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Report on the 1st inter-laboratory study C3-I

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Author: Anja Duffek

Contact person:

Peter Lepom
Tel : +49 (0) 30 89 03 57 24
Fax : +49 (0) 30 89 03 29 65
E-mail : peter.lepom@uba.de

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PP	Restricted to other programme participants (including the Commission Services)	
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Contents

1 Objective

The main objective of this study is to validate and harmonise the analytical methodology for the determination of decabromodiphenyl ether (decaBDE) in environmental samples, and to transfer this knowledge from expert/research laboratories to laboratories involved in routine monitoring. DecaBDE, an emerging pollutant that belongs to the group of brominated flame retardants, seems to be an ideal example for this case study. On the one hand there is the need for monitoring decaBDE according to the outcome of the recently completed risk assessment (EUR 20402 EN) and on the other hand there is still a need for improvement in the analysis of decaBDE in many laboratories as demonstrated by the results of recent inter-laboratory comparisons on the determination of PBDE in biota and sediment (de Boer & Cofino 2002, de Boer & Wells 2006).

To achieve the above-mentioned objectives a sequential approach will be applied. Starting with an inter-laboratory study of expert laboratories, followed by a second round of intercomparison with monitoring laboratories from EU Member States to test the developed harmonised protocol for the analysis of decaBDE in environmental samples at routine level.

The first round aims at identifying the crucial steps in the analysis of decaBDE. On the basis of the results of this exercise a detailed method description will be elaborated to enable monitoring laboratories not specialised in the analysis of brominated flame retardants to determine decaBDE in environmental samples with reasonable accuracy.

This report summarises the outcome of the 1st inter-laboratory study including statistical evaluation of the results and a critical assessment of the analytical procedures used. The influences of analytical methodology and experiences in BDE analyses on the variability of the results are discussed.

2 Participants

Seven expert laboratories from six European countries participated in this study. In addition, two laboratories involved in the NORMAN project expressed their interest in participating already in the first round even though they were not familiar with PBDE analysis in order to establish a method for the determination of PBDEs in environmental samples.

IVW	Institute for Environmental Studies, Amsterdam, The Netherlands
JRC-IES	Joint Research Centre - Institute for Environment and Sustainability, Ispra, Italy
INERIS	French National Institute for Industrial Environment and Risks, Verneuil-en-Halatte, France
CSIC	Environmental Chemistry Department**,** Barcelona, Spain
CIEMAT	Departamento de Medio Ambiente**,** Madrid, Spain
ITM	Department of Applied Environmental Science, Stockholm, Sweden
UBA	Federal Environment Agency, Berlin, Germany

3 Methodology

In preparation of the first inter-laboratory study all participants met in conjunction with the NORMAN workshop in Stresa on 20 June 2006. Extraction, clean-up and detection techniques to be included in the inter-laboratory study were discussed aiming at the harmonisation of those analytical steps, which had been identified to be critical. Recognising the apparent difficulties in the analysis of decaBDE in environmental samples, documented by the results of recent intercomparison studies, existing experiences in the analysis of decaBDE with special emphasis on QA/QC issues were exchanged between the participating laboratories. There was a common understanding among the participants to use $^{13}C_{12}$-labelled decaBDE as internal standard and to allow for various extraction, clean-up as well as detection techniques including low and high-resolution electron ionisation mass spectrometry as well as electron capture negative ionisation mass spectrometry. This denotes each laboratory applied its own fully validated method with which it had long practical experience.

The inter-laboratory study took place between September and November 2006. A standard solution and a dust sample were provided on 25th September 2006 including a questionnaire on experimental conditions, an instruction protocol and a standard form for reporting of results. The deadline for returning results and additional information was 17th November 2006.

