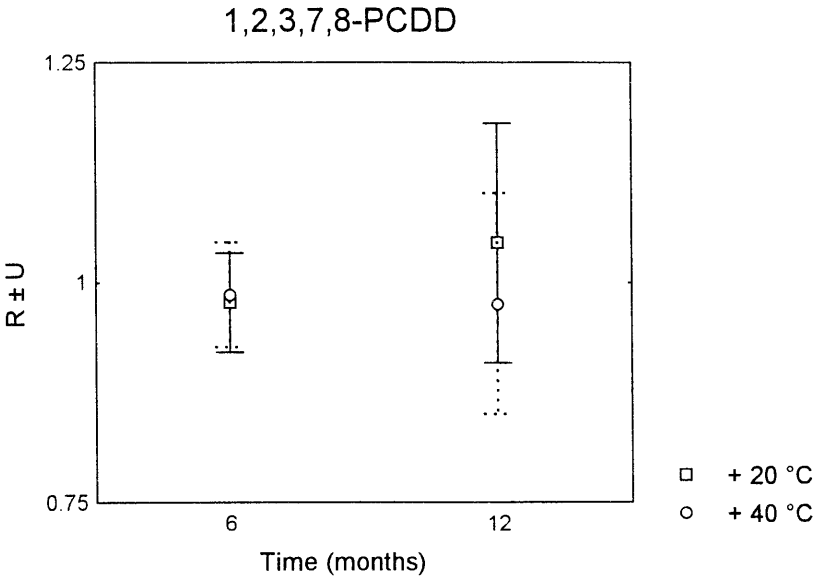
$$U_{(6\ m,\ 20\ °C)} = (CV^2_{(6\ m,\ 20\ °C)} + CV^2_{(6\ m,\ -20\ °C)})^{1/2} \cdot R_{(6\ m,\ 20\ °C)}$$

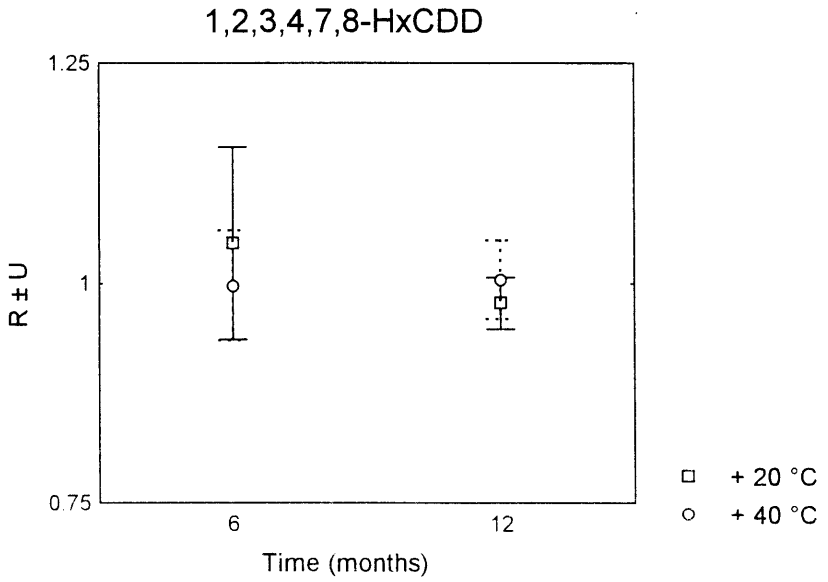
For each of the 17 PCDDs and PCDFs in the fly ash candidate CRM 490, the ratios of the mean values (R) are listed in table 4-3. A graphical representation of the mean values (R) and the corresponding uncertainties U is presented in figure 4-1. In case of ideal stability, R should be 1; in practice, however, some random variation due to the uncertainty of the measurements is to be expected. In all cases the value 1 was found between R-U and R+U; consequently it may be concluded that the PCDD and PCDF content in the material remained stable after 6 and 12 months storage at 20 or 40 °C.

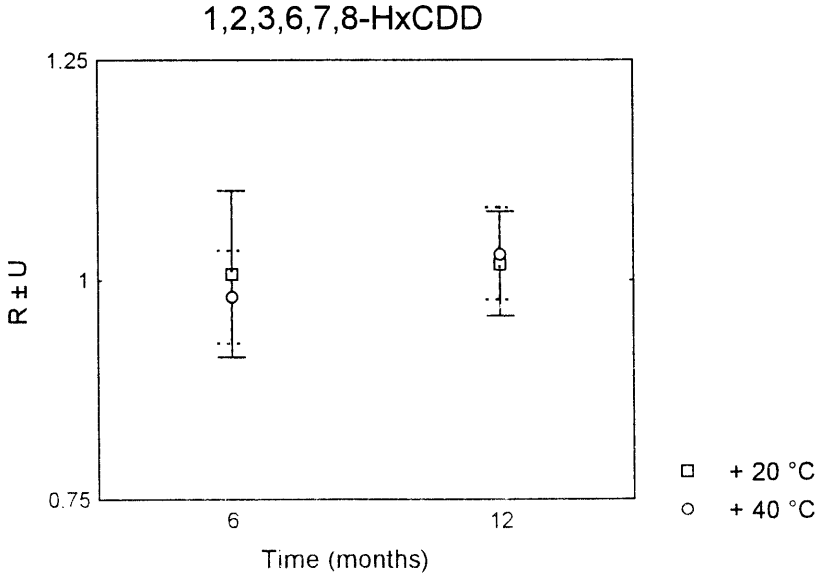
Table 4-3: Ratios of the mean values (R) obtained from the stability experiments at the various temperatures after 6 and 12 months storage; each time the mean value after storage at -20 °C was used as the reference level.

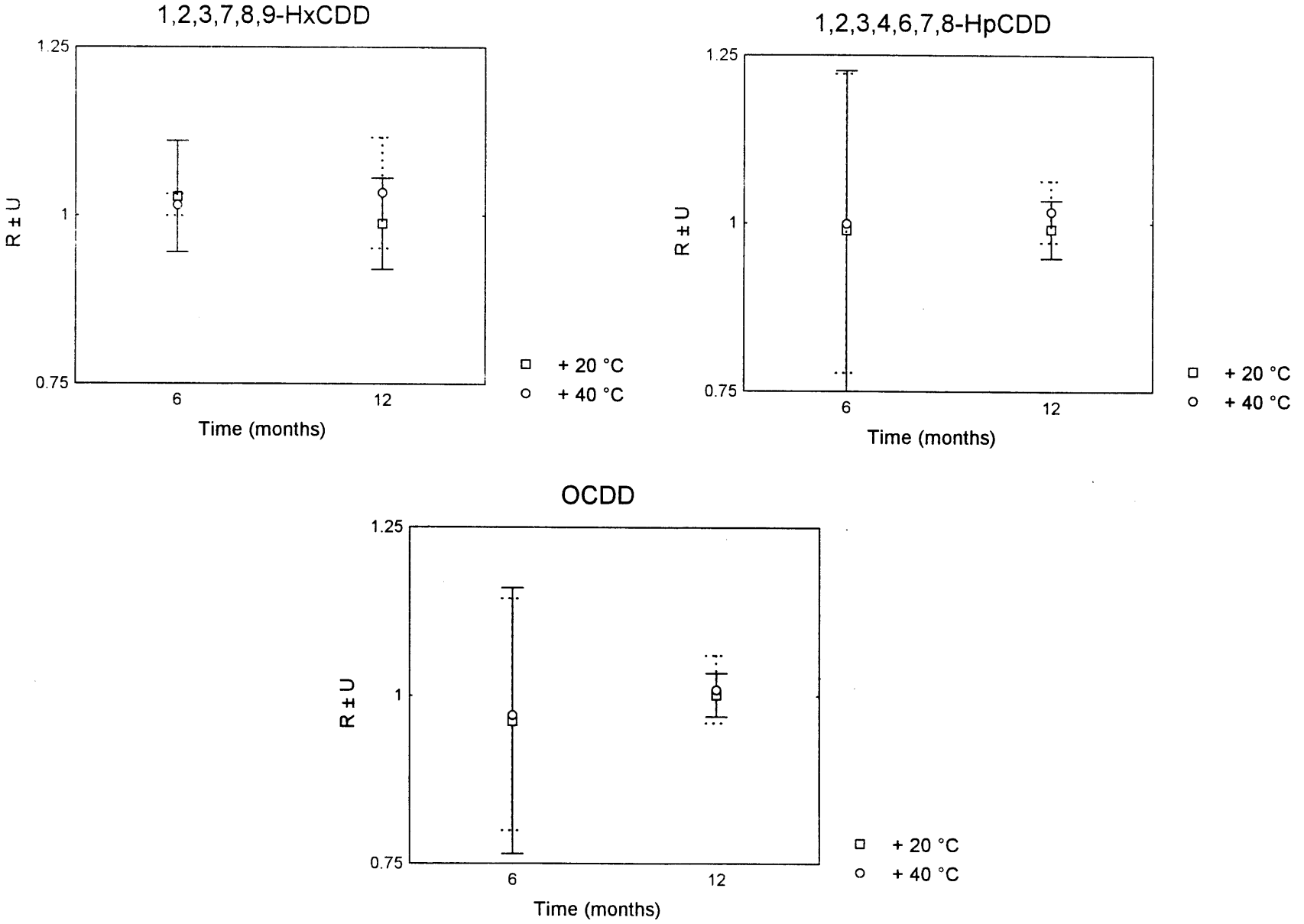
CONGENER	$R_{(6\ m,\ 20\ °C)}$	$R_{(6\ m,\ 40\ °C)}$	$R_{(12\ m,\ 20\ °C)}$	$R_{(12\ m,\ 40\ °C)}$
2,3,7,8-T4CDD	1.02	1.02	0.97	1.00
1,2,3,7,8-P5CDD	0.98	0.99	1.05	0.98
1,2,3,4,7,8-H6CDD	1.05	1.00	0.98	1.00
1,2,3,6,7,8-H6CDD	1.01	0.98	1.02	1.03
1,2,3,7,8,9-H6CDD	1.03	1.02	0.99	1.03
1,2,3,4,6,7,8-H7CDD	0.99	1.00	0.99	1.02
OCDD	0.96	0.97	1.00	1.01
2,3,7,8-T4CDF	1.00	0.96	1.00	1.00
1,2,3,7,8-P5CDF	0.98	0.98	1.00	1.01
2,3,4,7,8-P5CDF	0.98	0.99	0.91	0.95
1,2,3,4,7,8-H6CDF	0.97	0.99	0.99	1.03
1,2,3,6,7,8-H6CDF	0.94	0.95	1.01	1.03
1,2,3,7,8,9-H6CDF	0.98	1.01	0.95	0.96
2,3,4,6,7,8-H6CDF	0.94	0.96	0.98	0.99
1,2,3,4,6,7,8-H7CDF	0.99	1.03	0.98	1.00
1,2,3,4,7,8,9-H7CDF	1.01	0.99	1.00	1.02
OCDF	1.00	0.98	0.97	0.99

