# Fly ash CRM 490: a new BCR certified reference material for PCDD/PCDF analysis

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## 1. Introduction

Municipal waste incineration is one of the main known sources of environmental pollution with dioxins, and world-wide legislative and technical efforts are aiming at the reduction of these emissions. 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 polychlorinated dibenzo-p-dioxins (PCDDs) and dibenzofurans (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.

Following the successful preparation and certification of a crude fly ash extract for PCDDs and PCDFs in 1993 [1], which enables to check clean-up and instrumental procedures, a project was issued under the EC third framework (Measurements and Testing) programme to proceed to a certified fly ash. Fly ash has a PCDD and PCDF pattern that is representative for emission samples, and is an inexpensive and stable material. A certified value for the content of each of the tetra-, penta- and hexachlorinated congeners with 2,3,7,8-chlorine substitution was aimed at. The underlying principle on which the certification was based 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 that were selected for the interlaboratory study already had been involved in an earlier step-by-step feasibility study supported by the EC [2].

## 2. Participants in the project

*Preparation of the material:* Vlaamse Instelling voor Technologisch Onderzoek (VITO), Mol (BE); Institute for Reference Materials and Measurements (IRMM), Geel (BE)

*Certification measurements:* BASF, Ludwigshafen (DE); Bayer, Leverkusen (DE); CARSO, Vernaison (FR); Centro de Investigación y Desarrollo (CID-CSIC), Barcelona (ES); Elf Atochem, Levallois-Perret (FR); ENEL - Centro Ricerca Termica, Pisa (IT); The Finnish Pulp and Paper Research Institute, Espoo (FI); Istituto di Ricerche Farmacologiche "Mario Negri", Milano (IT); Ministry of Agriculture, Fisheries and Food (MAFF) - CSL, Norwich (GB); Solvay Duphar, Weesp (NL); TNO - Instituut voor Milieu-wetenschappen (IMW), Delft (NL); Institute of Occupational Health, Helsinki (FI); Universiteit van Amsterdam, Amsterdam (NL); Universität Ulm, Ulm (DE);

University of Umeå - Institute of Environmental Chemistry, Umeå (SE); Vlaamse Instelling voor Technologisch Onderzoek (VITO), Mol (BE); Zeneca Specialties, Manchester (GB)

3. Preparation of the material

The fly ash was collected end February 1991 from an at the time relatively modern municipal waste incinerator. After preliminary analysis and homogenisation using a Turbula mixer, the whole amount was sieved to less than 1 mm. Fine grinding of the fly ash was carried out using a jet mill; this resulted in a total amount of ground fly ash powder < 125 µm of about 36 kg. After further homogenisation, the powder was finally divided in batches using a laboratory sample divider and bottled in 30 g amounts into 60 ml brown glass bottles.

A homogeneity study was set up using 20 units in their final packaged form, randomly selected during the bottling process. From each of 2 bottles, five sub-samples were analysed to obtain an estimate of the within-bottle homogeneity. One sub-sample from each of the remaining 18 bottles was analysed to estimate the between-bottle homogeneity. A validated laboratory procedure was applied, consisting of treatment with 4% hydrochloric acid, addition of $^{13}C$-labeled isomers, soxhlet extraction with toluene, clean-up on basic alumina and determination by gas chromatography - high resolution mass spectrometry on two polar columns with different stationary phases. In both within-bottle homogeneity tests the coefficient of variation (CV) amounted to 2 - 10 % for the various congeners. Generally the between-bottle CV values appeared slightly higher, ranging between 4 and 14 %. When for each congener the between-bottle variance was compared with the average of both within-bottle values, a one-tailed F-test at the 95 % confidence level demonstrated that any increase of the variability was not significant. It was concluded that the homogeneity at a 1 g sample intake level was satisfactory for certification of PCDDs and PCDFs.

