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CERTIFICATION REPORT

The certification of the mass fractions of polybrominated diphenyl ethers (PBDEs) and α-, β- and γ-hexabromocyclododecane (HBCD) in freshwater sediment: ERM®-CC537a

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Abstract

This report describes the production of ERM®-CC537a, a sediment certified for the mass fraction of polybrominated diphenyl ethers PBDE 28, 47, 99, 100, 153, 154, 183 and 209 (further referred as BDEs) and of α-, β- and γ-hexabromocyclododecane (HBCD) on a dry mass basis. This material was produced following ISO Guide 34:2009 [ ] and is certified in accordance with ISO Guide 35:2006.
The starting material is a freshwater sediment originating from a small Belgian river. It was air-dried, sieved, jet-milled and finally homogenised. The obtained powdered sediment was bottled under argon atmosphere and sterilised by γ-irradiation.
Between unit-homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2006. Within-unit homogeneity was quantified to determine the minimum sample intake.
The material was characterised by an interlaboratory comparison of laboratories of demonstrated competence and adhering to ISO/IEC 17025:2005. Technically invalid results were removed but no outlier was eliminated on statistical grounds only.
Uncertainties of the certified values were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) and include uncertainties related to possible inhomogeneity, instability and characterisation.
The material is intended for the quality control and assessment of method performance. As any reference material, it can also be used for control charts or validation studies. The Certified Reference Material (CRM) is available in amber glass bottles containing 40 g of sediment. The minimum amount of sample to be used is 750 mg.

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Disclaimer

Certain commercial equipment, instruments, and materials are identified in this paper to specify adequately the experimental procedure. In no case does such identification imply recommendation or endorsement by the European Commission, nor does it imply that the material or equipment is necessarily the best available for the purpose.

Summary

This report describes the production of ERM®-CC537a, a sediment certified for the mass fraction of polybrominated diphenyl ethers PBDE 28, 47, 99, 100, 153, 154, 183 and 209 (further referred as BDEs) and of α-, β- and γ-hexabromocyclododecane (HBCD) on a dry mass basis. This material was produced following ISO Guide 34:2009 [1] and is certified in accordance with ISO Guide 35:2006 [2].

The starting material is a freshwater sediment originating from a small Belgian river. It was air-dried, sieved, jet-milled and finally homogenised. The obtained powdered sediment was bottled under argon atmosphere and sterilised by γ-irradiation.

Between unit-homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2006 [2]. Within-unit homogeneity was quantified to determine the minimum sample intake.

The material was characterised by an interlaboratory comparison of laboratories of demonstrated competence and adhering to ISO/IEC 17025:2005 [3]. Technically invalid results were removed but no outlier was eliminated on statistical grounds only.

Uncertainties of the certified values were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) [4] and include uncertainties related to possible inhomogeneity, instability and characterisation.

The material is intended for the quality control and assessment of method performance. As any reference material, it can also be used for control charts or validation studies. The Certified Reference Material (CRM) is available in amber glass bottles containing 40 g of sediment. The minimum amount of sample to be used is 750 mg.

The following values were assigned:

Mass Fraction (dry mass basis)	Certified value [3)]	Uncertainty [4)]
BDE-28 (2,4,4'-tribromodiphenyl ether)[1)]	0.28 µg/kg	0.05 µg/kg
BDE-47 (2,2',4,4'-tetrabromodiphenyl ether)[1)]	16.5 µg/kg	1.8 µg/kg
BDE-99 (2,2',4,4',5-pentabromodiphenyl ether)[1)]	34 µg/kg	4 µg/kg
BDE-100 (2,2',4,4',6-pentabromodiphenyl ether)[1)]	5.8 µg/kg	0.6 µg/kg
BDE-153 (2,2',4,4',5,5'-hexabromodiphenyl ether)[1)]	6.6 µg/kg	0.9 µg/kg
BDE-154 (2,2',4,4',5,6'-hexabromodiphenyl ether)[1)]	3.5 µg/kg	0.5 µg/kg
BDE-183 (2,2',3,4,4',5',6-heptabromodiphenyl ether)[1)]	1.41 µg/kg	0.21 µg/kg
BDE-209 (decabromodiphenyl ether)[1)]	7.8 mg/kg	0.7 mg/kg
α-HBCD (1,2,5,6,9,10-hexabromocyclododecane)[2)]	8.3 µg/kg	1.6 µg/kg
β-HBCD (1,2,5,6,9,10-hexabromocyclododecane)[2)]	2.3 µg/kg	0.5 µg/kg
γ-HBCD (1,2,5,6,9,10-hexabromocyclododecane)[2)]	60 µg/kg	16 µg/kg

1) as obtained by analytical procedures using gas chromatography.

2) as obtained by analytical procedures using liquid chromatography.

3) Certified values are values that fulfil the highest standards of accuracy and represent the unweighted mean value of the means of accepted sets of data, each set being obtained in a different laboratory and/or with a different method of determination. The certified values and their uncertainties are traceable to the International System of Units (SI).

4) The uncertainty of the certified value is the expanded uncertainty with a coverage factor $k = 2$ corresponding to a level of confidence of about 95 % estimated in accordance with ISO/IEC Guide 98-3, Guide to the Expression of Uncertainty in Measurement (GUM:1995), ISO, 2008.

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Glossary

ANOVA	Analysis of variance
ACN	Acetonitrile
ASE	Accelerated solvent extraction
b	Slope in the equation of linear regression $y = a + bx$
CI	Confidence interval
CRM	Certified reference material
ECNI	Electron capture negative ionisation
EI	Electron ionisation
ERM®	Trademark of European Reference Materials
EU	European Union
GC	Gas chromatography
GC-ECNI-MS	Gas chromatography-electron capture negative ionization-mass spectrometry
GC-HRMS	Gas chromatography-high resolution mass spectrometry
GPC	Gel permeation chromatography
GUM	Guide to the Expression of Uncertainty in Measurements [4]
HBCD	Hexabromocyclododecane
HPLC	High performance liquid chromatography
ID	Isotope dilution
IDMS	Isotope dilution mass spectrometry
IEC	International Electrotechnical Commission
ISO	International Organization for Standardization
JRC	Joint Research Centre of the European Commission
k	Coverage factor
LC	Liquid chromatography
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LLE	Liquid-liquid extraction
LOQ	Limit of quantification
MS	Mass spectrometry
$MS_{between}$	Mean of squares between-unit from an ANOVA
MS_{within}	Mean of squares within-unit from an ANOVA
n	Number of replicates per unit
N	Number of samples (units) analysed
n.a.	Not applicable
n.c.	Not calculated

NIST	National Institute of Standards and Technology (USA)
(P)BDE	(Poly)brominated diphenyl ether
QC	Quality control
rel	Index denoting relative figures (uncertainties etc…)
RM	Reference material
RM Unit	Reference Materials Unit
RSD	Relative standard deviation
s	Standard deviation
s_{bb}	Between-unit standard deviation; an additional index "rel" is added when appropriate
$s_{between}$	Standard deviation between groups as obtained from ANOVA; an additional index "rel" is added as appropriate
SI	International System of Units
SPE	Solid phase extraction
s_{within}	Standard deviation within groups as obtained from ANOVA; an additional index "rel" is added as appropriate
s_{wb}	Within-unit standard deviation
T	Temperature
t	Time
t_i	Time elapsed at time point i
$\bar{t}$	Mean of all time t_i
t_{sl}	Proposed shelf life
t_{tt}	Chosen transport time
TOC	Total organic carbon
u	Standard uncertainty
U	Expanded uncertainty
u^*_{bb}	Standard uncertainty related to a maximum between-unit inhomogeneity that could be hidden by method repeatability; an additional index "rel" is added as appropriate
u_{bb}	Standard uncertainty related to a possible between-unit inhomogeneity; an additional index "rel" is added as appropriate
u_c	Combined standard uncertainty; an additional index "rel" is added as appropriate
u_{char}	Standard uncertainty of the material characterisation; an additional index "rel" is added as appropriate
u_{CRM}	Combined standard uncertainty of the certified value; an additional index "rel" is added as appropriate
U_{CRM}	Expanded uncertainty of the certified value; an additional index "rel" is added as appropriate
u_Δ	Combined standard uncertainty of measurement result and certified

	value
u_{lts}	Standard uncertainty of the long-term stability; an additional index "rel" is added as appropriate
u_{meas}	Standard measurement uncertainty
U_{meas}	Expanded measurement uncertainty
u_{sts}	Standard uncertainty of the short-term stability; an additional index "rel" is added as appropriate
UPLC	Ultra performance liquid chromatography
VMM	Flemish Environment Agency
WFD	Water Framework Directive
X_y	particle diameter corresponding to y % of the cumulative undersize distribution (*i.e.* y % by volume of the particles are smaller than this diameter and y % are larger)
$\bar{y}$	mean of all results of the homogeneity study
Δ_{meas}	Absolute difference between mean measured value and the certified value
$\nu_{MSwithin}$	Degrees of freedom of MS_{within}

1 Introduction

1.1 Background

Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecane (HBCD, sometimes abbreviated as HBCDD) were, until relatively recently, widely used [5, 6, 7] as flame retardants in many combustible commercial and household products, such as polymers, electrical and electronic equipment, textiles, furniture, building and packaging materials. PBDEs and HBCD are additive-type brominated flame retardants, meaning that they are not chemically bound but only physically mixed/dissolved in the material. Due to the absence of covalent bonds, the release of these compounds into the environment can occur not only when they are manufactured but also when products containing them are used and disposed of [8, 9]. Environmental contamination by PBDEs and HBCD has attracted public attention and concern in recent years due to their widespread use, ubiquity (potential for long-range atmospheric transport [10]) and high persistence, bioaccumulation and toxicity, thus presenting a potential threat to wildlife and human health [11]. Due to their high octanol-water partition coefficient (log K_{ow}) [12], they are mostly found bound to air particles, to suspended and bed-sediments or to the lipids in aquatic organisms [13, 14]. The presence of PBDEs and HBCD has been reported in a range of environmental media and biota including fish, treated sewage sludge and household dust [15, 16, 17, 18].

PBDEs 28, 47, 99, 100, 153 and 154 and HBCD (diastereoisomers α, β and γ) are considered of primary interest for the environment all over the world. The European Commission has listed them as priority substances under the EU Water Framework Directive (WFD) and related Daughter Directives [19, 20]: Member States are expected to assess, monitor and control them in European water bodies.

The quality and comparability of the analytical results reported by the Member States is the subject of another EU Directive [21] adopted in 2009, which sets minimum analytical performance criteria for the analytical methods applied in the implementation of the WFD. This Directive also prescribes the use of reference materials (RMs) for guaranteeing the competence of the environmental laboratories which are officially appointed by the Member States to the chemical analysis and monitoring of water status.

1.2 Choice of the material

The WFD focuses on the pollution prevention and control of the whole aquatic environment including not only water, but also sediment and biota. It is known that chemical pollutants are portioned among these compartments, also depending on their hydrophobicity. Persistent, bioaccumulative and toxic substances, like flame retardants, have shown evidence for long-term ubiquity in the environment. EU Member States shall arrange for the long-term trend analysis of those priority substances, including PBDEs and HBCD, that tend to accumulate in sediment and/or biota and shall take measures aimed at ensuring that their levels do not significantly increase along the years [17].

ERM-CC537a is a dried freshwater sediment which contains, besides other environmental contaminants, PBDEs and HBCD at levels typically found in environmental samples.

1.3 Design of the project

The certification of ERM-CC537a was performed by interlaboratory comparison involving analytical methods based on gas chromatography (GC) and liquid chromatography (LC) for PBDEs and for HBCD, respectively, but differing in the sample preparation, clean-up and detection steps.

2 Participants

2.1 Project management and evaluation

European Commission, Joint Research Centre, Directorate F - Health, Consumers and Reference Materials, Reference Materials Unit, Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM)

2.2 Processing

European Commission, Joint Research Centre, Directorate F - Health, Consumers and Reference Materials, Reference Materials Unit, Geel, BE
(accredited to ISO Guide 34 for production of certified reference materials, BELAC No. 268-RM)

2.3 Homogeneity study

Universiteit Antwerpen, Toxicologie, Antwerpen, BE

2.4 Stability study

VITO NV, Vlaamse Instelling voor Technologisch Onderzoek, Mol, BE

Vrije Universiteit Amsterdam, Institute for Environmental Studies (IVM), Amsterdam, NL

2.5 Characterisation

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(measurements under the scope of ISO/IEC 17025 Czech Accreditation Institute; 319/2016)

Centre for Environment, Fisheries & Aquaculture Science (Cefas), Suffolk, UK

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(measurements under the scope of ISO/IEC 17025 accreditation BELAC No. 268-TEST)

Fera Science Ltd, York, UK
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Universiteit Antwerpen, Toxicologie, Antwerpen, BE

Wageningen University & Research, Wageningen Marine Research, Chemical Laboratory of the Fish Division, IJmuiden, NL
(measurements under the scope of ISO/IEC 17025 accreditation Raad voor Accreditatie/Dutch Accreditation Council; L097)

3 Material processing and process control

3.1 Origin of the starting material

The starting material was freshwater sediment originating from a small Belgian river (classified as unnavigable river of category 2 by the Flemish Environment Agency) included in the Flemish sediment monitoring network [22]. The sampling of the sediment was executed on 30/11/2011 by JRC-Geel staff under supervision and in co-operation with the division "Reporting Water" of the Flemish Environment Agency.

The sampling site was chosen after a careful selection based on the levels of PBDEs and HBCD occurring in the sediment and on the site accessibility. About 700 kg of sediment top layer (to about 20 cm depth) was collected into high-density polyethylene containers and transported to JRC-Geel where further treatments took place, after discarding the segregated water.

3.2 Processing of ERM-CC537a

The wet sediment was placed in steel trays and subjected to air-drying at 35 °C for several days in ventilated drying cabinets (Hereaus, model UT 6760, Langenselbold, DE). During this time, the material was stirred and mixed repetitively to break up lumps and was subjected to repeated sieving over a 1 mm sieve to remove large biota elements and other coarse fractions. After about one week of drying, the sediment was manually crushed and sieved again over a 1 mm stainless steel sieve (Russel Finex, London, UK). The obtained material was spread out onto trays for a final drying step overnight and then stored in drums at room temperature. After about one month of storage, the bulk sediment was jet-milled (Alpine, Augsburg, DE) to obtain approximately 160 kg of jet-milled material. The jet-milled sediment was homogenised in a Turbula mixer (WAB, Postfach, CH) for about one hour and subsequently dispensed through a Cone Mixer into 60 mL amber glass bottles with screw caps having an aluminium disc as insert. The 1567 bottles produced, containing about 40 g of dried sediment each, were sterilised by γ-irradiation (average dose of 10 kGy) and stored at +4 °C. The homogeneity analyses of the target measurands performed on this candidate reference material evidenced the presence of outlier values causing a too high between-bottle heterogeneity. The material was carefully checked with two different laser diffraction methods and sieve-analysis and the presence of a small portion of coarse particles was confirmed. This fraction of coarser particles was assumed to be the cause for the presence of outliers in the homogeneity dataset. Therefore, it was decided to subject the jet-milled candidate reference material to a further sieving step using a < 125 µm stainless steel sieve (Russel Finex, London, UK). The additional sieving step yielded 67 kg of finer fraction sediment which was transferred to a stainless steel container and homogenised using a Dynamix-200 CM mixer (WAB, Postfach, CH) for 2 h. Thereafter, the sediment was transferred to a Cone Mixer for final mixing and filling in bottles. After labelling, a total of 1500 units (60 mL amber glass bottles containing about 40 g of sediment each) were produced as the final batch of ERM-CC537a. Sterilisation of ERM-CC537a was carried out by γ-irradiation using a dose between 7 kGy and 12.5 kGy. Afterwards, ERM-CC537a was placed for storage at +4 °C awaiting further tests.

3.3 Process control

The additional step of sieving using the Russel sieve described in Section 3.2 had been previously applied to produce a small batch of test material to be used in the key comparison and pilot study CCQM-K102/P138 run under the activities of the Organic Analysis Working Group (OAWG) of the Consultative Committee for Amount of Substance – Metrology in

Chemistry (CCQM). On this test batch, homogeneity and short-term stability studies were performed, which confirmed the suitability of such approach for the preparation of the candidate CRM.

Particle size analysis using laser diffraction (Sympatec Helos laser light diffraction instrument, Clausthal Zellerfeld, DE) was performed in duplicate on six bottles (n=12) of ERM-CC537a. The particle size data are displayed in Figure 1 and Table 1.

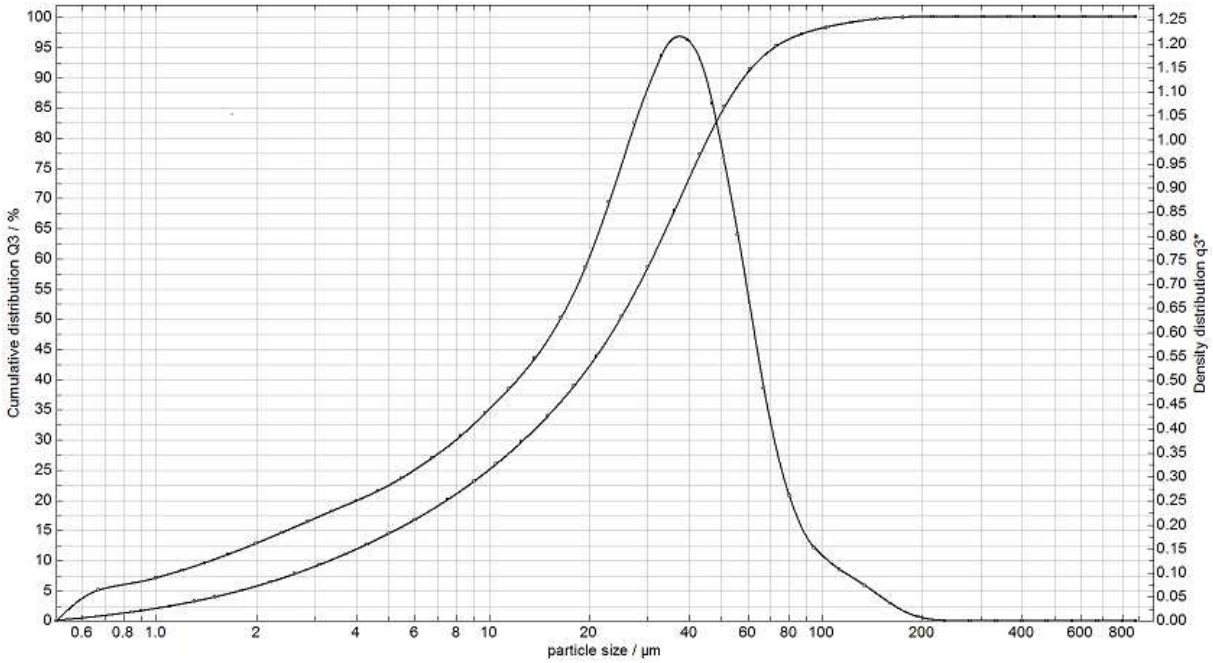

Figure 1: Average particle size distribution in ERM-CC537a using 2-propanol as dispersant (n=12).

Table 1: Particle size data for ERM-CC537a (n=12)

Upper band limit	Average particle size (µm)	s (µm)	RSD (%)
X_{10}	3.4	0.2	5.1
X_{50}	24.8	0.7	2.8
X_{90}	58.8	2.5	4.3

As an overall assessment of comparability of the particle size distribution between the different units, the average of the deviation for X_{10}, X_{50} and X_{90} from their respective average values is calculated. Results with an average deviation for X_{10}, X_{50} and X_{90} below 20 % are considered as acceptable.

As can be seen in Table 1, the RSDs are all well below 10 %. Consequently the material is considered to be homogeneous and uniform over the whole batch with respect to particle size distribution.

The water content of ERM-CC537a was measured using volumetric Karl-Fischer titration in triplicate on six bottles, yielding an average value of 0.62 ± 0.06 g/100g (mean ± *U*, *k*=2).

4 Homogeneity

A key requirement for any reference material aliquoted in units is the equivalence between those units. In this respect, it is relevant whether the variation between units is significant compared to the uncertainty of the certified value, but it is not relevant if this variation between units is significant compared to the analytical variation. Consequently, ISO Guide 34 requires RM producers to quantify the between unit variation. This aspect is covered in between-unit homogeneity studies.

The within-unit inhomogeneity does not influence the uncertainty of the certified value when the minimum sample intake is respected, but determines the minimum size of an aliquot that is representative for the whole unit. Quantification of within-unit inhomogeneity is therefore necessary to determine the minimum sample intake.

4.1 Between-unit homogeneity

The between-unit homogeneity was evaluated to ensure that the certified values of the CRM are valid for all bottles of the material, within the stated uncertainties.

The number of selected units corresponds to approximately the cubic root of the total number of units produced. Ten bottles of ERM-CC537a were selected using a random stratified sampling scheme covering the whole batch for the between-unit homogeneity test. For this, the batch was divided into 10 groups (with a similar number of units) and one unit was selected randomly from each group. Four independent samples of at least 750 mg were taken from each selected unit, and analysed by GC-ECNI-MS for PBDEs (internal standards: BDE-77 and -128, ^{13}C-BDE-209) and by LC-MS/MS for HBCD (internal standards: ^{13}C-α-HBCD, ^{13}C-β-HBCD and ^{13}C-γ-HBCD), see Annex A for more details on the sample preparation. The measurements were performed under repeatability conditions, and in a randomised manner to be able to separate a potential analytical drift from a trend in the filling sequence. The results were reported on a dry mass basis (i.e., corrected by determining the dry matter content in duplicate on each unit (for details regarding the prescribed drying procedure refer to Section 6.3). The results are shown graphically in Annex A.

Regression analyses were performed to evaluate potential trends in the analytical sequence as well as trends in the filling sequence. No trends in the filling sequence were visible. One significant trend (95 % confidence level) in the analytical sequence was visible for BDE-47, possibly pointing at a signal drift in the analytical system. The correction of biases, even if they are statistically not significant, was found to combine the smallest uncertainty with the highest probability to cover the true value [23]. Correction of trends is therefore expected to improve the sensitivity of the subsequent statistical analysis through a reduction in analytical variation without masking potential between-unit heterogeneities. As the analytical sequence and the unit numbers were not correlated, trends significant on at least a 95 % confidence level were corrected as shown below:

$$x_{i\_corr} = x_i - b \cdot i \qquad \textbf{Equation 1}$$

b = slope of the linear regression

i = position of the result in the analytical sequence

All datasets (corrected for analytical trend in the case of BDE-47) were assessed for consistency using Grubbs outlier tests at a confidence level of 99 % on the individual results and the unit means. No outlying individual results and no outlying unit means were detected.

Quantification of between-unit inhomogeneity was undertaken by analysis of variance (ANOVA), which separates the between-unit variation (s_{bb}) from the within-unit variation (s_{wb}). The latter is equivalent to the method repeatability if the individual samples are representative for the whole unit.

Evaluation by ANOVA requires mean values per unit which follow at least a unimodal distribution and results for each unit that follow unimodal distributions with approximately the same standard deviations. The distribution of the mean values per unit was visually tested using histograms and normal probability plots. Too few data are available for the unit means to make a clear statement of the distribution. Therefore, it was checked visually whether all individual data follow a unimodal distribution using histograms and normal probability plots. Minor deviations from unimodality of the individual values do not significantly affect the estimate of between-unit standard deviations. The results of all statistical evaluations are given in Table 2.

Table 2: Results of the statistical evaluation of the homogeneity studies

Parameter	Trends[1] (before correction)		Outliers[2]		Distribution	
	Analytical sequence	Filling sequence	Individual results	Unit means	Individual results	Unit means
BDE-28	no	no	none	none	normal unimodal	normal unimodal
BDE-47	yes	no	none	none	normal unimodal	normal unimodal
BDE-99	no	no	none	none	normal unimodal	slightly skewed unimodal
BDE-100	no	no	none	none	normal unimodal	slightly skewed unimodal
BDE-153	no	no	none	none	normal unimodal	normal unimodal
BDE-154	no	no	none	none	normal unimodal	normal unimodal
BDE-183	no	no	none	none	normal unimodal	normal unimodal
BDE-209	no	no	none	none	normal unimodal	normal unimodal
α-HBCD	no	no	none	none	normal unimodal	normal unimodal
β-HBCD	no	no	none	none	normal unimodal	normal unimodal
γ-HBCD	no	no	none	none	normal unimodal	normal unimodal

1) 95 % confidence level
2) 99 % confidence level

It should be noted that $s_{bb,rel}$ and $s_{wb,rel}$ are estimates of the true standard deviations and therefore subject to random fluctuations. Therefore, the mean square between groups ($MS_{between}$) can be smaller than the mean squares within groups (MS_{within}), resulting in negative arguments under the square root used for the estimation of the between-unit variation, whereas the true variation cannot be lower than zero. In this case, u^*_{bb}, the maximum inhomogeneity that could be hidden by method repeatability, was calculated as described by Linsinger *et al.* [24]. u^*_{bb} is comparable to the limit of quantification of an analytical method, yielding the maximum inhomogeneity that might be undetected by the given study setup.

Method repeatability ($s_{wb,rel}$), between–unit standard deviation ($s_{bb,rel}$) and $u^*_{bb,rel}$ were calculated as:

$$s_{wb,rel} = \frac{\sqrt{MS_{within}}}{\bar{y}}$$ **Equation 2**

$$s_{bb,rel} = \frac{\sqrt{\frac{MS_{between} - MS_{within}}{n}}}{\bar{y}}$$ **Equation 3**

$$u^{*}_{bb,rel} = \frac{\sqrt{\frac{MS_{within}}{n}\sqrt[4]{\frac{2}{v_{MSwithin}}}}}{\bar{y}}$$ **Equation 4**

MS_{within} mean of squares within-unit from an ANOVA

$MS_{between}$ mean of squares between-unit from an ANOVA

$\bar{y}$ mean of all results of the homogeneity study

n number of replicates per unit

$v_{MSwithin}$ degrees of freedom of MS_{within}

The results of the evaluation of the between-unit variation are summarised in Table 3. The resulting values from the above equations were converted into relative uncertainties. In almost half of the cases, the uncertainty contribution for homogeneity was determined by the method repeatability.

Table 3: Results of the homogeneity study

Parameter	$s_{wb,rel}$ [%]	$s_{bb,rel}$ [%]	$u^{*}_{bb,rel}$ [%]	$u_{bb,rel}$ [%]
BDE-28	7.6	1.1	1.9	1.9
BDE-47	5.5	1.6	1.4	1.6
BDE-99	8.9	1.6	2.3	2.3
BDE-100	7.1	1.9	1.8	1.9
BDE-153	9.3	3.8	2.4	3.8
BDE-154	10.2	3.1	2.6	3.1
BDE-183	11.0	5.2	2.8	5.2
BDE-209	3.8	n.c.[1)]	1.0	1.0
α-HBCD	13.3	3.6	3.4	3.6
β-HBCD	16.2	0.4	4.1	4.1
γ -HBCD	15.3	1.6	3.9	3.9

[1)] n.c.: cannot be calculated as $MS_{between} < MS_{within}$

The homogeneity study showed no outlying unit means or trends in the filling sequence. Therefore the between-unit standard deviation can be used as estimate of u_{bb}. As u^{*}_{bb} sets

the limits of the study to detect inhomogeneity, the larger value of s_{bb} and u^*_{bb} is adopted as uncertainty contribution to account for potential inhomogeneity.

4.2 Within-unit homogeneity and minimum sample intake

The within-unit homogeneity is closely correlated to the minimum sample intake. The minimum sample intake is the minimum amount of sample that is representative for the whole unit and thus should be used in an analysis. Using sample sizes equal or above the minimum sample intake guarantees the certified value within its stated uncertainty.

To estimate the minimum sample intake, a series of measurements with decreasing amounts of sample were performed. The following sample intakes were tested: 750 mg, 500 mg and 200 mg. Two randomly selected units were measured in quadruplicate for the 500 mg and 200 mg sample intakes, while the 40 measurements results of the homogeneity study were used for the evaluation of the 750 mg sample intake. The samples were measured by GC-ECNI-MS for PBDEs and LC-MS/MS for HBCD (same analytical method applied as for the homogeneity study, see Annex A for details) under repeatability conditions, and in a randomised manner. The results were reported on a dry mass basis *i.e.*, corrected by determining the dry matter content in duplicate on each unit (for details regarding the prescribed drying procedure refer to Section 6.3). The measurement method was robust over the whole range of the sample intake tested and its repeatability was either in the same range or better than the repeatability achieved during the material characterisation (Section 6).

The obtained data sets (the results from the 500 mg and 200 mg sample intakes were evaluated together, while the statistical evaluation of the 750 mg sample intake results was carried out within the scope of homogeneity assessment) were first tested whether they follow a normal, or at least a unimodal distribution. This was done by visual inspection of normal probability plots and histograms (if the data do not follow at least a unimodal distribution, the calculation of standard deviations is doubtful or impossible). The combined results from the 500 mg and 200 mg sample intakes were normally and unimodally distributed (for the 750 mg sample intake refer to Table 2).