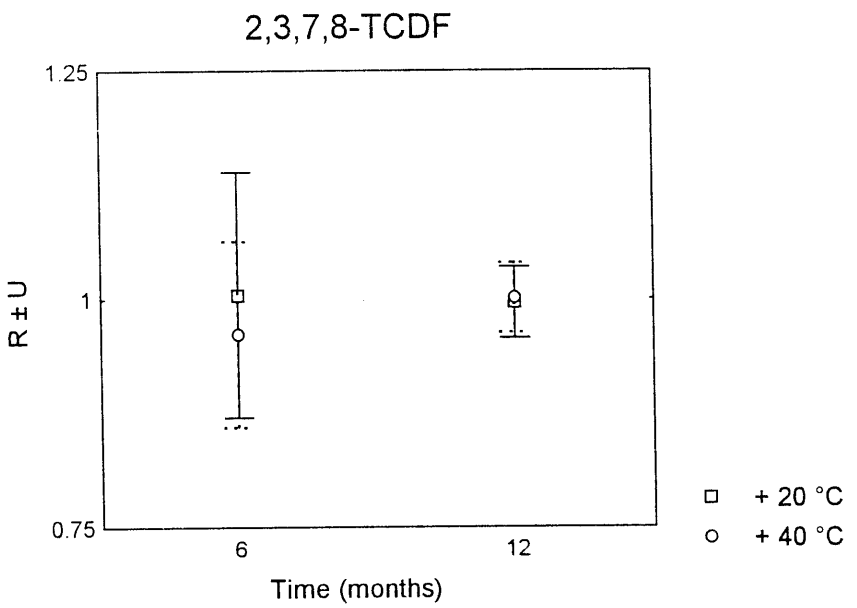

2,3,7,8-TCDD
1.25
1
0.75
R ± U
6
12
Time (months)
+ 20 °C
+ 40 °C

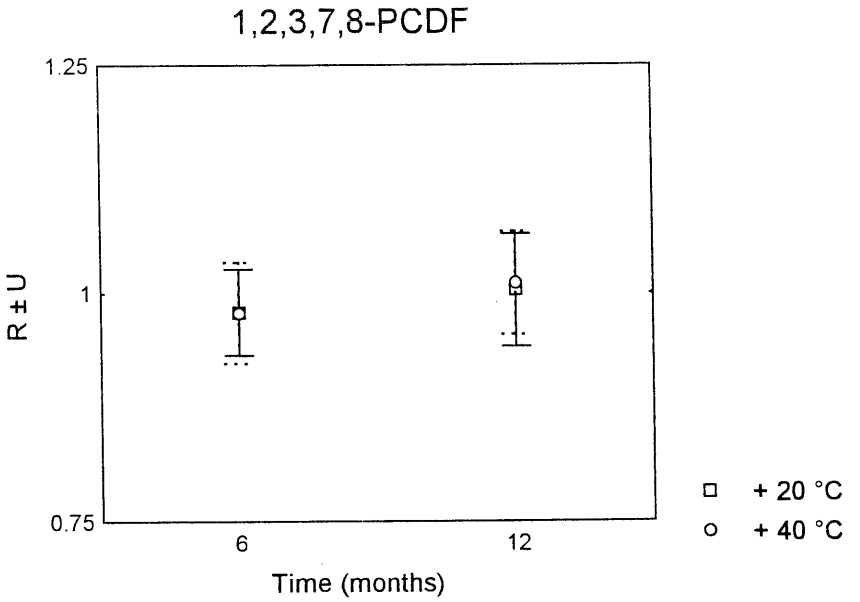

1,2,3,7,8-PCDD
1.25
1
0.75
R ± U
6
12
Time (months)
+ 20 °C
+ 40 °C

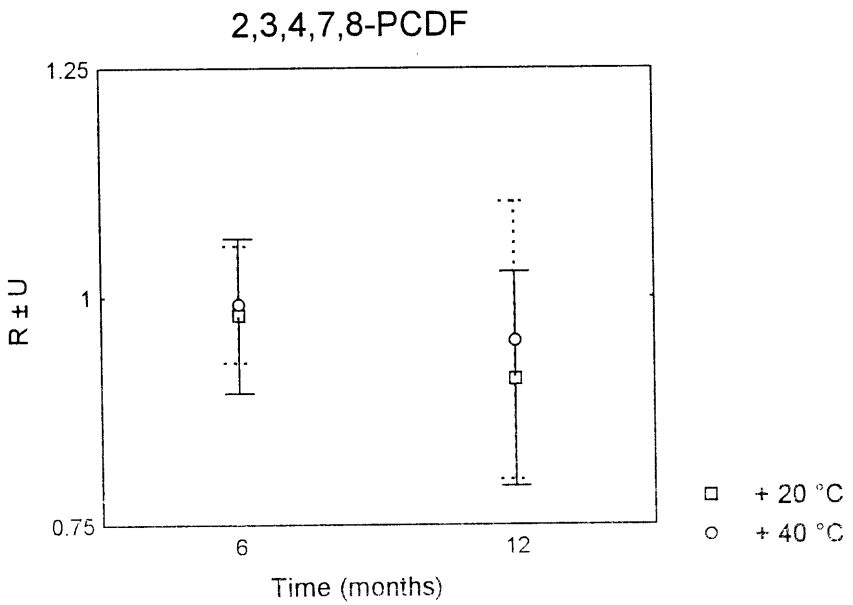

1,2,3,4,7,8-HxCDD
1.25
1
0.75
R ± U
6
12
Time (months)
+ 20 °C
+ 40 °C

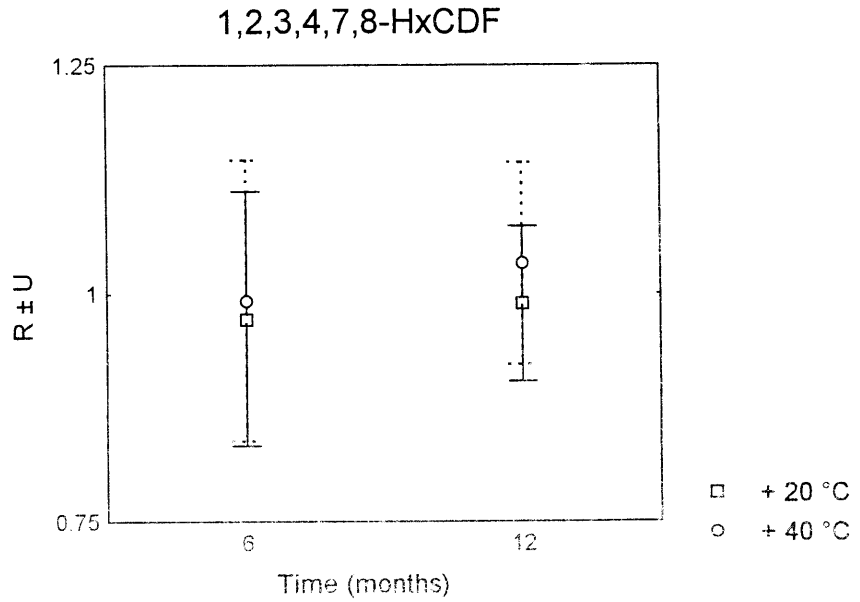

1,2,3,6,7,8-HxCDD
1.25
1
0.75
R ± U
6
12
Time (months)
+ 20 °C
+ 40 °C

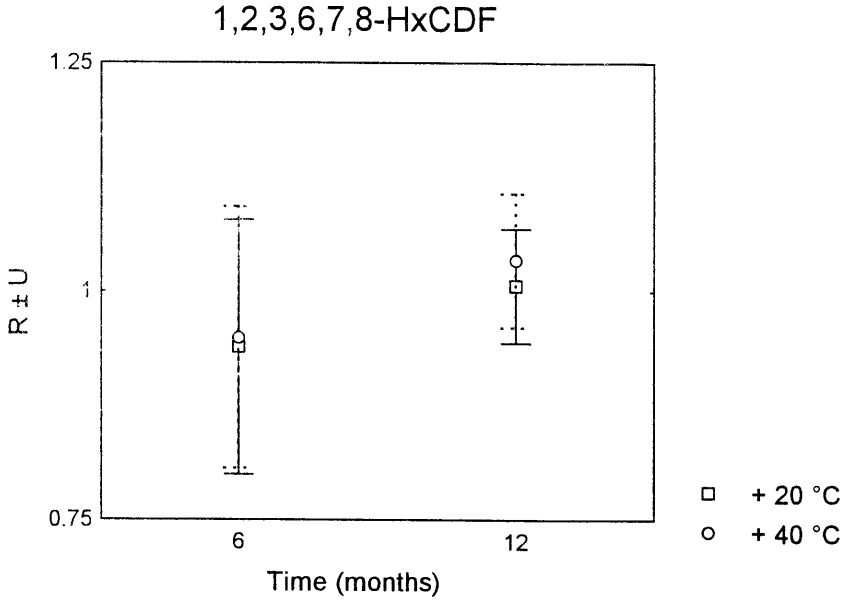

1,2,3,7,8,9-HxCDD
1.25
1
0.75
R ± U
6
12
Time (months)
+ 20 °C
+ 40 °C
1,2,3,4,6,7,8-HpCDD
1.25
1
0.75
R ± U
6
12
Time (months)
+ 20 °C
+ 40 °C
OCDD
1.25
1
0.75
R ± U
6
12
Time (months)
+ 20 °C
+ 40 °C

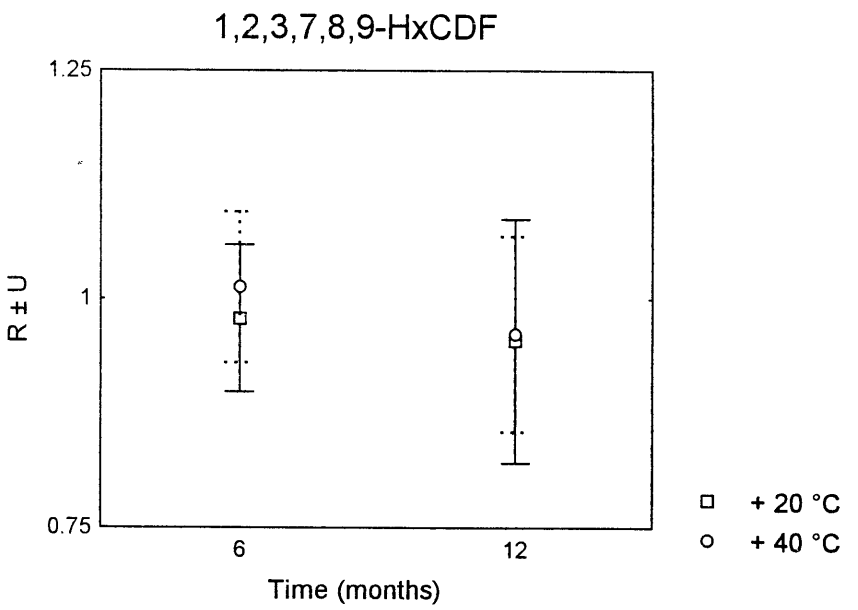

2,3,7,8-TCDF
1.25
1
0.75
R ± U
6
12
Time (months)
+ 20 °C
+ 40 °C

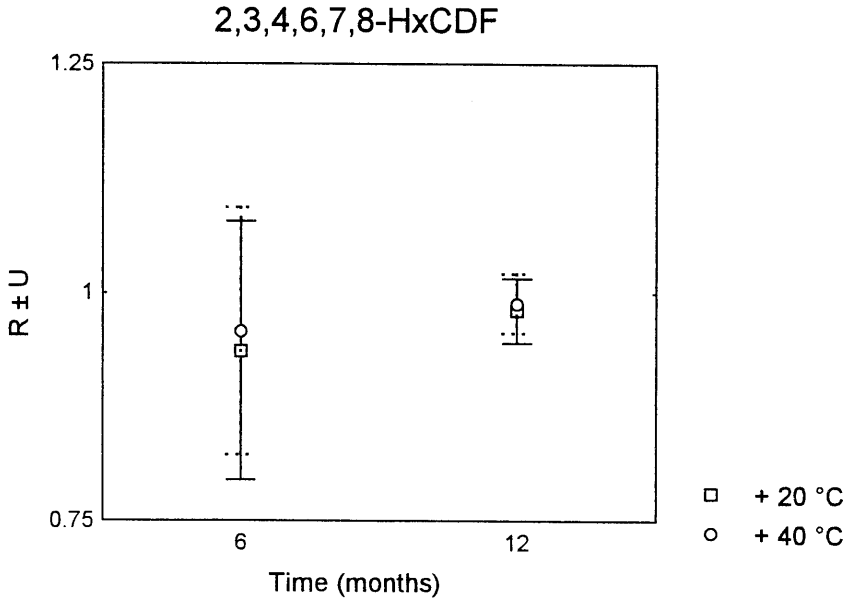

1,2,3,7,8-PCDF
1.25
1
0.75
R ± U
6
12
Time (months)
+ 20 °C
+ 40 °C

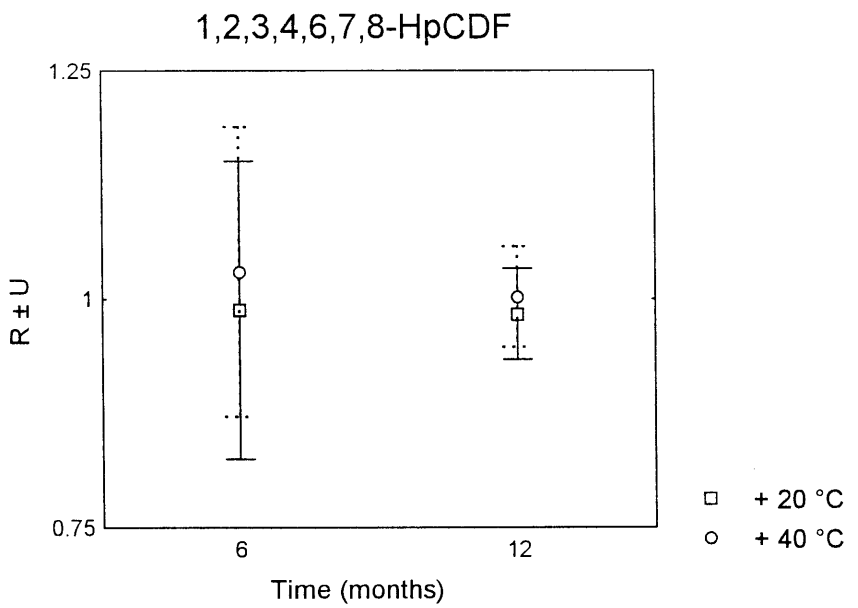

2,3,4,7,8-PCDF
1.25
1
0.75
R ± U
6
12
Time (months)
+ 20 °C
+ 40 °C

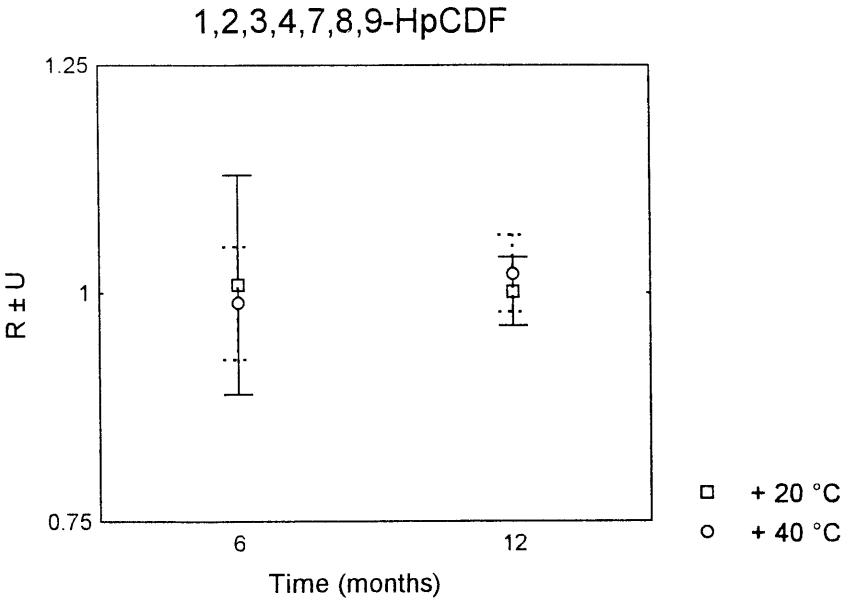

1,2,3,4,7,8-HxCDF
1.25
1
0.75
R ± U
6
12
Time (months)
+ 20 °C
+ 40 °C

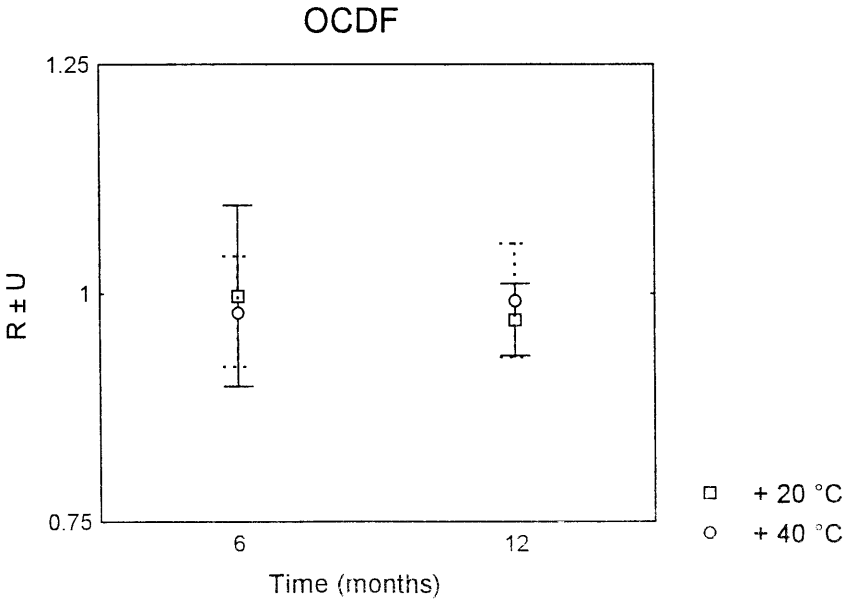

1,2,3,6,7,8-HxCDF
1.25
1
0.75
R±U
6
12
Time (months)
+ 20 °C
+ 40 °C


1,2,3,7,8,9-HxCDF
1.25
1
0.75
R±U
6
12
Time (months)
+ 20 °C
+ 40 °C


2,3,4,6,7,8-HxCDF
1.25
1
0.75
R±U
6
12
Time (months)
+ 20 °C
+ 40 °C


1,2,3,4,6,7,8-HpCDF
1.25
1
0.75
R ± U
6
12
Time (months)
+ 20 °C
+ 40 °C


1,2,3,4,7,8,9-HpCDF
1.25
1
0.75
R ± U
6
12
Time (months)
+ 20 °C
+ 40 °C


OCDF
1.25
1
0.75
R ± U
6
12
Time (months)
+ 20 °C
+ 40 °C

5. CERTIFICATION MEASUREMENTS

An interlaboratory study has been set up to certify the dioxin content in the fly ash candidate reference material. The underlying principle of such a certification is the agreement between a range of widely different methods of demonstrated reliability, applied in laboratories working independently and providing appropriate internal quality control, and consequently the reduction of the risk of a common systematic error. Many of the expert laboratories which were selected for the interlaboratory study already had been involved in an earlier step-by-step feasibility study supported by the European Commission [2]. To assist them in supplying all information and data in a common format, so that the traceability and quality of the data could be confirmed and compared, a detailed protocol for analysis and reporting results was discussed and distributed. Each laboratory was requested to determine the twelve target tetra-, penta- and hexa- CDD/CDF and possibly the five supplementary hepta- and octa- CDD/CDF congeners.

