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CERTIFICATION REPORT

The certification of the mass fractions of PBDEs in Fish Tissue: ERM®-CE102

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Abstract

This report describes the production of ERM®-CE102, which is a fish tissue certified for the mass fraction of polybrominated diphenyl ethers (PBDEs) on a wet weight basis. This material was produced following ISO 17034:2016 and is certified in accordance with ISO Guide 35:2017.
The Certified Reference Material is a fish tissue homogenate prepared from wild Wels catfish (*Silurus glanis*) originating from the Flix reservoir of the Ebro river (Spain) and farmed rainbow trout (*Oncorhynchus mykiss*) sourced in Belgium. The fish fillets were cut, shock-frozen in liquid nitrogen and cryogenically milled. After a pre-cooking step, step-wise mixing and homogenisation were carried out. The resulting material was filled into jars, autoclaved for sterilisation and labelled as ERM-CE102.
Between-unit homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2017. 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. Technically invalid results were removed but no outlier was eliminated on statistical grounds only.
Uncertainties of the certified values were calculated in accordance 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 with any reference material, it can be used for establishing control charts or in validation studies. ERM-CE102 is available in glass jars with twist-off lids containing at least 40 g of fish paste. The minimum amount of sample to be used is 8 g.

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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®-CE102, which is a fish tissue certified for the mass fraction of polybrominated diphenyl ethers (PBDEs) on a wet weight basis. This material was produced following ISO 17034:2016 [1] and is certified in accordance with ISO Guide 35:2017 [2].

The Certified Reference Material is a fish tissue homogenate prepared from wild Wels catfish (*Silurus glanis*) originating from the Flix reservoir of the Ebro river (Spain) and farmed rainbow trout (*Oncorhynchus mykiss*) sourced in Belgium. The fish fillets were cut, shock-frozen in liquid nitrogen and cryogenically milled. After a pre-cooking step, step-wise mixing and homogenisation were carried out. The resulting material was filled into jars, autoclaved for sterilisation and labelled as ERM-CE102.

Between-unit homogeneity was quantified and stability during dispatch and storage were assessed in accordance with ISO Guide 35:2017 [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 [3]. Technically invalid results were removed but no outlier was eliminated on statistical grounds only.

Uncertainties of the certified values were calculated in accordance 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 with any reference material, it can be used for establishing control charts or in validation studies. ERM-CE102 is available in glass jars with twist-off lids containing at least 40 g of fish paste. The minimum amount of sample to be used is 8 g.

The following values were assigned:

	Mass Fraction (relative to wet weight)	
	Certified value [2] [µg/kg]	Uncertainty [3] [µg/kg]
BDE-47 (2,2',4,4'-tetrabromodiphenyl ether)[1]	0.227	0.019
BDE-49 (2,2',4,5'-tetrabromodiphenyl ether)[1]	0.033	0.007
BDE-99 (2,2',4,4',5-pentabromodiphenyl ether)[1]	0.123	0.013
BDE-100 (2,2',4,4',6-pentabromodiphenyl ether)[1]	0.060	0.006
BDE-153 (2,2',4,4',5,5'-hexabromodiphenyl ether)[1]	0.069	0.008
BDE-154 (2,2',4,4',5,6'-hexabromodiphenyl ether)[1]	0.109	0.008

[1] as obtained by gas chromatography coupled to mass spectrometry.

[2] 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).

[3] The uncertainty of the certified values 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
ASE	Accelerated solvent extraction
b	Slope in the equation of linear regression $y = a + bx$
BFR	Brominated flame retardant
CI	Confidence interval
CRM	Certified reference material
EI	Electron ionisation
EC	European Commission
ECNI	Electron capture negative ionisation
EQS	Environmental quality standard
ERM®	Trademark of European Reference Materials
EU	European Union
GC-MS	Gas chromatography mass spectrometry
GUM	Guide to the Expression of Uncertainty in Measurements [ISO/IEC Guide 98-3:2008]
HRMS	High resolution mass spectrometry
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
LLE	Liquid liquid extraction
LOD	Limit of detection
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
MQC	Method quality control
n	Number of replicates per unit
n.a.	Not applicable
n.c.	Not calculated
NIST	National Institute of Standards and Technology (USA)
PBDE	Polybrominated diphenyl ether
QA	Quality assurance

QC	Quality control
rel	Index denoting relative figures (uncertainties etc.)
RM	Reference material
RM Unit	Reference Materials Unit of Directorate F
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
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 point for each replicate
t_{sl}	Proposed shelf life
u	standard uncertainty
U	expanded uncertainty
u^*_{bb}	Standard uncertainty related to a maximum between-unit inhomogeneity that could be hidden by method repeatability or intermediate precision; 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_{char}	Standard uncertainty of the material characterisation; 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_{rec}	Standard uncertainty related to possible between-unit inhomogeneity modelled as rectangular distribution; an additional index "rel" is added as appropriate
u_{sts}	Standard uncertainty of the short-term stability; an additional index "rel" is added as appropriate
WFD	Water Framework Directive
Δ_{meas}	Absolute difference between mean measured value and the certified value

$\nu_{MSwithin}$	Degrees of freedom of MS_{within}
X_{10}	Particle diameter corresponding to 10 % of the cumulative undersize distribution (here by volume)
X_{50}	Particle diameter corresponding to 50 % of the cumulative undersize distribution (here by volume)
X_{90}	Particle diameter corresponding to 90 % of the cumulative undersize distribution (here by volume)