For stability monitoring, 3 times 10 units were stored in the dark at -20, 20 and 40 °C, respectively. After 6 months and after 12 months a set of 5 bottles at each temperature was taken for analysis. To verify the stability, both after 6 and 12 months the ratio R of the mean value 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. The uncertainty U was derived from the coefficient of variation of each set of measurements, according to the following example:

$$U_{(6\ m.\ 20\ °C)} = (CV^2_{(6\ m.\ 20\ °C)} + CV^2_{(6\ m.\ -20\ °C)})^{1/2} \cdot R_{(6\ m.\ 20\ °C)} / 100$$

In all cases the interval [R-U, R+U] contained the value 1, which is the expected value for ideal stability; consequently it was concluded that the PCDD and PCDF content in the material remained stable after 6 and 12 months of storage at 20 or 40 °C.

4. Certification measurements

*General*

To assist the participants in the interlaboratory study 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 beforehand. Each laboratory was requested to determine at least the twelve most toxic PCDDs and PCDFs and invited to determine also the five hepta- and octachlorinated 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 to be 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. Pre-treatment, 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, but each

laboratory optimised its own 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 the (non-certified) reference materials BCR RMs 432 to 443 [3] and an ampoule of a mixture of 6 $^{13}C$- labelled isomers, one for each product class and each chlorination degree. They were used for calibration or for checking the laboratory's own calibrants. 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 was provided.

*Analytical methods*

All participants applied an acid treatment, prior to and/or during extraction, to destroy the matrix structure of the fly ash. Table 1 summarises the acid pre-treatment and extraction procedures, and gives details on the use of internal standards. 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. The techniques applied were similar to the ones for fly ash extract BCR CRM 429 [1]: chromatography (using single- or multi-layer columns, open or flow-controlled) on alumina, silica, florisil, carbon (with celite or glass fibres), acid- or base-impregnated silica or celite, silica impregnated with silver nitrate, C18-silica, and batch treatment with acid or base.

Instrumental analysis of the purified extracts was based on capillary gas chromatography with mass spectrometric detection (GC-MS). At least two GC columns with different stationary phase 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 GC stationary phases applied were: BPX-5, CP SIL 8, CP SIL 88, DB 5, DB 5 MS, DB DIOXIN, RTX 5, RTX 2330, SP 2330 and SP 2331. A wide variety of injection techniques (splitless, on column, programmed temperature vaporisation), and both low and high resolution mass spectrometric detection were employed. All mass spectrometers were used in the electron impact mode, with selected ion monitoring of at least two abundant masses for each native and labeled congener.

*Quality assurance and quality control*

The congeners were identified by comparing the relative retention times of the peaks in the sample chromatograms and the calibration chromatograms. 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. 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 relied on peak area measurement, taking into account two isotope peaks. It was performed according to the internal standardisation method, using within each isomeric group at least one $^{13}C$-labeled isomer. Congener-specific relative response factors (RRF's) were derived from each measurement of a calibration solution. As a quality control parameter, the recovery of the $^{13}C$- labeled internal standards was estimated directly from the GC-MS run of the sample replicate. This was done either by quantification versus one or more additional $^{13}C$- labelled internal standards (such as $^{13}C$-1,2,3,4-TCDD) spiked immediately before GC-MS injection, or by external calibration. It was also requested to check, by a separate standard addition experiment, whether the recovery of 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

Table 1: Summary of acid treatment and extraction procedures applied

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

Legend:

- number of internal standards: for target congeners (+ for supplementary congeners, if analysed)
- stage of introduction of I.S.:
  - I: prior to the acid treatment
  - II: prior to extraction but after acid treatment
  - III: prior to clean-up but after extraction
- methods for acid treatment:
  - A1: pre-treatment with dilute (1-3 molar) hydrochloric acid
  - A2: treatment with hydrochloric acid during extraction
  - A3: pre-treatment with concentrated hydrochloric acid
  - A4: pre-treatment with glacial acetic acid
- isolation of fly ash and/or water:
  - I1: filtration, rinsing, drying
  - I2: 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

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.

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.

A voluntary control of the clean-up and quantification procedures could be carried out by analysis of the certified fly ash extract BCR CRM 429, which was made available to all participants. 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.

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 mean water content determined in the different laboratories, amounted to 1.7 % with a standard deviation of 0.5 %.