Five independent replicate analyses (from sub-sampling of the fly ash to the final determination, including calibration) were carried out, spread over no less than two separate days. None of the major steps in the analytical procedure were carried out as a single series on one day. At least two sub-samples were taken from each of the two bottles delivered to each participant.

Pretreatment, extraction and clean-up methods for the analysis were chosen and optimised by each laboratory. Gas chromatography with mass spectrometric detection was the common approach for final determination of the PCDDs and PCDFs. Each laboratory optimised the instrumental parameters (e.g. method of injection, capillary columns, ions monitored, etc.). In addition to the samples each laboratory received a set of calibration solutions of the individual target congeners, consisting of an ampoule of each of BCR RMs 432 to 443, and an ampoule with a ^{13}C- internal standard solution. They were used for calibration or for checking the laboratory's own calibrants. The fly ash extract BCR CRM 429 was made available as internal quality control sample.

5.1. Internal standards

The ampoule with a ^{13}C- internal standard solution contained one ^{13}C-labelled isomer for each product class and each chlorination degree; its composition is shown in table 5-1.

The solution was prepared gravimetrically from individual 50 μg/ml stock solutions in n-nonane, purchased from Cambridge Isotope Laboratories (Woburn, U.S.A.). The chemical and isotopic purity had been investigated by the manufacturer and was used as stated. Iso-octane was used for the dilution. The solvent was previously verified for the absence of any impurities by gas chromatography with flame ionisation detection.

Generally, participants aiming at determination of the tetra- to hexachlorinated congeners directly started from this ^{13}C- internal standard solution to prepare the necessary working solutions for spiking samples and calibrating the instrument. The others applied the laboratory's own internal standard stock solution(s), with adequate measures to avoid systematic calibration errors (e.g. referencing vs. BCR Rms 432-443).

Table 5-2 gives the number and the mass of internal standards added by each participant and indicates the stage of the procedure at which they were introduced.

Table 5-1: Composition of the ^{13}C- labelled internal standard mixture.

^{13}C LABELLED CONGENER	CONCENTRATION (ng/μl)
2,3,7,8-T4CDD	1.016
1,2,3,7,8-P5CDD	1.025
1,2,3,7,8,9-H6CDD	0.939
2,3,7,8-T4CDF	0.977
2,3,4,7,8-P5CDF	0.735
1,2,3,7,8,9-H6CDF	0.941

Table 5-2: Summary of internal standards applied for the certification measurements

LAB Nr	NUMBER AND MASS OF INTERNAL STANDARDS				STAGE OF PROCEDURE
	^{13}C T4-P5-H6- CDD/CDF		^{13}C H7-O- CDD/CDF		
1	6	0.1-0.3 ng	n.a.		II
2	11	1 ng	4	1-2 ng	II
3	11	4 ng	n.a.		III
4	11	1 ng	5	1-2 ng	I
5	12	4-6 ng	n.a.		I
6	12	2-5 ng	5	3-5 ng	II
7	12	5-7 ng	5	6-9 ng	I
8	11	5 ng	4	5-10 ng	I
9	11	1 ng	4	1-2 ng	I
10	6	3-6 ng	2	100-200 ng	I
11	6	2-3 ng	4	3-5 ng	II
12	11	1-4 ng	4	2-5 ng	II
13	6	10-30 ng	n.a.		II
14	6	1-3 ng	n.a.		II
15	8	30-70 ng	4	60-200 ng	I
16	9	0.4-2 ng	4	1-20 ng	I
17	6	1-3 ng	n.a.		II

I: prior to the acid treatment
II: prior to extraction but after acid treatment
III: prior to clean-up but after extraction

n.a.: not analyzed

5.2. Acid treatment and extraction

All participants applied an acid treatment, prior to and/or during extraction, to destroy the matrix structure of the fly ash. Table 5-3 summarises the acid pretreatment and extraction procedures. In the technical discussion doubts were expressed on the use of sulfuric acid for the acid treatment; an excluded set of data containing systematically low values confirmed the insufficient strength of this acid to break down the fly ash structure.

5.3. Clean-up

The extracts obtained were cleaned up by a variety of established techniques to remove compounds that could interfere with the gas chromatographic determination of the PCDDs and PCDFs, either by altering the gas chromatographic response or by degrading the performance of the instrument. The techniques used by the participating laboratories are listed in table 5-4. The clean-up steps were carried out either on classic open columns, on flow-controlled columns or as a batch treatment.

For the concentration of raw and purified extracts rotary evaporation, nitrogen blow-down or distillation was applied. Care was taken to avoid solvent evaporation to complete dryness.

5.4. GC-MS measurement

Instrumental analysis of the purified extracts was based on capillary gas chromatography with high or low resolution mass spectrometric detection. At least two GC columns with different stationary phases and polarity were used by each laboratory, thus enabling confirmation of the identity of each analyte and the absence of interfering peaks. Only one result, i.e. that one giving the best estimate (to be judged by the laboratory itself), was submitted for each analyte/replicate. The congeners were identified by comparing the relative retention times of the peaks in the sample chromatograms and the calibration chromatograms. The final quantification relied on relative peak area, taking into account two isotope peaks. To further confirm the identity of the analytes, the isotopic ratios of the selected isotope peaks were verified against the expected values.

All participants checked the linearity of their detection system for each of the congeners to be determined. This was done by injecting a series of standard solutions of different concentrations.

The most relevant gas chromatographic conditions used by the participating laboratories have been summarised in table 5-5, showing a wide variety of columns and injection techniques. In addition, the use of helium as carrier gas was common to all laboratories. In table 5-6 the most relevant mass spectrometric conditions are given. All mass spectrometers were used in the electron impact mode, with selected ion monitoring of at least two abundant masses of the molecular ion cluster for each native and labelled congener. For peak quantification, all participants started from the software provided with their GC-MS system but redrew baselines whenever these were considered inappropriately drawn.

Table 5-3: Summary of acid treatment and extraction procedures applied

LAB Nr	SAMPLE INTAKE (g)	ACID TREATMENT AND ISOLATION PROCEDURE	EXTRACTION PROCEDURE	EXTRACTION DURATION (h)
1	1	A1 + A2	E1	24
2	2	A1 + I1	E2	20
3	5	A1 + I1	E2	20
4	1-2	A1 + I1	E2	30
5	5	A1 + I1	E2	52-62
6	1	A1 + I1	E2	48
7	1	A2	E3	16
8	5	A3 + A2	E4	20
9	4	A1 + I1	E2	48
10	3	A1 + I1 + I2	E2 + E5	24
11	8	A1 + I1	E2	48
12	2	A1 + I1	E2	40
13	5	A1 + I1	E2	35
14	1	A1 + I1	E2	48
15	6-11	A3 + I1	E2	24
16	1.5	A4 + I2	E2	24
17	2.5	A1 + I1	E2	46

methods for acid treatment:

A1: pretreatment with dilute hydrochloric acid (1-3 mol/l)
A2: treatment with hydrochloric acid during extraction
A3: pretreatment with concentrated hydrochloric acid
A4: pretreatment with glacial acetic acid

methods for isolation of fly ash and/or water removal:

I1: filtration, rinsing, drying
I2: use of Dean-Stark trap during extraction

extraction methods:

E1: Soxhlet extraction with toluene + ethanol
E2: Soxhlet extraction with toluene
E3: Reflux extraction with toluene + ethoxyethanol
E4: Reflux extraction with toluene + methoxyethanol
E5: Soxhlet extraction with toluene + methoxyethanol

Table 5-4: Summary of clean-up techniques applied

LAB Nr	STEP 1	STEP 2	STEP 3	STEP 4	STEP 5
1	P+D+F+P	D+F	O/M	F+E+P	J
2	N+J	D			
3	O/H	F+E+G+P	B+P		
4	D+F+D+E+D	B+P			
5	D	O/H	D+F+D+E+D	G	P+B
6	B+P				
7	P+D+F+D+E+D- +L+D+G	P+C	P+D+F+D+E+D+ L+D+G		
8	D	G	B		
9	G+D+F+D+E+D+P	J+P			
10	D+F+D+E+D	G	B		
11	F+E+E	G+B	K	S	
12	B				
13	A				
14	R	Q	P+D+E+P	B	O/H
15	D+P+I	B			
16	D+F+E+P	A+P	O/H		
17	D+F+E	C	O/H		

methods:

A: alumina
B: basic alumina
C: acidic alumina
D: silicagel
E: acid (H_2SO_4)- impregnated silicagel
F: base (NaOH or KOH)- impregnated silicagel
G: $AgNO_3$- impregnated silicagel
H: celite
I: acid (H_2SO_4)- impregnated celite
J: florisil
K: HPLC C18-silica
L: CsOH- impregnated silica
M: glass fibres
N: $(NH_4)_2SO_4$ anhydrous
O: carbon
P: Na_2SO_4 anhydrous
Q: H_2SO_4
R: KOH
S: $NaHCO_3$

+: in layers
/: mixed bed

Table 5-5: Summary of gas chromatographic conditions applied

LAB Nr	COLUMN				INJECTION			
	TYPE	L	ID	FT	TYPE	V	T	RG
1	RTX 2330 RTX 5	60 60	0.25 0.25	0.10 0.10	splitless (1.6 min)	1.5	280	-
2	RTX 2330 DB 5 MS	60 60	0.25 0.25	0.20 0.25	splitless (1.5 min)	2	260 280	-
3	RTX 2330 DB 5	30 60	0.25 0.25	0.20 0.10	on column	1 2	70 130	1
4	DB 5 MS DB DIOXIN	50 60	0.22 0.25	0.25 0.15	splitless (1 min)	1.5	270	2
5	CP SIL 88 DB 5	50 60	0.25 0.25	0.20 0.25	PTV (12 °C s^{-1})	1	70-330 70-350	-
6	DB DIOXIN CP SIL 88 DB 5 MS	60 50 60	0.25 0.25 0.25	0.25 0.20 0.25	splitless (1 min)	1	270 275 275	-
7	RTX 2330 DB 5 MS	60 30	0.32 0.25	0.10 0.25	splitless (1.2 min)	3	290	-
8	SP 2331 DB DIOXIN DB 5	60 60 50	0.32 0.32 0.25	0.20 0.17 0.25	on column	2.5 2 1	80	2
9	DB 5 CP SIL 88	60 50	0.25 0.25	0.25 0.20	splitless	2	280	-
10	SP 2331 DB 5 DB DIOXIN	60 30 60	0.32 0.25 0.25	0.20 0.25 0.25	splitless (1 min) splitless (0.5 min) splitless (1 min)	2 1 2	270 280 280	-
11	SP 2331 DB 5	60 60	0.32 0.32	0.25 0.25	on column, secon- dary cooling 20 s	2	80	2
12	CP SIL 88 DB 5	50 60	0.25 0.24	0.20 0.25	splitless (1 min)	1-2	270	-
13	DB DIOXIN CP SIL 88	60 50	0.25 0.25	0.25 0.20	splitless (1 min)	2	270 240	1
14	SP 2331 DB 5	60 60	0.25 0.24	0.20 0.25	splitless (2 min)	1 0.5-1	280 270	-
15	DB DIOXIN CP SIL 8	60 30	0.25 0.25	0.25 0.25	splitless (0.9 min)	1	300	-
16	SP 2330 DB 5	60 60	0.32 0.32	0.20 0.25	splitless (2 min)	1	250	-
17	SP 2331 DB DIOXIN	60 60	0.25 0.25	0.20 0.25	splitless (0.5 min)	2	275	-

L: length (in m) ID: internal diameter (in mm) FT: film thickness (in µm) V: injection volume (in µl) T: injectortemperature (in °C)
RG: retention gap length (in m) PTV: programmed temperature vaporisation

Table 5-6: Summary of mass spectrometric conditions applied

LAB Nr	MS TYPE	INTERFACE	MS SOURCE	
		T (°C)	T (°C)	ELECTRON ENERGY (eV)
1	HR	250	250	40
2	HR	280-300	280	37
3	LR	275-325	200	70
4	HR	280	250	28
5	LR	300	165	70
6	HR	275-260-300	250	28
7	HR	250	270	70
8	LR	250-270-300	200	70
9	HR	280-250	250	37
10	HR	270	250	35
11	LR	250	180	70
12	HR	240	240	35
13	LR	300-240	200	32
14	HR	270	260	30
15	LR	250-300	180-250	70
16	HR	250	250	36
17	HR	260	260	30

abbreviations used:

HR: high resolution MS
LR: low resolution MS
T: temperature (whenever several interface temperatures are presented, they refer to the various GC columns as indicated in table 5-5)

5.5. Calibration and quantification

As a basis for calibration, all participants received a set of reference solutions (BCR Rms 432 to 443) which each contain one of the target congeners at a mass fraction of 1 - 6 μg/g in iso-octane. They were to be used for calibration or for checking the laboratory's own calibrants. The production and verification of the content of these (non-certified) reference materials has been described in detail elsewhere [3]. It included the evaluation of the purity of each individual congener on the initial crystals, using several analytical methods (reversed phase HPLC, GC-FID, GC-MS in both selected ion monitoring and full scan mode). Also the solvents used were checked for impurities. The entire procedure of preparation of the twelve solutions was performed under strict metrological conditions [4].