Furthermore, the results (500 mg and 200 mg sample intakes evaluated together, for the 750 mg sample intake refer to Table 2) were scrutinised for outliers using the single Grubbs-test at a 99 % confidence level.

The minimum sample intake was established by comparison of variances obtained for 500 mg and 200 mg sample intakes with the variance obtained for 750 mg sample intake. It was done using the F-test for equality of two-sample variances at a confidence level of 95 %.

The RSDs of the results per sample intake are presented in Annex B and the minimum sample intakes are summarised in Table 4.

Table 4: Results of the minimum sample intake determination

Parameter	Minimum sample intake [mg]
BDE-28	750
BDE-47, -99, -100, -209, α-HBCD	200
BDE-153, -154, -183, β-HBCD, γ-HBCD	500

As shown above, the minimum sample intake to be taken as representative for all analytes is 750 mg. In addition, a 750 mg sample intake was used for the homogeneity study giving acceptable repeatability and demonstrating that the within-unit inhomogeneity does no longer contribute to the analytical variation at this sample intake.

5 Stability

Time, temperature and light (including ultraviolet radiation) were regarded as the most relevant influences on the stability of the material. The influence of ultraviolet or visible light was minimised by storing the material in containers which reduces light exposure. In addition, materials are stored in the dark and dispatched in boxes, thus eliminating any possibility of degradation by light. Additionally the material was sterilized by γ-irradiation to eliminate microbial growth. Therefore, only the influences of time and temperature needed to be investigated.

Stability testing is necessary to establish conditions for storage (long-term stability) as well as the conditions for dispatch of the materials to the customers (short-term stability). During transport, especially in summer time, temperatures up to 60 °C could be reached and stability under these conditions must be demonstrated if the samples are to be transported without any additional cooling.

The stability studies were carried out using an isochronous design [25]. In this approach, samples were stored for a particular length of time at different temperature conditions. Afterwards, the samples were moved to conditions where further degradation can be assumed to be negligible (reference conditions). At the end of the isochronous storage, the samples were analysed simultaneously under repeatability conditions. Analysis of the material (after various exposure times and temperatures) under repeatability conditions greatly improves the sensitivity of the stability tests.

5.1 Short-term stability study

For the short-term stability study, samples were stored at 18 °C and 60 °C for 0, 1, 2 and 4 weeks (at each temperature). The reference temperature was set to -20 °C. Two units per storage time were selected using a random stratified sampling scheme. From each unit, three samples of about 1.5 g each were measured by GC-HRMS for PBDEs and UPLC-MS/MS for HBCD (quantification was performed using isotopically labelled BDE congeners and HBCD isomers as internal standards, see Annex C for more details on the sample preparation). The measurements were performed under repeatability conditions, and a randomised sequence was used to differentiate any potential analytical drift from a trend over storage time. The results were reported on a dry mass basis i.e., corrected by determining the dry matter content in duplicate on each unit (for details regarding the prescribed drying procedure refer to Section 6.3). Significant trends (95 % confidence level) in the analytical sequence were visible for BDE-154 at 18 °C and for BDE-99 at 60 °C and were corrected (see Equation 1 in Section 4.1) before proceeding further with the evaluation.

The data were evaluated individually for each temperature. The results were screened for outliers using the single and double Grubbs test on a confidence level of 99 %. No outlying individual results were found (Table 5).

In addition, the data were evaluated against storage time, and regression lines of mass fraction versus time were calculated, to test for potential increasing/decreasing trend of the individual parameters due to shipping conditions. The slopes of the regression lines were tested for statistical significance. For all parameters, none of the trends was statistically significant at a 95 % confidence level at 18 °C. On the other hand, the slopes of the regression lines were significantly different from zero on at least a 95 % confidence level at 60 °C for BDE-28, -47, -153, -154, -183, -209, α- and γ-HBCD. The trend observed in the

case of α-HBCD at 60 °C was positive. As the analyte cannot be created in the sample, a positive trend could only be due to the degradation of the matrix. This, however, should be seen for all measurands, which is not the case. The observed trend was therefore regarded as statistical artefact.

The results of the measurements are shown as graphs in Annex C. The results of the statistical evaluation of the short-term stability are summarised in Table 5.

Table 5: Results of the short-term stability tests

Parameter	Number of individual outlying results[1)]		Trends [2)]	
	18 ºC	60 ºC	18 ºC	60 ºC
BDE-28	none	none	no	yes (also on 99 % confidence level)
BDE-47	none	none	no	yes
BDE-99	none	none	no	no
BDE-100	none	none	no	no
BDE-153	none	none	no	yes
BDE-154	none	none	no	yes
BDE-183	none	none	no	yes (also on 99 % confidence level)
BDE-209	none	none	no	yes (also on 99 % confidence level)
α-HBCD	none	none	no	yes (also on 99 % confidence level)
β-HBCD	none	none	no	no
γ-HBCD	none	none	no	yes (also on 99 % confidence level)

1) 99 % confidence level
2) 95 % confidence level

No outliers (neither technical nor statistical) were detected for any of the analytes. None of the trends was statistically significant on a 95 % confidence level at 18 °C.

On the other hand, since significant trends were observed for most of the parameters at 60 °C, the material shall be shipped under cooled conditions to make sure that it is not exceeding 18 °C.

5.2 Long-term stability study

For the long-term stability study, samples were stored at 4 °C and 18 °C for 0, 8, 16 and 24 months (at each temperature). The reference temperature was set to -20 °C. Two samples per storage time were selected using a random stratified sampling scheme. From each unit, three samples were measured by GC-ECNI-MS for PBDEs and HPLC-MS/MS for HBCD (quantification was performed using BDE-58, ^{13}C-BDE-209 and ^{13}C-HBCD isomers, respectively). More details on the sample preparation are reported in Annex D.

In the case of BDE-28, results of the 2-year long-term stability study were reported by the laboratory as just below the LOQ. Therefore, for this compound, the dataset of 1–year long-term stability study was used instead to assess the stability of the CRM. For the 1-year long-term stability, samples were stored at 4 °C and 18 °C for 0, 4, 8 and 12 months (at each temperature). The reference temperature was set to -20 °C.

Two samples per storage time were selected using a random stratified sampling scheme. From each unit, three samples between 1.1 g and 1.5 g each were measured by GC-MS/MS for PBDEs and by UPLC-MS/MS for HBCD (quantification was performed using ^{13}C-PBDEs and ^{13}C-γ-HBCD, respectively). The same sample preparation as for the short-term stability study was applied, see Annex C for details.

The measurements were performed under repeatability conditions, in a random sequence to be able to separate any potential analytical drift from a trend over storage time. The results were reported on a dry mass basis i.e., corrected by determining the dry matter content in duplicate on each unit (for details refer to Section 6.3).

The long-term stability data were evaluated individually for each temperature. The results were screened for outliers using the single and double Grubbs test at a confidence level of 99 %. Some outlying individual results were found (Table 6). As no technical reason for the outliers could be found all data were retained for statistical analysis.

Furthermore, the data were plotted against storage time and linear regression lines of mass fraction versus time were calculated. The slopes of the regression lines were tested for statistical significance (loss/increase due to storage). No significant trend was detected for all analytes at a 95 % confidence level, except for β-HBCD at 4 °C (but not at 18°C).

The results of the long term stability measurements are shown in Annex D. The results of the statistical evaluation of the long-term stability study are summarised in Table 6.

Table 6: Results of the long-term stability tests

Parameter	Number of individual outlying results[1)]		Significance of the trend [2)]	
	4 ºC	18 ºC	4 ºC	18 ºC
BDE-28	none	none	yes	no
BDE-47	one	one	no	no
BDE-99	one	one	no	no
BDE-100	one	one	no	no
BDE-153	two	none	no	no
BDE-154	none	one	no	no
BDE-183	one	none	no	no
BDE-209	none	none	no	no
α-HBCD	none	none	no	no
β-HBCD	none	none	yes	no
γ-HBCD	one	none	no	no

1) 99 % confidence level
2) 95 % and 99 % confidence level

Statistical outliers were observed for BDE-47, -99, -100, -153, -154, -183 and γ-HBCD. As no technical reason for the outliers could be found, all data were retained for statistical analysis.

A significant trend at 4 °C was found for BDE-28 and β-HBCD, but the material appeared to be stable at 18 °C. As it is unlikely that the material degrades faster at lower temperature than at higher one, and given that the results of the long-term stability study lasting 1 year did not evidence any significant instability for β-HBCD, this was regarded as statistical artefact. The confirmation with another long-term stability study was unfortunately not possible for BDE-28.

The material can be stored at 18 °C.

5.3 Estimation of uncertainties

Due to the intrinsic variation of measurement results, no study can entirely rule out degradation of materials, even in the absence of statistically significant trends. It is therefore necessary to quantify the potential degradation that could be hidden by the method repeatability i.e., to estimate the uncertainty of stability. This means that, even under ideal conditions, the outcome of a stability study can only be that there is no detectable degradation within an uncertainty to be estimated.

The uncertainties of stability during dispatch and storage were estimated as described in [26] for each analyte. In this approach, the uncertainty of the linear regression line with a slope of zero was calculated. The uncertainty contributions u_{sts} and u_{lts} were calculated as the product of the chosen transport time/shelf life and the uncertainty of the regression lines as:

$$u_{sts,rel} = \frac{s_{rel}}{\sqrt{\sum\left(t_i - \bar{t}\right)^2}} \cdot t_{tt} \qquad \textbf{Equation 5}$$

$$u_{lts,rel} = \frac{s_{rel}}{\sqrt{\sum\left(t_i - \bar{t}\right)^2}} \cdot t_{sl} \qquad \textbf{Equation 6}$$

s_{rel} relative standard deviation of all results of the stability study

t_i time elapsed at time point *i*

$\bar{t}$ mean of all time t_i

t_{tt} chosen transport time (1 week at 18 ºC)

t_{sl} chosen shelf life (18 months at 18 ºC)

The following uncertainties were estimated:

- $u_{sts,rel}$, the uncertainty of degradation during dispatch. This was estimated from the 18 °C studies. The uncertainty describes the possible change during a dispatch at 18 °C lasting one week.
- $u_{lts,rel}$, the stability during storage. This uncertainty contribution was estimated from the 18 °C studies. The uncertainty contribution describes the possible degradation during 18 months storage at 18 °C.

The results of these evaluations are summarised in Table 7.

Table 7: Uncertainties of stability during dispatch and storage. $u_{sts,rel}$ was calculated for a temperature of 18 °C and 1 week; $u_{lts,rel}$ was calculated for a storage temperature of 18 °C and 18 months

Parameter	$u_{sts,rel}$ [%]	$u_{lts,rel}$ [%]
BDE-28	1.1	6.1
BDE-47	0.9	2.9
BDE-99	0.5	3.0
BDE-100	0.8	3.3
BDE-153	0.7	3.2
BDE-154	0.7	3.9
BDE-183	0.7	3.2
BDE-209	0.9	2.0
α-HBCD	1.1	5.5
β-HBCD	1.2	5.2
γ-HBCD	1.0	9.2

The material showed significant degradation at 60 °C but no significant degradation was observed for transport up to 18 °C. Cooled shipment is therefore necessary.

The material can be stored at 18 °C.

After the certification campaign, the material will be included in the JRC's regular stability monitoring programme to control its further stability.

6 Characterisation

The material characterisation is the process of determining the property values of a reference material.

The material characterisation was based on an interlaboratory comparison of expert laboratories, i.e. the properties of the material were determined in different laboratories that applied different measurement procedures to demonstrate the absence of a measurement bias. This approach aims at randomisation of laboratory bias, which reduces the combined uncertainty. Due to the nature of the analytes however, all participants used GC-based methods to measure the PBDEs and LC-based methods to measure the HBCD, respectively.

6.1 Selection of participants

Thirteen laboratories were selected based on criteria that comprised both technical competence and quality management aspects. Each participant was required to operate a quality system and to deliver documented evidence of its laboratory proficiency in the field of PBDEs and HBCD measurements in sediment (or similar matrices) by submitting results of interlaboratory comparison exercises and/or method validation. Having a formal accreditation

was not mandatory, but meeting the requirements of ISO/IEC 17025 was obligatory. Where measurements are covered by the scope of accreditation, the accreditation number is stated in the list of participants (Section 2).

6.2 Study setup

Each laboratory received two units of ERM-CC537a and was requested to provide six independent results, three per unit. The units for material characterisation were selected using a random stratified sampling scheme and covered the whole batch. The sample preparations (and preferably also the measurements) had to be spread over at least two days to ensure intermediate precision conditions. Fresh calibration standards had to be prepared on each day of measurement. The water and volatiles' content had to be determined on each unit in duplicate (according to a prescribed oven-drying procedure). PBDEs and HBCD results had thus to be corrected for the latter and reported on a dry mass basis.

Each participant received a sample of NIST SRM 1944, New York/New Jersey Waterway Sediment, as a blind quality control (QC) sample. The results for this sample were used to support the evaluation of the characterisation results.

Laboratories were also requested to give estimations of the expanded uncertainties of the mean value of the six results. No approach for the estimation was prescribed, i.e. top-down and bottom-up were regarded as equally valid procedures.

6.3 Methods used

A variety of extraction (e.g., Soxhlet, ASE, SPE) and clean-up methods [e.g., alumina and (acidic) silica gel column, GPC] with different quantification steps (GC-MS, GC-HRMS, GC-MS/MS, HPLC-MS/MS, UPLC-MS/MS) were used to characterise the material for the analytes of interest. The combination of results from methods based on different principles mitigates undetected method bias.

All methods used during the characterisation study are summarised in Annex E. The laboratory code (e.g., L01) is a random number and does not correspond to the order of laboratories in Section 2. The lab-method code consists of a number assigned to each laboratory (e.g., L01) and abbreviation of the measurement method used (e.g., GC-MS).

6.3.1 Dry mass correction

For all measurements carried out during certification (homogeneity, stability and characterisation studies) the following protocol for dry matter content determination was prescribed:

"A correction for dry mass shall be performed at the same time of the analysis by taking 2 separate portions of at least 1 g from each bottle analysed, drying them in an oven at 105 °C ± 2 °C until constant mass is attained (subsequent weightings should not differ more than 0.5 mg)."

The water and volatiles' content determined by the laboratories according to the above procedure was in the range of 4 g/kg to 15 g/kg, with the majority of values (10 out of 14) between 4 g/kg and 8 g/kg.

6.4 Evaluation of results

The characterisation campaign resulted in fourteen and eight datasets for the PBDEs and HBCD, respectively. All individual results of the participants, grouped per class of analytes are displayed in tabular form in Annex F.

6.4.1 Technical evaluation

The data obtained were first checked for compliance with the requested analysis protocol and for their validity based on technical reasons. The following criteria were considered during the evaluation:

- appropriate validation of the measurement procedure
- compliance with the analysis protocol: sample preparations and measurements performed on two days, according to the prescribed analytical sequence and volatiles' content determination.
- absence of values given as below limit of detection or below limit of quantification
- method performance,
    1. agreement of the measurement results with the assigned values of the QC sample (corresponding to the reference values for PBDE congeners and information values for the HBCD isomers in NIST SRM 1944, the only exception being BDE-28 for which the consensus value among all laboratories was used as assigned value, given that no value is reported in the certificate) applying the ERM Application Note 1 [27],
    2. coherence between method repeatability values as provided by the laboratory *a priori* (based on method validation data) and extrapolated from the characterisation measurement dataset.

Based on the above criteria, the following datasets were rejected as not technically valid.

L00: the measurement results for the HBCD isomers were excluded because the laboratory reported "less than" values for the QC sample, thus the assessment of the method performance was not possible.

L01: the measurement results for BDE-99, BDE-154 and BDE-209 were excluded because the results obtained on the QC sample did not agree with the assigned values.

L04: the measurement result for BDE-209 was excluded because the results obtained on the QC sample did not agree with the assigned value.

L05: the measurement result for BDE-154 was excluded because the results obtained on the QC sample did not agree with the assigned value.

L07: the measurement results for BDE-100, BDE-154, BDE-183 and BDE-209 were excluded because the results obtained on the QC sample did not agree with the assigned values. The measurement results for the HBCD isomers were excluded because the laboratory reported "less than" values both for the QC sample and for ERM-CC537a.

L08: the complete dataset was excluded because of the high variability of the results shown in an extended dataset of measurements provided by the laboratory, for which no technical reason could be given (according to point 2. of the method performance criteria).

L09: the measurement results for BDE-47, BDE-99, BDE-100 and BDE-209 were excluded because the results obtained on the QC sample did not agree with the assigned values (contamination issues were reported by the laboratory).

L10: the measurement result for BDE-183 was excluded on the basis of a technical reason reported by the laboratory (overestimation caused by the possible degradation of BDE-209).

L11: the measurement results for BDE-209 and for the β- and γ-HBCD isomers were excluded because the results obtained on the QC sample did not agree with the assigned values. The measurement result for the β-HBCD was excluded on the basis of a technical reason reported by the laboratory (chromatographic co-elution).

L12: the measurement results for BDE-28 and BDE-100 were excluded because the results obtained on the QC sample did not agree with the assigned values.

L13: the measurement results for BDE-47, BDE-153 and BDE-154 were excluded because they did not agree with the assigned values of the QC sample. The measurement results for BDE-209 were excluded because the results for the QC sample were reported as "outside the working range".

6.4.2 Statistical evaluation

The datasets accepted based on technical reasons were tested for normality of dataset means using kurtosis/skewness tests (at a 99 % confidence level) and normal probability plots and were tested for outlying means using the Grubbs test and using the Cochran test for outlying standard deviations, (both at a 99 % confidence level). Standard deviations within (s_{within}) and between ($s_{between}$) laboratories were calculated using one-way ANOVA. The results of these evaluations are shown in Table 7.

Table 7: Statistical evaluation of the technically accepted datasets for ERM-CC537a.
p: number of technically valid datasets

Parameter	*p*	Outliers		Normally distributed	Statistical parameters			
		Means	Variances		Mean [µg/kg]	*s* [µg /kg]	$s_{between}$ [µg /kg]	s_{within} [µg /kg]
BDE-28	11	--	--	yes	0.281	0.050	0.052	0.022
BDE-47	10	--	--	yes	16.499	2.126	2.100	0.822
BDE-99	11	--	--	yes	34.282	4.119	3.940	2.946
BDE-100	10	--	--	yes	5.757	0.526	0.507	0.345
BDE-153	12	L00	--	no	6.641	0.993	0.933	0.537
BDE-154	9	--	--	yes	3.475	0.403	0.384	0.337
BDE-183	9	--	--	yes	1.414	0.174	0.162	0.116
BDE-209	6	--	L00	insufficient data	7751.882	688.831	646.046	585.386
α-HBCD	6	--	L11	insufficient data	8.337	1.365	1.306	0.977
β-HBCD	5	--	--	insufficient data	2.263	0.361	0.352	0.194
γ-HBCD	5	--	L10	insufficient data	59.682	10.994	9.244	14.578

The laboratory means follow normal distributions, except for BDE-153. The non-normality (according to the kurtosis/skewness tests) of the BDE-153 dataset can be traced back to the presence of L00, flagged as outlier for BDE-153 by the statistical evaluation, while no technical reason could be identified for excluding the result. However, it must be borne in mind that outlier tests do not take uncertainty information into consideration. A closer investigation reveals that the difference between the mean value of laboratory L00 and the other results is covered by the measurement uncertainty of laboratory L00 (see Annex F, Figure F5). There is therefore no evidence that the result of laboratory L00 does not agree with the other results and it is therefore retained for the calculation of the certified value. An additional check by applying ERM Application Note 1 [27] confirms that there is no significant difference between the mean of L00 and the certified value.

The statistical evaluation flags laboratories L00, L10 and L11 as outlying variance for BDE-209, γ-HBCD and α-HBCD, respectively. This merely reflects the fact that different methods have different intrinsic variability. As all measurement methods were found technically sound, all results were retained.

The datasets are consistent and the mean of laboratory means is a good estimate of the true value.

The uncertainty related to the characterisation is estimated as the standard error of the mean of laboratory means (Table 8).

Table 8: Uncertainty of characterisation for ERM-CC537a

Parameter	*p*	Mean [µg/kg]	*s* [µg/kg]	u_{char} [µg/kg]
BDE-28	11	0.281	0.050	0.015
BDE-47	10	16.499	2.126	0.672
BDE-99	11	34.282	4.119	1.242
BDE-100	10	5.757	0.526	0.166
BDE-153	12	6.641	0.993	0.287
BDE-154	9	3.475	0.403	0.134
BDE-183	9	1.414	0.174	0.058
BDE-209	6	7751.882	688.831	281.214
α-HBCD	6	8.337	1.365	0.557
β-HBCD	5	2.263	0.361	0.161
γ-HBCD	5	59.682	10.994	4.917

7 Value Assignment

Certified and additional material information values were assigned.

Certified values are values that fulfil the highest standards of accuracy. Procedures at JRC require generally pooling of not less than 6 datasets to assign certified values. Full uncertainty budgets in accordance with the 'Guide to the Expression of Uncertainty in Measurement' [4] were established.

Additional material information refers to values that were obtained in the course of the study. For example, results reported from only one or two laboratories or in cases where individual measurement uncertainty is high, would fall under this category.

7.1 Certified values and their uncertainties

The unweighted mean of the means of the accepted datasets as shown in Table 7 was assigned as certified value for each parameter.

The assigned uncertainty consists of uncertainties related to characterisation, u_{char} (Section 6), potential between-unit inhomogeneity, u_{bb} (Section 4.1) and potential degradation during transport (u_{sts}) and long-term storage, u_{lts} (Section 5). These different contributions were combined to estimate the expanded, relative uncertainty of the certified value ($U_{CRM, rel}$) with a coverage factor *k* as:

$$U_{CRM,rel} = k \cdot \sqrt{u_{char,rel}^2 + u_{bb,rel}^2 + u_{sts,rel}^2 + u_{lts,rel}^2}$$ **Equation 7**

- u_{char} was estimated as described in Section 6
- u_{bb} was estimated as described in Section 4.1.
- u_{sts} and u_{lts} were estimated as described in Section 5.3.

Because of the sufficient numbers of the degrees of freedom of the different uncertainty contributions, a coverage factor k = 2 was applied to obtain the expanded uncertainties. Only five datasets were accepted for β- and γ-HBCD following the technical evaluation, while procedures at JRC require generally pooling of not less than 6 datasets to assign certified values. Nevertheless, considering that certified values for these analytes would be extremely useful for the laboratories working in environmental monitoring (ERM-CC537a will be the first sediment RM certified for HBCD), it was finally decided to include β- and γ-HBCD in the list of certified analytes. Because of the low number of datasets accepted for the characterisation, the effective number of degrees of freedom of the different uncertainty contributions was calculated using the Welch-Sattertwaithe equation [4]. The number of degrees of freedom was found to be 14 and 11 for β- and γ-HBCD, respectively, therefore sufficiently high to apply a coverage factor k = 2 to obtain the expanded uncertainties [2].

The certified values and their uncertainties are summarised in Table 9.

Table 9: Certified values and their uncertainties for ERM-CC537a

Parameter	Certified value[1)]	$u_{char,\ rel}$ [%]	$u_{bb,\ rel}$ [%]	$u_{sts,\ rel}$ [%]	$u_{lts,\ rel}$ [%]	$U_{CRM,\ rel}$ [%]	U_{CRM}[2)]
BDE-28	0.28 µg/kg	5.4	1.9	1.1	6.1	16.8	0.05 µg/kg
BDE-47	16.5 µg/kg	4.1	1.6	0.9	2.9	10.7	1.8 µg/kg
BDE-99	34 µg/kg	3.6	2.3	0.5	3.0	10.5	4 µg/kg
BDE-100	5.8 µg/kg	2.9	1.9	0.8	3.3	9.7	0.6 µg/kg
BDE-153	6.6 µg/kg	4.3	3.8	0.7	3.2	13.2	0.9 µg/kg
BDE-154	3.5 µg/kg	3.9	3.1	0.7	3.9	12.7	0.5 µg/kg
BDE-183	1.41 µg/kg	4.1	5.2	0.7	3.2	14.8	0.21 µg/kg
BDE-209	7.8 mg/kg	3.6	1.0	0.9	2.0	8.7	0.7 mg/kg
α-HBCD	8.3 µg/kg	6.7	3.6	1.1	5.5	18.9	1.6 µg/kg
β-HBCD	2.3 µg/kg	7.1	4.1	1.2	5.2	19.6	0.5 µg/kg
γ-HBCD	60 µg/kg	8.2	3.9	1.0	9.2	26	16 µg/kg

[1)] reported on a dry mass basis (Section 6.3.1)
[2)] expanded (k = 2) and rounded uncertainty.

7.2 Additional material information

The data provided in this section should be regarded as informative only on the general composition of the material and cannot be, in any case, used as certified or indicative value.

Total organic carbon (TOC) measurements, in accordance to ISO 10694:1995 *Soil quality - Determination of organic and total carbon after dry combustion (elementary analysis)* and sulfur measurements by combustion and infrared detection were carried out in triplicate on two bottles of ERM-CC537a. The mean values obtained for TOC and the total sulfur were 0.691 m/m % and 0.073 m/m % (equivalent to 10^{-2} g/g), respectively on an air-dried basis.

Another useful information about ERM-CC537a is that the presence of BB153 was not detected. This information was obtained during the characterisation exercise by one of the participating laboratory which specifically checked for the BDE154/BB153 co-elution, using a chromatographic column with a stationary phase capable of separating these two compounds (HT8 GC).

8 Metrological traceability and commutability

8.1 Metrological traceability

Identity

PBDEs and HBCD are chemically clearly defined analytes. Identity was confirmed by mass spectrometry. The participants used different methods for the sample preparation as well as for the final determination, demonstrating to a great extent the absence of measurement bias. Nevertheless, since all participants used a GC separation step for the determination of the PBDEs, and an LC separation step for the determination of the HBCD, the measurands are operationally defined by GC and LC, respectively.

Quantity value

Only validated methods were used for the determination of the assigned values. Different calibrants of known purity and specified traceability of their assigned values were used and all relevant input parameters were calibrated. All technically accepted datasets are therefore traceable to the same reference, namely the SI. This traceability to the same reference is also confirmed by the agreement of results within their respective uncertainties. As the assigned values are combinations of agreeing results individually traceable to the SI, the assigned quantity values themselves are traceable to the SI as well.

8.2 Commutability

Many measurement procedures include one or more steps, which are selecting specific (or specific groups of) analytes from the sample for the subsequent steps of the whole measurement process. Often the complete identity of these 'intermediate analytes' is not fully known or taken into account. Therefore, it is difficult to mimic all the analytically relevant properties of real samples within a CRM. The degree of equivalence in the analytical behaviour of real samples and a CRM with respect to various measurement procedures (methods) is summarised in a concept called 'commutability of a reference material'. There are various definitions expressing this concept. For instance, the CSLI Guideline C-53A [28] recommends the use of the following definition for the term *commutability*:

> "The equivalence of the mathematical relationships among the results of different measurement procedures for an RM and for representative samples of the type intended to be measured."

The commutability of a CRM defines its fitness for use and, thus, is a crucial characteristic in case of the application of different measurement methods. When commutability of a CRM is not established in such cases, the results from routinely used methods cannot be legitimately compared with the certified value to determine whether a bias does not exist in calibration, nor can the CRM be used as a calibrant.

ERM-CC537a was produced from a naturally contaminated freshwater sediment by drying, milling and mixing. The methods used in the characterisation of ERM-CC537a are methods routinely applied for measuring brominated flame retardants in sediment and alike matrices. The agreement of results from different methods demonstrates that the processing step did not affect any properties relevant for these methods and that the analytical behaviour of ERM-CC537a is the same as of a real sediment sample.

9 Instructions for use

9.1 Safety information

The usual laboratory safety measures apply.

9.2 Storage conditions

The materials shall be stored at 18 °C ± 5 °C in the dark. The user is reminded to close bottles immediately after taking a sample.

Please note that the European Commission cannot be held responsible for changes that happen during storage of the material at the customer's premises, especially of opened bottles.

9.3 Preparation and use of the material

The units shall be shaken by turning upside down for at least 2 min before opening to ensure material re-homogenisation.

9.4 Minimum sample intake

The minimum sample intake representative for all certified parameters is 750 mg.

9.5 Dry mass correction

Dry mass determination shall be carried out on two separate portions of at least 1 g, by drying them in an oven at 105 ^{o}C ± 2 ^{o}C until constant mass (successive weighing should not differ by more than 0.5 mg) is attained. Weighing of the samples for dry mass determination and weighing for the analysis shall be done at the same time to avoid differences due to possible take up of moisture by the material.

9.6 Use of the certified value

The main purpose of this material is to assess method performance, i.e. for checking accuracy of analytical results. As any reference material, it can also be used for control charts or validation studies.