## 5. Certified values and uncertainties

All methods and results were scrutinised at a technical evaluation meeting. Good analytical quality control, in accordance with the demands of certification, and implementation of the guidelines outlined in the protocol for analysis were a prerequisite for acceptance of data for certification. The most common explanations for rejection of data were chromatographic interferences, insufficient signal for accurate quantification, and unconvincing evidence on the control of the GC-MS instrumental performance or on the reliability of the calibration.

The sets of results accepted after the technical evaluation were further submitted to the following statistical tests: Kolmogorov-Smirnov-Lilliefors (conformity of the distributions of laboratory means to normal distributions), Dixon and Nalimov (outlying laboratory means), Cochran (outlying variances), Bartlett (overall consistency of the variance values obtained in the participating laboratories), Snedecor F-test (significance of between laboratory variance), Scheffe (two by two compatibility of individual data sets). For the Cochran test, the criterion was adopted that an outlier of variance would be discarded only if the standard error of the mean of the set of data exceeds the standard deviation of the distribution of all laboratory mean values.

The statistical analysis confirmed the feasibility of certification for all twelve target congeners. The certified values for the mass fraction (in $\mu g\ kg^{-1}$, on dry weight basis) are shown in table 2. 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 2, are expressed as the half width of the 95 % confidence interval.

The quality of the available data for hepta- and octachlorinated congeners, which could be determined on a free 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. In view of the reasonable between-laboratory agreement, indicative (non-certified) values were assigned to these congeners. The calculated indicative values (unweighed arithmetic mean of the laboratory means) and uncertainties (half width of the 95 % confidence interval) are listed in table 3.

Table 2: Certified PCDD and PCDF content (mass fraction expressed as μg $kg^{-1}$) in fly ash CRM 490

| COMPOUND | CERTIFIED VALUE | UNCERTAINTY |
|---|---|---|
| 2,3,7,8-TCDD | 0.169 | 0.012 |
| 1,2,3,7,8-PCDD | 0.67 | 0.04 |
| 1,2,3,4,7,8-HxCDD | 0.95 | 0.11 |
| 1,2,3,6,7,8-HxCDD | 4.8 | 0.4 |
| 1,2,3,7,8,9-HxCDD | 2.84 | 0.17 |
| 2,3,7,8-TCDF | 0.90 | 0.05 |
| 1,2,3,7,8-PCDF | 1.71 | 0.12 |
| 2,3,4,7,8-PCDF | 1.85 | 0.11 |
| 1,2,3,4,7,8-HxCDF | 2.37 | 0.12 |
| 1,2,3,6,7,8-HxCDF | 2.64 | 0.14 |
| 1,2,3,7,8,9-HxCDF | 0.34 | 0.05 |
| 2,3,4,6,7,8-HxCDF | 2.47 | 0.17 |

Table 3: Indicative values (mass fraction expressed as μg $kg^{-1}$) for hepta- and octachlorinated congeners in fly ash CRM 490

| COMPOUND | INDICATIVE VALUE | UNCERTAINTY |
|---|---|---|
| 1,2,3,4,6,7,8-HpCDD | 31 | 3 |
| OCDD | 49 | 5 |
| 1,2,3,4,6,7,8-HpCDF | 9.2 | 1.0 |
| 1,2,3,4,7,8,9-HpCDF | 1.58 | 0.22 |
| OCDF | 4.3 | 0.8 |

6. References


[1] Maier E.A., Griepink B., Hinschberger J., Rymen T. (1993): The certification of five polychlorodibenzo-p-dioxins (D48, D54, D66, D67, D70) and six polychlorodibenzofurans (F83, F94, F114, F121, F124, F130) in a fly ash extract. EUR report, 15038, CEC Brussels

[2] Rymen T., Hinschberger J., Maier E.A., Griepink B. (1992): The quantitative determination of PCDD and PCDF: improvement of the analytical quality up to a level acceptable for certification of certified reference materials. EUR report, 14357, CEC Brussels

[3] Rymen T., Belliardo J.J., Griepink B., Maier E.A., Mal N., Lindsey A.S. (1994): Reference materials for PCDD and PCDF analysis: production and verification of the contents of twelve congeners in iso-octane reference solutions. Fresenius' J. Anal. Chem., 348, 31-36