Each laboratory adopted its own approach for the preparation of working solutions, to spike samples and calibrate the instrumental response. With regard to the supplementary hepta- and octachlorinated congeners, no common basis for calibration could be provided; each laboratory had to rely on its own calibrants.

Instrumental calibration for the sample analyses was based on at least one calibration solution within the demonstrated linear range of the GC-MS system. The mass of determinants in the sample aliquot was adjusted by concentrating or diluting the sample extracts to fall within the demonstrated linear range.

Quantification was performed according to the method generally referred to as internal standardisation, using within each isomeric group at least one ^{13}C-labelled isomer as an internal standard. Congener-specific relative response factors (RRF's) were derived from each measurement of a calibrating solution, according to the formula:

$$RRF_x = \frac{\Sigma \text{ area}_x \text{ (2 isotope peaks)}}{\Sigma \text{ area}_{i.s.} \text{ (2 isotope peaks)}} * \frac{\text{mass}_{i.s.}}{\text{mass}_x}$$

with 'x' representing the analyte and 'i.s.' the corresponding internal standard, being the structurally identical ^{13}C-labelled congener or else a ^{13}C-labelled congener belonging to the same isomeric group. In order to minimise the contribution of the inherent ion statistics to the overall uncertainty of the results, the peak areas used for RRF calculations were always the sum of two of the most abundant peaks of the molecular ion cluster.

From the measurement of the samples, compound concentrations were subsequently calculated using the formula:

$$(\text{mass/mass})_x = \frac{\Sigma \text{ area}_x \text{ (2 isotope peaks)}}{\Sigma \text{ area}_{i.s.} \text{ (2 isotope peaks)}} * \frac{1}{RRF_x} * \frac{\text{mass}_{i.s. \text{ added}}}{\text{mass}_{\text{sample intake}}}$$

A separate calibration run for each replicate sample analysis was requested from the participants, the calibration solution(s) being injected just before and/or after the sample solution.

5.6. Recovery and extraction efficiency

The internal standardisation adequately takes into account losses during the analytical procedure, provided that the internal standards are selected and introduced properly; this means the application of structurally identical ^{13}C- labelled congeners early in the analytical procedure, with appropriate conditioning after introduction. In such case separate recovery experiments may be considered unnecessary; as a quality control parameter, the recovery of the ^{13}C- labelled internal standards can be estimated directly from the GC-MS run of the sample replicate. The latter was done either by quantification versus one or more additional ^{13}C- internal standards (such as ^{13}C- 1,2,3,4-T4CDD) spiked immediately before GC-MS injection, or occasionally (lab 10) by external calibration.

If some of the structurally identical ^{13}C- labelled H6CDF congeners were not available (e.g., when the ^{13}C internal standard solution described in table 5-1 was used), it was recommended to the participants to set up at least one separate standard addition experiment. This aimed at investigating whether the recovery of the different congeners of an isomeric group, quantified versus a particular internal standard congener, was constant (within the determination uncertainty). In case the difference between isomers was larger than the determination uncertainty, the laboratory had to carry out three replicate recovery estimations to allow a precise recovery correction upon calculation of the final results for the unknown.

The recovery data, measured as the ratio of masses of added versus recovered compounds, for the various congeners and expressed as mean ± standard deviation, are listed in table 5-7. When a separate experiment was carried out only once, the single recovery values are given without standard deviation. Missing values for ^{13}C-1,2,3,7,8,9-H6CDD are due to the use of this labelled congener as 'recovery' standard, added after the sample preparation to estimate directly the recovery of the internal standards.

All the participants verified the efficiency of their extraction procedure by re-extracting one or several samples already extracted. The amounts in the re-extracts were generally found below the limit of determination or small (< 5 %) compared to the amounts in the original extract. Depending on the particular analytical approach chosen, the incompleteness of extraction was already corrected by the internal standardisation or by a supplementary correction of the data.

5.7. Additional quality control

The control of the clean-up and quantification procedures was assessed by analysis of the certified fly ash extract BCR CRM 429. The results obtained were used in the technical discussion afterwards for tracing or confirming systematic errors due to, e.g., chromatographic interferences.

Prior to, or within, each of the two separate series of sample preparations at least one procedure blank was determined. These analytical blanks covered the complete procedure, except the sample intake, and had to be blank at the concentration levels of interest.

5.8. Determination of the water content

At each occasion of analysis, the water content of the material was determined on a separate sub-sample, spread in a layer of less than 1 cm thickness and dried in a well-ventilated oven at 105 °C until constant mass. The PCDD and PCDF content of the fly ash was corrected for the water content.

The water content of the material, calculated from the mean water content determined in the different laboratories, amounted to 1.7 $\pm$ 0.5 % (mass fraction).

Table 5-7: Summary of the recovery values obtained

LAB Nr	TYPE	RECOVERY (mean ± standard deviation)						
		D48	D54	D66	D67	D70	D73	D75
1	I II	117 ± 38	68 ± 16	 89 ± 12	 90 ± 6	150 ± 44 100	n.a.	n.a.
2	I II	73 ± 9	84 ± 6	73 ± 7 97 ± 2	62 ± 5 84 ± 0	 99 ± 4	104 ± 14	169 ± 65
3	I II	88 ± 4	86 ± 12	68 ± 11 126 ± 12	83 ± 6 117 ± 14	 105 ± 1	n.a.	n.a.
4	I II	103 ± 4	80 ± 10	67 ± 6 95 ± 5	102 ± 23 92 ± 19	 90 ± 16	100 ± 17	95 ± 26
5	I	83 ± 4	85 ± 2	86 ± 4	85 ± 4	87 ± 4	n.a.	n.a.
6	I	94 ± 12	114 ± 13	83 ± 12	86 ± 12	85 ± 14	89 ± 12	92 ± 15
7	I	64 ± 8	79 ± 8	70 ± 9	73 ± 10	73 ± 9	62 ± 7	68 ± 5
8	II	91 ± 6	95 ± 14	91 ± 13	92 ± 12	89 ± 11	80	61
9	I II	99 ± 18	70 ± 18	64 ± 17 73	57 ± 18 87	 101	55 ± 23	59 ± 32
10	I II	94 ± 12 94	91 ± 12 101	 109	 120	94 ± 11 103		168 ± 24
11	I II	61 ± 2 53	66 ± 6 73	 77	66 ± 3 78	 79	65 ± 8	66 ± 9
12	I II	102 ± 4	101 ± 8	96 ± 6 94 ± 6	98 ± 13 100 ± 3	 95 ± 5	154 ± 10	157 ± 20
13	II	89 ± 2	97 ± 6	95 ± 5	108 ± 7	98 ± 5	n.a.	n.a.
14	I II	65 ± 4 108 ± 4	106 ± 4 105 ± 4	 79 ± 8	 79 ± 6	69 ± 10 61 ± 13	n.a.	n.a.
15	I II	79 ± 7 100 ± 10	64 ± 14 129 ± 9	57 ± 11 98 ± 12	 123 ± 5	75 ± 8 100 ± 8	74 ± 11	75 ± 14
16	I II	83 ± 6	92 ± 6	 97	97 ± 5 97	 103	89 ± 7	98 ± 16
17	I II	69 ± 4 61 ± 6	72 ± 3 77 ± 2	85 ± 17 100 ± 9	85 ± 17 101 ± 12	71 ± 8 99 ± 11	n.a.	n.a.

type I: recovery of the ^{13}C- labelled internal standards, as determined directly from the GC-MS run of the sample replicate type II: recovery from separate experiments n.a.: not analyzed

Table 5-7 (cont.): Summary of the recovery values obtained

LAB Nr	TYPE	RECOVERY (mean ± standard deviation)									
		F83	F94	F114	F118	F121	F124	F130	F131	F134	F135
1	I II	173 ± 72	101 ± 10	93 ± 23 100	98 ± 12	92 ± 24	140 ± 58 100	97 ± 14	n.a.	n.a.	n.a.
2	I	64 ± 5	84 ± 6	78 ± 7	73 ± 5	64 ± 6	108 ± 7	67 ± 4	85 ± 12		
3	I	91 ± 5	104 ± 6	100 ± 7	88 ± 12	97 ± 7	97 ± 14	98 ± 16	n.a.	n.a.	n.a.
4	I	84 ± 4	95 ± 10	92 ± 20	99 ± 11	100 ± 14	85 ± 10	92 ± 16	106 ± 19	103 ± 13	94 ± 20
5	I	83 ± 4	86 ± 3	86 ± 3	87 ± 6	85 ± 5	86 ± 11	93 ± 4	n.a.	n.a.	n.a.
6	I	94 ± 18	81 ± 11	97 ± 8	94 ± 10	88 ± 8	82 ± 11	82 ± 13	93 ± 10	86 ± 11	90 ± 14
7	I	70 ± 4	110 ± 11	109 ± 9	78 ± 9	86 ± 11	73 ± 9	79 ± 12	69 ± 6	62 ± 6	70 ± 8
8	II	89 ± 7	95 ± 8	90 ± 6	93 ± 11	93 ± 11	90 ± 19	88 ± 14	85	76	62
9	I	72 ± 13	72 ± 22	72 ± 15	63 ± 21	58 ± 20	53 ± 17	53 ± 20	47 ± 22	54 ± 22	
10	I II	93 ± 18 94	112	95 ± 12 121	159	109	98 ± 9 78	112			86 ± 22
11	I II	61 ± 4 76	75	62 ± 3 79	66 ± 5 81	81	81	87	63 ± 8		
12	I	98 ± 11	98 ± 11	83 ± 11	101 ± 7	101 ± 10	91 ± 10	108 ± 10	143 ± 13	149 ± 14	
13	II	94 ± 8	76 ± 2	88 ± 4	89 ± 9	94 ± 6	94 ± 3	100 ± 12	n.a.	n.a.	n.a.
14	I II	37 ± 2 35 ± 1	58	50 ± 1 49 ± 0	90 ± 1	81 ± 5	83 ± 6 80 ± 6	82 ± 7	n.a.	n.a.	n.a.
15	I II	74 ± 4 117 ± 15	103 ± 4	68 ± 7 124 ± 7	51 ± 9 147 ± 7	98 ± 4	72 ± 7 97 ± 25	101 ± 3	44 ± 8		51 ± 7
16	I II	92 ± 4	93 ± 5	67 ± 7	103 ± 4	94 ± 5	80	81 ± 4 81	82 ± 6 82	74	85 ± 7
17	I II	76 ± 8 64 ± 7	86 ± 9 78 ± 15	71 ± 3 82 ± 9	97 ± 14 90 ± 10	71 ± 8 93 ± 9	95 ± 16 100 ± 14	89 ± 16 97 ± 14	n.a.	n.a.	n.a.

type I: recovery of the ^{13}C- labelled internal standards, as determined directly from the GC-MS run of the sample replicate
type II: recovery from separate experiments
n.a.: not analyzed

6. TECHNICAL AND STATISTICAL EVALUATION

6.1. Technical discussion

All methods and results were discussed extensively at a technical evaluation meeting in order to confirm the reliability of the analytical procedures and to verify the traceability of the data. Good analytical quality control, in accordance with the demands of certification, and implementation of the guidelines outlined in the protocol for analysis (see chapter 5) were a prerequisite for acceptance of data for certification.

The results submitted by the participants and accepted for certification are listed in Annex I, preceded by a laboratory code. Details of the analytical methods used by the individual laboratories are given in chapter 5, together with the corresponding laboratory code.

6.1.1. 2,3,7,8-T4CDD (D48)

The data for 2,3,7,8-T4CDD from laboratories nr. 2, 7 and 15 were not accepted for certification because of unproper tuning of the HRMS instrument (Lab 7) which led to an unreliable quantification. Lab 7 confirmed this explanation by running parallel determinations of the PCDDs and PCDFs with LRMS. Lab 2 and 15 reported an unexplainable discrepancy between the results of the apolar (DB 5MS) and the polar GC column (RTX 2330). An individual value with outlying isotopic ratio was rejected from the data sets submitted by laboratory 13.

6.1.2. 1,2,3,7,8-P5CDD (D54)

The results from laboratory nr. 7 could not be accepted, for the same reason as indicated in section 6.1.1. Laboratory nr. 9 withdrew its results because of a possible interference on the DB 5 column used. The data from laboratory nr. 15 were not accepted for certification because of a known interference on the DB DIOXIN column. Laboratory nr. 16 reported a significant deviation between the laboratory calibration solution used for the quantification and BCR RM 433; its results were not included for calculation of the certified mass fraction of 1,2,3,7,8-P5CDD.

6.1.3. 1,2,3,4,7,8-H6CDD (D66)

Only the results from laboratory nr. 7 could not be accepted for certification (for the reason described in section 6.1.1).

6.1.4. 1,2,3,6,7,8-H6CDD (D67)

All the data sets submitted by the participants listed in chapter 2 met the criteria for acceptance for certification, except that from laboratory nr. 16 because of the significant deviation between the laboratory calibration solution used for the quantification and BCR RM 435. Its results were not included for calculation of the certified mass fraction of 1,2,3,6,7,8-H6CDD.

6.1.5. 1,2,3,7,8,9-H6CDD (D70)

In the chromatograms from laboratory nr. 2 a peak impurity was noticed, and therefore the data were not accepted for certification. Laboratory nr. 9 considered its within-laboratory standard deviation for 1,2,3,7,8,9-H6CDD unreliably high and thus withdrew the results.