Use as a calibrant

It is not recommended to use this matrix material as calibrant.

Comparing an analytical result with the certified value

A result is unbiased if the combined standard uncertainty of measurement and certified value covers the difference between the certified value and the measurement result (see also ERM Application Note 1 https://crm.jrc.ec.europa.eu [27].

For assessing the method performance, the measured values of the CRMs are compared with the certified values. The procedure is described here in brief:

- Calculate the absolute difference between mean measured value and the certified value (Δ_{meas}).
- Combine measurement uncertainty (u_{meas}) with the uncertainty of the certified value (u_{CRM}): $u_{\Delta} = \sqrt{u_{meas}^2 + u_{CRM}^2}$
- Calculate the expanded uncertainty (U_{Δ}) from the combined uncertainty ($u_{\Delta,}$) using an appropriate coverage factor, corresponding to a level of confidence of approximately 95 %
- If $\Delta_{meas} \leq U_{\Delta}$ no significant difference between the measurement result and the certified value, at a confidence level of about 95 % exists.

Use in quality control charts

The materials can be used for quality control charts. Different CRM units will give the same result as inhomogeneity was included in the uncertainties of the certified values.

10 Acknowledgments

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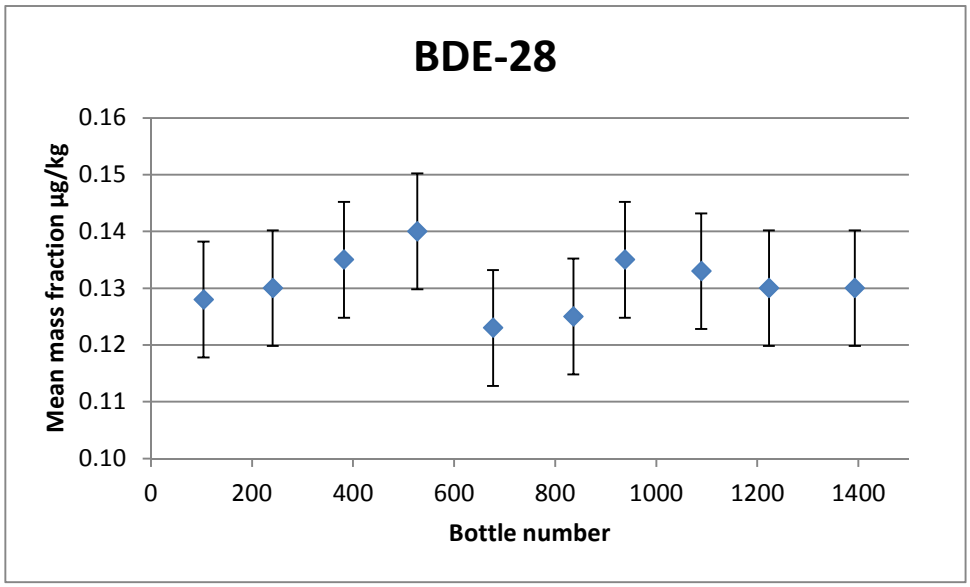
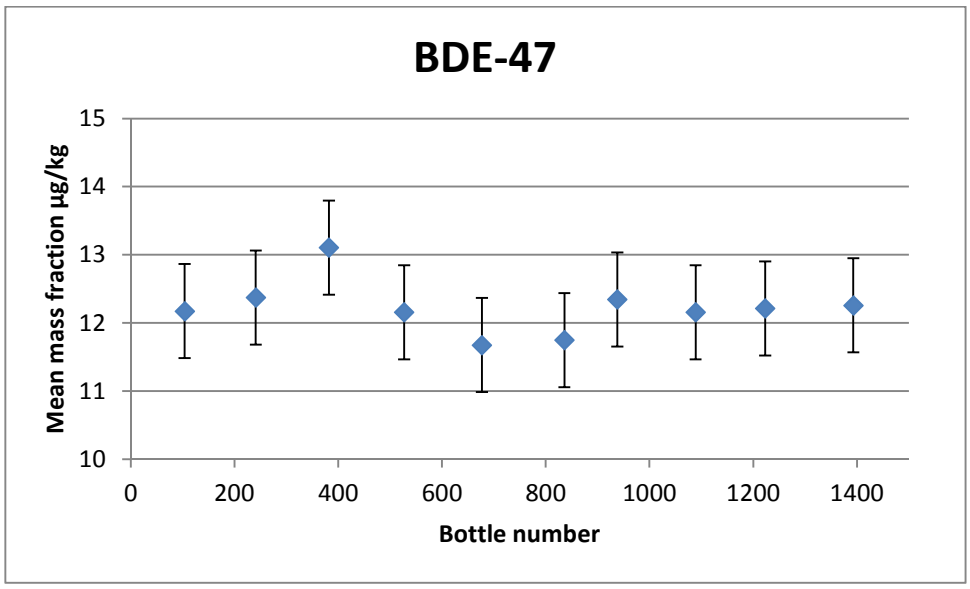
Annexes

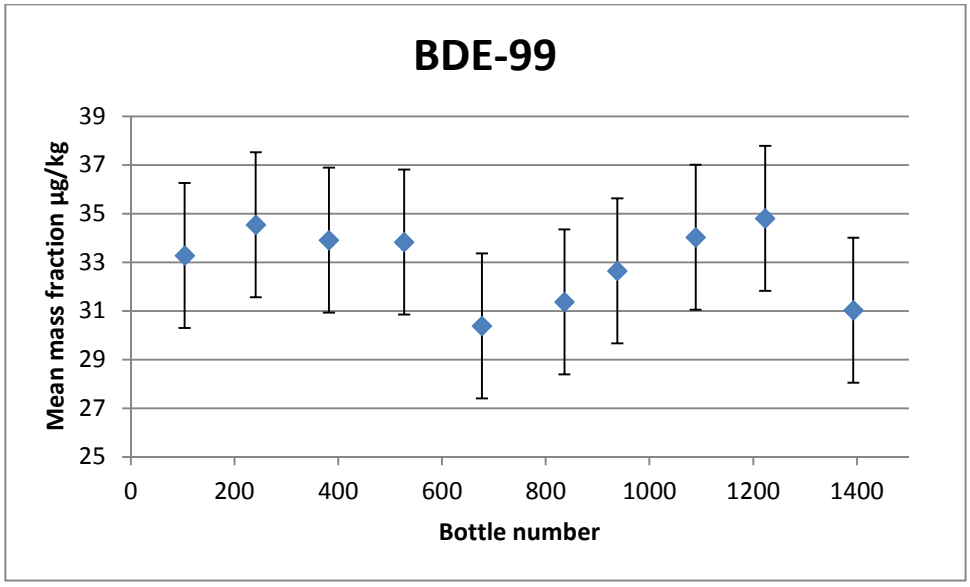
Annex A. Results of the homogeneity measurements

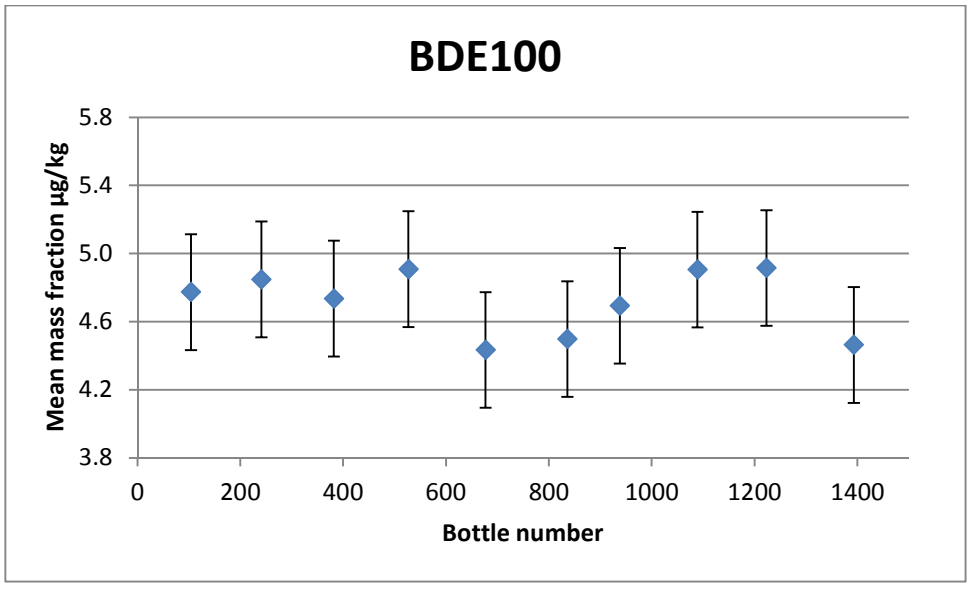
Analytical method applied: GC-ECNI-MS for PBDEs and by LC-MS/MS for HBCD after Soxhlet extraction using hexane and acetone (3/1 v/v), clean-up on acidified silica (44 % H_2SO_4 conc. w/w; elution with 20 mL hexane and 15 mL CH_2Cl_2) and further fractionation on an SPE cartridge (1st fraction containing PBDE eluted with hexane and 2nd fraction containing HBCD eluted with CH_2Cl_2).

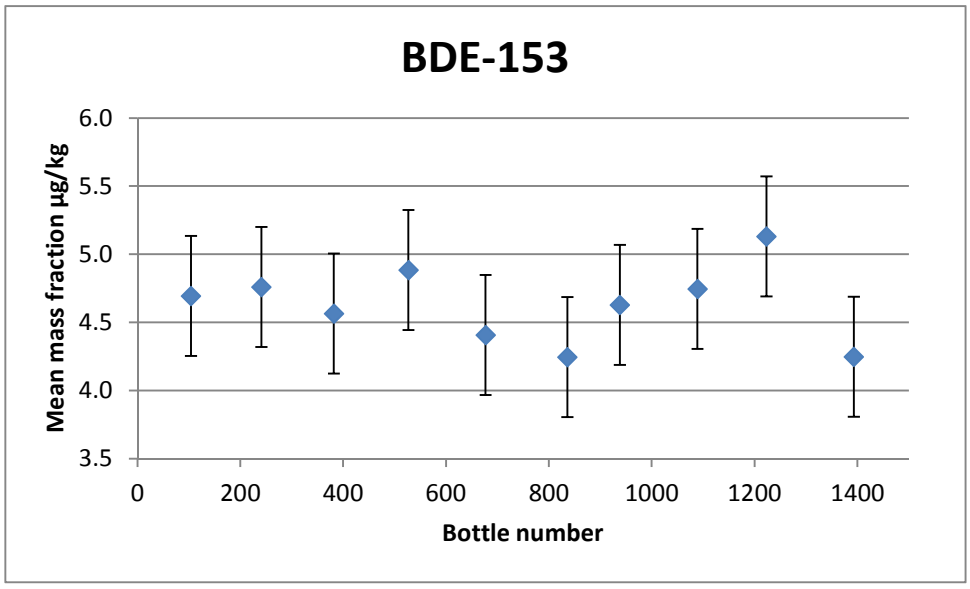
- The graphs report unit means ± 95 % confidence interval (CI) of the means expressed as mass fraction on a dry mass basis.

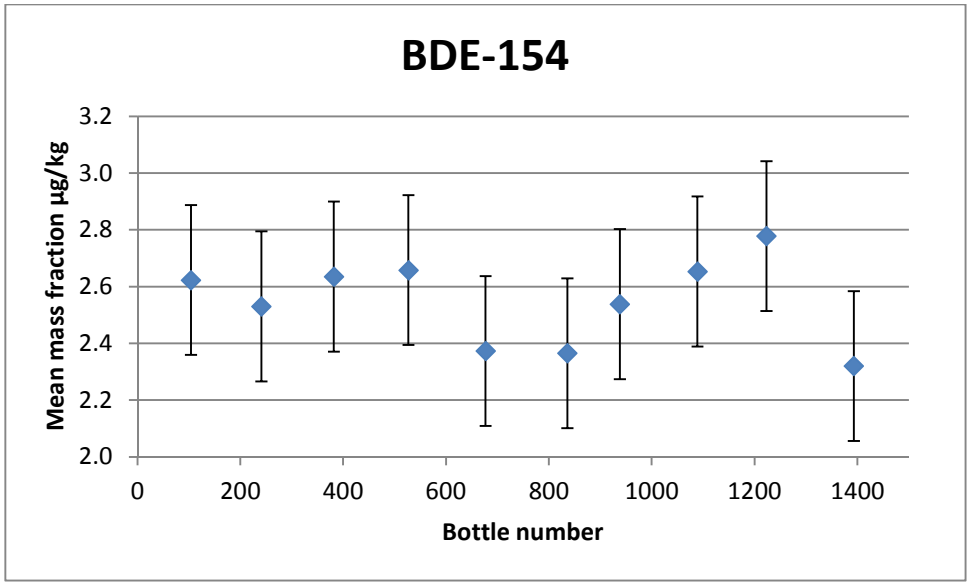


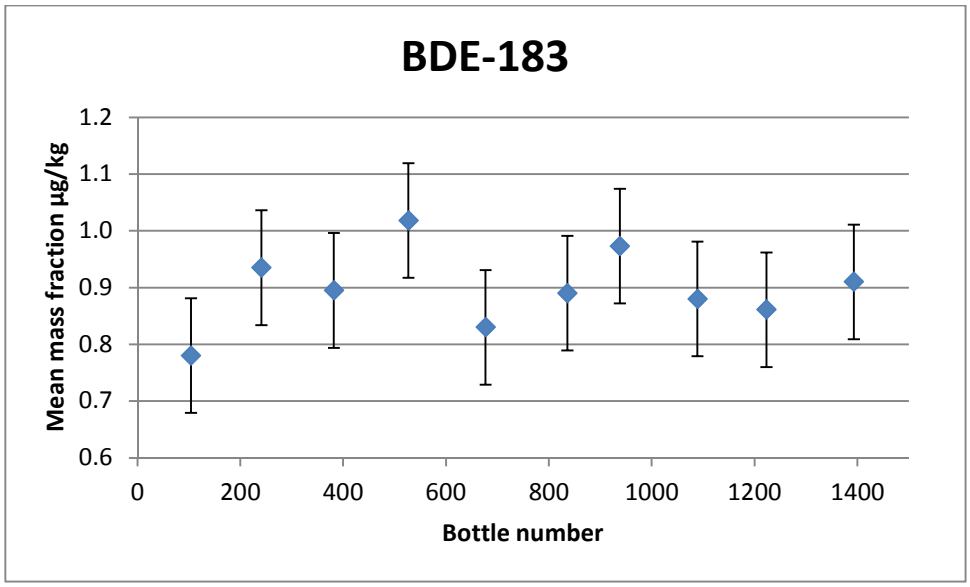


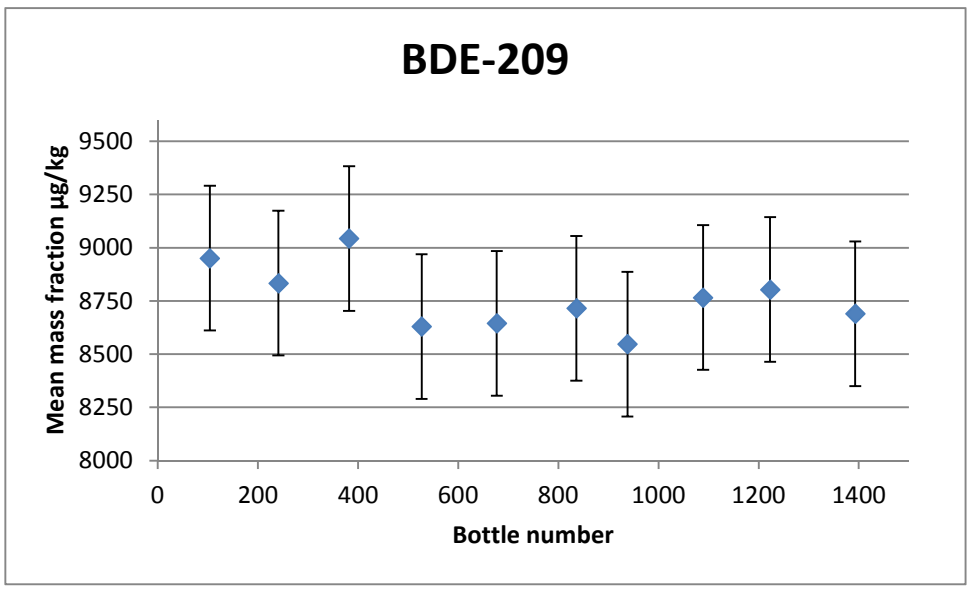

BDE-99
Mean mass fraction µg/kg
39
37
35
33
31
29
27
25
0
200
400
600
800
1000
1200
1400
Bottle number

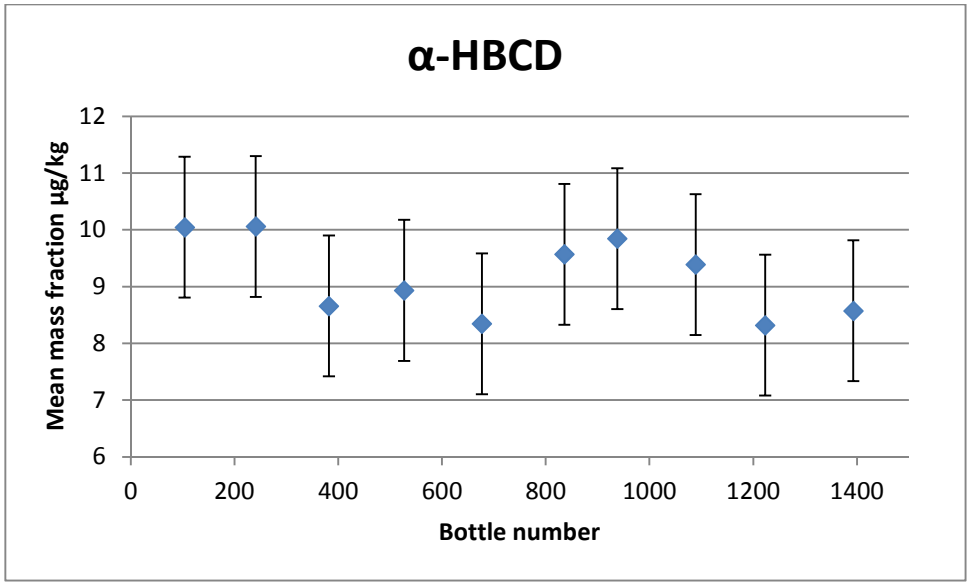

BDE100
Mean mass fraction µg/kg
5.8
5.4
5.0
4.6
4.2
3.8
0
200
400
600
800
1000
1200
1400
Bottle number

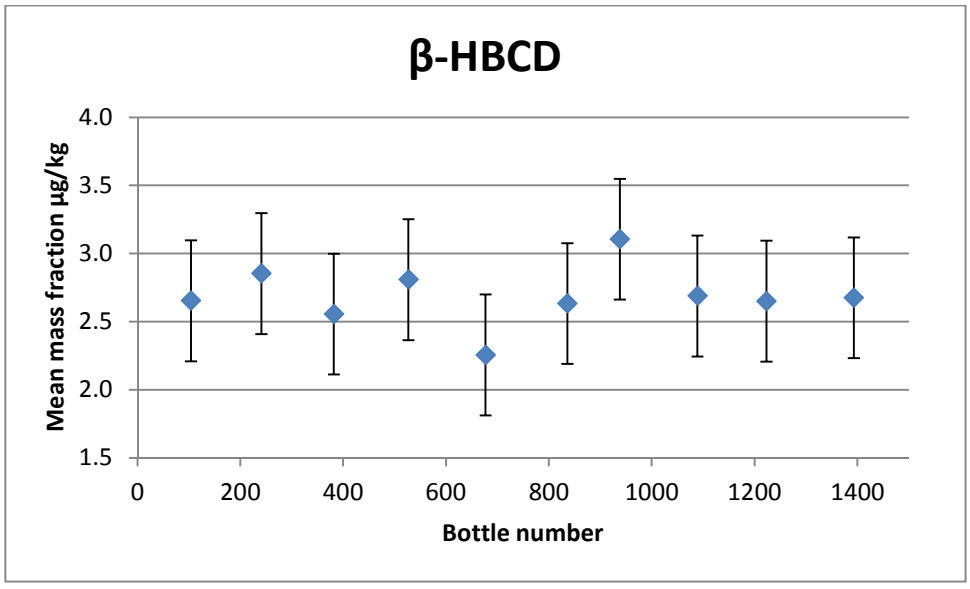

BDE-153
Mean mass fraction µg/kg
6.0
5.5
5.0
4.5
4.0
3.5
0
200
400
600
800
1000
1200
1400
Bottle number

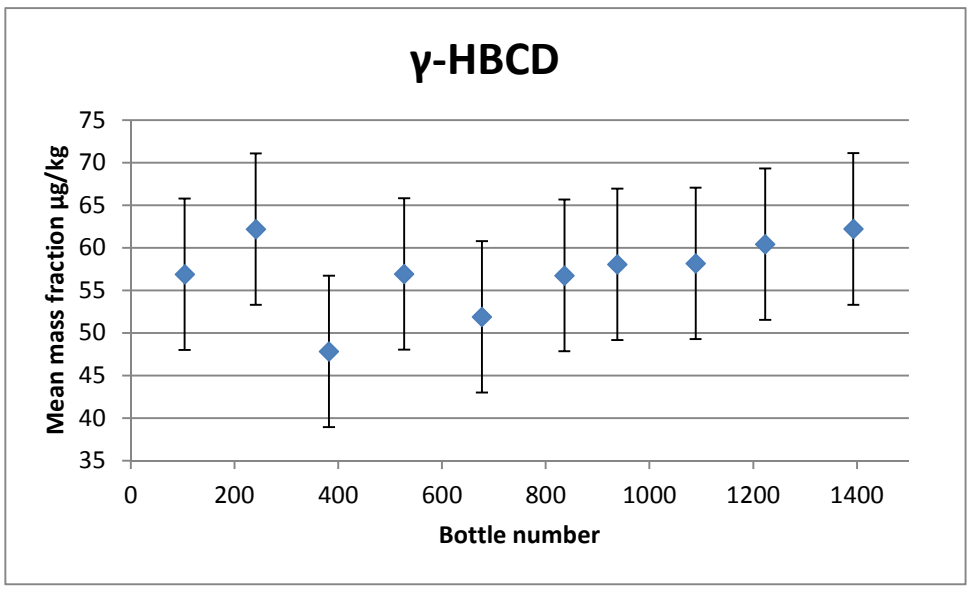

BDE-154
Mean mass fraction µg/kg
3.2
3.0
2.8
2.6
2.4
2.2
2.0
0
200
400
600
800
1000
1200
1400
Bottle number


BDE-183
Mean mass fraction µg/kg
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0
200
400
600
800
1000
1200
1400
Bottle number


BDE-209
Mean mass fraction µg/kg
9500
9250
9000
8750
8500
8250
8000
0
200
400
600
800
1000
1200
1400
Bottle number


α-HBCD
Mean mass fraction µg/kg
12
11
10
9
8
7
6
0
200
400
600
800
1000
1200
1400
Bottle number


β-HBCD
Mean mass fraction µg/kg
4.0
3.5
3.0
2.5
2.0
1.5
0
200
400
600
800
1000
1200
1400
Bottle number


γ-HBCD
Mean mass fraction µg/kg
75
70
65
60
55
50
45
40
35
0
200
400
600
800
1000
1200
1400
Bottle number

Annex B. Minimum sample intake study

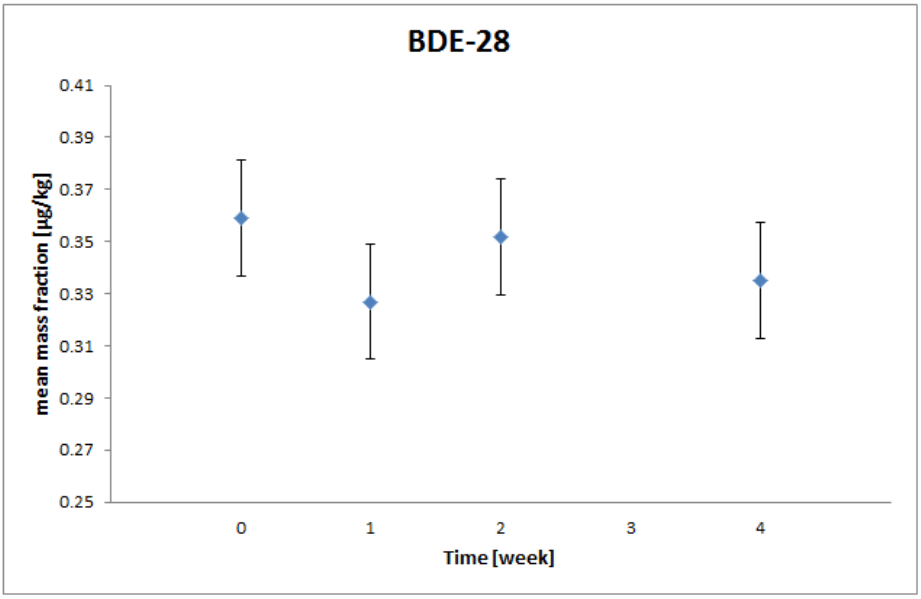
- Relative standard deviations (RSD) of measurement results for different sample intakes
  (n = number of independent replicates)

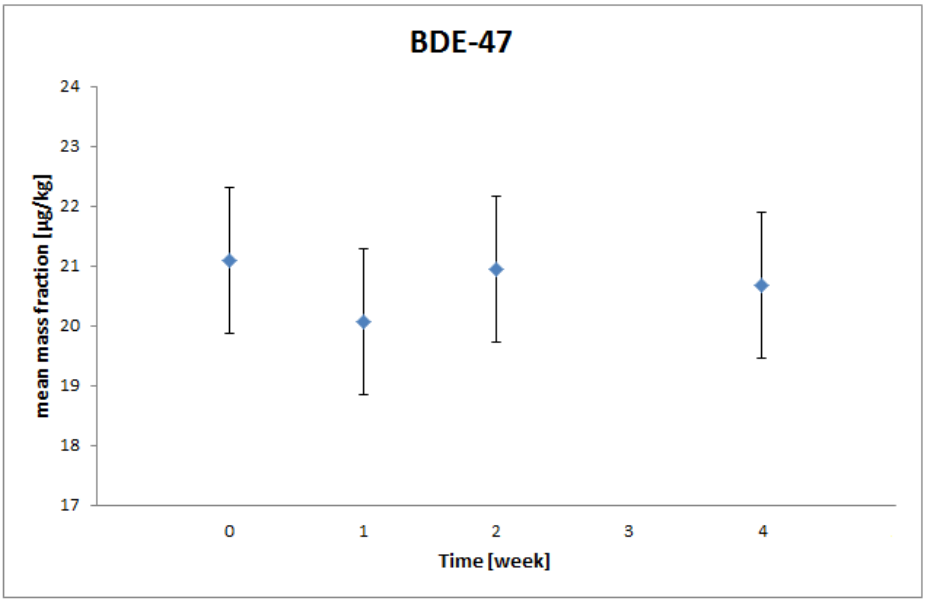
	RSD %		
	750 mg (n=40)	**500 mg** (n=8)	**200 mg** (n=8)
BDE 28	7.6	11.6	16.9
BDE 47	5.7	3.8	7.5
BDE 99	9.0	5.8	13.6
BDE 100	7.3	5.2	10.4
BDE 153	10.0	8.0	18.0
BDE 154	10.6	8.2	18.7
BDE 183	12.1	7.4	23.9
BDE 209	3.7	2.7	3.5
α-HBCD	13.7	14.8	17.5
β-HBCD	16.2	18.0	34.9
γ-HBCD	15.3	16.6	24.4

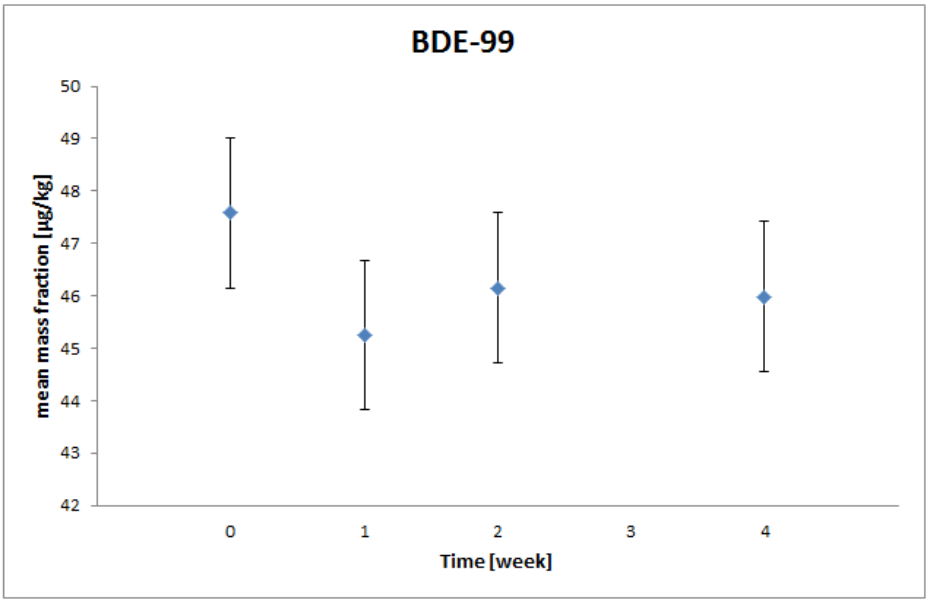
Annex C. Results of the short-term stability measurements

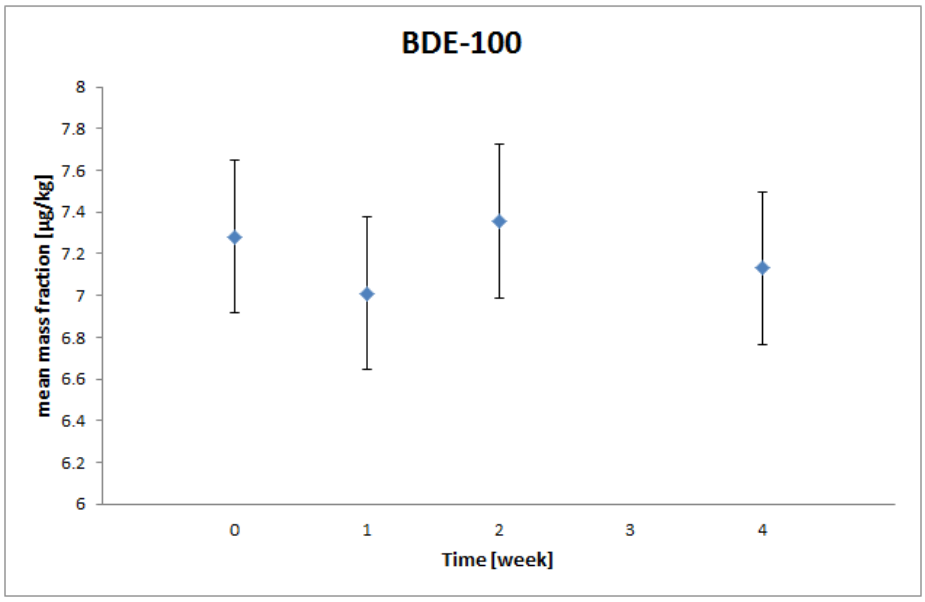
Analytical method applied: GC-HRMS for PBDEs and UPLC-MS/MS for HBCD after clean-up on an SPE cartridge (elution with methanol and *n*-hexane).