For the inter-laboratory study, the house dust reference material NIST 2585 recently certified for its PBDE content (Stapleton et al. 2006) was chosen as test sample. This reference material is a sterilized, freeze-dried and sieved (< 100 µm) house dust collected from vacuum cleaner bags from homes, motels, and hotels. It contains various polycyclic hydrocarbons, polychlorinated biphenyl congeners, chlorinated pesticides, and polybrominated diphenyl ether congeners. In addition, a standard solution containing DECABDE in undisclosed concentration was distributed. This solution was prepared by diluting a certified standard solution of decaBDE in toluene purchased by Wellington Laboratories Inc. (Guelph, Ontario, Canada).

Each laboratory used its own analytical methodology. For the final determination GC/MS operated in either electron ionisation (GC/EI-MS) or electron capture negative ionization (GC-ECNI-MS) mode was used. The sample intake ranged from 0.1 to 0.5 g. Four replicate analyses of each sample were requested. Because of known blank problems in decaBDE analysis, participants were asked to determine four independent blank replicates. As agreed at the preparatory meeting all participants used isotpe dilution technique for quantification. This was regarded a fundamental requirement for reliable analytical results. During the analysis of the test material the participants were also requested to record each single step of the whole procedure and any circumstances that might have influenced the results by filling out the provided questionnaire on experimental conditions.

Statistical evaluation of the results submitted was carried out pursuant to the requirements of ISO 5725-2 using the software ProLab (quo data Ltd., Dresden, Germany). Data were checked for outliers according to Grubbs and Cochran.

The 2nd meeting of the participants of the inter-laboratory study C3-I is planned to be held in conjunction with the first meeting of the participants of the inter-laboratory study C3-II in June 2007 in Amsterdam.

4 Results and Discussion

Valid data and detailed method descriptions were received from six laboratories. One laboratory submitted obviously erroneous results. After further enquiry the error was identified. The laboratory had added different quantities of internal standard to calibration solutions/control extracts and sample extracts, respectively, which resulted in false quantification results. All results have been re-calculated based on the correct amounts of internal standard added to the various sample types and were included in the statistical evaluation. The two laboratories with no previous experience in the analysis of PBDEs had problems with the analysis and submitted no results whereas one laboratory reported only one result for each sample, which was not included in the statistical evaluation.

The variety of possible options to analyse PBDEs is reflected in our study (Figure 1). Each laboratory applied a different analytical method. The internal standard $^{13}C_{12}$-BDE-209 was added by all participants' prior extraction. However, amounts added ranged from 2 to 1000 ng. Different extraction techniques like accelerated solvent extraction, shaking and ultrasonic extraction were applied using different solvents and mixtures of solvents including toluene, hexane/acetone and hexane/dichloromethane. Normally, the obtained extracts were purified applying various clean-up techniques. One laboratory did not undertake any clean-up at all.
The laboratories used non-polar GC columns with a length of 15 m or less, an internal diameter of 0.25 mm, and a film thickness of 0.1 µm. PTV/splitless with or without pressure pulse or splitless injection, predominantly moderate injector and column temperatures < 300 °C were applied. This denotes that all participants used separation conditions specifically optimised for the analysis of decaBDE in accordance with the recommendations given in the literature (e.g. Covaci et al. 2003, Björklund et al. 2004, Stapelton 2006). Five laboratories used GC-ECNI-MS and one GC-HRMS.