6.1.6. 2,3,7,8-T4CDF (F83)

The results from laboratory nr. 7 could not be accepted for certification, for the same reason as indicated in section 6.1.1. As the selective determination of 2,3,7,8-T4CDF, free from interferences, requires an extremely good resolution, chromatographic records were screened for a sufficient separation; the data from laboratory nr. 14 were not accepted because of an apparent chromatographic interference. An individual value with outlying isotopic ratio was rejected from the data set submitted by laboratory nr. 2.

6.1.7. 1,2,3,7,8-P5CDF (F94)

The selective determination of 1,2,3,7,8-P5CDF in fly ash cannot be performed on some common polar capillary columns such as RTX 2330 or SP 2331. This was confirmed by the insufficient chromatographic separation obtained by laboratory nr. 2 on a RTX 2330 column; consequently these results were not accepted for certification. In addition to the DB DIOXIN column, most of the commonly used non polar capillary columns were found to perform well. The results from laboratory nr. 1 however, obtained on a RTX 5 column, suffered from an interference and were not accepted. In the chromatograms from laboratory nr. 3 an interference on the ^{13}C masses was detected, which led to rejection of the results.

6.1.8. 2,3,4,7,8-P5CDF (F114)

The results from laboratory nr. 7 could not be accepted, for the same reason as indicated in section 6.1.1. The results obtained on a DB DIOXIN column suffered from a chromatographic interference and therefore were rejected (laboratory nr. 15); the problem can be overcome by using a common polar capillary column such as CP SIL 88 or SP 2331.

6.1.9. 1,2,3,4,7,8-H6CDF (F118)

In view of the problems to resolve this congener chromatographically [1], it was decided to accept only results which either were backed up by a chromatogram showing a reasonable separation between the analyte and the interfering peak (laboratories nr. 4, 6, 8, 13 and 17), or else were corrected for the co-eluting peak after quantification of the latter on a second GC column (laboratories 5, 11 and 16). Only good DB DIOXIN or DB 5 MS columns were found to yield an acceptable separation. For the fly ash involved, the interference apparently implies only a small correction, as illustrated by six eliminated data sets (laboratories 1, 2, 3, 10, 12 and 14) with results ranging between 2.6 and 3.1 μg/kg.

6.1.10. 1,2,3,6,7,8-H6CDF (F121)

Only the results from laboratory nr. 7 could not be accepted for certification (for the reason described in section 6.1.1).

6.1.11. 1,2,3,7,8,9-H6CDF (F124)

The separation of 1,2,3,7,8,9-H6CDF from interfering peaks on some common polar GC columns is known to be difficult, so the chromatograms were critically examined. This pointed out that, for the fly ash involved, also on non-polar columns such as DB 5 or DB 5 MS an unacceptable resolution is often obtained. The data from laboratories 2, 3, 7 and 14 were not accepted for certification because of chromatographic interference. The measurements by laboratory nr. 15 were rejected because of a too low signal to noise ratio for reliable quantification. Laboratory nr. 16 reported a significant deviation between the laboratory calibration solution used for the quantification and BCR RM 442; its results were not included for calculation of the certified mass fraction of 1,2,3,7,8,9-H6CDF.

6.1.12. 2,3,4,6,7,8-H6CDF (F130)

The data from laboratory nr. 7 were not accepted for certification because of a probable calibration error, confirmed by the overestimation reported for control measurements of the fly ash extract BCR CRM 429.

6.1.13. Hepta- and octa- CDD/CDF (D73, D75, F131, F134, F135)

In general the quality of the available data for hepta- and octachlorinated congeners, which could be determined on a voluntary basis, was considered insufficient to envisage certification. The main reason was the inability to demonstrate in a traceable manner the purity of the calibrants used. Furthermore, recovery correction and linearity of the instrumental response in the working range were usually not backed up by all the supporting data requested in the protocol for analysis. Nevertheless it was felt that, in view of the reasonable between-laboratory agreement, sufficient confidence could be given to the majority of the results to assign indicative (non-certified) values.

It was agreed in the technical discussion that data sets which were not based on the use of the identical ^{13}C-labelled congener as internal standard were to be excluded for the calculation of indicative values. The same was done with results that appreciably disagreed with those from the other laboratories, probably due to calibration or quantification problems.

The laboratory means withstanding the above criteria are listed in Table 6-1, together with the calculated indicative values (unweighed arithmetic mean of the laboratory means) and uncertainties (half width of the 95 % confidence interval).

Table 6-1: Individual laboratory means and indicative values for the hepta- and octa-chlorinated congeners

	MASS FRACTION (µg/kg)				
	D73	D75	F131	F134	F135
LAB Nr 2	30.8	52.6	8.34		
LAB Nr 4	28.6	43.6	8.83	1.71	4.90
LAB Nr 6	34.8	42.8	9.13	1.63	4.75
LAB Nr 7	34.8	55.4	9.99	1.46	4.39
LAB Nr 8	33.9	54.1	10.61	1.76	
LAB Nr 9	21.8		6.90	1.21	
LAB Nr 10		55.1			2.98
LAB Nr 11	29.3	44.5	9.16		4.84
LAB Nr 12	33.0	49.9	11.00	1.73	
LAB Nr 16	29.0	38.6	8.59		4.18
INDICATIVE VALUE STANDARD DEVIATION	31 4	49 6	9 1	1.6 0.2	4.3 0.7

6.2. Statistical Discussion

6.2.1 General

The certification of the polychlorinated dioxins and furans in fly ash (CRM 490) has been performed according to the recommendations provided in ISO GUIDE 35 - 1985 and in particular in section 8: Certification by interlaboratory testing.
The determination of PCDD and PCDF in a solid matrix requires the use of complex analytical procedures including numerous sources of possible systematic errors. Different methods used in laboratories working independently and which had proven a priori their ability to perform the requested task were applied in the certification. Consequently, it may be accepted that the remaining systematic errors which cannot be detected and quantified are randomised.
As on purpose different methods were used, the certification of the PCDD and PCDF in the fly ash was based on the laboratory means rather than on all individual data.

Prior to the statistical treatment of the results a technical evaluation has been performed to assess the reliability of the applied methods and exclude technically explainable outliers.

The sets of results accepted after the technical evaluation have been further submitted to the following statistical tests:

- Kolmogorov-Smirnov-Lilliefors tests to assess the conformity of the distributions of laboratory means to normal distributions;
- Dixon and Nalimov tests to detect "outlying" values in the population of laboratory means;
- Cochran test to detect "outlying" values in the laboratory variances;
- Bartlett test to assess the overall consistency of the variance values obtained in the participating laboratories;
- Snedecor F-test to check if the between laboratory variance is significant;
- Scheffe test to estimate the two by two compatibility of individual data sets.

a) For each compound the unweighted arithmetic mean value of laboratory means, the 95% confidence interval and the 95% tolerance interval were calculated.

b) In case the Dixon and Nalimov tests identify an outlying set of data after the technical evaluation of the results, the property to which this set belongs is not certified.

c) For the Cochran test, the criterion was adopted that an outlier of variance would be discarded only if the standard error of the mean ($s/\sqrt{n_i}$) of the set of data exceeds the standard deviation of the distribution of all laboratory mean values.

A summary of the data and of the results of all the statistical tests is given in Table 6.2. The sets of results found acceptable on technical and statistical grounds are presented in Annex 1.

6.2.2 Detailed statistical discussion

No outlying mean values were detected (Dixon and Nalimov tests).
Outliers of variance were detected (Cochran test) for 2,3,7,8 - T4CDD; 1,2,3,6,7,8 - H6CDD; 1,2,3,7,8,9 - H6CDD; 2,3,4,7,8 - P5CDF; 1,2,3,6,7,8 -H6CDF; 1,2,3,7,8,9 - H6CDF and 2,3,4,6,7,8 - H6CDF.
All outliers except Lab 14 for 2,3,7,8 -T4CDD and Lab 15 for 1,2,3,6,7,8 -H6CDF satisfied the criteria for acceptance (see above section 6.2.1 c). The data of the excluded outliers are given in the corresponding tables but are not shown in the accompanying figure.

TABLE 6.2 : SUMMARY OF STATISTICAL RESULTS

CERTIFIED PROPERTY	2,3,7,8-T4CDD	1,2,3,7,8-P5CDD	1,2,3,4,7,8-H6CDD	1,2,3,6,7,8-H6CDD	1,2,3,7,8,9-H6CDD
Number of data sets	13	13	16	16	15
Number of individual data	68	70	85	85	80
Compatibility of data sets two by two - Scheffe's multiple t-test[1]	3/78	3/78	19/120	0/120	0/105
Outlying data sets (Dixon, Nalimov tests)[1]	NO	NO	NO	NO	NO
Outlying variances (Cochran test)[1]	YES	NO	NO	YES	YES
Mean of means of data sets (µg/kg)	0.16850	0.67196	0.94529	4.76987	2.83595
Within data sets standard deviation (µg/kg)	0.01333	0.04481	0.09819	0.48007	0.27428
Between data sets standard deviation (µg/kg)	0.01998	0.06639	0.19630	0.55415	0.27120
Homogeneity of variance (Bartlett test)[1]	NO	[2]	NO	NO	[2]
Standard deviation of the data set means (µg/kg)	0.01955	0.06495	0.19714	0.60082	0.29082
Normality of the distribution of the data set of means (Kolmogorov-Smirnov-Lilliefors test)[1]	YES	YES	YES	YES	YES
Half-width of the 95% confidence interval (µg/kg)	0.01181	0.03925	0.10505	0.32015	0.16105
Half-width of the 95% tolerance interval (µg/kg)	0.06526	0.14152	0.42020	1.28060	0.62374

[1]: tests performed at the 0.05 and 0.01 significance levels [2]: yes at 0.01 not at 0.05 significance level

TABLE 6.2 (contd): SUMMARY OF STATISTICAL RESULTS

CERTIFIED PROPERTY	2,3,7,8 T4CDF	1,2,3,7,8 P5CDF	2,3,4,7,8 P5CDF	1,2,3,4,7,8 H6CDF	1,2,3,6,7,8 H6CDF	1,2,3,7,8,9 H6CDF	2,3,4,6,7,8 H6CDF
Number of data sets	15	14	15	8	15	11	16
Number of individual data	79	74	80	40	79	59	85
Compatibility of data sets two by two - Scheffe's multiple t-test[1]	2/105	1/91	1/105	0/28	0/105	4/55	1/120
Outlying data sets (Dixon, Nalimov tests)[1]	NO	NO	NO	NO	NO	NO	NO
Outlying variances (Cochran test)[1]	NO	NO	YES	NO	NO	YES	YES
Mean of means of data sets (µg/kg)	0.89874	1.70611	1.85022	2.37266	2.64251	0.33609	2.47285
Within data sets standard deviation (µg/kg)	0.070370	0.17260	0.13909	0.20899	0.25455	0.04975	0.27537
Between data sets standard deviation (µg/kg)	0.08057	0.18390	0.18357	0.10129	0.21891	0.07708	0.28633
Homogeneity of variance (Bartlett test)[1]	YES	NO	NO	NO	NO	YES	NO
Standard deviation of the data set means (µg/kg)	0.08617	0.20413	0.19674	0.13782	0.25145	0.07286	0.31506
Normality of the distribution of the data set of means (Kolmogorov-Smirnov-Lilliefors test)[1]	YES	YES	YES	YES	YES	YES	YES
Half-width of the 95% confidence interval (µg/kg)	0.04772	0.11786	0.10895	0.11522	0.13925	0.04895	0.16789
Half-width of the 95% tolerance interval (µg/kg)	0.184826	0.44099	0.42196	0.32589	0.53931	0.1623	0.67156

[1]: tests performed at the 0.05 and 0.01 significance levels [2]: yes at 0.01 not at 0.05 significance level

7. CERTIFIED VALUES AND UNCERTAINTIES

The certified values for the mass fraction (in $\mu g\ kg^{-1}$, on dry weight basis) of five PCDDs (D48, D54, D66, D67, D70) and seven PCDFs (F83, F94, F114, F118, F121, F124, F130) in fly ash CRM 490 are shown in table 7-1. They correspond to the unweighed arithmetic mean of means of data sets that were found acceptable on technical and statistical grounds. The uncertainties of the certified values, also shown in table 7-1, are expressed as the half width of the 95 % confidence interval.

Table 7.1 Certified PCDD and PCDF content (mass fraction expressed as $\mu g/kg$) in fly ash CRM 490.

Compound	Certified value	Uncertainty (*)
2,3,7,8-T4CDD (D48)	0.169	0.012
1,2,3,7,8-P5CDD (D54)	0.67	0.04
1,2,3,4,7,8-H6CDD (D66)	0.95	0.11
1,2,3,6,7,8-H6CDD (D67)	4.8	0.4
1,2,3,7,8,9-H6CDD (D70)	2.84	0.17
2,3,7,8-T4CDF (F83)	0.90	0.05
1,2,3,7,8-P5CDF (F94)	1.71	0.12
2,3,4,7,8-P5CDF (F114)	1.85	0.11
1,2,3,4,7,8-H6CDF (F118)	2.37	0.12
1,2,3,6,7,8-H6CDF (F121)	2.64	0.14
1,2,3,7,8,9-H6CDF (F124)	0.34	0.05
2,3,4,6,7,8-H6CDF (F130)	2.47	0.17

(*) half width of the 95% confidence interval

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(4) Winand M., Mal N., Deblaton M.
Etude de faisabilité pour la préparation de solutions étalonnées de dioxines et autres substances toxiques analogues.
EUR report, **15258**, EC Brussels (1994)

9. INSTRUCTIONS FOR USE

Please consult these notes before opening the bottle of CRM 490. The guidelines given below are intended as an aid for the analytical chemist using CRM 490 for quality control purposes or method development.

- The reference material is a fly ash, ground to a particle size of < 125 μm. The bottle contains about 30 g of the material. The approximate total content of PCDDs and PCDFs, expressed as toxicity equivalents (TEC), amounts to 3.7 $\mu g\ kg^{-1}$.

- It should be realised that, due to the pretreatment (jet-milling etc.) of fly ash CRM 490, its physico-chemical behaviour may differ appreciably from that of the fly ash samples routinely measured in the laboratory. For a meaningful comparison the latter should also, to the extent possible, be pretreated in a similar way as CRM 490.

- Before attempting any sampling, the material should be allowed to attain room temperature and should be rehomogenised thoroughly, e.g. by manual or mechanical shaking during several minutes.

- It cannot be ruled out completely that uptake of water may give rise to 'sticking' of particles in incompletely tightened vials. Particularly in such case the water content should be verified on a separate portion of the material at each occasion of analysis.