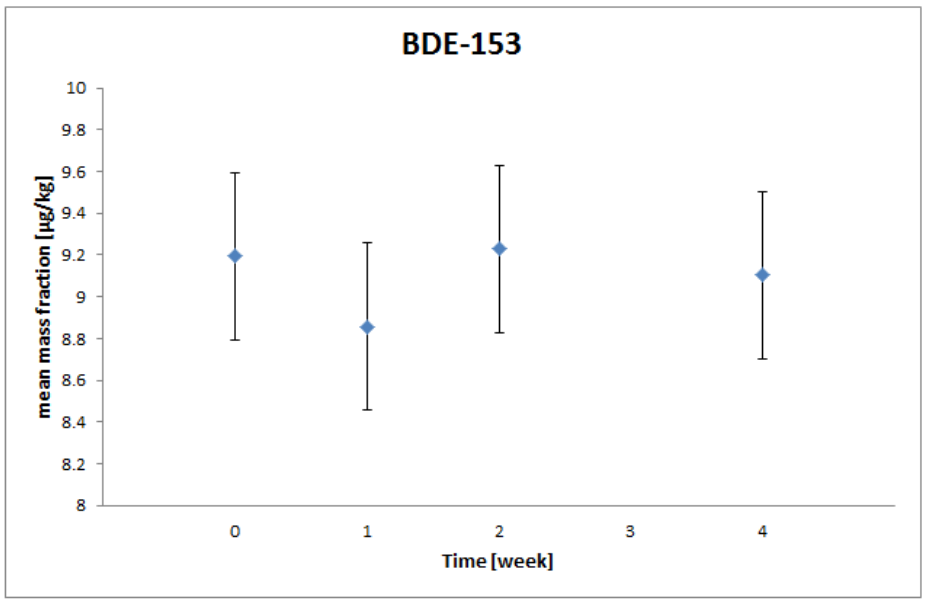
- Data for the short-term stability study at **18 °C**. The graphs report means per time point ± 95 % CI of the means expressed as mass fraction on a dry mass basis.

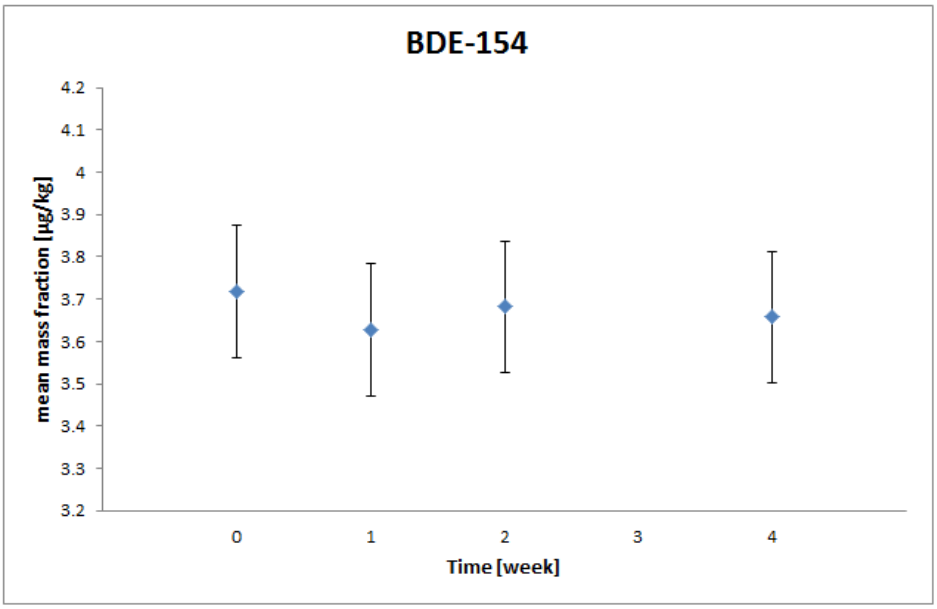


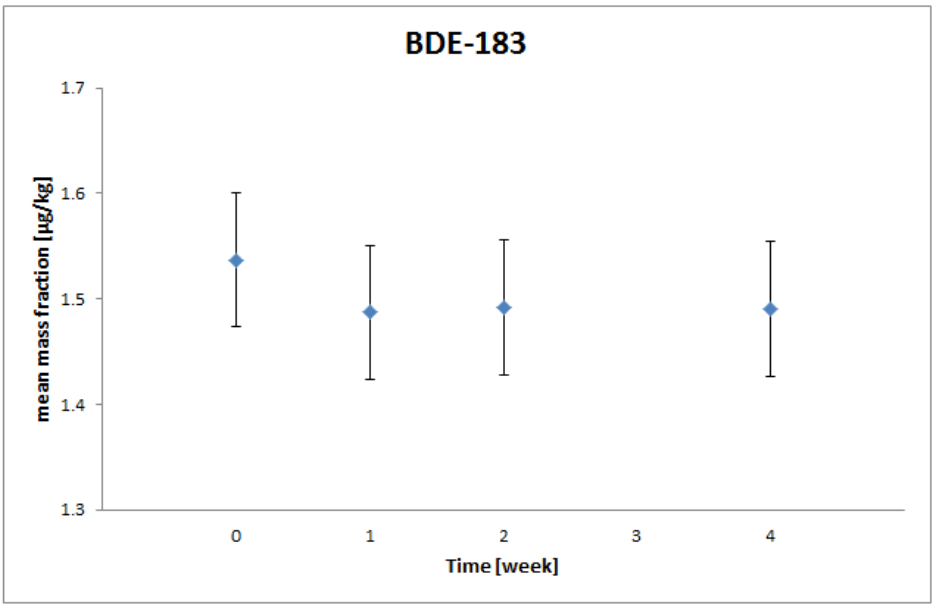


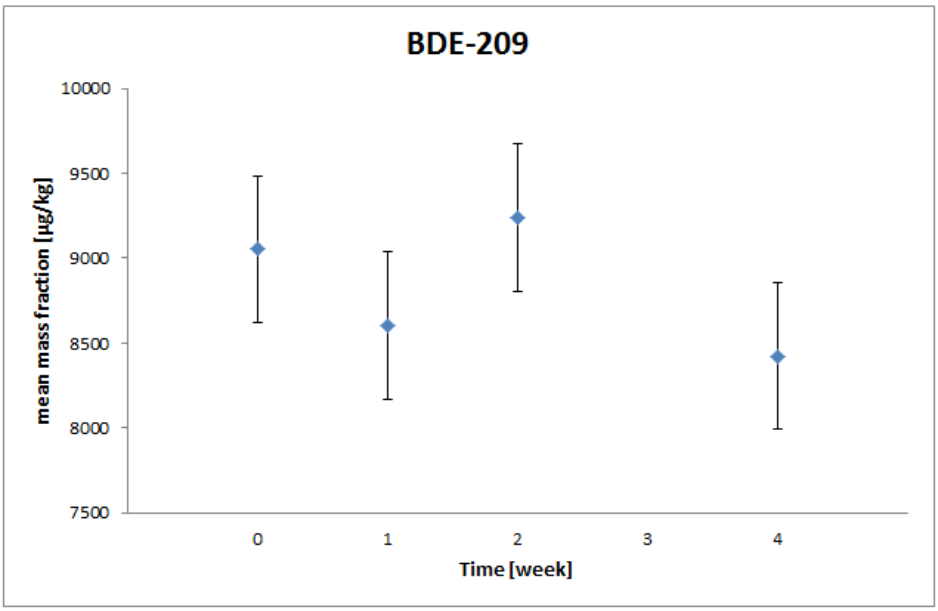

BDE-99
mean mass fraction [µg/kg]
50
49
48
47
46
45
44
43
42
0
1
2
3
4
Time [week]

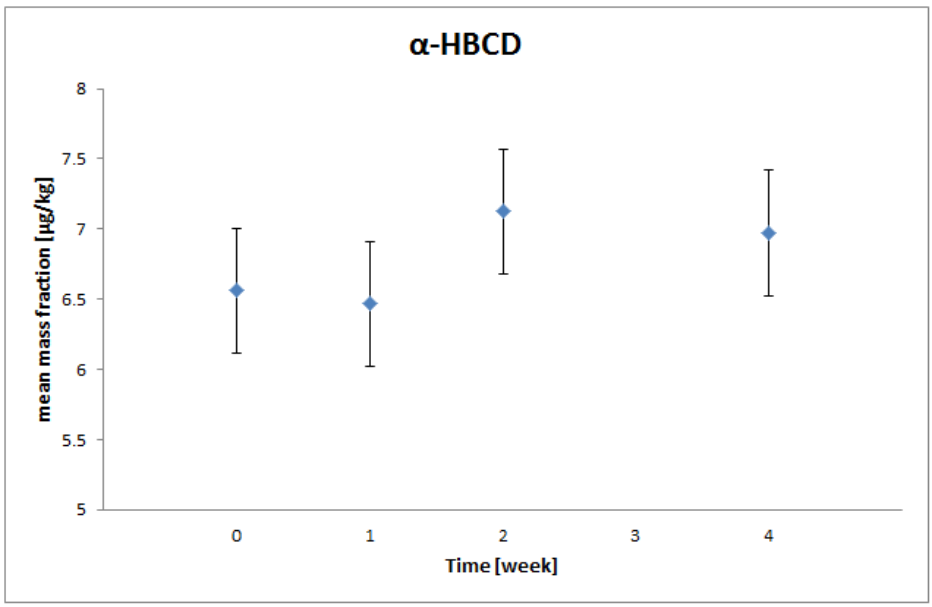

BDE-100
mean mass fraction [µg/kg]
8
7.8
7.6
7.4
7.2
7
6.8
6.6
6.4
6.2
6
0
1
2
3
4
Time [week]

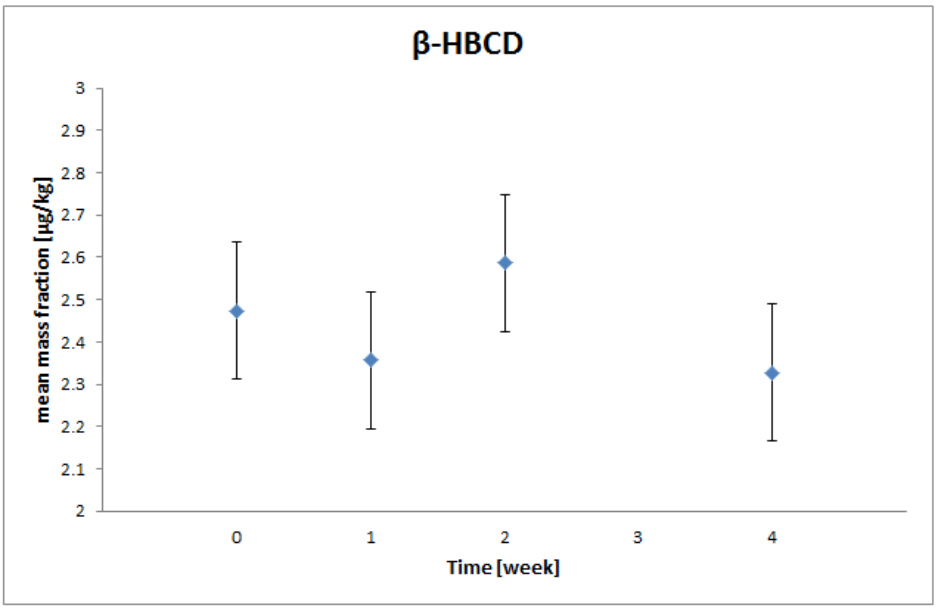

BDE-153
mean mass fraction [µg/kg]
10
9.8
9.6
9.4
9.2
9
8.8
8.6
8.4
8.2
8
0
1
2
3
4
Time [week]

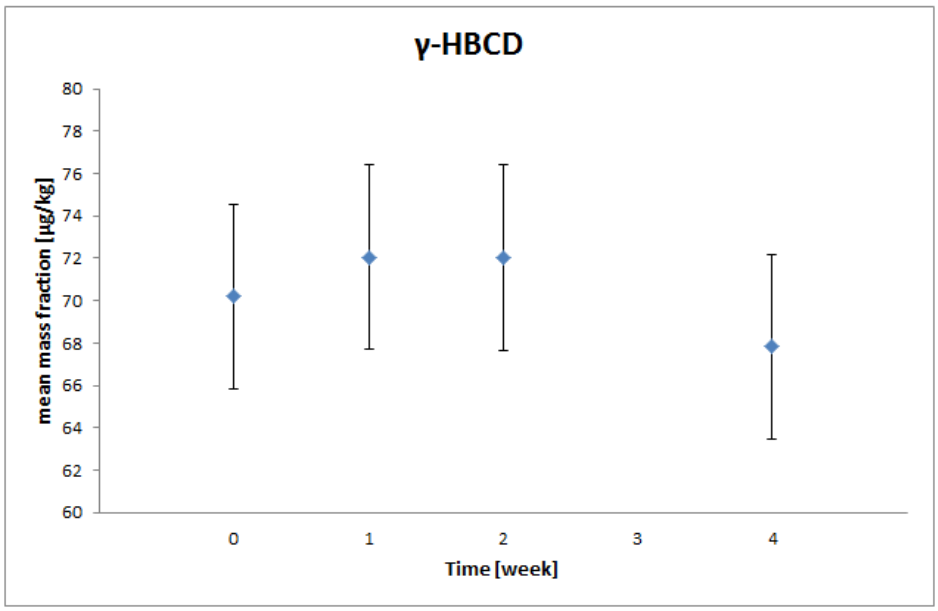

BDE-154
mean mass fraction [µg/kg]
4.2
4.1
4
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
0
1
2
3
4
Time [week]


BDE-183
mean mass fraction [µg/kg]
1.7
1.6
1.5
1.4
1.3
0
1
2
3
4
Time [week]


BDE-209
mean mass fraction [µg/kg]
10000
9500
9000
8500
8000
7500
0
1
2
3
4
Time [week]

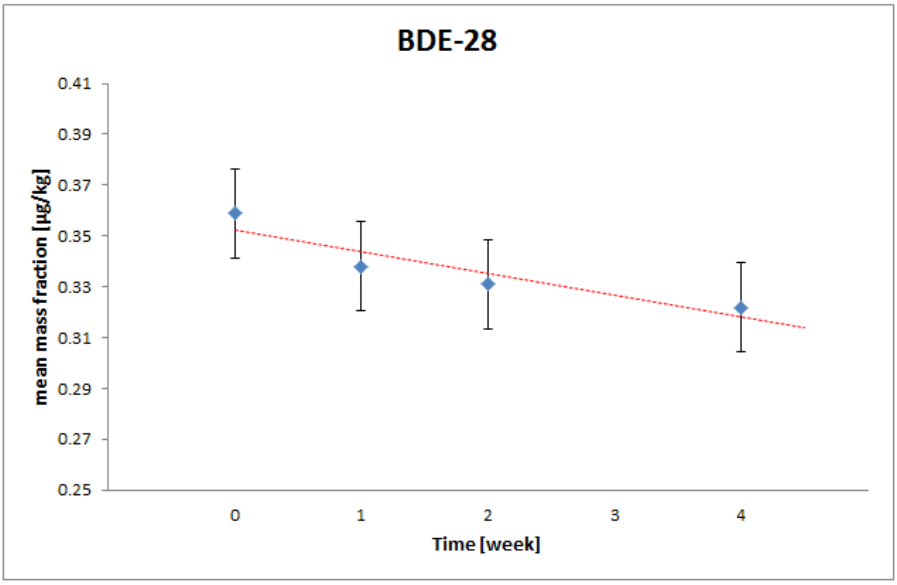

α-HBCD
mean mass fraction [µg/kg]
8
7.5
7
6.5
6
5.5
5
0
1
2
3
4
Time [week]

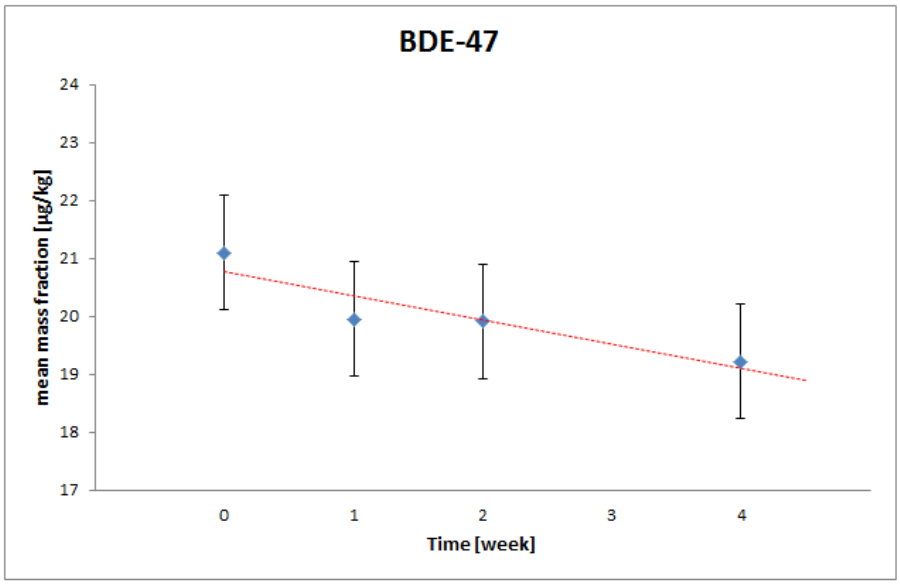

β-HBCD
mean mass fraction [µg/kg]
3
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2
0
1
2
3
4
Time [week]

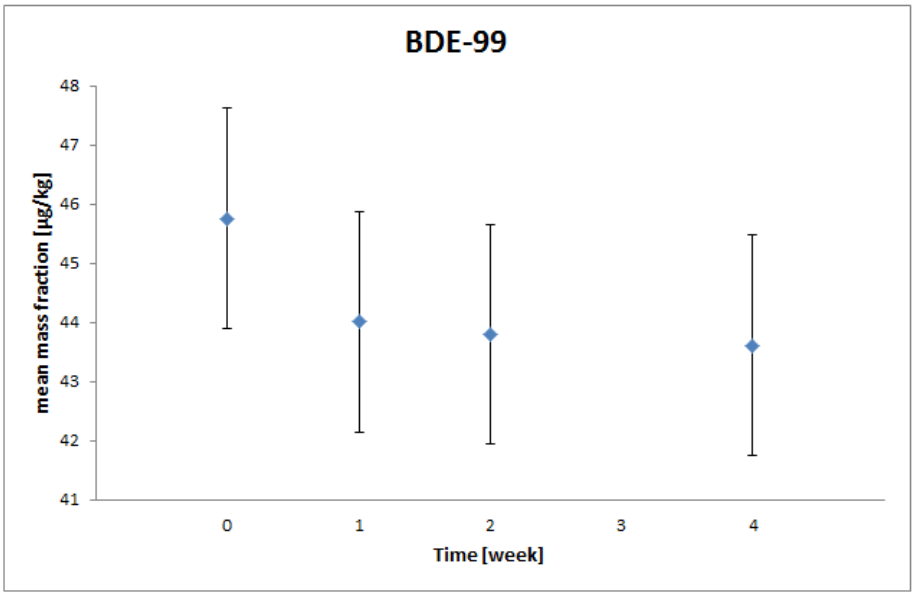

γ-HBCD
mean mass fraction [µg/kg]
80
78
76
74
72
70
68
66
64
62
60
0
1
2
3
4
Time [week]

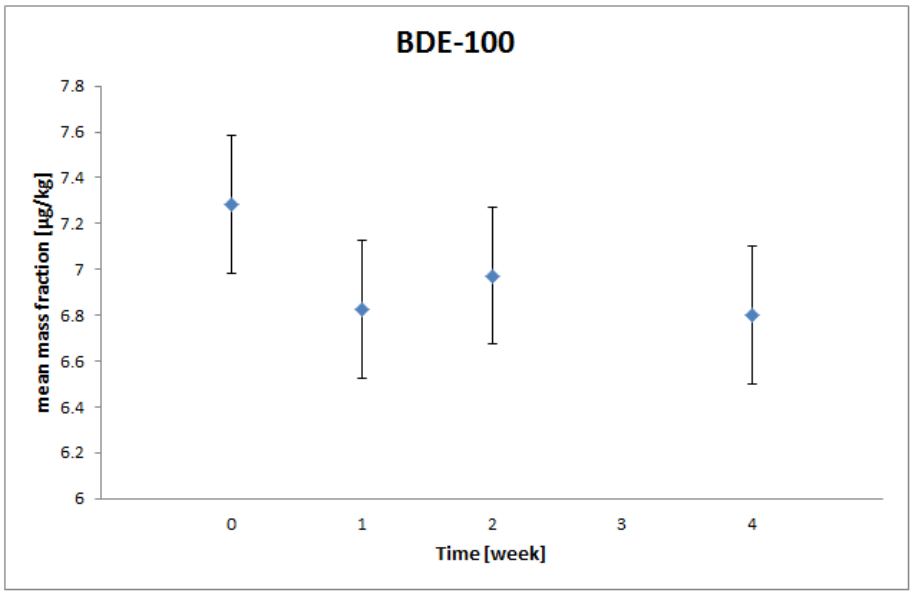
- Data for the short-term stability study at **60 °C**. The graphs report means per time point ± 95 % CI of the means expressed as mass fraction on a dry mass basis.

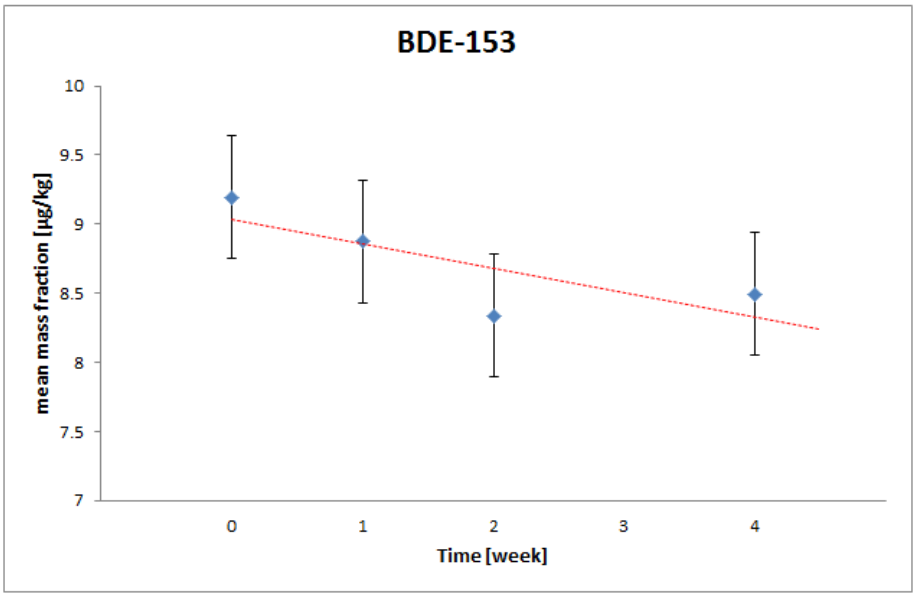
  The trend line is shown when significant.