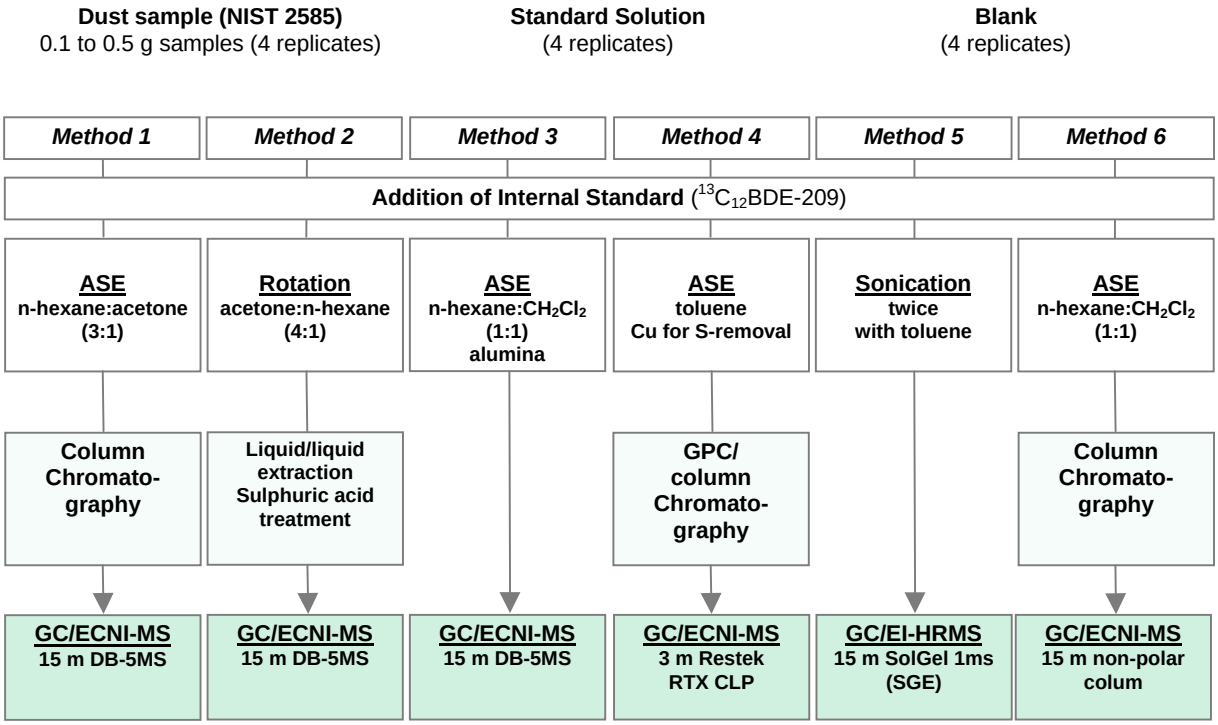

Figure 1: **Applied procedures for the determination of decaBDE in dust (NIST 2585), standard solution and blank reported by six laboratories**

Two individual within-laboratory outliers (one out of four results deviated significantly) were eliminated. The results of the laboratories were within a narrow range indicating that they followed the recommendations on how to recognise and avoid possible sources of errors. A summary of the results is given in Figure 2.

The evaluation of the results of this study did not reveal any significant difference in decaBDE concentration compared to the certified value. The average recovery for all laboratories was 107 %. After elimination of outliers the reproducibility and repeatability variation coefficients were less than 10 % for both samples. This study demonstrates that laboratories experienced in the analysis of PBDEs are able to determine decaBDE in the provided dust sample accurately even though they applied a variety of methods. However, a tendency to slightly higher decaBDE concentrations compared to the certified value was observed.

Recent international inter-laboratory studies have shown that until now satisfactory results are difficult to achieve especially for inexperienced laboratories (de Boer & Cofino 2002, de Boer et al. 2005, de Boer & Wells 2006). The Fifth International Laboratory Performance Study on the Analysis of Brominated Flame Retardants in Environmental Samples organised by QUASIMEME has still shown relatively high coefficients of variation (50-60 %) for DECABDE in sediments, even though advice with regard to specific analytical difficulties, such as blank problems, has repeatedly given (de Boer et al. 2005).