- Storage of the bottles in the dark at 18 °C is recommended.

- The homogeneity of the fly ash was demonstrated at a 1 g sample intake level and it is therefore recommended that at least this mass of material should be used for analysis.

- Apart from the fact that adequate methods should be used regarding good laboratory practices, including blank controls, detector linearity checks, recovery determination and bracketing calibration runs, the precise and accurate determination of PCDDs and PCDFs in fly ash requires the availability of reliable gravimetrically prepared standards and a stringent control of integration and calculation procedures.

- To ensure complete extraction of the analytes, an acid pretreatment using hydrochloric or acetic acid followed by a prolonged (16-48 h) Soxhlet or reflux extraction with toluene or a toluene-based solvent mixture is recommended.

- Although the clean-up is linked to the use of low or high resolution mass spectrometric detection, there is in both cases a rather broad range of valid clean-up procedures, provided that appropriate capillary GC columns are used for the final measurement.

- The evaporation of extracts down to dryness is discouraged.

- With the actually available column technology, the chromatographic separation of the twelve certified congeners from other isomers and accompanying impurities requires the analysis to be performed on at least two capillary columns with different polarity. It has been experienced that the performance of commonly used columns such as SP 2331, CP SIL 88, DB DIOXIN or DB 5MS, is variable so that critical separations need consequent checking.

- If the reference material is used for quality control, the user should first check, by calculating the standard deviation of a set of measurements on identical samples, whether the repeatability achieved with his procedure satisfies the technical, economic or legal requirements. Guidance for comparing the obtained average with the certified value is given in ISO Guide 33.

THIS MATERIAL SHOULD NOT BE USED FOR CALIBRATION

ANNEX I

TABLES OF INDIVIDUAL RESULTS AND GRAPHICAL PRESENTATIONS

Figures

The length of a horizontal dotted bar corresponds to the 95% confidence interval
The vertical dotted line features the certified value (mean of means).

Tables

The laboratory code corresponds to the codes used in Tables in the text.

Table 1

2,3,7,8 - T4CDD (D48) in µg/kg

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
01	5	.17300	.14500	.16600	.15000	.14700	.15620	.01252
03	5	.17000	.13000	.17000	.16000	.15000	.15600	.01673
04	5	.17300	.17300	.17300	.17300	.17800	.17400	.00224
05	5	.16400	.18600	.19700	.19600	.16900	.18240	.01524
06	5	.14900	.14500	.15200	.14500	.15700	.14960	.00508
08	5	.18200	.17800	.17900	.19300	.17200	.18080	.00773
09	5	.15200	.15800	.13900	.11200	.12500	.13720	.01897
10	9	.12900 .13300	.13200 .14500	.14600 .11500	.14200 .13000	.13600	.13422	.00961
11	5	.19900	.18600	.18000	.17500	.17200	.18240	.01069
12	5	.17600	.16600	.19700	.19700	.18700	.18460	.01354
13	4	.20900	.13400	.18400	.19000		.17925	.03199
14	5	.21000	.12000	.20000	.14000	.24000	.18200	.05020
16	5	.18200	.18300	.17600	.17700	.17400	.17840	.00391
17	5	.19770	.19070	.20770	.19290	.18810	.19542	.00772

Figure 1

BAR-GRAPHS FOR LABORATORY MEANS AND 95% CI

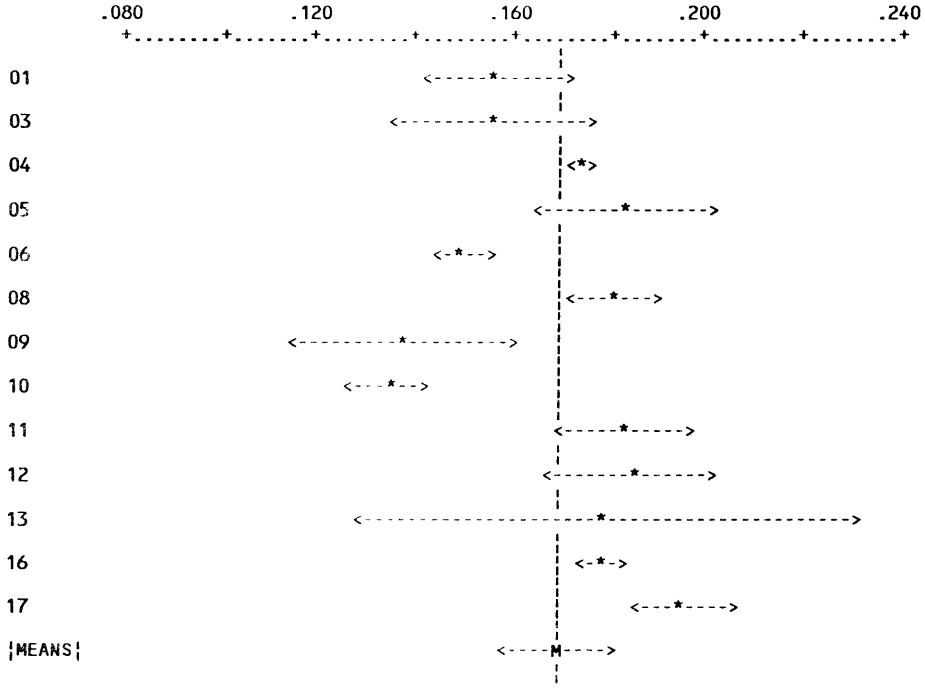


Table 2

1,2,3,7,8 - P5CDD (D54) in µg/kg

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
01	5	.69700	.60700	.57100	.50700	.59000	.59440	.06872
02	5	.74000	.74000	.75000	.76000	.73000	.74400	.01140
03	6	.52000 .49000	.69000	.62000	.60000	.63000	.59167	.07414
04	5	.61000	.56000	.62000	.68000	.72000	.63800	.06261
05	5	.61300	.65700	.67500	.69800	.60800	.65020	.03909
[illegible]	5	.68000	.65300	.62500	.67700	.57800	.64260	.04235
[illegible]	5	.75000	.72900	.77500	.74700	.75200	.75060	.01641
[illegible]	9	.51500 .56000	.55300 .57200	.62900 .46200	.56700 .54400	.60400	.55622	.04834
[illegible]	5	.63300	.70800	.73700	.70200	.65700	.68740	.04178
[illegible]	5	.68000	.70000	.73000	.76000	.71000	.71600	.03050
[illegible]	5	.70100	.69800	.75000	.72500	.72600	.72000	.02125
[illegible]	5	.74000	.69000	.66000	.68000	.71000	.69600	.03050
[illegible]	5	.74710	.71310	.75650	.74140	.78380	.74838	.02557

Figure 2

BAR-GRAPHS FOR LABORATORY MEANS AND 95% CI

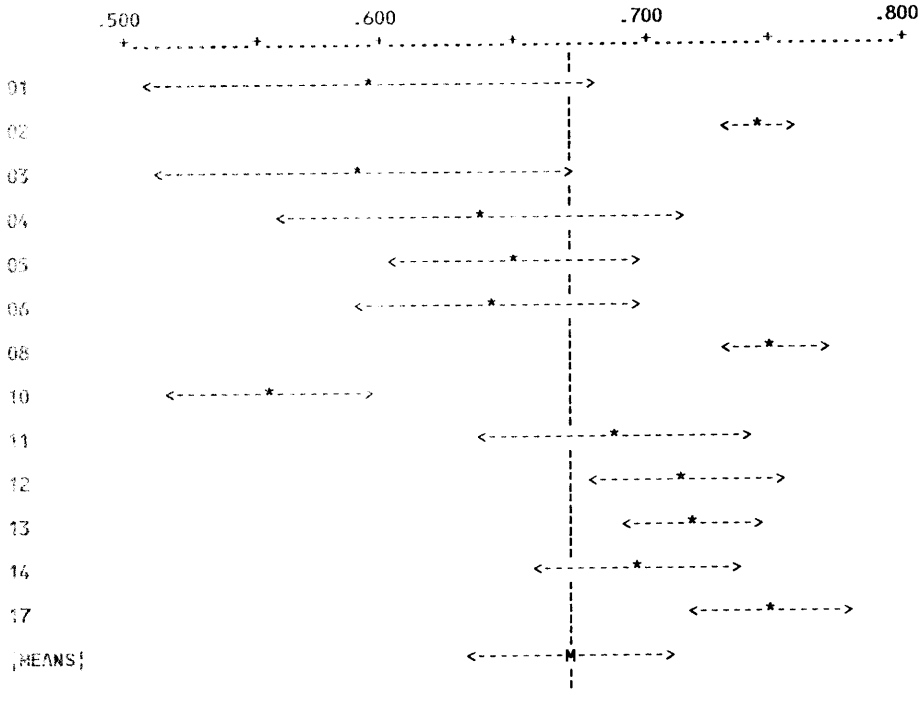


Table 3

1,2,3,4,7,8 - H6CDD (D66) in µg/kg

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
01	5	1.27400	1.25300	1.37500	1.05400	1.18800	1.22880	.11856
02	5	1.11000	1.06000	1.11000	1.06000	1.00000	1.06800	.04550
03	6	1.14000 1.23000	1.02000	.99000	1.11000	1.07000	1.09333	.08687
04	5	.69000	.70000	.57000	.75000	.61000	.66400	.07266
05	5	.80700	.90500	.84900	.89100	.77100	.84460	.05624
06	5	.83500	.82600	.77600	.89100	.92700	.85100	.05891
08	5	.99100	.99300	1.02000	.97200	.95700	.98660	.02380
09	5	.80500	.82300	.65100	.51400	.54600	.66780	.14290
10	9	.65000 .70700	.64900 .68600	.77300 .56400	.67300 .65400	.73400	.67667	.05945
11	5	.89900	.82200	.88700	.85300	.86000	.86420	.03023
12	5	1.01000	1.07000	1.14000	1.12000	.97000	1.06200	.07190
13	5	1.23200	1.10700	1.11100	.96600	1.16500	1.11620	.09806
14	5	.94000	.72000	.79000	.78000	.89000	.82400	.08905
15	5	1.33000	1.03000	1.27000	1.45000	1.55000	1.32600	.19769
16	5	.94100	.87200	.85700	.90500	.76300	.86760	.06682
17	5	1.04600	1.15240	.71570	1.12410	.88100	.98384	.18332

Figure 3

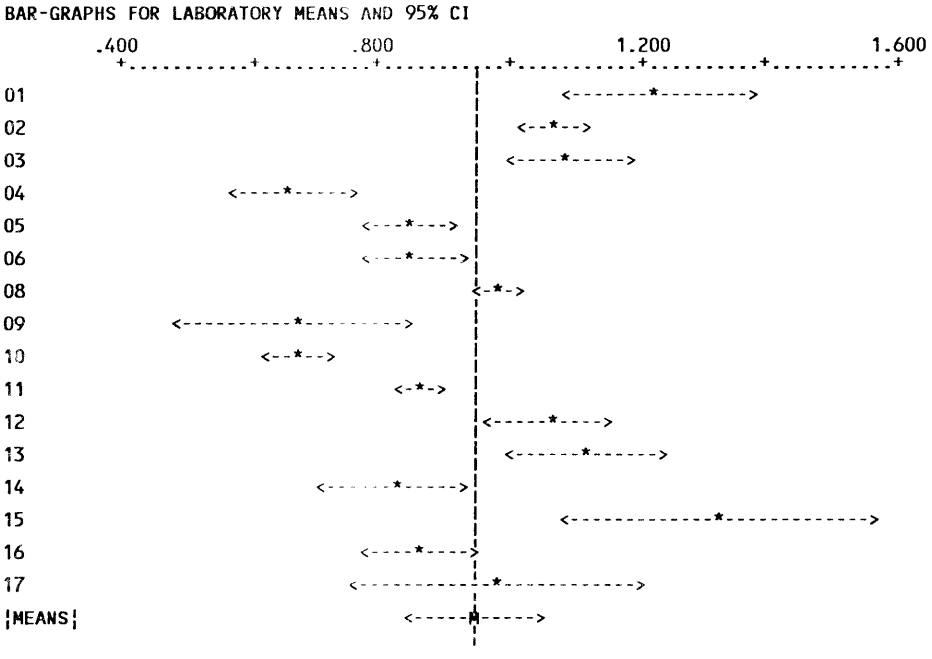


Table 4

1,2,3,6,7,8 - H6CDD (D67) in µg/kg

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
01	5	4.69100	4.79500	5.25100	4.14000	4.59800	4.69500	.39893
02	5	4.53000	4.29000	4.45000	4.50000	4.43000	4.44000	.09274
03	6	4.21000 4.70000	4.88000	4.30000	5.04000	5.45000	4.76333	.46616
04	5	5.13000	5.04000	5.01000	4.88000	5.04000	5.02000	.09028
05	5	4.23900	5.00900	4.84400	4.77200	4.20200	4.61320	.36887
06	5	4.26500	4.07200	3.72700	4.40400	4.33400	4.16040	.27206
07	5	4.93000	5.25000	5.25000	5.89000	5.25000	5.31400	.35054
08	5	5.24000	5.39000	5.62000	5.20000	5.30000	5.35000	.16703
09	5	4.22700	4.18200	3.96400	3.42200	3.59000	3.87700	.35794
10	9	3.68000 4.53000	4.11000 4.47000	4.33000 4.45000	4.13000 4.27000	4.70000	4.29667	.29904
11	5	3.93300	3.91600	4.11700	3.70300	4.01900	3.93760	.15357
12	5	5.70000	5.29000	5.91000	6.01000	5.60000	5.70200	.28208
13	5	5.16200	4.79200	4.76200	4.99700	4.92700	4.92800	.16246
14	5	5.06000	3.77000	3.25000	4.42000	4.22000	4.14400	.68193
15	5	5.31000	4.45000	4.95000	7.07000	6.51000	5.65800	1.09559
17	5	5.93260	6.57750	3.85710	5.68680	5.03960	5.41872	1.03212