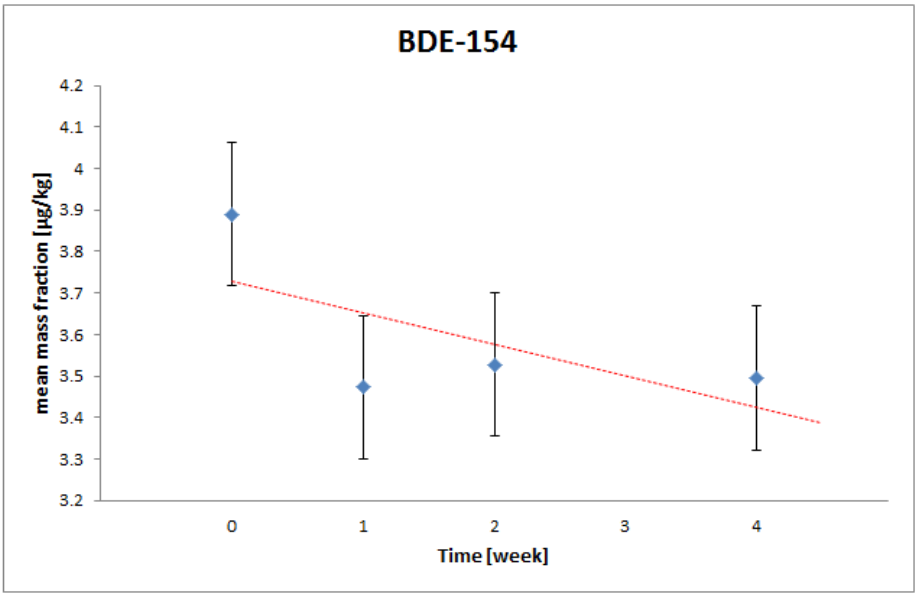


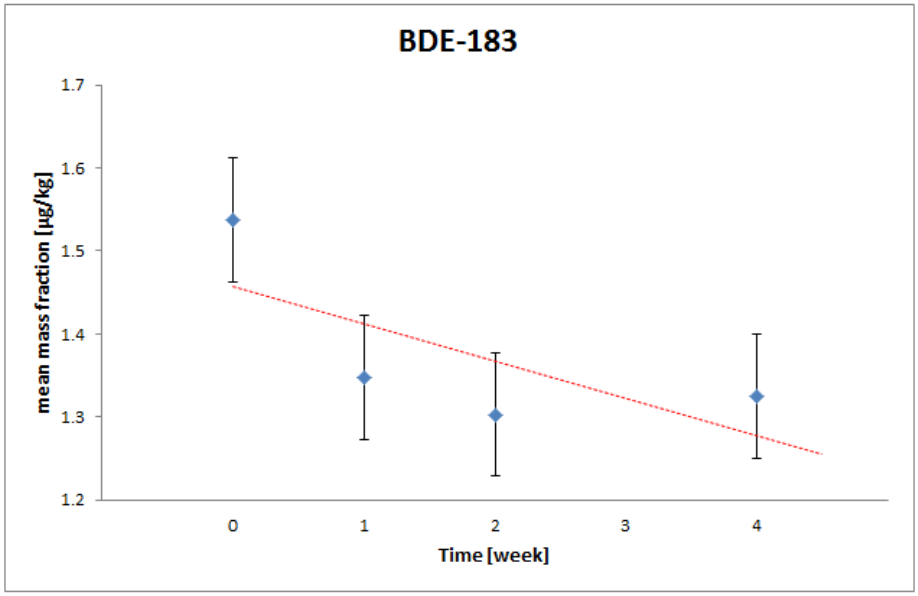


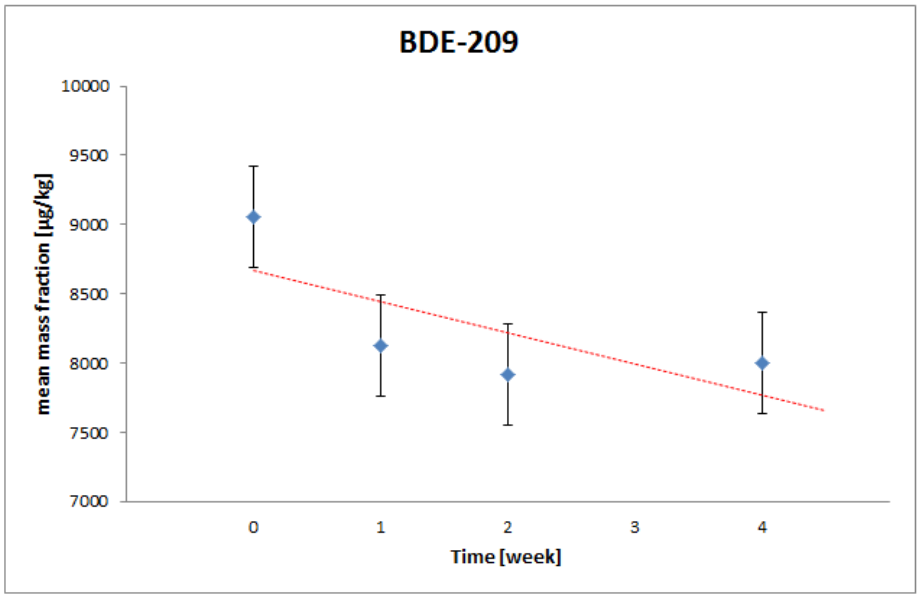

BDE-99
mean mass fraction [µg/kg]
48
47
46
45
44
43
42
41
0
1
2
3
4
Time [week]

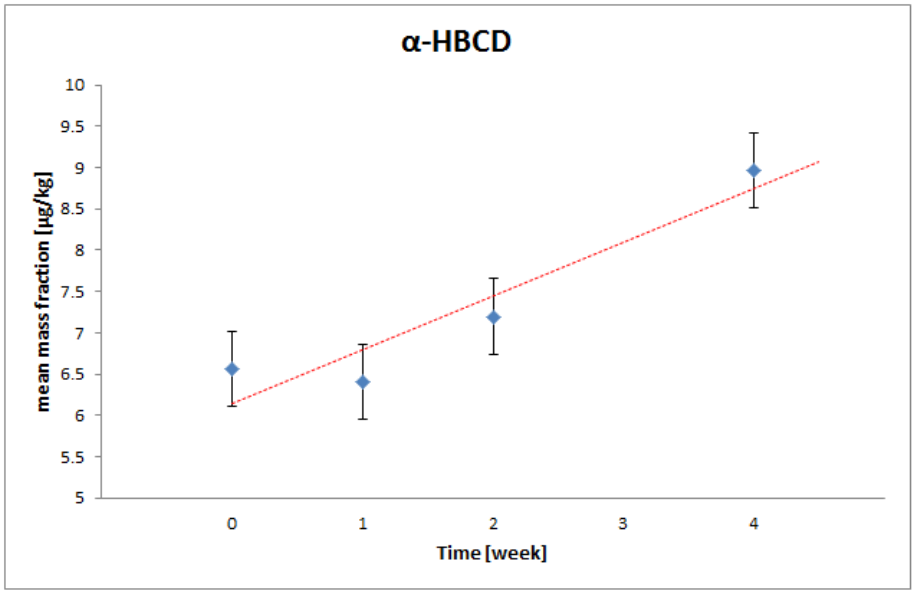

BDE-100
mean mass fraction [µg/kg]
7.8
7.6
7.4
7.2
7
6.8
6.6
6.4
6.2
6
0
1
2
3
4
Time [week]

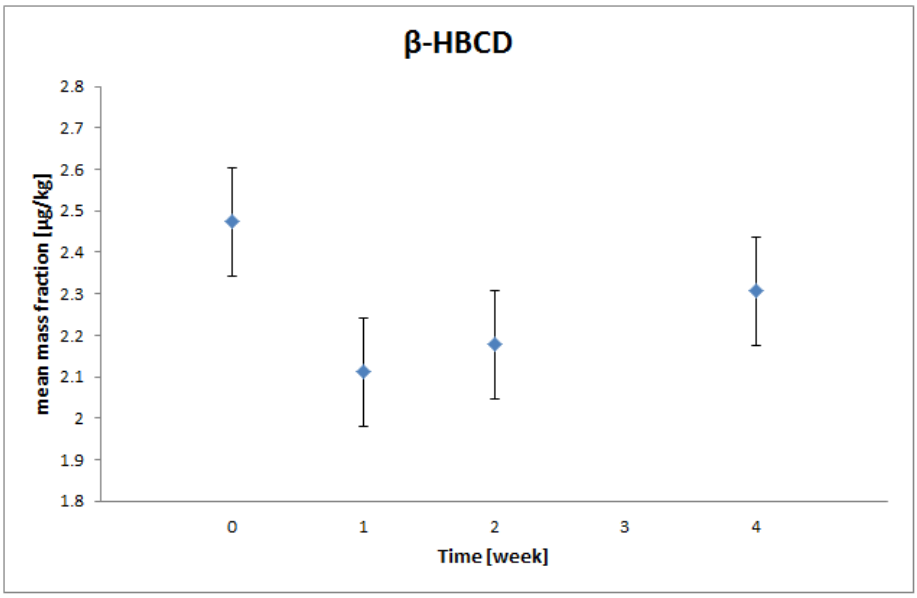

BDE-153
mean mass fraction [µg/kg]
10
9.5
9
8.5
8
7.5
7
0
1
2
3
4
Time [week]

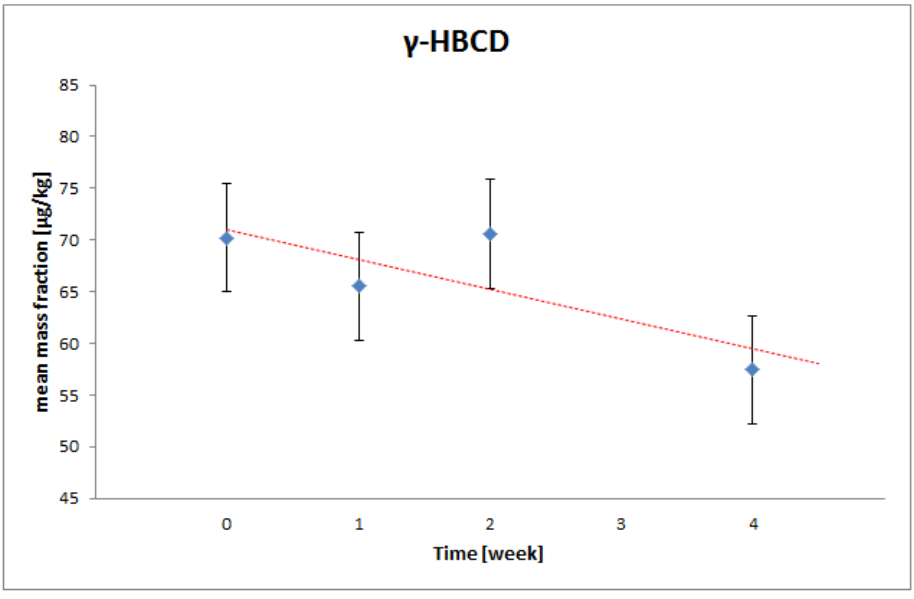

BDE-154
mean mass fraction [µg/kg]
4.2
4.1
4
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
0
1
2
3
4
Time [week]


BDE-183
mean mass fraction [µg/kg]
1.7
1.6
1.5
1.4
1.3
1.2
0
1
2
3
4
Time [week]


BDE-209
mean mass fraction [µg/kg]
10000
9500
9000
8500
8000
7500
7000
0
1
2
3
4
Time [week]


α-HBCD
mean mass fraction [µg/kg]
10
9.5
9
8.5
8
7.5
7
6.5
6
5.5
5
0
1
2
3
4
Time [week]


β-HBCD
mean mass fraction [µg/kg]
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2
1.9
1.8
0
1
2
3
4
Time [week]

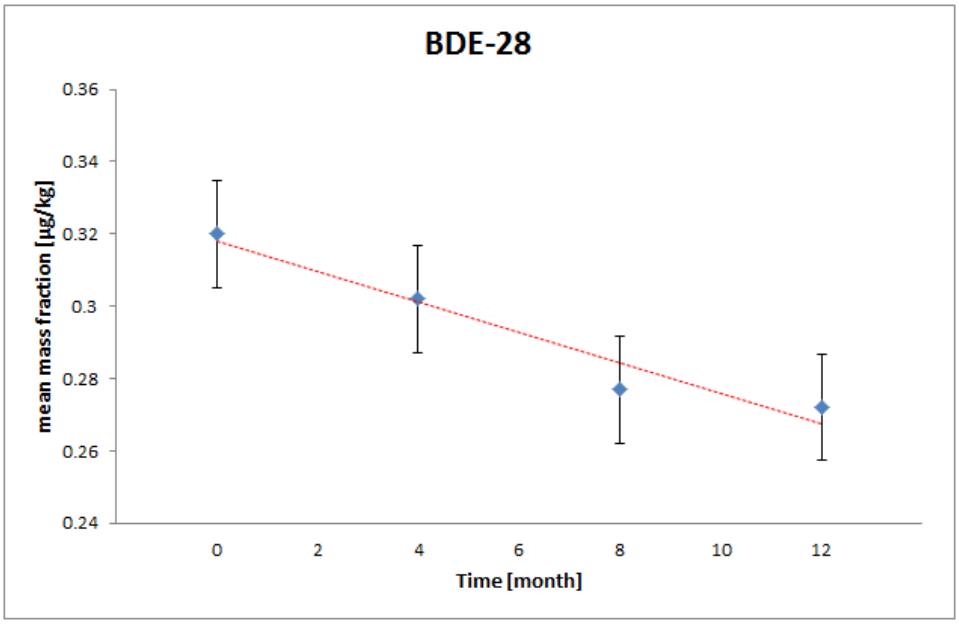

γ-HBCD
mean mass fraction [µg/kg]
85
80
75
70
65
60
55
50
45
0
1
2
3
4
Time [week]

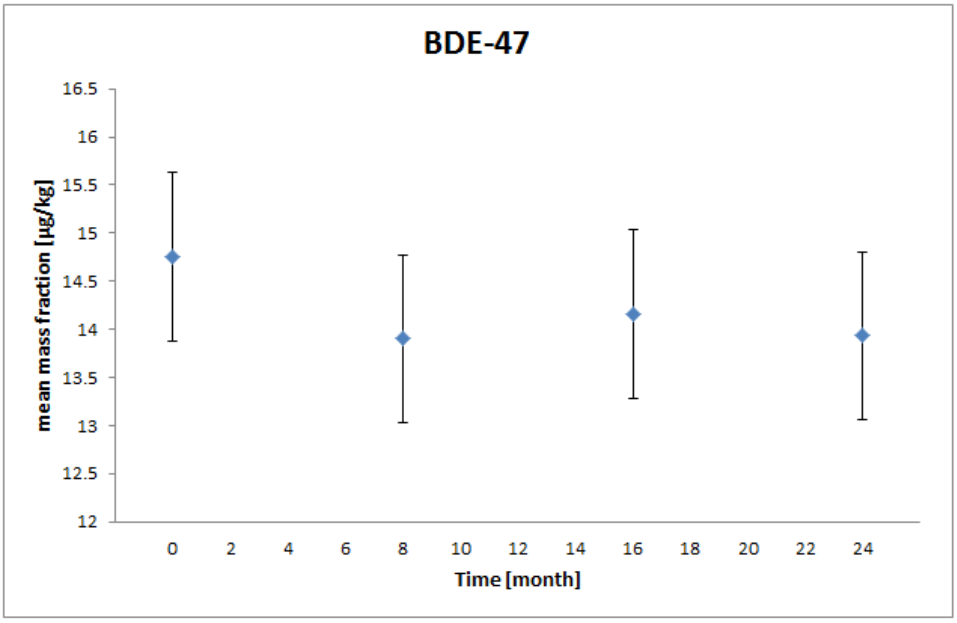
Annex D: Results of the long-term stability measurements

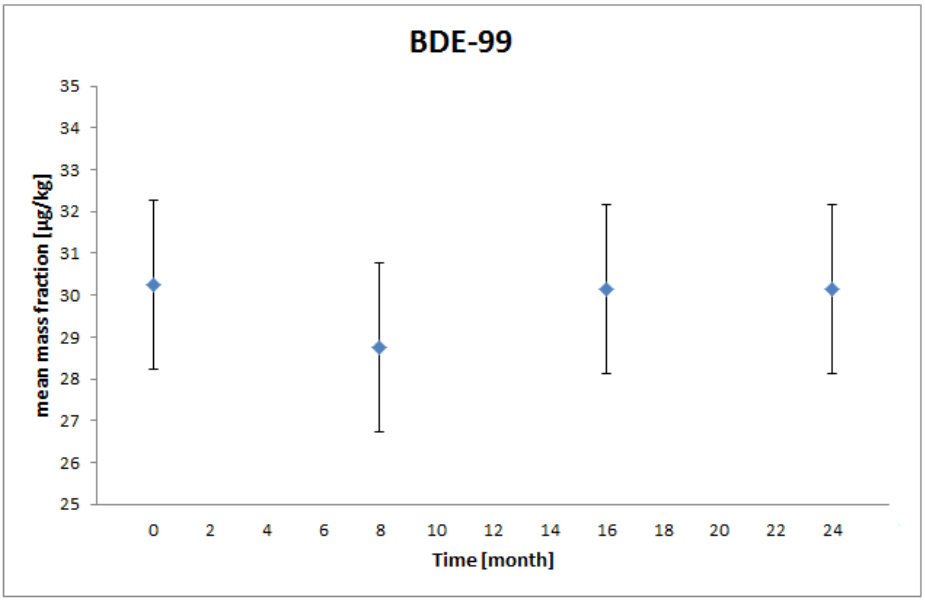
Analytical method applied: GC-ECNI-MS for PBDEs and HPLC-MS/MS for HBCD after ASE extraction using hexane and acetone (3/1 v/v), clean-up on H_2SO_4-treated silica (40:60 w/w) eluting with 150 mL of hexane/ CH_2Cl_2 (70:30 v/v) and GPC to eliminate sulfur. Further fractionation on an SPE cartridge was carried out eluting the PBDEs in the 1st fraction with hexane and the HBCD in the 2nd fraction with acetone.

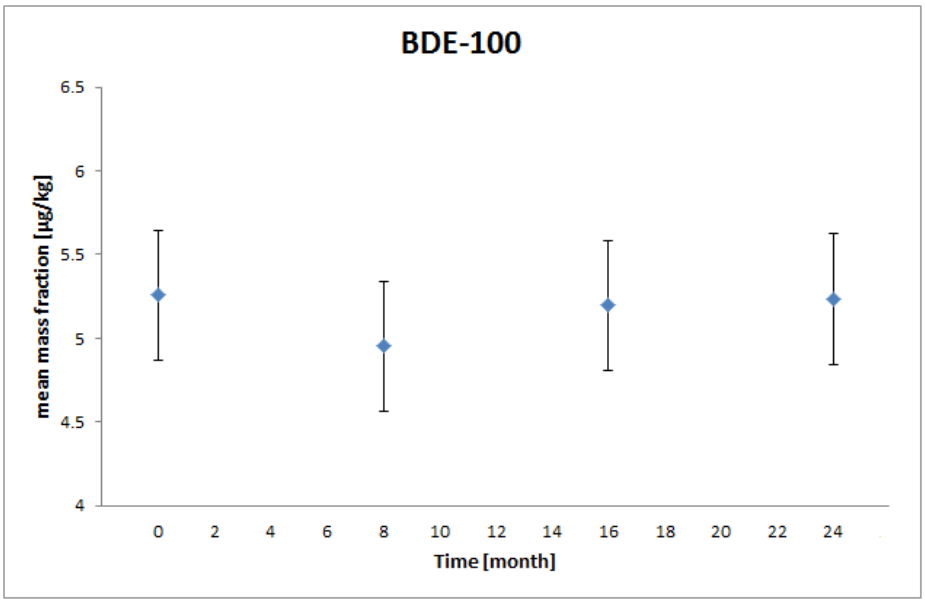
- Data for the long-term stability study at **4 °C**. The graphs report means per time point ± 95 % CI of the means expressed as mass fraction on a dry mass basis.

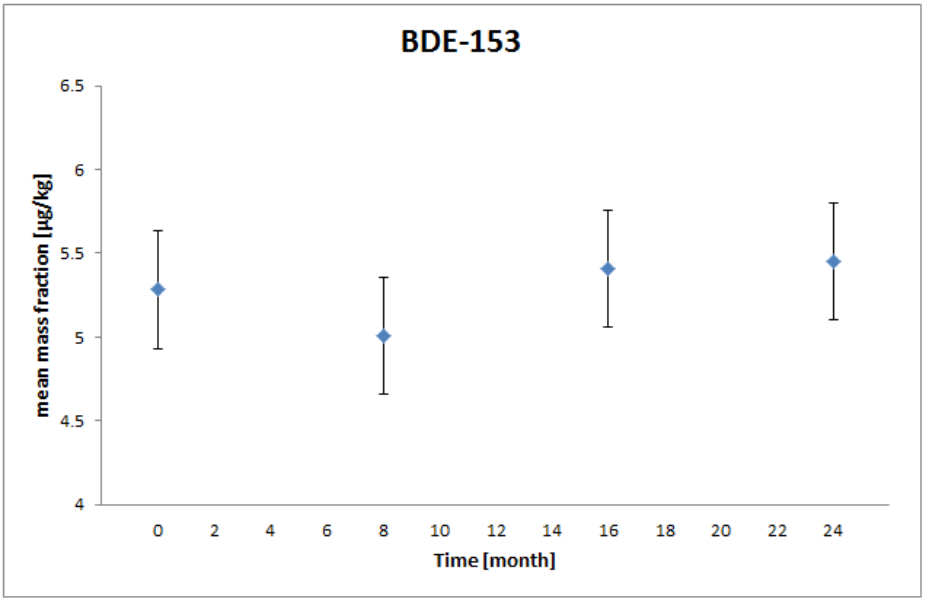
  The trend line is shown when significant.

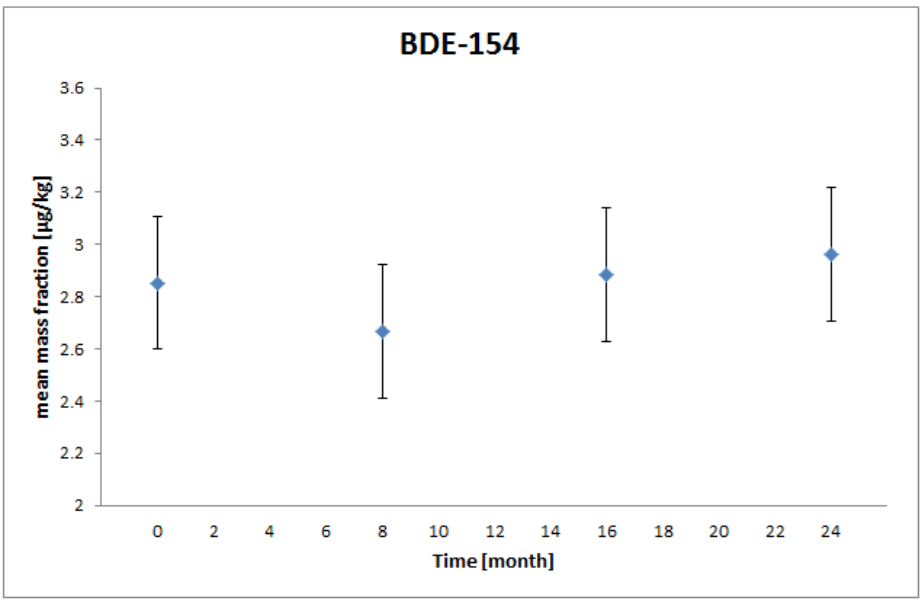


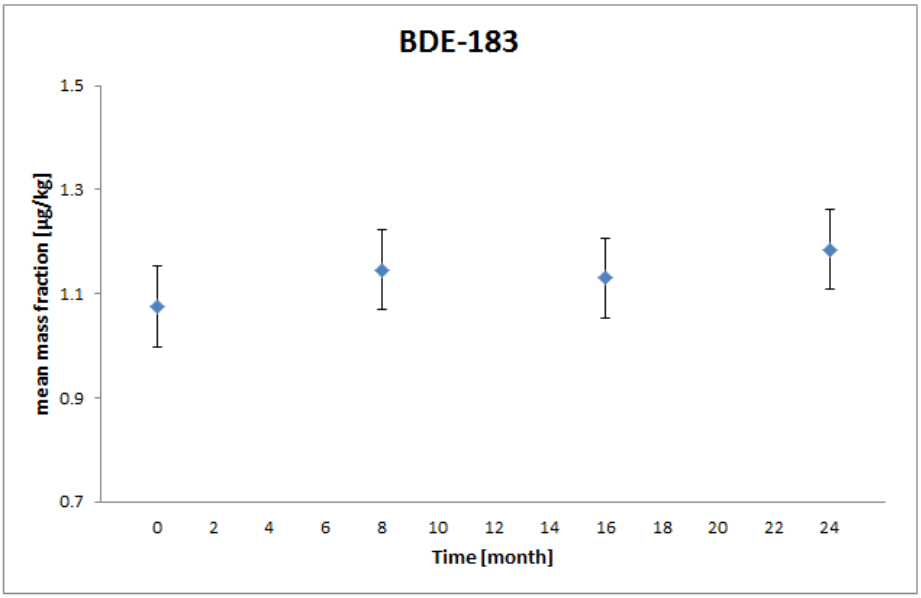


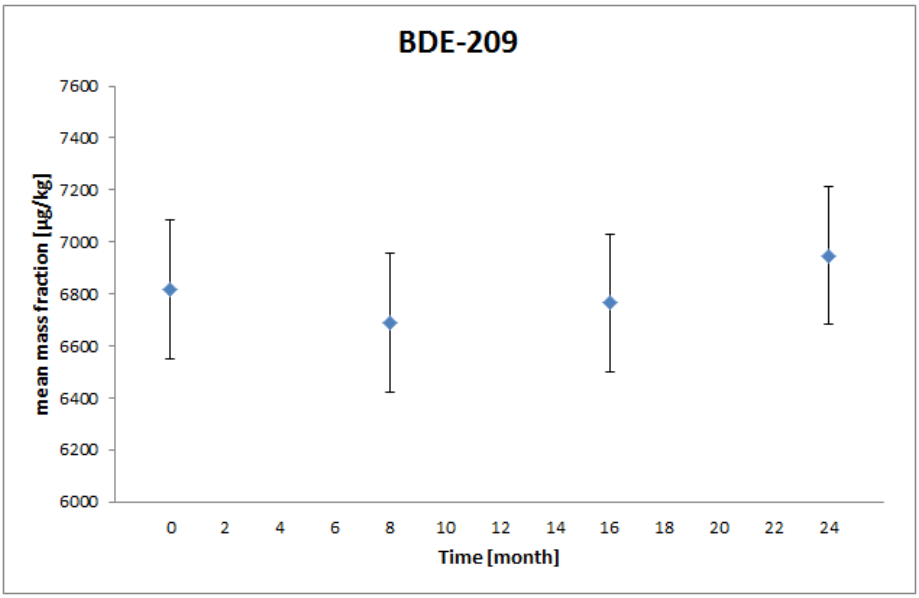

BDE-99
mean mass fraction [µg/kg]
35
34
33
32
31
30
29
28
27
26
25
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]

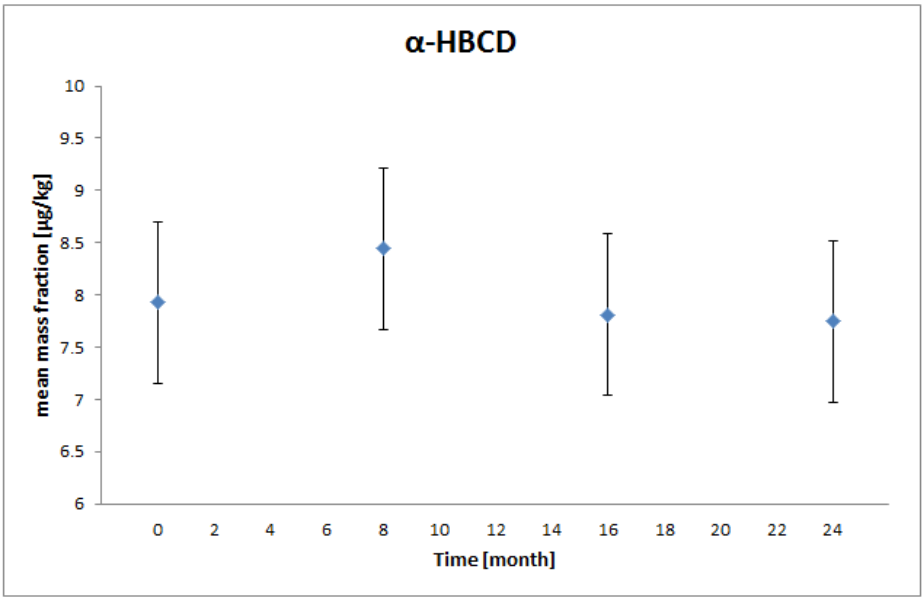

BDE-100
mean mass fraction [µg/kg]
6.5
6
5.5
5
4.5
4
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]

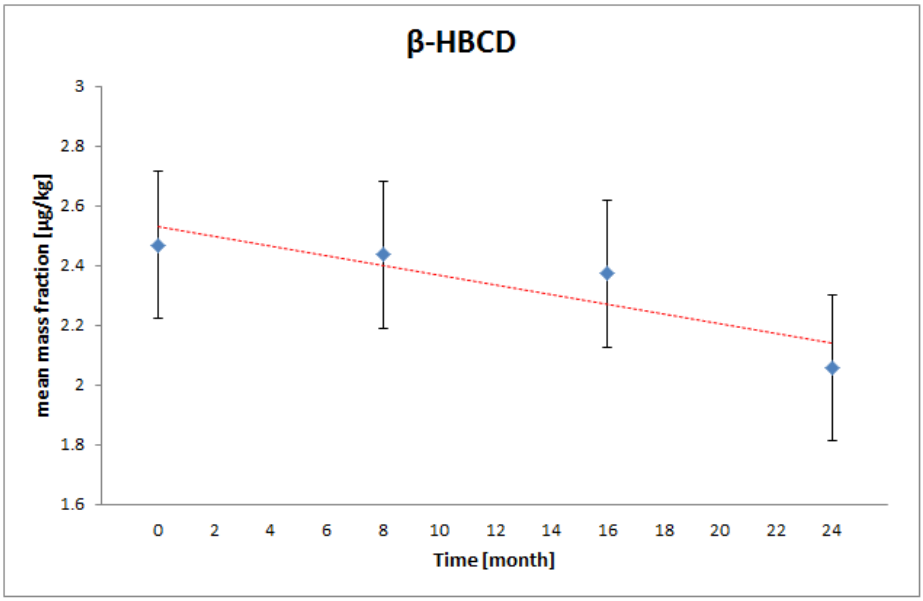

BDE-153
mean mass fraction [µg/kg]
6.5
6
5.5
5
4.5
4
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]

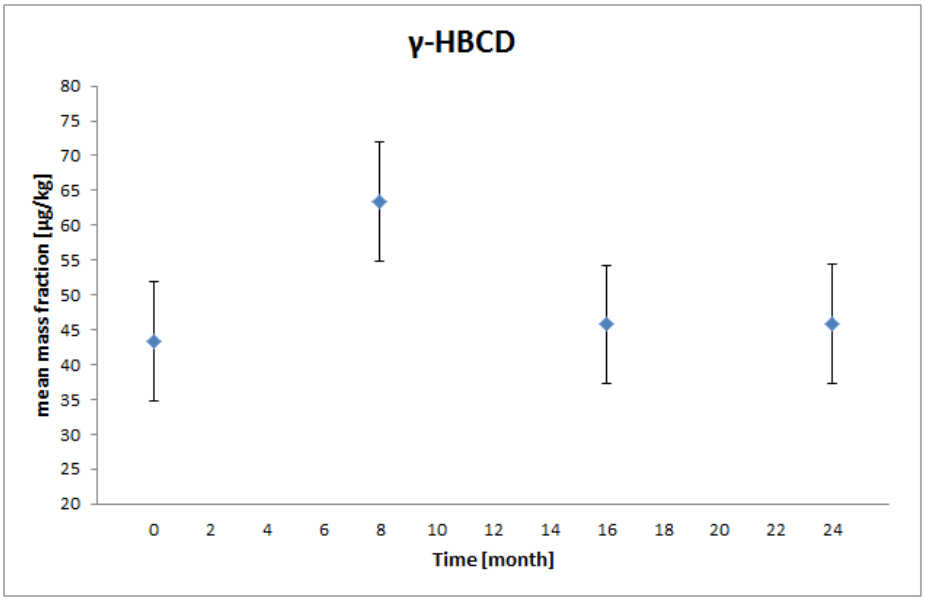

BDE-154
mean mass fraction [µg/kg]
3.6
3.4
3.2
3
2.8
2.6
2.4
2.2
2
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]