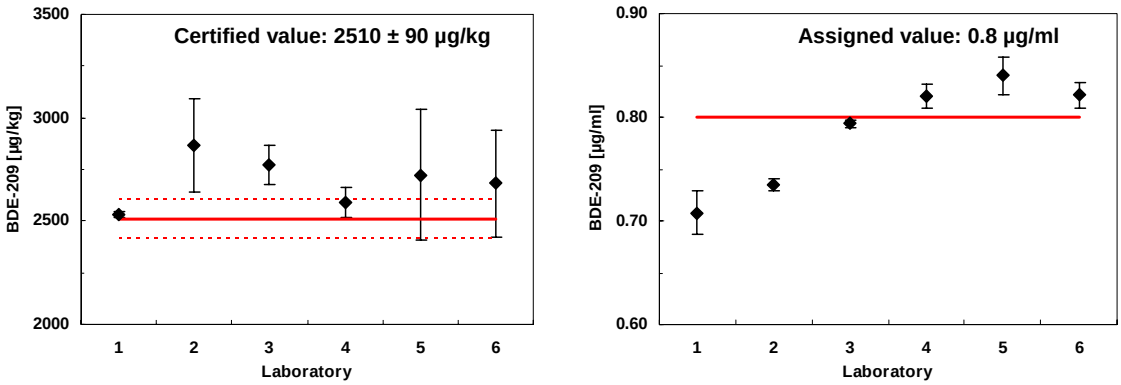

Figure 2: **Means of four replicates and standard deviations of decaBDE concentrations in dust (NIST 2585) (to the left) and standard solution (to the right) reported by six laboratories (no elimination of outliers)**

Sample	*l*	*n*	n_{AP} %	x	s_R	CV_R %	s_r	CV_r %
Dust	6	24	8.3	2692	207.7	7.7	203.8	7.6
Solution	6	24	0	0.79	0.05	6.9	0.01	1.7

l *Number of laboratories*
n *Number of single results*
n_{AP} *Percentage of outliers*
x *Total mean after elimination of outliers in µg/kg for the dust sample and µg/ml for the test solution*
s_R *Reproducibility standard deviation in µg/kg for the dust sample and µg/ml for the test solution*
CV_R *Reproducibility variation coefficient [%]*
s_r *Repeatability standard deviation in µg/kg for the dust sample and µg/ml for the test solution*
CV_r *Repeatability variation coefficient [%]*

Table 1: **Performance Characteristics for the NORMAN Inter-laboratory Study "Determination of DecaBDE in Dust"**

The first evaluation of the present method performance study showed that several methods for extraction and clean-up are appropriate for the determination of decaBDE in dust. The approach to offer various methodological options is also adopted in the International Standard (ISO 22032) for the determination of PBDE in sediment and sewage sludge. Obviously, the choice of the analytical method is less important than the experience of the laboratories and the careful control of critical factors like thermal and photochemical degradation of decaBDE as well as blanks. Analytical solutions to avoid possible errors are described in the literature (Covaci et al. 2003, de Boer & Wells 2006). In the present study the provided instruction protocol (see Annex) with advice to all critical factors as well as the reference to the ISO standard 22032 was not sufficient to enable inexperienced users to establish a fully validated method for the determination of decaBDE within the given time frame. In view of all the critical factors in the analysis of decaBDE in environmental samples QA/QC measures are of utmost importance. An internal standard, preferably $^{13}C_{12}$-BDE-209 as in the present study, should always be used to compensate for the losses throughout the analytical procedure and for inter-injection fluctuations.

On the basis of all findings of this method performance study, a detailed method description for the determination of decaBDE in dust will be prepared, which shall be applicable for the second round of the case study with the collaboration of routine laboratories.

5 References

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6 Annex

1st Inter-laboratory Study CASE 3

6.1 Instructions for Analysis

Test materials

The **dust material** is a sterilized; freeze dried and sieved (< 100 µm) house dust taken from vacuum cleaner bags collected from homes, motels, and hotels. Besides polybrominated diphenylether congeners, this material comprises selected polycyclic hydrocarbons, polychlorinated biphenyl congeners, and chlorinated pesticides. The bottle contains approximately 5 g of the material. The moisture content is 2.11 % ± 0.06 % (95 % confidence level).

The **GC - test solution** contains BDE-209, dissolved in toluene.

Homogeneity, stability and storage

The material has been shown to be homogeneous and stable for the purpose of the test. The dust material must be stored in its original bottle at temperatures less than 15 °C to 30 °C away from direct sunlight.

Analysis

This material is naturally occurring house dust from a number of locations and may contain constituents of unknown toxicities; therefore, caution and care should be exercised during its handling and use.