Figure 4

BAR-GRAPHS FOR LABORATORY MEANS AND 95% CI

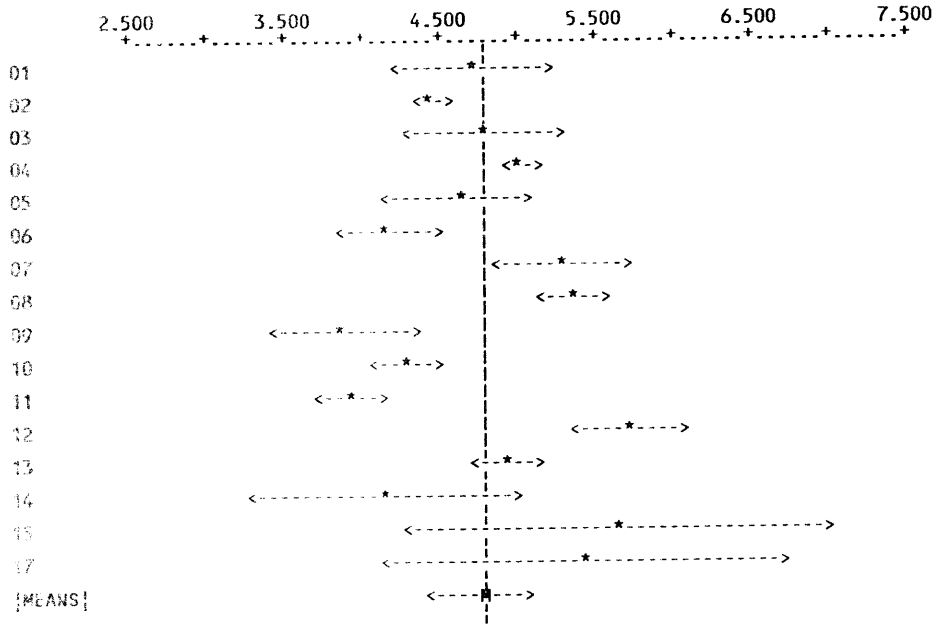


Table 5

1,2,3,7,8,9 - H6CDD (D70) in µg/kg

DATA SET	NUM	REPLICATES.....					MEAN	ST.DEV
01	5	3.02500	3.00000	3.31400	2.56700	2.82600	2.94640	.27506
03	6	2.17000 2.24000	2.34000	2.11000	3.01000	3.09000	2.49333	.43866
04	5	3.43000	2.94000	2.93000	3.06000	3.23000	3.11800	.21230
05	5	2.48800	2.83000	2.77500	2.88100	2.44400	2.68360	.20274
06	5	2.62800	2.47400	2.37600	2.68800	2.85100	2.60340	.18542
07	5	2.97000	2.97000	3.25000	3.65000	3.25000	3.21800	.27914
08	5	2.91000	2.97000	3.09000	2.92000	2.88000	2.95400	.08264
10	9	2.41000 2.59000	2.54000 2.34000	2.78000 2.27000	2.42000 2.20000	2.64000	2.46556	.18709
11	5	2.22500	2.45800	2.49800	2.12500	2.17500	2.29620	.17027
12	5	3.13000	3.07000	3.36000	3.10000	3.22000	3.17600	.11718
13	5	3.07100	2.72600	2.61700	3.17200	2.90300	2.89780	.23107
14	5	3.05000	2.46000	2.42000	2.75000	2.67000	2.67000	.25367
15	5	3.33000	2.12000	2.57000	3.57000	2.94000	2.90600	.58149
16	5	3.66000	3.18000	3.21000	3.24000	2.86000	3.23000	.28496
17	5	2.87800	2.99900	2.77800	2.57500	3.17500	2.88100	.22617

Figure 5

BAR-GRAPHS FOR LABORATORY MEANS AND 95% CI

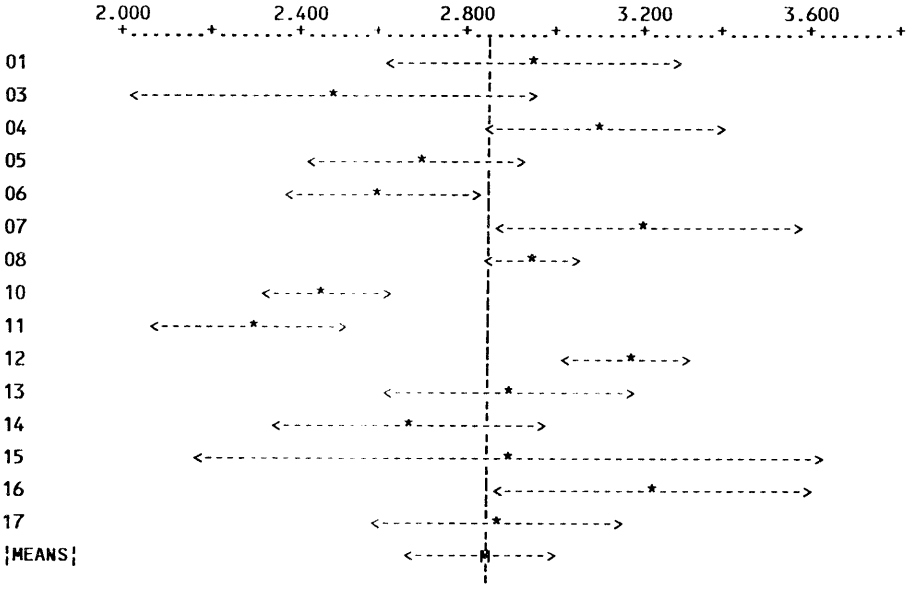


Table 6

2,3,7,8 - T4CDF (F83) in µg/kg

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
01	5	.87600	.84800	.92600	.86800	.88700	.88100	.02891
02	4	.95000	.90000	.93000	.94000		.93000	.02160
03	6	.88000 .76000	.72000	.90000	.92000	.93000	.85167	.08909
04	5	.71000	.70000	.75000	.80000	.86000	.76400	.06656
05	5	.76700	.86600	.83600	.84100	.73200	.80840	.05633
06	5	.89800	.85400	.87100	.92600	.99100	.90800	.05385
08	5	1.06000	1.14000	1.15000	1.15000	1.05000	1.11000	.05050
09	5	.92000	.85600	.95100	.78400	.78100	.85840	.07730
10	9	.76500 .84900	.74000 .76900	.83900 .70000	.80200 1.04000	.81700	.81344	.09745
11	5	.85200	.90800	.89300	.87700	.80300	.86660	.04116
12	5	.98000	.90000	1.03000	.86000	.98000	.95000	.06856
13	5	1.09800	.89800	.96100	.94400	1.11300	1.00280	.09669
15	5	1.01000	.88000	.77000	1.01000	.96000	.92600	.10213
16	5	.89600	.87000	.86600	.83800	.80200	.85440	.03579
17	5	1.02210	.98750	.90150	.90880	.96190	.95636	.05146

Figure 6

BAR-GRAPHS FOR LABORATORY MEANS AND 95% CI

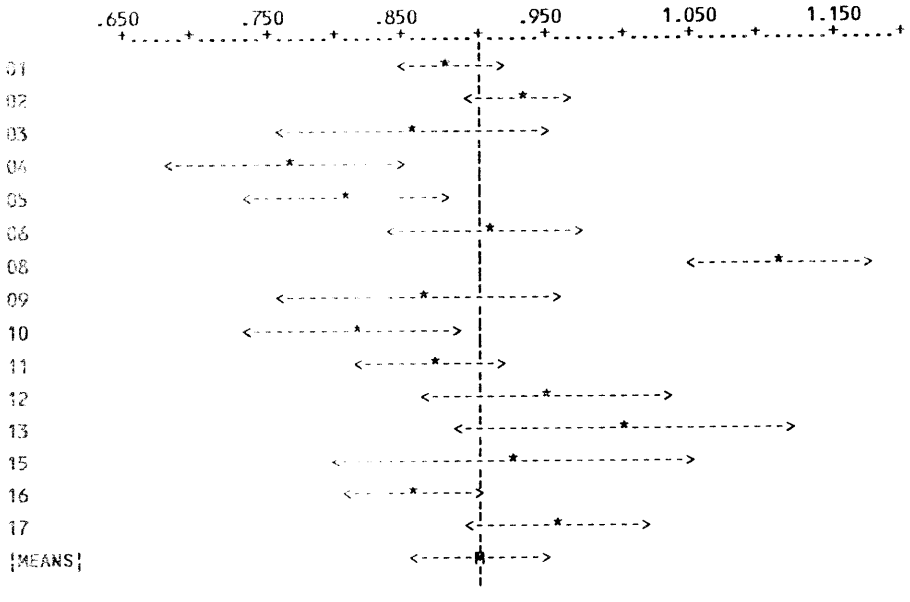


Table 7

1,2,3,7,8 - P5CDF (F94) in µg/kg

DATA SET	NUM	REPLICATES.....					MEAN	ST.DEV
04	5	1.50000	1.48000	1.58000	1.42000	1.66000	1.52800	.09338
05	5	1.58600	1.64700	1.74600	1.53300	1.43600	1.58960	.11680
06	5	1.67900	1.60400	1.67600	1.67700	1.68000	1.66320	.03313
07	5	1.50000	1.31000	1.77000	1.77000	1.77000	1.62400	.21090
08	5	1.84000	1.81000	1.86000	2.00000	1.93000	1.88800	.07662
09	5	1.57000	1.58900	1.28700	.90000	.92900	1.25500	.33321
10	9	1.52000 1.85000	1.57000 1.66000	1.54000 1.67000	1.79000 1.73000	1.79000	1.68000	.11906
11	5	1.71600	1.52100	1.76200	1.76300	1.57700	1.66780	.11186
12	5	1.81000	1.67000	1.72000	2.00000	1.91000	1.82200	.13517
13	5	1.82600	1.95400	1.78400	1.63300	1.79500	1.79840	.11462
14	5	1.94000	2.05000	1.98000	2.34000	2.07000	2.07600	.15662
15	5	2.42000	1.82000	1.72000	1.91000	1.56000	1.88600	.32555
16	5	2.07000	2.01000	1.85000	1.79000	1.65000	1.87400	.16935
17	5	1.31020	1.49710	1.44360	1.73770	1.67940	1.53360	.17482

Figure 7

BAR-GRAPHS FOR LABORATORY MEANS AND 95% CI

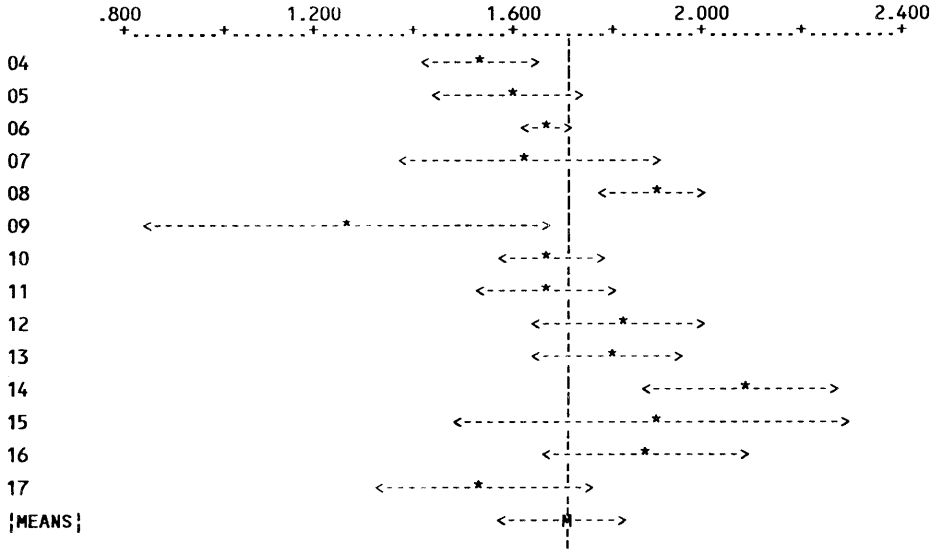


Table 8

2,3,4,7,8 - P5CDF (F114) in µg/kg

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
01	5	2.20000	1.87900	2.07100	1.94300	1.97100	2.01280	.12545
02	5	1.83000	1.63000	1.81000	1.85000	1.72000	1.76800	.09176
03	6	1.44000 1.53000	1.68000	1.63000	1.79000	1.56000	1.60500	.12276
04	5	1.96000	1.80000	1.36000	1.45000	1.98000	1.71000	.28879
05	5	1.76900	1.97000	1.87300	1.83100	1.73600	1.83580	.09198
06	5	1.79600	1.73500	1.67600	1.66700	1.62200	1.69920	.06743
08	5	2.12000	2.11000	2.23000	2.09000	2.10000	2.13000	.05701
09	5	1.77000	1.88900	1.63900	1.19800	1.34100	1.56740	.29039
10	9	1.66000 1.86000	1.82000 1.80000	1.66000 1.77000	1.82000 1.72000	1.89000	1.77778	.08258
11	5	1.63500	1.70600	1.66900	1.64100	1.58100	1.64640	.04610
12	5	1.87000	1.83000	2.09000	2.17000	1.93000	1.97800	.14601
13	5	2.10700	2.11100	2.05700	2.24800	2.37500	2.17960	.13026
14	5	2.25000	1.91000	1.95000	2.10000	2.00000	2.04200	.13627
16	5	1.88000	1.85000	1.79000	1.72000	1.61000	1.77000	.10840
17	5	2.06550	2.02180	2.09490	1.94890	2.02550	2.03132	.05506