BDE-183
mean mass fraction [µg/kg]
1.5
1.3
1.1
0.9
0.7
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]

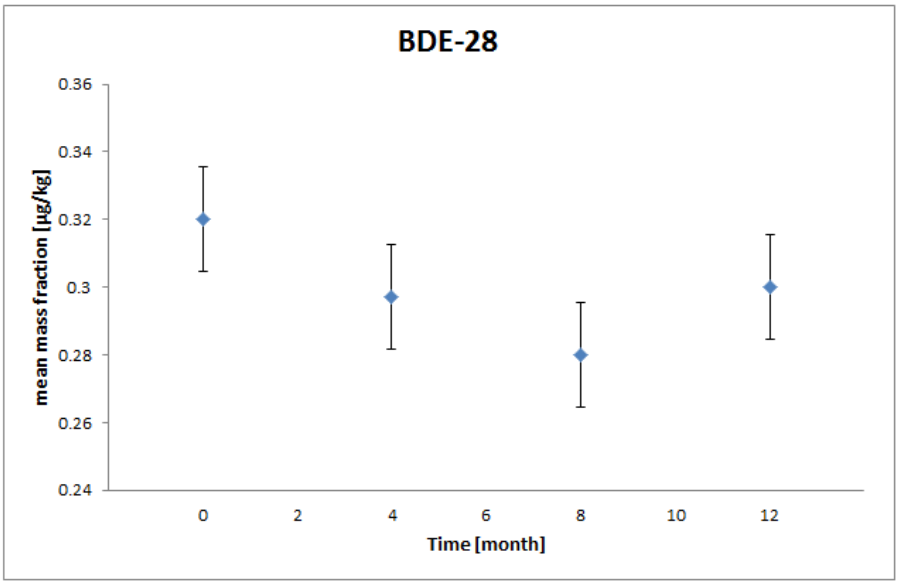

BDE-209
mean mass fraction [µg/kg]
7600
7400
7200
7000
6800
6600
6400
6200
6000
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]

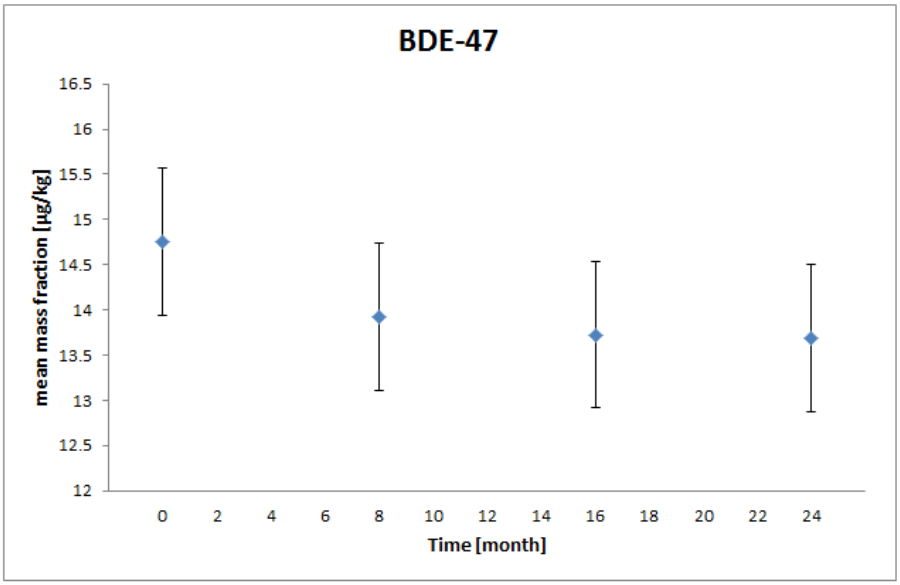

α-HBCD
mean mass fraction [µg/kg]
10
9.5
9
8.5
8
7.5
7
6.5
6
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]

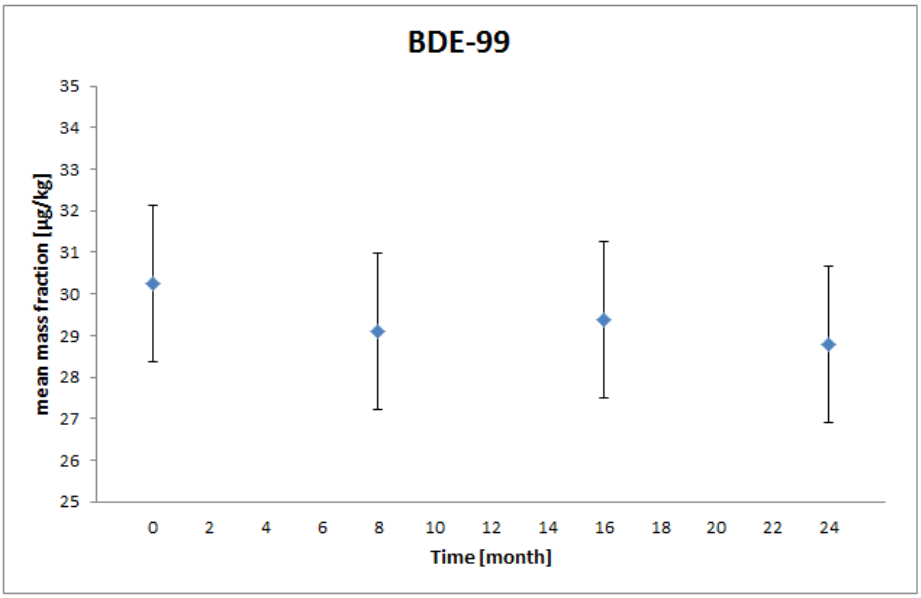

β-HBCD
mean mass fraction [µg/kg]
3
2.8
2.6
2.4
2.2
2
1.8
1.6
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]

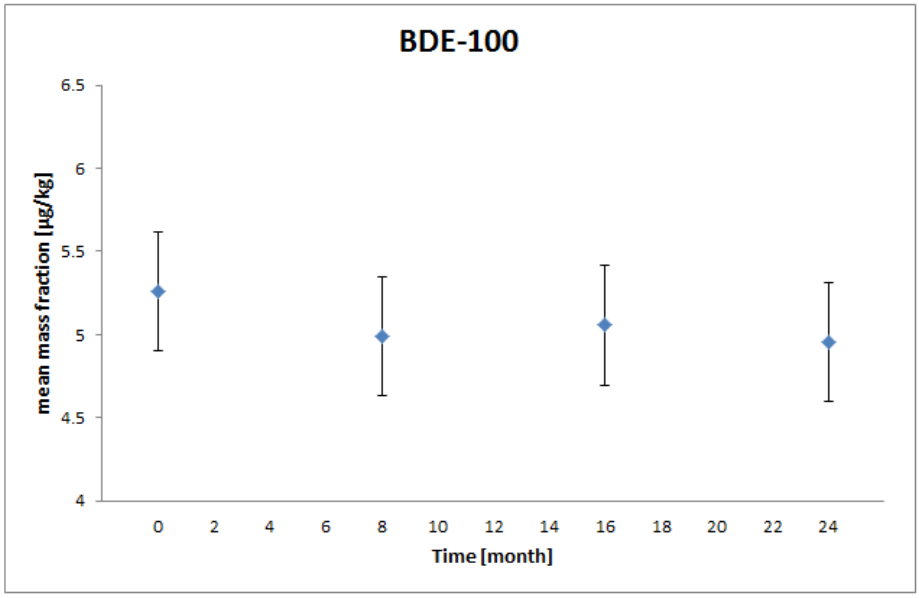

γ-HBCD
mean mass fraction [µg/kg]
80
75
70
65
60
55
50
45
40
35
30
25
20
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]

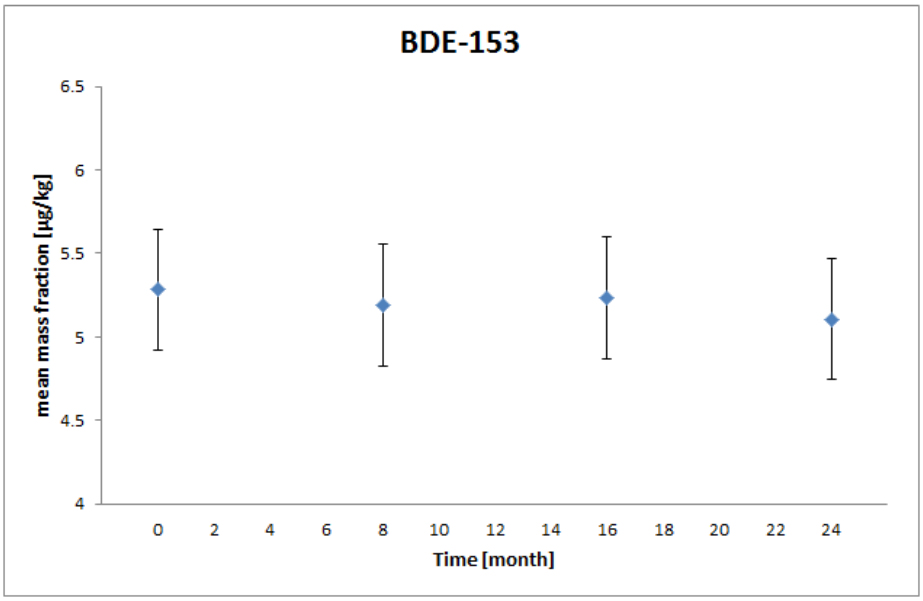
- Data for the long-term stability study at **18 °C**. The graphs report means per time point ± 95 % CI of the means expressed as mass fraction <u>on a dry mass basis</u>.

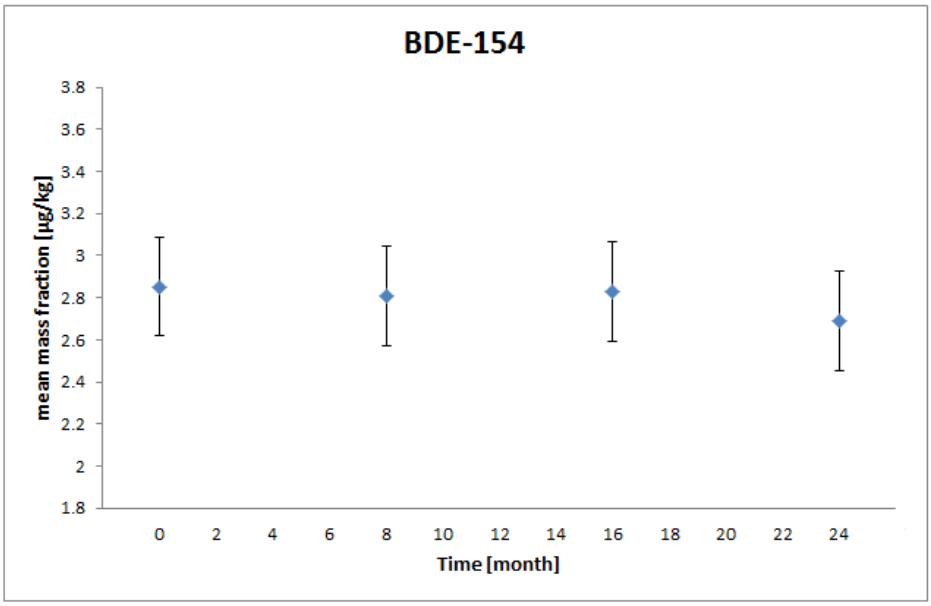


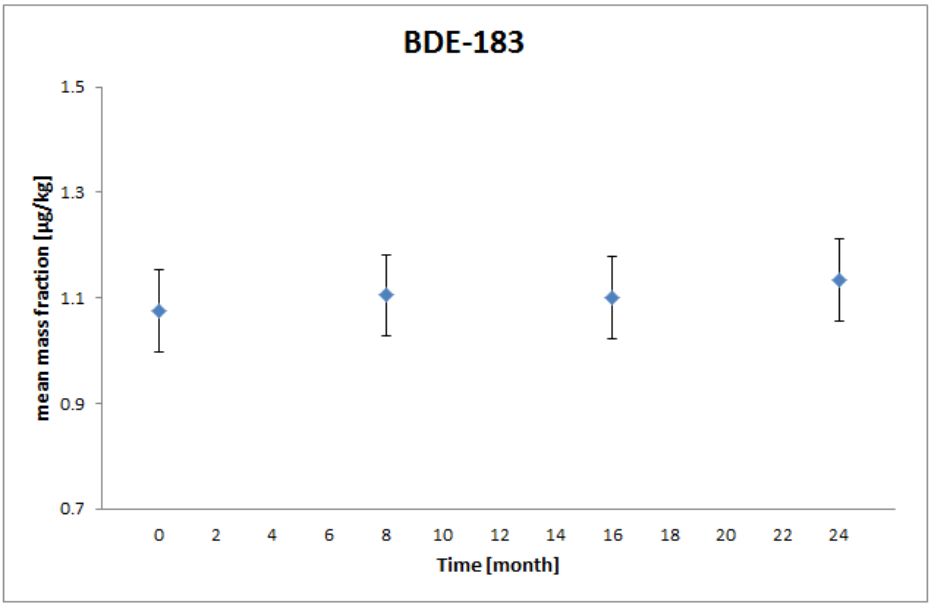


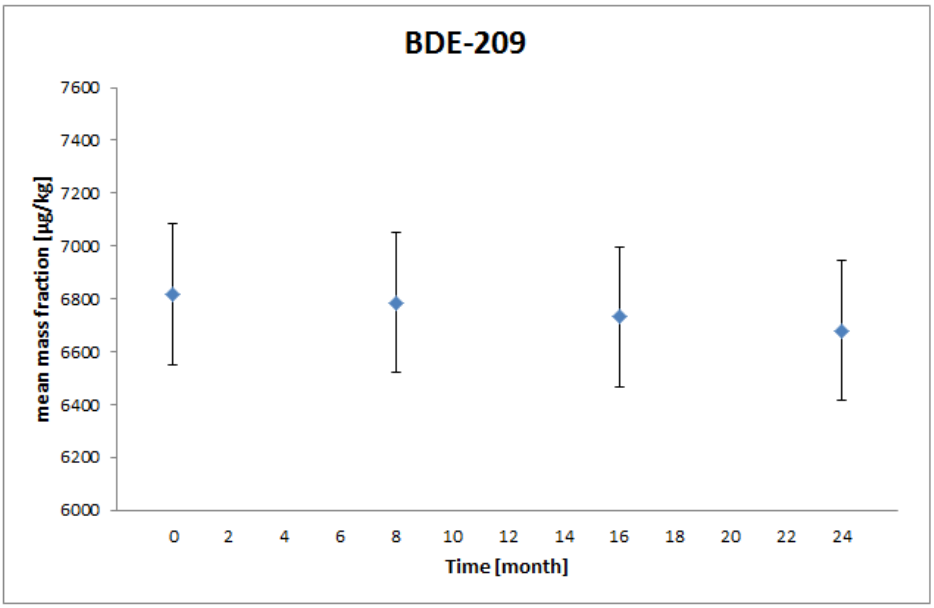

BDE-99
mean mass fraction [µg/kg]
35
34
33
32
31
30
29
28
27
26
25
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]

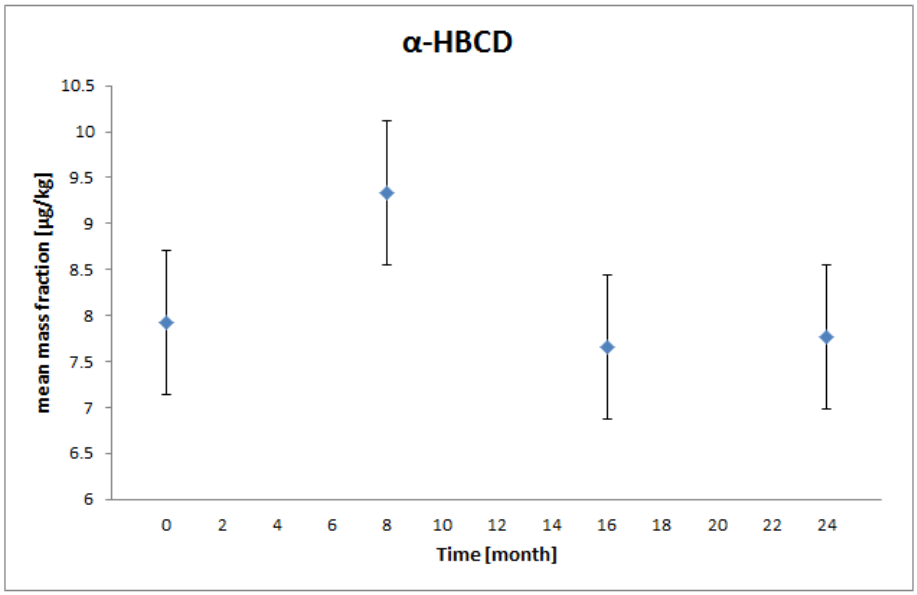

BDE-100
mean mass fraction [µg/kg]
6.5
6
5.5
5
4.5
4
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]

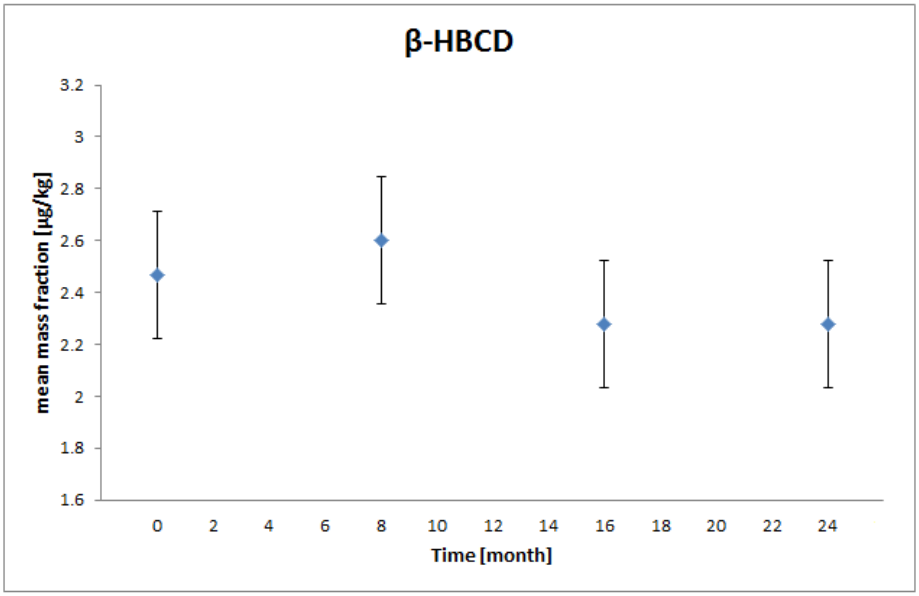

BDE-153
mean mass fraction [µg/kg]
6.5
6
5.5
5
4.5
4
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]

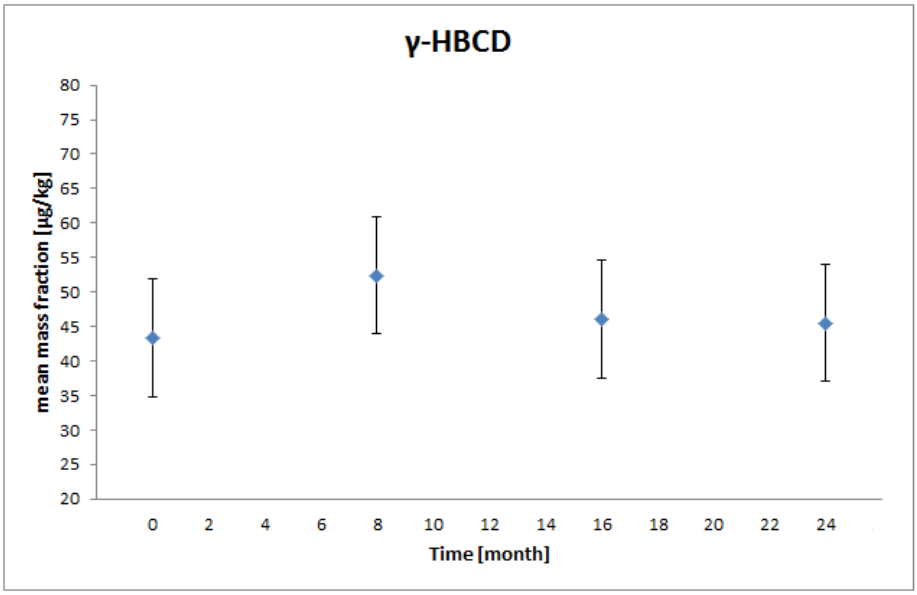

BDE-154
mean mass fraction [µg/kg]
3.8
3.6
3.4
3.2
3
2.8
2.6
2.4
2.2
2
1.8
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]


BDE-183
mean mass fraction [µg/kg]
1.5
1.3
1.1
0.9
0.7
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]


BDE-209
mean mass fraction [µg/kg]
7600
7400
7200
7000
6800
6600
6400
6200
6000
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]


α-HBCD
mean mass fraction [µg/kg]
10.5
10
9.5
9
8.5
8
7.5
7
6.5
6
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]


β-HBCD
mean mass fraction [µg/kg]
3.2
3
2.8
2.6
2.4
2.2
2
1.8
1.6
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]


γ-HBCD
mean mass fraction [µg/kg]
80
75
70
65
60
55
50
45
40
35
30
25
20
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]

Annex E: Summary of methods used in the characterisation study

- Method information is reported as given by the laboratories

PBDEs analysis

Laboratory code–method	Sample pre-treatment	Detection method Internal standards' details	Type of calibration Calibrants' details (purity)	LOQs [µg/kg dry mass basis]
L00-GC-HRMS	Soxhlet extraction with toluene, clean-up by LLE with conc. H_2SO_4 and multilayer silica column	GC-EI-IDMS MBDE-209, MBDE-MXFS (mass labelled PBDE surrogate stock) by Wellington Laboratories (WL)	5 points calibration BDE-CSV-G by WL (solution > 98 %)	0.025 – 0.8
L01-GC-MS	Soxhlet extraction with toluene, clean-up by acidic and basic silica gel and alumina column	GC-EI-IDMS Method 1614 labelled surrogate stock solution by Cambridge Isotope Laboratories (CIL)	6 points calibration ROHS PBDE native PAR spike by CIL (solution ≥ 97.4 %)	0.05 - 5
L02-GC-HRMS	ASE with hexane/acetone 3:1 (v/v), clean-up (except for BDE 209) with silica + alumina and C18-modified silica columns	GC-EI-IDMS BFR-LCS by WL	Single point calibration BFR-PAR, WL (solution > 95 %)	0.002 – 0.01
L03-GC-MS	Soxhlet extraction with hexane/acetone 3:1, clean-up with acidic silica column	GC-ECNI-MS BDE 77, BDE 128 and ^{13}C-BDE 209 by Accustandard and WL	6 points calibration BDE-MXF and BDE 209 by WL (solution, purity not specified)	0.05 - 1
L04-GC-HRMS	Soxhlet extraction, clean-up with automatic MIURA system	GC-EI-IDMS Single congener ^{13}C labelled standard solutions by CIL	5 points calibration Single congener native standards by CIL (solutions > 98 %)	0.01
L05-GC-MS/MS	Solid phase extraction (SPE) with MeOH and hexane, clean-up with multilayer silica column and Cu	GC-EI IDMS/MS ^{13}C labelled BDE 28, 47, 99, 153,183, 209 by WL	2 points bracketing calibration Single congener native standards by Accustandard and WL (solutions, purity not specified)	0.1 15 (BDE 209)
L06-GC-HRMS	Soxhlet extraction with toluene, clean-up by adsorption chromatography	GC-EI-IDMS Single congener ^{13}C labelled standard solutions by CIL	Single point calibration Single congener native standards by CIL (solutions > 98 %)	0.002

PBDEs analysis cont.

L07-GC-MS	Soxhlet extraction with pentane/CH_2Cl_2, clean-up with acidic silica column	GC-ECNI-MS BDE 58 and ^{13}C-BDE 209 by Accustandard and CIL	10 points calibration NIST SRM 2257 and 2258 (certified solutions)	0.03 – 0.8
L09-GC-HRMS	Extraction on a Büchi automated extraction system with toluene, clean-up with multilayer silica column	GC-EI-IDMS/MS BFR-LCS-STK (^{13}C labelled BDEs stock standard) by WL	10 points calibration BFR-CVS by WL (solution > 98 %)	not reported
L10-GC-MS	ASE with hexane-acetone 3:1 (v/v), clean-up with acidic silica column and gel permeation chromatography	GC-ECNI-MS BDE 58 and ^{13}C-BDE 209 by WL	8 points calibration BDE-MXE by WL (solution, 1 to 5 µg/mL ± 5 %)	0.1-0.3
L11-GC-MS	Soxhlet extraction with hexane/acetone 4/1, clean-up with alumina and acidic silica column	GC-ECNI-MS ^{13}C-Polychlorinated naphthalenes and ^{13}C-BDE 209 by CIL	10 points calibration Single congener native standards by CIL (solutions > 98 %)	0.05-0.15
L12-GC-MS	ASE with hexane/acetone 3:1, clean-up by SPE	GC-ECNI-MS BDE 77 by Accustandard	6 points calibration NIST SRM 2257 (certified solution)	0.1-1.2
L13-GC-MS/MS	ASE, hexane/acetone 3:1, clean-up by SPE	GC-EI-IDMS/MS Single congener ^{13}C labelled standard solutions by CAMPRO	6 points calibration Single congener native standards by Chiron (solutions > 97.5 %, except BDE 209 > 95 %)	0.04-10.7
Not used in certification				
L08-GC-MS/MS	Soxhlet extraction, clean-up with deactivated alumina column	GC-EI-MS/MS F-BDE 69 and F-BDE160	10 points calibration BDE-MXE by WL (solution, purity not specified)	0.1, 0.2

HBCD analysis

Laboratory code–method	Sample pre-treatment	Detection method Internal standard(s) details	Type of calibration Calibrants' details (purity)	LOQ [µg/kg dry mass basis]
L03-LC-MS/MS	Soxhlet extraction with hexane/acetone, clean-up with acidic silica	HPLC-ESI negative-IDMS/MS Single isomers ^{13}C-HBCD by WL	6 points calibration Single isomers HBCD by WL (solution > 98 %)	0.5
L04- LC-MS/MS	QuEChERS extraction	HPLC-ESI negative-IDMS/MS Single isomers ^{13}C-HBCD by WL	10 points calibration Single isomers HBCD by WL (solutions, purity not specified)	0.2
L05-LC-MS/MS	Solid phase extraction (SPE) with MeOH and hexane	UPLC-ESI negative-IDMS/MS ^{13}C-γ-HBCD	7 points calibration Single isomers HBCD by WL (solutions, purity not specified)	0.3
L06- LC-MS/MS	Extraction by sonication in CH_2Cl_2, clean-up by adsorption chromatography	UPLC-ESI negative-IDMS/MS Single isomers ^{13}C-HBCD by WL	4 points calibration Single isomers HBCD by WL (solution > 98 %)	0.2
L10-LC- MS/MS	ASE with hexane-acetone 3:1 (v/v), clean-up with acidic silica column and gel permeation chromatography	HPLC-ESI negative-IDMS/MS Single isomers ^{13}C-HBCD by WL	8 points calibration Single isomers HBCD by WL (solutions, purity not specified)	0.1
L11- LC-MS/MS	Soxhlet extraction with hexane/acetone 4/1, clean-up with alumina and acidic silica column	HPLC-ESI negative-IDMS/MS Single isomers ^{13}C-HBCD by CIL	10 points calibration Single isomers HBCD by CIL (solutions: α- and β-HBCD > 98 %, γ-HBCD > 97 %)	0.05
Not used in certification				
L00-LC-MS/MS	Extraction by shaking with MeOH/ACN, centrifugation, dilution 1:1 with milliQ water	UPLC-ESI-IDMS/MS Deuterium labelled γ-HBCD	8 points calibration Single isomer standards by WL (solution, > 98 %)	0.8 - 1.5

HBCD analysis cont.