Prior to removal of subsamples for analysis, the contents of the bottle should be mixed. The dust sample should be dried to a constant mass before weighing for analysis, or a separate subsample of the dust should be removed from the bottle at the time of analysis and dried to determine the concentration on a dry-mass basis.

The samples should be analysed for DecaBDE using the analytical procedure, which is applied in your laboratory routinely for the determination of PBDEs. Any suitable extraction and cleanup procedure may be used. All measurements shall be performed using GC/MS operated in either electron ionisation (GC/EI-MS) or electron capture negative ionisation (GC/ECNI-MS) mode. Advice on how recognise and avoid possible sources of error is given in the Standard Protocol.

Please analyse three independent replicates. Sample intake should be 0.5 to 1 g.
The GC - Test solution shall be analysed directly by GC-MS (EI or ECNI mode) without dilution or concentration to avoid any losses of DecaBDE. The GC - Test solution shall be injected twice. For calibration and integration please refer to the Standard Protocol.

Results should be expressed on a dry weight basis (µg/kg).

Reporting of results

Please feed the technical details of the method applied and the performance characteristics in the provided template. The results of the Test dust and the GC – Test solution are to report by using Excel-file “Results of Test dust and GC – Test solution.xls” and Word-document “Experimental conditions.doc”. In addition, please provide typical chromatograms of the Test dust sample and the GC-test solution with the drawn integration marks (either by email or a printed copy).

6.2 Standard Protocol

Determination of DECABDE in house dust

Principle

Dried dust samples are extracted with an organic solvent or a mixture of organic solvents using pressurised liquid extraction, Soxhlet extraction, sonication, or shaking. Sample extracts are cleaned to remove interfering components. Clean-up procedures may include treatment with acid and/or base, treatment with copper, alumina, silica, gel permeation chromatography. After clean-up the extract is concentrated near to dryness. The analytes are separated by high-resolution gas chromatography and detected by mass spectrometry operated in the electron ionisation (EI) or electron capture negative ionisation mode (ECNI). Quantification is performed using selected ion monitoring (SIM) areas. The concentration of DecaBDE is determined using the isotope dilution technique.

The International Standard ISO 22032:2006 "Water quality - Determination of selected polybrominated diphenylethers in sediment and sewage sludge - Method using extraction and gas chromatography/mass spectrometry" is recommend as guidance for those who have not yet established a fully validated analytical procedure in their laboratories for the determination of decaBDE.

Special attention should be paid on following issues:

Problems	**Solutions**
Photodegradation under influence of daylight	Use of UV filters at laboratory windows and at fluorescent lightings Use of amber glassware (or glassware covered with e.g. aluminium foil)
Poor solubility	Check solubility in the organic solvent you intend to use before preparing stock solutions or concentrated extracts It should be avoided without fail to evaporate extracts or solutions of DecaBDE to dryness, because decaBDE does strongly adsorb to glass surfaces and may not be re-dissolve completely. Add toluene or another solvent of similar boiling point as a keeper before concentrating extracts/solutions.
High background concentrations (decaBDE may be present in dust in the laboratory or as contamination of the glassware)	The laboratory in which samples are handled should be as far as possible free of dust. Keep out any kind of plastic material and packaging that might contain PBDEs. Open glassware should be covered, e.g. by aluminium foil, to prevent dust particles to enter. If possible, samples should be handled in a clean bench or at least on a pre-cleaned work surface in a fume hood. Blank analyses should be carried out frequently (e.g. within each sample batch). The treatment of the blanks should be identical to that of the sample (e.g. residence time at the bench). The use of a $^{13}C_{12}$ internal standard is compulsory; the sensitivity of the detector should be fully optimised.
Thermal degradation	Use Short (< 15 m) and narrow (< 0.25 mm) GC columns with thin films (0.1 µm), moderate injector and column temperatures (< 300 °C), and short injector residence times, or cold injectors Splitless injection is critical and can only be applied successfully when combined with pressure pulse or by using short splitless time. On column injection may a suitable alternative.