Figure 8

BAR-GRAPHS FOR LABORATORY MEANS AND 95% CI

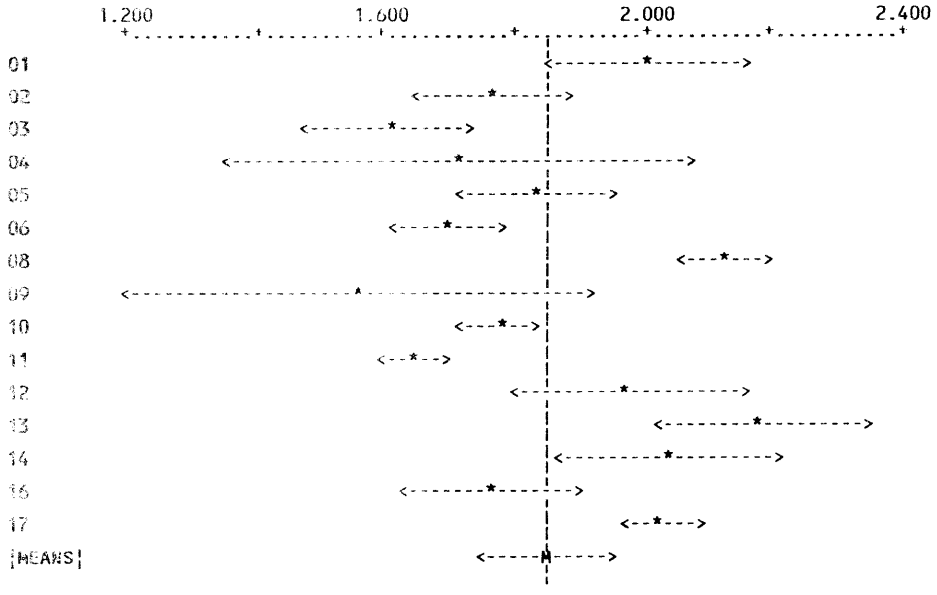


Table 9

1,2,3,4,7,8 - H6CDF (F118) in µg/kg

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
04	5	2.22000	2.32000	2.35000	2.30000	2.27000	2.29200	.04970
05	5	2.18000	2.34000	2.26000	2.20000	1.97000	2.19000	.13784
06	5	2.45400	2.39100	2.31500	2.55700	2.49600	2.44260	.09356
08	5	2.64000	2.72000	2.50000	2.54000	2.53000	2.58600	.09154
11	5	2.12200	2.44000	2.38000	2.23100	2.43700	2.32200	.14035
13	5	2.81800	2.64200	2.59700	1.96900	2.59100	2.52340	.32334
16	5	2.59000	2.47000	2.39000	2.42000	2.05000	2.38400	.20169
17	5	1.87060	2.09440	1.97320	2.78260	2.48540	2.24124	.38192

Figure 9

BAR-GRAPHS FOR LABORATORY MEANS AND 95% CI

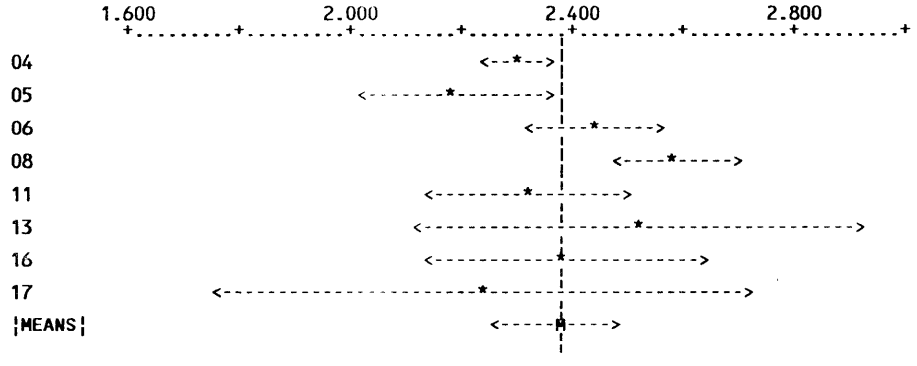


Table 10

1,2,3,6,7,8 - H6CDF (F121) in µg/kg

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
01	5	2.55200	2.41000	2.62800	2.25700	2.43000	2.45540	.14247
02	5	2.49000	2.47000	2.46000	2.64000	2.59000	2.53000	.08031
03	6	2.73000 2.29000	2.85000	3.05000	3.09000	2.72000	2.78833	.28972
04	5	2.46000	2.46000	2.51000	2.47000	2.56000	2.49200	.04324
05	5	2.64100	2.99600	2.74000	2.80400	2.49400	2.73500	.18697
06	5	2.48900	2.46900	2.53000	2.73000	2.65000	2.57360	.11214
08	5	3.00000	2.98000	3.16000	3.20000	2.99000	3.06600	.10526
09	5	2.61400	2.61800	1.94000	1.44900	1.82700	2.08960	.51379
10	9	2.42000 2.67000	2.80000 2.85000	2.52000 2.37000	2.73000 2.39000	2.48000	2.58111	.18415
11	5	2.51200	2.48500	2.48400	2.28000	2.57800	2.46780	.11172
12	5	2.91000	2.88000	3.04000	3.11000	2.83000	2.95400	.11675
13	4	2.79300	2.75700	2.15800	2.27100		2.49475	.32721
14	5	3.12000	3.32000	3.26000	2.59000	2.74000	3.00600	.32401
15	5	3.24000	2.35000	1.24000	2.21000	1.60000	2.12800	.76810
16	5	3.01000	2.86000	2.70000	2.75000	2.38000	2.74000	.23377
17	5	2.66410	3.33150	1.95090	2.81300	2.56050	2.66400	.49673

Figure 10

BAR-GRAPHS FOR LABORATORY MEANS AND 95% CI

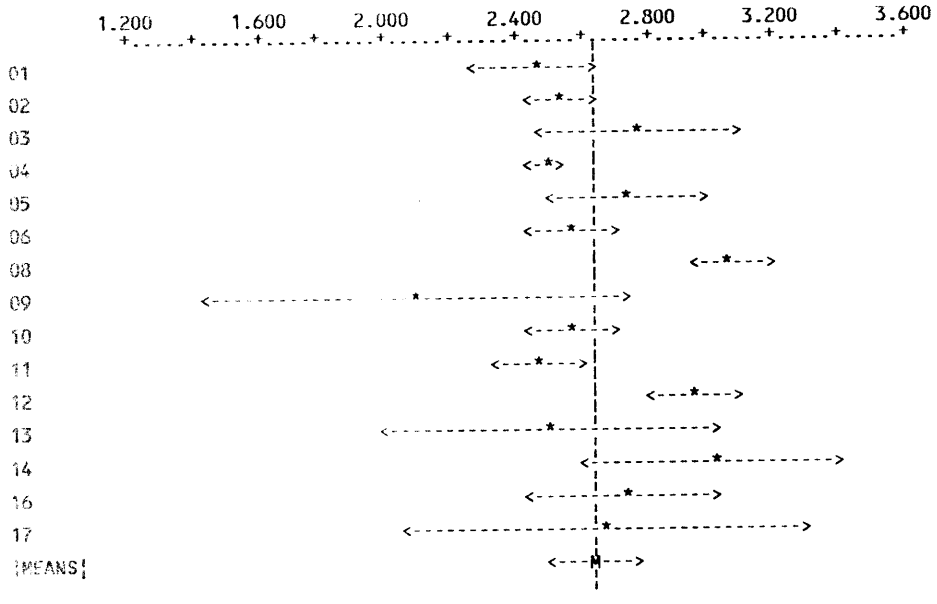


Table 11

1,2,3,7,8,9 - H6CDF (F124) in µg/kg

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
01	5	.34100	.31600	.37100	.29500	.32000	.32860	.02878
04	5	.36000	.33000	.35000	.33000	.35000	.34400	.01342
05	5	.28600	.31400	.30000	.30500	.27200	.29540	.01655
06	5	.34800	.30500	.33200	.32000	.30500	.32200	.01843
08	5	.37400	.41600	.41600	.37700	.36200	.38900	.02528
09	5	.33800	.34300	.24400	.22500	.26500	.28300	.05439
10	9	.17200 .24300	.19400 .14400	.19300 .18000	.16300 .21000	.22400	.19144	.03090
11	5	.28400	.29600	.30400	.29300	.29200	.29380	.00722
12	5	.42000	.41000	.42000	.50000	.39000	.42800	.04207
13	5	.64900	.51600	.43300	.28100	.37400	.45060	.14016
17	5	.35430	.37110	.42730	.34140	.36140	.37110	.03323

Figure 11

BAR-GRAPHS FOR LABORATORY MEANS AND 95% CI

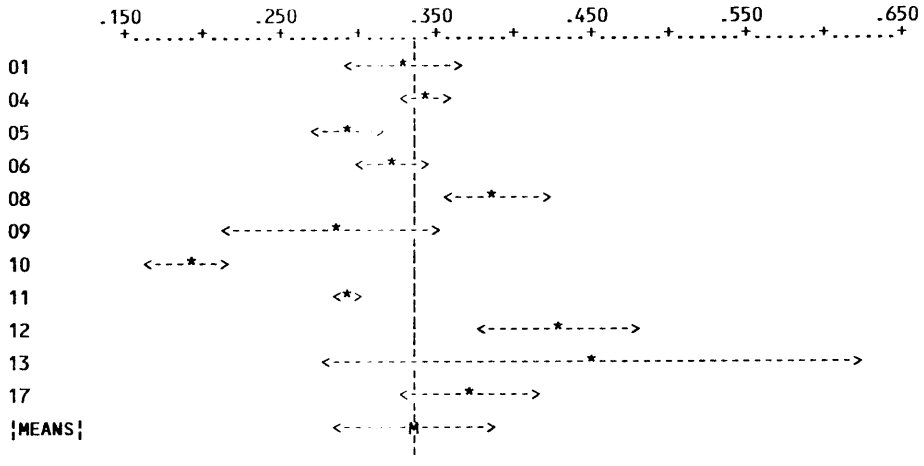


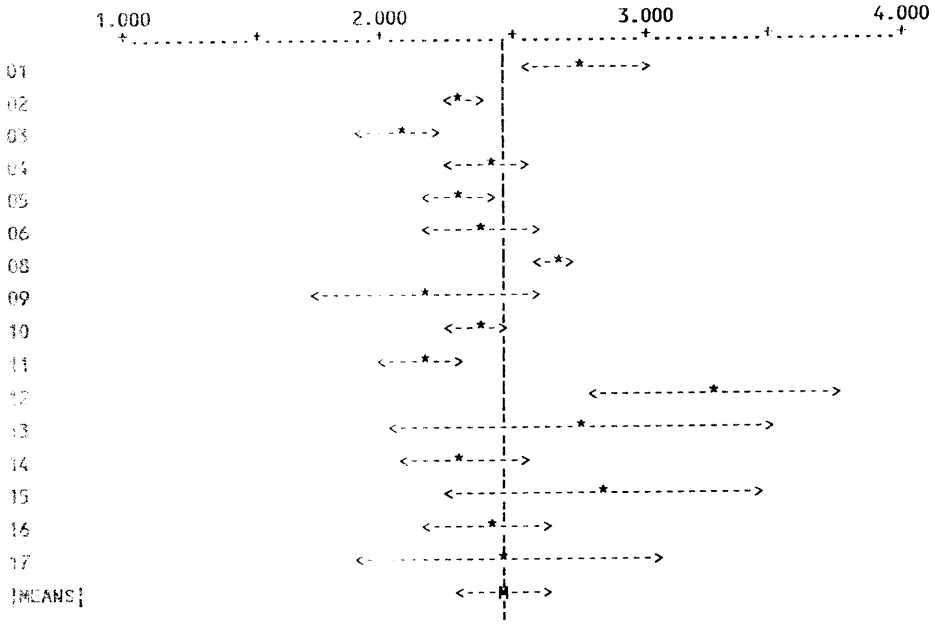
Table 12

2,3,4,6,7,8 - H6CDF (F130) in µg/kg

DATA SET	NUM	REPLICATES					MEAN	ST.DEV
01	5	2.88800	2.76200	3.00800	2.53700	2.66300	2.77160	.18463
02	5	2.35000	2.36000	2.28000	2.31000	2.22000	2.30400	.05683
03	6	1.98000 2.20000	2.04000	1.86000	2.06000	2.18000	2.05333	.12691
04	5	2.58000	2.29000	2.42000	2.30000	2.45000	2.40800	.11946
05	5	2.23100	2.39900	2.31800	2.40700	2.17200	2.30540	.10317
06	5	2.39200	2.11700	2.30900	2.42900	2.56600	2.36260	.16571
08	5	2.61000	2.64000	2.70000	2.69000	2.62000	2.65200	.04087
09	5	2.45200	2.58900	1.92700	1.78900	2.07100	2.16560	.34242
10	9	2.16000 2.45000	2.42000 2.48000	2.27000 2.62000	2.33000 2.27000	2.16000	2.35111	.15415
11	5	2.25000	2.14400	2.19200	1.96300	2.15400	2.14060	.10764
12	5	3.40000	3.61000	3.56000	3.10000	2.70000	3.27400	.37760
13	5	3.78600	2.65700	2.65900	2.27300	2.41200	2.75740	.59827
14	5	2.55000	2.28000	2.45000	2.10000	2.15000	2.30600	.19217
15	5	3.14000	2.42000	2.27000	3.02000	3.40000	2.85000	.48394
16	5	2.58000	2.50000	2.36000	2.41000	2.12000	2.39400	.17487
17	5	2.67510	2.96700	1.84080	2.73680	2.12980	2.46990	.46687

Figure 12

BAR-GRAPHS FOR LABORATORY MEANS AND 95% CI



European Commission

EUR 16888

The certification of the contents (mass fractions) of five Polychlorodibenzo-p-Dioxins (D48, D54, D66, D67, D70) and seven Polychlorodibenzo Furans (F83, F94, F114, F118, F121, F124, F130) in fly ash - CRM 490

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BCR information series

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This report describes the preparation, homogeneity and stability of a fly ash reference material and the subsequent certification of the mass fraction of five polychlorodibenzo-p-dioxins (D48, D54, D66, D67, D70) and seven polychlorodibenzo furans (F83, F94, F114, F118, F121, F124, F130).

The material, CRM 490, is intended for use in the method validation and quality control of the complete analytical procedure for congener-specific determination of dioxins.

The certification was based on an interlaboratory study. All individual results which were found acceptable after detailed technical and statistical evaluation are listed, together with a summary of the analytical techniques used and the quality control guidelines followed.

The certified PCDD and PCDF content (mass fraction expressed as μg/kg) in fly ash CRM 490 is given in the table below, together with the uncertainty (half width of the 95 % confidence interval).

COMPOUND	CERTIFIED VALUE	UNCERTAINTY
2,3,7,8-T4CDD	0.169	0.012
1,2,3,7,8-P5CDD	0.67	0.04
1,2,3,4,7,8-H6CDD	0.95	0.11
1,2,3,6,7,8-H6CDD	4.8	0.4
1,2,3,7,8,9-H6CDD	2.84	0.17
2,3,7,8-T4CDF	0.90	0.05
1,2,3,7,8-P5CDF	1.71	0.12
2,3,4,7,8-P5CDF	1.85	0.11
1,2,3,4,7,8-H6CDF	2.37	0.12
1,2,3,6,7,8-H6CDF	2.64	0.14
1,2,3,7,8,9-H6CDF	0.32	0.05
2,3,4,6,7,8-H6CDF	2.47	0.17

An indicative (non-certified) value was assigned to the mass fraction of the hepta- and octachlorinated congeners D73, D75, F131, F134 and F135.