L07-LC-MS	Soxhlet extraction, clean-up with sulfuric acid	HPLC-ESI negative-IDMS Single isomers ^{13}C-HBCD	7 points calibration Single isomer standards by CIL (solutions, purity not specified)	1
L08-UPLC-MS/MS	Soxhlet extraction, clean-up with gel permeation chromatography and acidified silica column	UPLC-ESI-IDMS/MS Deuterium labelled single isomers HBCD by WL	7 points calibration Single isomer standards by WL (solution > 98 %)	0.75

Annex F: Results of the characterisation measurements

Note: *values as reported by the laboratories and expressed on a dry mass basis*

Table F1: BDE-28

laboratory code - method	replic. 1 [µg/kg]	replic. 2 [µg/kg]	replic. 3 [µg/kg]	replic. 4 [µg/kg]	replic. 5 [µg/kg]	replic. 6 [µg/kg]	replic. 7 [µg/kg]	replic. 8 [µg/kg]	replic.9 [µg/kg]	replic. 10 [µg/kg]	replic. 11 [µg/kg]	replic. 12 [µg/kg]	mean [µg/kg]	expanded uncertainty [µg/kg]
L00-GC-HRMS	0.29	0.32	0.32	0.29	0.33	0.32	--	--	--	--	--	--	0.31	0.09
L01-GC-MS	0.24	0.25	0.29	0.25	0.29	0.29	--	--	--	--	--	--	0.27	0.03
L02-GC-HRMS	0.26	0.28	0.28	0.27	0.26	0.24	--	--	--	--	--	--	0.27	0.08
L03-GC-MS	0.21	0.22	0.22	0.21	0.21	0.20	--	--	--	--	--	--	0.21	0.03
L04-GC-HRMS	0.30	0.32	0.31	0.28	0.29	0.31	--	--	--	--	--	--	0.30	0.08
L05-GC-MS/MS	0.270	0.262	0.273	0.281	0.261	0.269	--	--	--	--	--	--	0.269	0.027
L06-GC-HRMS	0.31	0.38	0.29	0.35	0.27	0.33	--	--	--	--	--	--	0.32	0.09
L07-GC-MS	0.16	0.18	0.17	0.21	0.21	0.21	--	--	--	--	--	--	0.19	0.09
L09-GC-HRMS	0.36	0.37	0.40	0.38	0.39	0.38	0.34	0.36	0.34	0.33	0.32	0.33	0.36	0.05
L10-GC-MS	0.34	0.33	0.35	0.27	0.32	0.35	--	--	--	--	--	--	0.33	0.07
L11-GC-MS	0.27	0.27	0.26	0.25	0.25	0.27	--	--	--	--	--	--	0.26	0.13
Results not used for certification														
L08-GC-MS/MS	*11.16*	*11.12*	*7.74*	*13.12*	*9.31*	*7.20*	--	--	--	--	--	--	*9.94*	*3.28*
L12-GC-MS	*0.41*	*0.43*	*0.43*	*0.44*	*0.48*	*0.42*	--	--	--	--	--	--	*0.44*	*0.05*

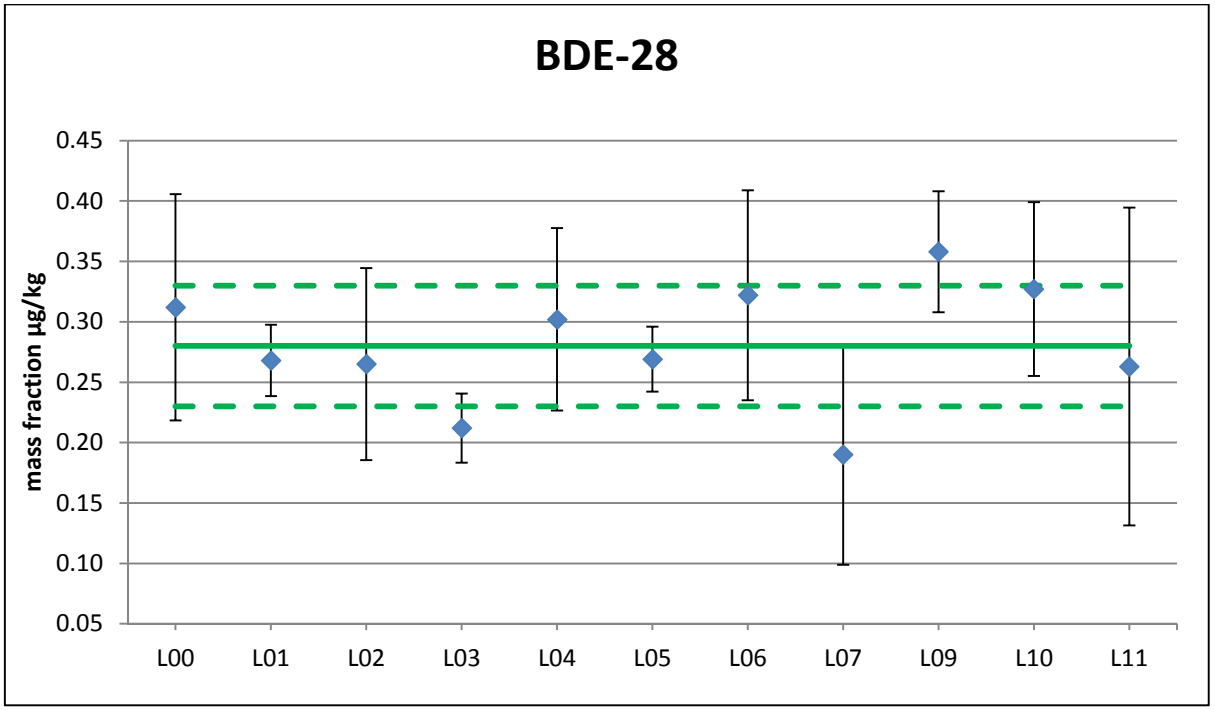


Figure F1: certified value (0.28 µg/kg, solid line) ± expanded uncertainty (0.05 µg/kg, dashed lines) for BDE-28; individual laboratory means (blue dots) are displayed with their expanded uncertainty (error bar)

Table F2: BDE-47

laboratory code - method	replic. 1 [µg/kg]	replic. 2 [µg/kg]	replic. 3 [µg/kg]	replic. 4 [µg/kg]	replic. 5 [µg/kg]	replic. 6 [µg/kg]	replic. 7 [µg/kg]	replic. 8 [µg/kg]	replic.9 [µg/kg]	replic. 10 [µg/kg]	replic. 11 [µg/kg]	replic. 12 [µg/kg]	mean [µg/kg]	expanded uncertainty [µg/kg]
L00-GC-HRMS	18.6	22.9	20.7	19.9	19.9	20.5	--	--	--	--	--	--	20.4	6.1
L01-GC-MS	19.00	19.00	20.70	18.68	18.54	18.96	--	--	--	--	--	--	19.15	2.11
L02-GC-HRMS	14.1	15.0	13.9	13.7	12.6	12.6	--	--	--	--	--	--	13.7	6.1
L03-GC-MS	13.70	14.13	13.98	14.21	14.12	13.42	--	--	--	--	--	--	13.93	3.48
L04-GC-HRMS	17	16	14	17	18	16	--	--	--	--	--	--	16	4
L05-GC-MS/MS	15.7	16.5	17.1	17.0	16.5	17.5	--	--	--	--	--	--	16.7	1.3
L06-GC-HRMS	16.59	16.27	16.07	16.62	16.49	17.33	--	--	--	--	--	--	16.56	1.99
L07-GC-MS	17.19	16.91	16.99	17.83	17.6	17.62	--	--	--	--	--	--	17.36	7.98
L10-GC-MS	15.96	16.52	16.64	16.06	14.96	15.6	--	--	--	--	--	--	15.96	5.27
L11-GC-MS	14.23	15.45	14.98	15.37	14.67	14.82	--	--	--	--	--	--	14.92	7.46
Results not used for certification														
L08-GC-MS/MS	*17.74*	*17.85*	*18.47*	*17.98*	*17.85*	*16.50*	--	--	--	--	--	--	*17.73*	*4.78*
L09-GC-HRMS	*23.57*	*25.78*	*24.73*	*24.96*	*25.21*	*24.73*	*22.06*	*23.58*	*22.92*	*22.52*	*21.76*	*23.11*	*23.74*	*7.26*
L12-GC-MS	*n.r.*	*n.r.*	*n.r.*	*n.r.*	*n.r.*	*n.r.*							*n.a.*	*n.a.*
L13-GC-MS/MS	*13.67*	*14.25*	*15.21*	*14,66*	*16.62*	*16.77*	--	--	--	--	--	--	*15.19*	*1.64*

n.r.= not reported
n.a.= not applicable

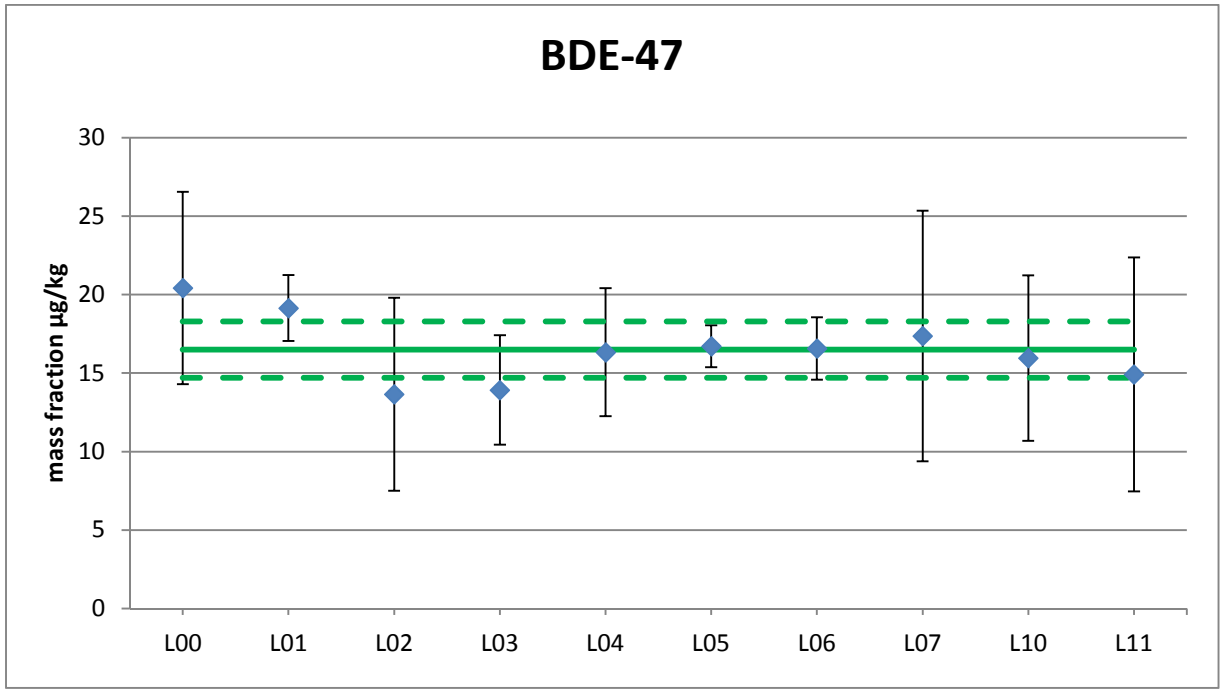


Figure F2: certified value (16.5 µg/kg, solid line) ± expanded uncertainty (1.8 µg/kg, dashed lines) for BDE-47; individual laboratory means (blue dots) are displayed with their expanded uncertainty (error bar)

Table F3: BDE-99

laboratory code - method	replic. 1 [µg/kg]	replic. 2 [µg/kg]	replic. 3 [µg/kg]	replic. 4 [µg/kg]	replic. 5 [µg/kg]	replic. 6 [µg/kg]	replic. 7 [µg/kg]	replic. 8 [µg/kg]	replic.9 [µg/kg]	replic. 10 [µg/kg]	replic. 11 [µg/kg]	replic. 12 [µg/kg]	mean [µg/kg]	expanded uncertainty [µg/kg]
L00-GC-HRMS	38.9	44.5	44.5	38.4	46.5	44.8	--	--	--	--	--	--	42.9	12.9
L02-GC-HRMS	23.5	26.9	26.5	27.6	22.8	27.5	--	--	--	--	--	--	25.8	12.1
L03-GC-MS	31.27	33.94	32.2	34.14	34.32	32.43	--	--	--	--	--	--	33.05	5.59
L04-GC-HRMS	37	28	27	37	39	29	--	--	--	--	--	--	33	8
L05-GC-MS/MS	30.1	31.4	35.2	33.4	30.3	31.9	--	--	--	--	--	--	32.1	3.2
L06-GC-HRMS	35.89	32.72	32.76	33.44	34.59	37.52	--	--	--	--	--	--	34.49	3.79
L07-GC-MS	36.93	36.77	35	30.69	33.88	29.27	--	--	--	--	--	--	33.76	12.8
L10-GC-MS	34.97	37.12	34.05	35.4	32.51	31.93	--	--	--	--	--	--	34.33	12.02
L11-GC-MS	34.05	36.18	35.21	46.37	39.67	35.74	--	--	--	--	--	--	37.87	18.94
L12-GC-MS	37.5	33.78	34.55	32.34	35.72	34.81	--	--	--	--	--	--	34.78	6.99
L13-GC-MS/MS	33.01	34.38	34.44	35.18	35.5	38.74	--	--	--	--	--	--	35.21	3.63
Results not used for certification														
L01-GC-MS	*39.61*	*39.42*	*39.88*	*39.02*	*39.25*	*38.92*	--	--	--	--	--	--	*39.35*	*5.12*
L08-GC-MS/MS	*43.21*	*43.04*	*36.11*	*46.72*	*37.74*	*33.85*	--	--	--	--	--	--	*40.11*	*15.24*
L09-GC-HRMS	*38.74*	*42.14*	*41.43*	*40.72*	*39.08*	*40.17*	*39.51*	*39.75*	*39.93*	*40.09*	*41.74*	*40.05*	*40.28*	*15.15*

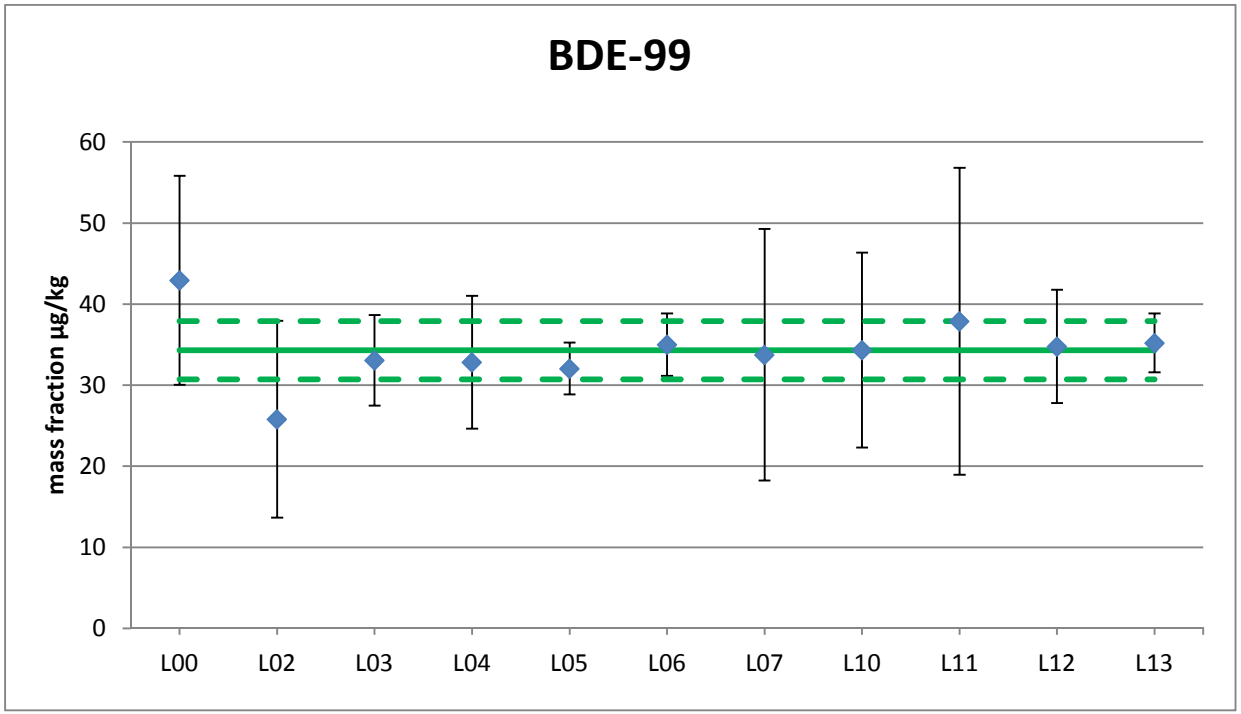


Figure F3: certified value (34 µg/kg, solid line) ± expanded uncertainty (4 µg/kg, dashed lines) for BDE-99; individual laboratory means (blue dots) are displayed with their expanded uncertainty (error bar)

Table F4: BDE-100

laboratory code - method	replic. 1 [µg/kg]	replic. 2 [µg/kg]	replic. 3 [µg/kg]	replic. 4 [µg/kg]	replic. 5 [µg/kg]	replic. 6 [µg/kg]	replic. 7 [µg/kg]	replic. 8 [µg/kg]	replic.9 [µg/kg]	replic. 10 [µg/kg]	replic. 11 [µg/kg]	replic. 12 [µg/kg]	mean [µg/kg]	expanded uncertainty [µg/kg]
L00-GC-HRMS	6.44	7.10	7.13	6.65	7.67	7.18	--	--	--	--	--	--	7.03	2.11
L01-GC-MS	5.00	5.22	5.53	5.18	5.30	5.53	--	--	--	--	--	--	5.29	0.69
L02-GC-HRMS	4.81	5.41	4.94	5.63	4.54	5.31	--	--	--	--	--	--	5.11	2.71
L03-GC-MS	5.26	5.70	5.49	5.76	5.93	5.47	--	--	--	--	--	--	5.60	1.13
L04-GC-HRMS	6.0	5.5	5.7	6.0	6.4	5.9	--	--	--	--	--	--	5.9	1.5
L05-GC-MS/MS	5.25	5.33	6.13	5.93	5.48	5.53	--	--	--	--	--	--	5.61	1.68
L06-GC-HRMS	6.08	5.68	5.63	5.71	5.75	6.12	--	--	--	--	--	--	5.83	0.64
L10-GC-MS	5.73	5.81	5.78	5.74	5.38	5.45	--	--	--	--	--	--	5.65	2.15
L11-GC-MS	5.66	5.93	5.86	7.19	5.78	5.84	--	--	--	--	--	--	6.04	3.02
L13-GC-MS/MS	5.02	5.45	5.30	5.60	5.56	6.03	--	--	--	--	--	--	5.49	0.62
Results not used for certification														
L07-GC-MS	*5.78*	*5.62*	*5.27*	*6.74*	*6.36*	*6.02*	--	--	--	--	--	--	*5.97*	*3.22*
L08-GC-MS/MS	*6.24*	*5.98*	*6.19*	*6.16*	*5.96*	*5.34*	--	--	--	--	--	--	*5.98*	*2.15*
L09-GC-HRMS	*6.31*	*7.11*	*6.94*	*7.41*	*6.43*	*7.08*	*6.57*	*6.74*	*6.45*	*6.56*	*6.70*	*6.55*	*6.74*	*1.06*
L12-GC-MS	*6.61*	*6.33*	*5.77*	*6.21*	*6.13*	*5.78*	--	--	--	--	--	--	*6.14*	*1.25*

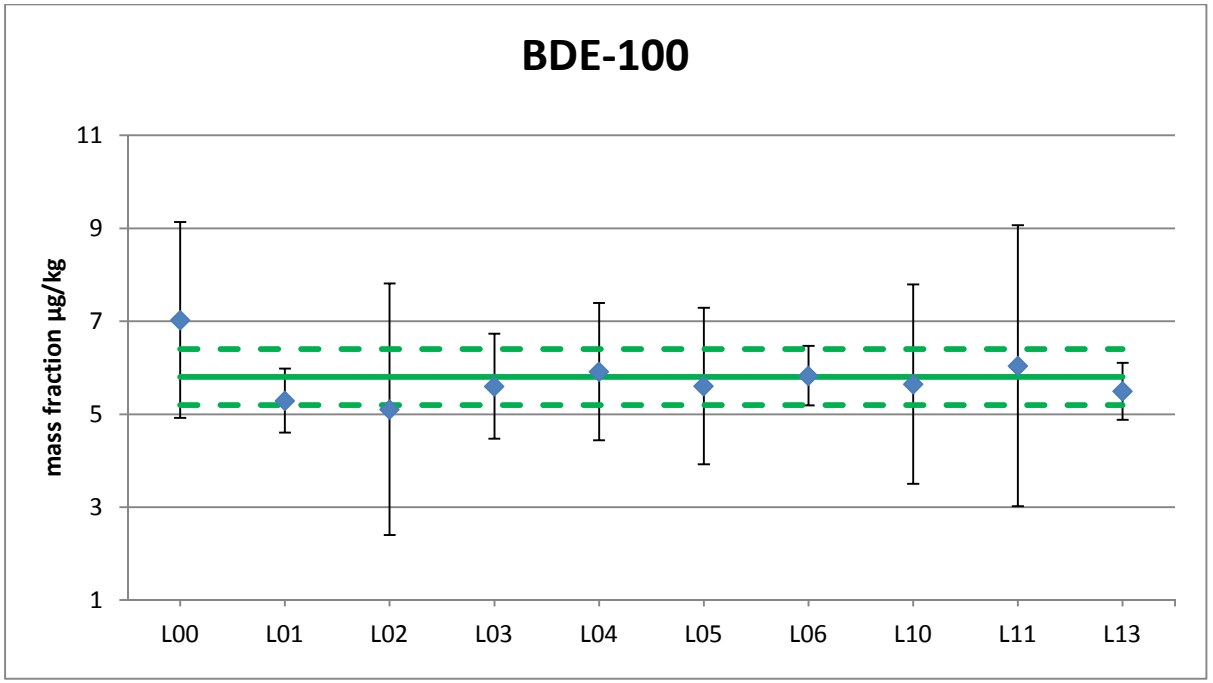


Figure F4: certified value (5.8 µg/kg, solid line) ± expanded uncertainty (0.6 µg/kg, dashed lines) for BDE-100; individual laboratory means (blue dots) are displayed with their expanded uncertainty (error bar)

Table F5: BDE-153

laboratory code - method	replic. 1 [µg/kg]	replic. 2 [µg/kg]	replic. 3 [µg/kg]	replic. 4 [µg/kg]	replic. 5 [µg/kg]	replic. 6 [µg/kg]	replic. 7 [µg/kg]	replic. 8 [µg/kg]	replic.9 [µg/kg]	replic. 10 [µg/kg]	replic. 11 [µg/kg]	replic. 12 [µg/kg]	mean [µg/kg]	expanded uncertainty [µg/kg]
L00-GC-HRMS	8.56	10.90	9.73	9.18	9.15	9.65	--	--	--	--	--	--	9.53	2.86
L01-GC-MS	7.26	7.14	7.18	7.56	7.07	7.30	--	--	--	--	--	--	7.25	1.16
L02-GC-HRMS	5.57	6.22	5.79	6.30	5.29	6.20	--	--	--	--	--	--	5.90	1.65
L03-GC-MS	5.08	5.96	5.42	5.57	5.89	5.78	--	--	--	--	--	--	5.62	1.68
L04-GC-HRMS	6.4	5.9	5.4	6.7	7.3	6.1	--	--	--	--	--	--	6.3	1.6
L05-GC-MS/MS	6.27	6.31	7.47	6.75	6.02	6.75	--	--	--	--	--	--	6.60	0.79
L06-GC-HRMS	7.12	6.03	6.03	6.25	6.06	6.72	--	--	--	--	--	--	6.37	0.70
L07-GC-MS	5.88	5.95	5.71	7.23	7.21	6.89	--	--	--	--	--	--	6.48	2.98
L09-GC-HRMS	6.56	6.70	6.81	6.26	6.31	6.84	6.47	6.55	6.86	6.36	6.93	6.28	6.58	1.05
L10-GC-MS	6.40	7.00	6.11	6.10	5.81	5.75	--	--	--	--	--	--	6.20	2.17
L11-GC-MS	5.73	6.26	6.20	8.56	6.56	6.15	--	--	--	--	--	--	6.58	3.29
L12-GC-MS	6.33	6.60	6.19	5.85	6.76	6.16	--	--	--	--	--	--	6.32	1.62
Results not used for certification														
L08-GC-MS/MS	*7.62*	*8.55*	*6.68*	*8.37*	*7.39*	*6.96*	--	--	--	--	--	--	*7.60*	*2.13*
L13-GC-MS/MS	*6.04*	*7.01*	*6.14*	*7.05*	*6.49*	*6.85*	--	--	--	--	--	--	*6.60*	*0.92*

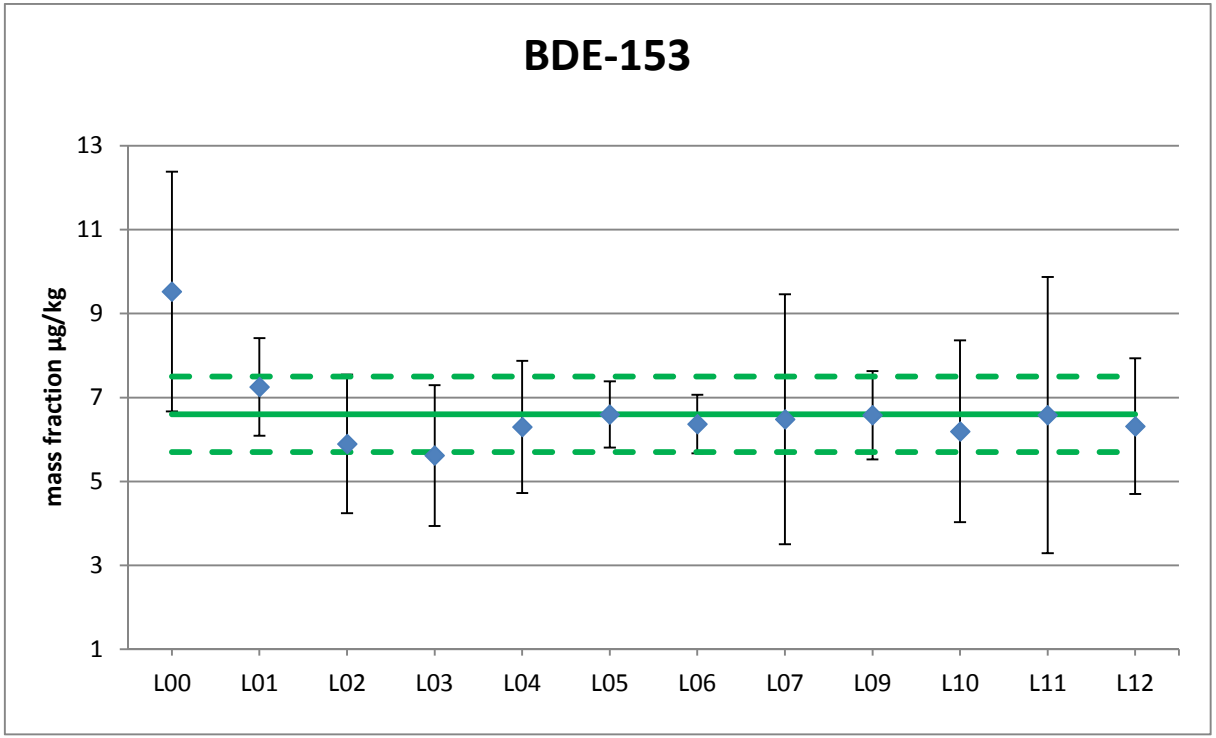


Figure F5: certified value (6.6 µg/kg, solid line) ± expanded uncertainty (0.9 µg/kg, dashed lines) for BDE-153; individual laboratory means (blue dots) are displayed with their expanded uncertainty (error bar)

Table F6: BDE-154

laboratory code - method	replic. 1 [µg/kg]	replic. 2 [µg/kg]	replic. 3 [µg/kg]	replic. 4 [µg/kg]	replic. 5 [µg/kg]	replic. 6 [µg/kg]	replic. 7 [µg/kg]	replic. 8 [µg/kg]	replic.9 [µg/kg]	replic. 10 [µg/kg]	replic. 11 [µg/kg]	replic. 12 [µg/kg]	mean [µg/kg]	expanded uncertainty [µg/kg]
L00-GC-HRMS	3.67	4.85	3.89	3.78	3.86	4.04	--	--	--	--	--	--	4.02	1.20
L02-GC-HRMS	3.31	3.20	3.31	3.22	2.90	3.44	--	--	--	--	--	--	3.23	1.68
L03-GC-MS	2.46	2.85	2.61	2.77	3.00	2.79	--	--	--	--	--	--	2.75	0.79
L04-GC-HRMS	3.8	3.9	3.1	3.9	4.3	4.2	--	--	--	--	--	--	3.9	1.0
L06-GC-HRMS	3.61	3.12	3.35	3.27	3.19	3.61	--	--	--	--	--	--	3.36	0.37
L09-GC-HRMS	3.50	4.05	4.12	4.22	3.77	4.02	3.73	3.76	3.92	3.82	4.10	3.60	3.88	0.76
L10-GC-MS	3.31	3.85	3.12	3.35	2.65	3.04	--	--	--	--	--	--	3.22	1.61
L11-GC-MS	3.22	3.30	3.30	4.84	3.44	3.28	--	--	--	--	--	--	3.56	1.78
L12-GC-MS	3.44	3.33	3.37	3.24	3.63	3.34	--	--	--	--	--	--	3.39	0.82
Results not used for certification														
L01-GC-MS	*3.47*	*3.62*	*3.52*	*3.73*	*3.51*	*3.59*	--	--	--	--	--	--	*3.57*	*0.57*
L05-GC-MS/MS	*2.65*	*2.69*	*3.26*	*2.97*	*2.72*	*2.66*	--	--	--	--	--	--	*2.83*	*0.40*
L07-GC-MS	*3.13*	*3.36*	*3.04*	*3.90*	*3.46*	*3.57*	--	--	--	--	--	--	*3.41*	*1.57*
L08-GC-MS/MS	*5.85*	*5.91*	*5.65*	*6.48*	*6.18*	*5.76*	--	--	--	--	--	--	*5.97*	*1.91*
L13-GC-MS/MS	*2.70*	*2.77*	*2.51*	*2.80*	*2.79*	*2.93*	--	--	--	--	--	--	*2.75*	*0.34*

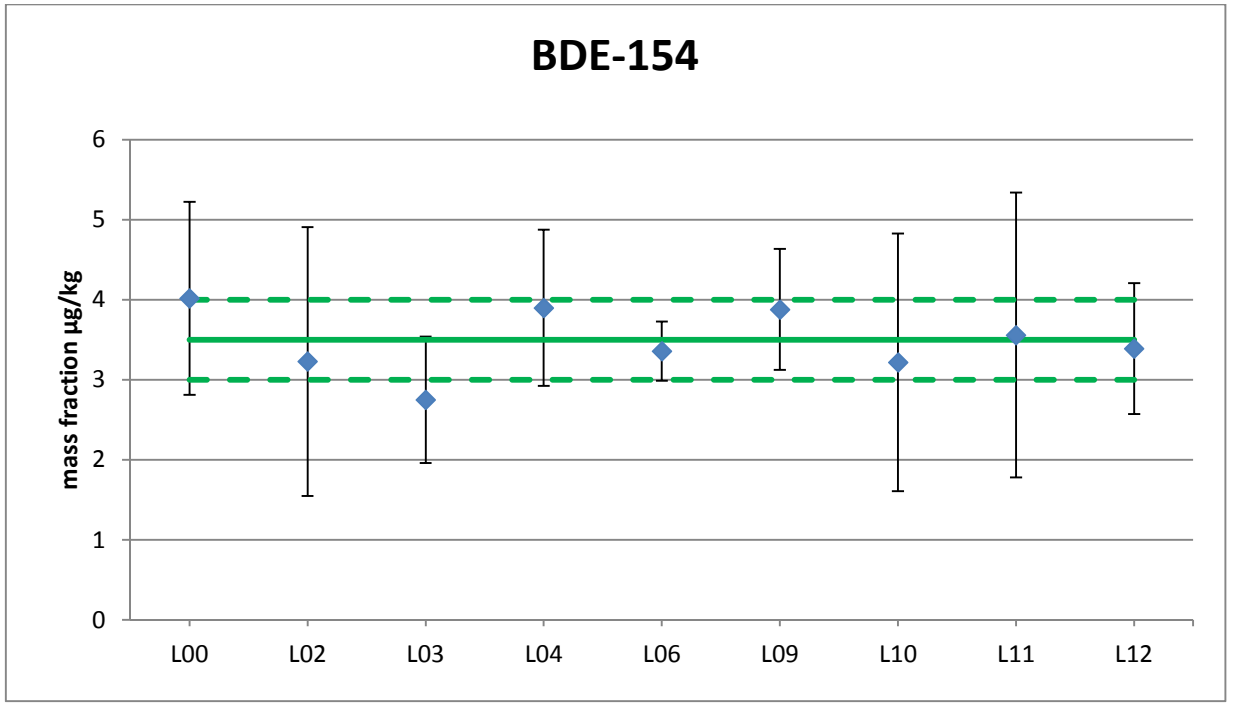


Figure F6: certified value (3.5 µg/kg, solid line ± expanded uncertainty (0.5 µg/kg, dashed lines) for BDE-154; individual laboratory means (blue dots) are displayed with their expanded uncertainty (error bar)

Table F7: BDE-183

laboratory code - method	replic. 1 [µg/kg]	replic. 2 [µg/kg]	replic. 3 [µg/kg]	replic. 4 [µg/kg]	replic. 5 [µg/kg]	replic. 6 [µg/kg]	replic. 7 [µg/kg]	replic. 8 [µg/kg]	replic.9 [µg/kg]	replic. 10 [µg/kg]	replic. 11 [µg/kg]	replic. 12 [µg/kg]	mean [µg/kg]	expanded uncertainty [µg/kg]
L00-GC-HRMS	1.48	1.23	1.45	1.13	1.62	1.09	--	--	--	--	--	--	1.33	0.40
L01-GC-MS	1.42	1.44	1.42	1.44	1.39	1.46	--	--	--	--	--	--	1.43	0.79
L02-GC-HRMS	1.24	1.39	1.63	1.27	1.42	1.47	--	--	--	--	--	--	1.40	0.66
L03-GC-MS	1.20	1.22	1.19	1.03	1.03	1.04	--	--	--	--	--	--	1.12	0.28
L04-GC-HRMS	1.6	1.4	1.4	1.5	1.5	1.3	--	--	--	--	--	--	1.5	0.4
L05-GC-MS/MS	2.00	1.61	1.78	2.02	1.61	1.66	--	--	--	--	--	--	1.78	0.53
L06-GC-HRMS	1.30	1.40	1.41	1.26	1.27	1.38	--	--	--	--	--	--	1.34	0.16
L09-GC-HRMS	1.43	1.57	1.57	1.58	1.41	1.46	1.41	1.54	1.46	1.52	1.50	1.49	1.50	0.21
L11-GC-MS	1.39	1.36	1.37	1.42	1.42	1.32	--	--	--	--	--	--	1.38	0.69
Results not used for certification														
L07-GC-MS	*1.67*	*1.43*	*1.82*	*1.71*	*2.05*	*1.84*	--	--	--	--	--	--	*1.75*	*1.23*
L08-GC-MS/MS	*36.16*	*32.64*	*18.00*	*40.39*	*19.88*	*17.48*	--	--	--	--	--	--	*27.43*	*7.13*
L10-GC-MS	*5.63*	*5.00*	*5.17*	*5.27*	*4.60*	*4.00*	--	--	--	--	--	--	*4.95*	*2.38*

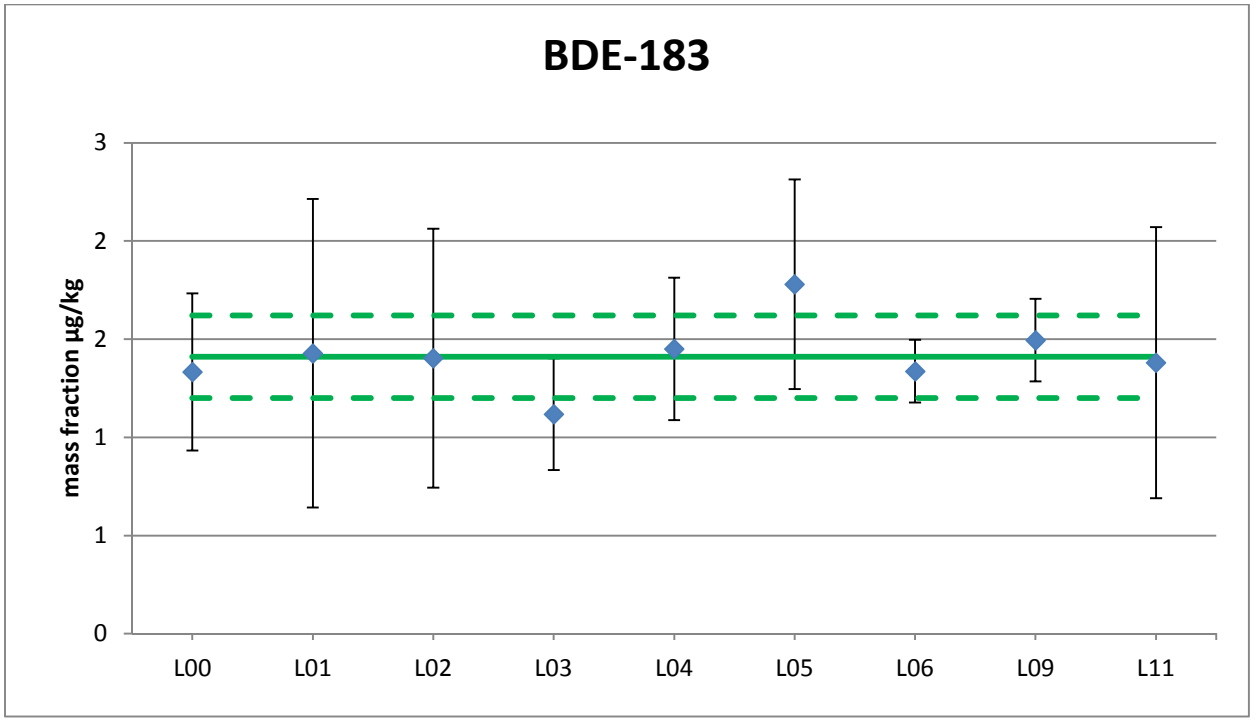


Figure F7: certified value (1.41 µg/kg, solid line) ± expanded uncertainty (0.21 µg/kg, dashed lines) for BDE-183; individual laboratory means (blue dots) are displayed with their expanded uncertainty (error bar)

Table F8: BDE-209

laboratory code - method	replic. 1 [µg/kg]	replic. 2 [µg/kg]	replic. 3 [µg/kg]	replic. 4 [µg/kg]	replic. 5 [µg/kg]	replic. 6 [µg/kg]	replic. 7 [µg/kg]	replic. 8 [µg/kg]	replic.9 [µg/kg]	replic. 10 [µg/kg]	replic. 11 [µg/kg]	replic. 12 [µg/kg]	mean [µg/kg]	expanded uncertainty [µg/kg]
L00-GC-HRMS	8339	8037	10960	8427	8063	8344	--	--	--	--	--	--	8695	2609
L02-GC-HRMS	6740	6408	6462	6158	7876	6383	--	--	--	--	--	--	6671	4870
L03-GC-MS	8234	7998	8351	8114	7926	8231	--	--	--	--	--	--	8142	822
L05-GC-MS/MS	8180	8249	7114	7209	7604	8019	--	--	--	--	--	--	7729	1082
L06-GC-HRMS	8011	7878	7769	7925	7902	7761	--	--	--	--	--	--	7874	3937
L10-GC-MS	7291.44	7485.02	6842.36	7966.48	7423.58	7296.88	--	--	--	--	--	--	7384.29	2289.13
Results not used for certification														
L01-GC-MS	*9920*	*9920*	*9790*	*10280*	*9940*	*10330*	--	--	--	--	--	--	*10030*	*3009*
L04-GC-HRMS	*8700*	*8500*	*7300*	*8700*	*6300*	*12000*	--	--	--	--	--	--	*8583*	*3433*
L07-GC-MS	*12819.86*	*8811.91*	*9971.01*	*8913.20*	*8465.18*	*7890.21*	--	--	--	--	--	--	*9478.56*	*2369.64*
L09-GC-HRMS	*9821.83*	*9983.41*	*9911.43*	*11259.53*	*10466.92*	*10014.14*	*9598.27*	*9715.44*	*10014.85*	*9772.59*	*9959.92*	*10070.61*	*10049.08*	*772.77*
L11-GC-MS	*9239.17*	*9219.45*	*8531.03*	*9604.59*	*10002.66*	*8872.36*	--	--	--	--	--	--	*9244.88*	*4622.44*
L13- GC-MS/MS	*8655.54*	*8848.45*	*8798.61*	*8569.45*	*8903.20*	*8710.03*	--	--	--	--	--	--	*8723.96*	*758.98*

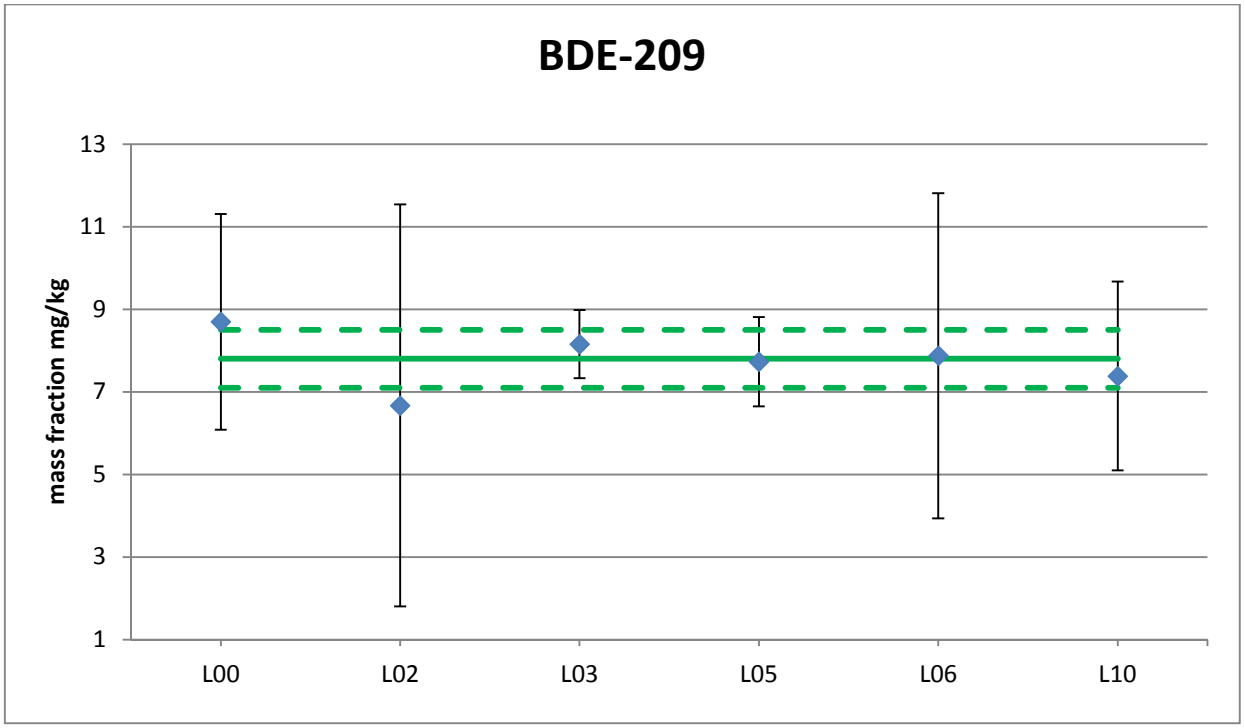


Figure F8: certified value (7.8 mg/kg, solid line) ± expanded uncertainty (0.7 mg/kg, dashed lines) for BDE-209; individual laboratory means (blue dots) are displayed with their expanded uncertainty (error bar)

Table F9: α-HBCD

laboratory code - method	replic. 1 [µg/kg]	replic. 2 [µg/kg]	replic. 3 [µg/kg]	replic. 4 [µg/kg]	replic. 5 [µg/kg]	replic. 6 [µg/kg]	mean [µg/kg]	expanded uncertainty [µg/kg]
L03-LC-MS/MS	8.61	8.82	9.49	9.15	10.54	9.47	9.35	2.60
L04- LC-MS/MS	8.8	7.7	9.4	8.5	8.4	8.0	8.5	2.1
L05- LC-MS/MS	5.50	5.56	6.38	4.78	7.50	5.80	5.9	1.2
L06- LC-MS/MS	8.30	7.64	9.04	8.60	8.08	9.58	8.54	1.62
L10- LC-MS/MS	7.34	9.02	7.92	7.65	7.90	7.69	7.92	3.80
L11- LC-MS/MS	10.77	9.73	8.40	12.78	7.74	9.56	9.83	4.92
Results not used for certification								
L00- LC-MS/MS	*8.70*	*8.00*	*7.76*	*9.19*	*9.31*	*8.08*	*8.51*	*2.55*
L07-LC-MS	*<16*	*<13*	*<22*	*<11*	*<15*	*<8.8*	*n.a.*	*n.a.*
L08-UPLC-MS/MS	*4.55*	*5.49*	*5.59*	*4.91*	*4.36*	*5.06*	*4.99*	*n.r.*

n.a.= not applicable
n.r.= not reported

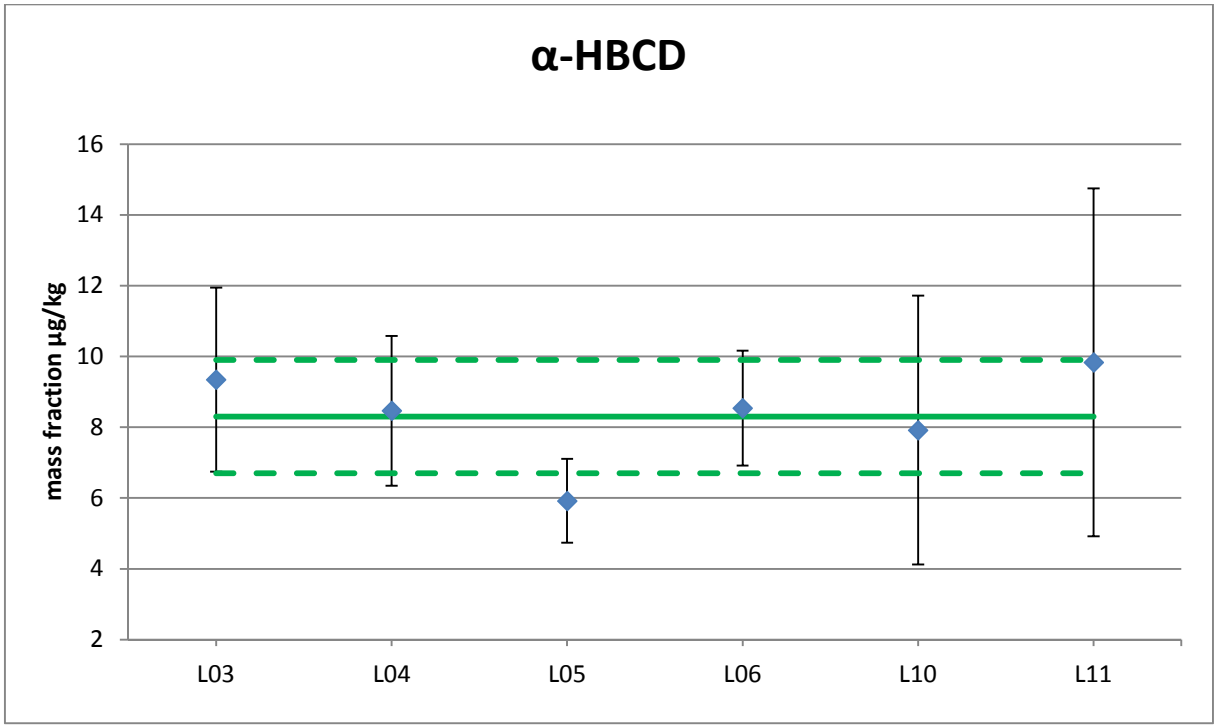


Figure F9: certified value (8.3 µg/kg, solid line) ± expanded uncertainty (1.6 µg/kg, dashed lines) for α-HBCD; individual laboratory means (blue dots) are displayed with their expanded uncertainty (error bar)

Table F10: β-HBCD

laboratory code - method	replic. 1 [µg/kg]	replic. 2 [µg/kg]	replic. 3 [µg/kg]	replic. 4 [µg/kg]	replic. 5 [µg/kg]	replic. 6 [µg/kg]	mean [µg/kg]	expanded uncertainty [µg/kg]
L03-LC-MS/MS	2.02	2.12	2.43	2.14	2.29	2.35	2.23	1.00
L04- LC-MS/MS	2.5	1.9	2.2	1.9	2.2	2.2	2.2	0.5
L05- LC-MS/MS	1.83	1.95	1.74	1.80	1.98	2.18	1.91	0.34
L06- LC-MS/MS	2.64	2.66	3.02	2.72	2.92	3.28	2.87	0.49
L10- LC-MS/MS	2.27	2.06	2.20	1.99	2.39	2.01	2.15	1.8
Results not used for certification								
L00- LC-MS/MS	*1.25*	*1.20*	*1.45*	*1.26*	*1.00*	*1.50*	*1.28*	*0.38*
L07-LC-MS	*<16*	*<13*	*<22*	*<11*	*<15*	*<8.8*	*n.a.*	*n.a.*
L08-UPLC-MS/MS	*1.03*	*0.81*	*1.06*	*1.12*	*1.08*	*1.06*	*1.03*	*n.r.*
L11- LC-MS/MS	*6.06*	*5.88*	*4.59*	*6.99*	*4.43*	*5.19*	*5.52*	*2.76*

n.a.= not applicable
n.r.= not reported

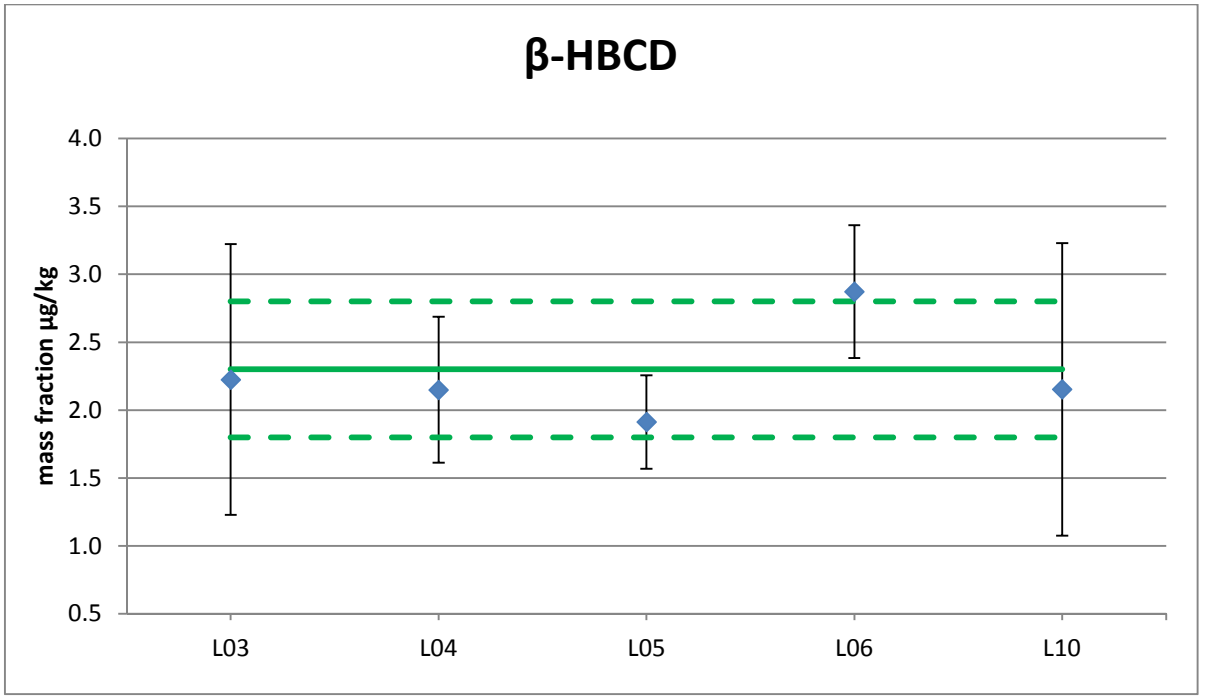


Figure F10: certified value (2.3 µg/kg, solid line) ± expanded uncertainty (0.5 µg/kg, dashed lines) for β-HBCD; individual laboratory means (blue dots) are displayed with their expanded uncertainty (error bar)

Table F11: γ-HBCD

laboratory code - Method	replic. 1 [µg/kg]	replic. 2 [µg/kg]	replic. 3 [µg/kg]	replic. 4 [µg/kg]	replic. 5 [µg/kg]	replic. 6 [µg/kg]	mean [µg/kg]	expanded uncertainty [µg/kg]
L03-LC-MS/MS	49.19	52.85	58.82	45.21	47.54	42.19	49.30	16.02
L04- LC-MS/MS	47	54	51	45	52	50	50	12
L05- LC-MS/MS	74.4	71.5	53.6	52.0	56.2	47.3	59.2	13.0
L06- LC-MS/MS	56.60	66.34	83.72	65.64	87.98	93.78	75.68	14.38
L10- LC-MS/MS	117.15	56.16	50.67	57.59	50.99	54.04	64.43	32.22
Results not used for certification								
L00- LC-MS/MS	*59.38*	*59.99*	*61.28*	*63.61*	*68.47*	*68.77*	*63.58*	*19.1*
L07-LC-MS	*73.10*	*102.93*	*59.38*	*82.23*	*62.52*	*81.07*	*76.87*	*29.21.*
L08-UPLC-MS/MS	*19.22*	*21.90*	*33.93*	*34.08*	*38.78*	*28.01*	*29.32*	*14.07*
L11- LC-MS/MS	*146.86*	*124.19*	*87.6*	*126.98*	*84.68*	*126.52*	*116.14*	*58.07*

n.a.= not applicable
n.r.= not reported

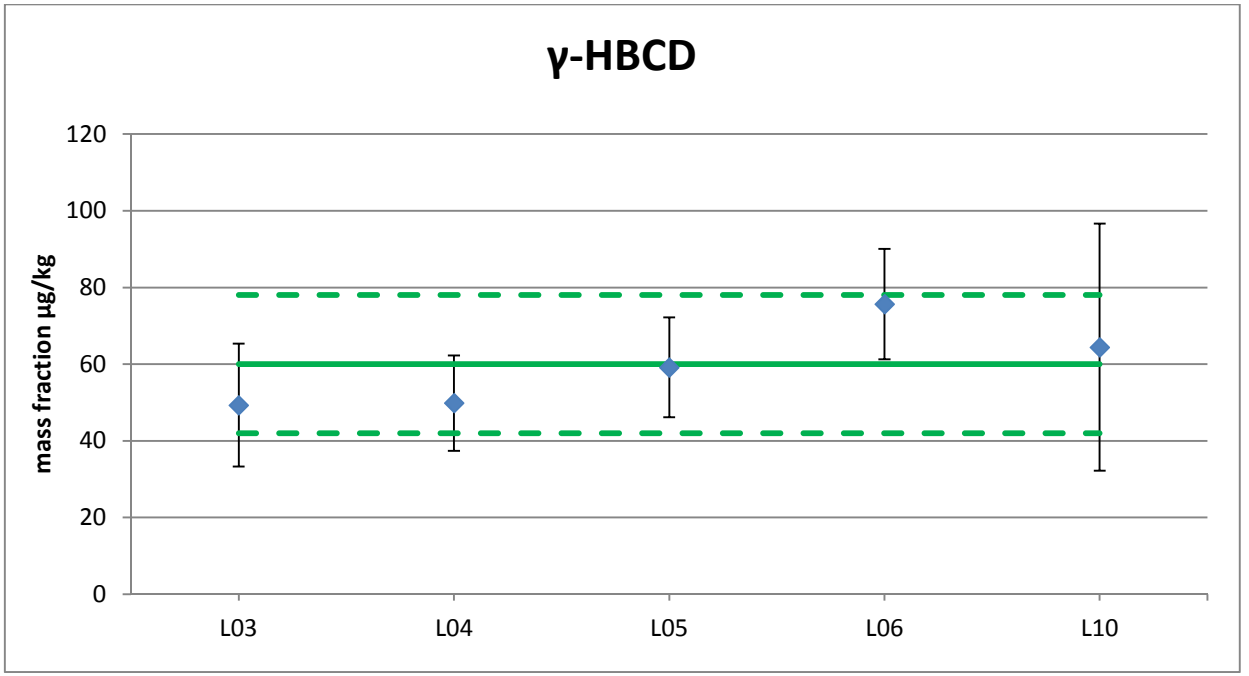


Figure F11: certified value (60 µg/kg, solid line) ± expanded uncertainty (16 µg/kg, dashed lines) for γ-HBCD; individual laboratory means (blue dots) are displayed with their expanded uncertainty (error bar)

European Commission

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