1 Introduction

1.1 Background

Polybrominated diphenyl ethers (PBDEs) are a major class of brominated flame retardants (BFRs), which ubiquity, persistence, bioaccumulation and biomagnification in the environment has been long observed and documented [5, 6]. Despite the ban and restriction on their production and use since 2003 [7,8,9], exposure to PBDEs via the environment and the food chain continues to be an indisputable threat to wildlife and human health [10]. For more than fifteen years, scientific evidence has been gathered on the endocrine-modulating effects of PBDEs, besides other recognised adverse health effects like neurodevelopmental toxicity and emerging indications of cancer [11,12,13]. The monitoring of BFRs in environmental and food samples is therefore an ongoing task for analytical laboratories all over the world which need to deliver reliable measurement results for compliance purposes. PBDEs are included in the list of priority substances of the EU Water Framework Directive (WFD) [14] with defined environmental quality standards (EQS) in water and biota [15] and they have to be monitored in fish and seafood (amongst other foodstuffs) following the Commission Recommendation 2014/118/EU [16]. In this respect, both in environmental and food-related legislation [17,18], the analysis of reference materials (RMs) is highly welcomed and recommended for ensuring the quality and comparability of measurement results.

1.2 Choice of the material

The choice of certifying a fish matrix for a range of PBDE congeners of primary interest answers to clear needs regarding the monitoring of these substances as laid out in EU legislation, both in the environmental sector and in the food control field. In 2013, the Directive 2013/39/EU was published, amending the WFD and setting EQS in biota on a wet weight basis for very hydrophobic priority substances among which the PBDE congeners 28, 47, 99, 100, 153 and 154. Biota (to be intended as fish, the only exception being crustaceans and molluscs to be analysed for PAHs) hence becomes the default matrix to be considered by the Member States in the mandatory monitoring of the EU surface water quality status. A year later, in 2014, a Commission recommendation on the monitoring of traces of brominated flame retardants in food was published. Following this recommendation, Members States should check the presence of PBDEs 28, 47, 49, 99, 100, 138, 153, 154 and 183 in a range of food commodities including fish (and other seafood). The ERM-CE102 is a fish paste produced as a fresh-like matrix to acknowledge the WFD setting of biota EQS on a wet weight basis and to enhance the commutability of the certified reference material (CRM) to environmental and food samples analysed routinely in the analytical laboratories. The levels of the certified PBDEs were constrained by the natural contamination of the starting material (wild Wels catfish) and the project planning. The aim was to balance the usefulness of the CRM in relation to the very low biota EQS (0.0085 µg/kg wet weight for the sum of congeners 28, 47, 99, 100, 153 and 154) and the performance needed by the analytical methods to be employed in the food monitoring (limit of quantification of 0.01 µg/kg wet weight) with a successful certification (also with regard to a reduced uncertainty of the certified values). In addition, ERM-CE102 is certified in the ng/kg - µg/kg mass fraction range, well representing the global environmental occurrence of PBDEs.

1.3 Design of the CRM project

The value assignment of PBDEs in ERM-CE102 was based on an interlaboratory comparison involving analytical methods all sharing gas chromatography (GC) separation (carried out with a range of different columns) and mass spectrometry (MS), but combining different sample extraction, clean-up and quantification principles.

2 Participants

2.1 Project management and evaluation

European Commission, Joint Research Centre, Geel, BE
(accredited to ISO 17034 for production of certified reference materials, BELAC No. 268-RM)

2.2 Processing

European Commission, Joint Research Centre, Geel, BE
(accredited to ISO 17034 for production of certified reference materials, BELAC No. 268-RM)

2.3 Homogeneity study

RIKILT Wageningen University & Research, Wageningen, NL

2.4 Stability study

RIKILT Wageningen University & Research, Wageningen, NL

Umweltbundesamt GmbH, Wien, AU
(measurements under the scope of ISO/IEC 17025 accreditation BMWFJ; AA 0200)

2.5 Characterisation

Aarhus Universitet, Institut for Miljøvidenskab, Roskilde, DK

ALS Czech Republic, Praha, CZ
(measurements under the scope of ISO/IEC 17025 CAI; 333/2018)

EMPA, Swiss Federal Laboratories for Materials Science and Technology, Laboratory for Advanced Analytical Technologies, Dübendorf, CH

Eurofins GfA Lab Service GmbH, Hamburg, DE
(measurements under the scope of ISO/IEC 17025 accreditation DAkkS; D-PL-14629-01-00)

Fera Science Ltd, York, UK
(measurements under the scope of ISO/IEC 17025 accreditation UKAS; 1642)

Helmholtz Zentrum München, Deutsches Forschungszentrum für Gesundheit und Umwelt, Neuherberg, DE

Ontario Ministry of Environment, Conservation and Parks, Laboratory Services Branch, Toxic Organics Section, Etobicoke, Ontario, Canada
(measurements under the scope of ISO/IEC 17025 accreditation CALA; 2081)

RIKILT Wageningen University & Research, Wageningen, NL

Umweltbundesamt GmbH, Wien, AT
(measurements under the scope of ISO/IEC 17025 accreditation BMWFJ; AA 0200)

University of Chemistry and Technology, Prague, Faculty of Food and Biochemical Technology, Department of Food Analysis and Nutrition, Prague, CZ
(measurements under the scope of ISO/IEC 17025 accreditation CAI; 202/2018)

VITO NV, Vlaamse Instelling voor Technologisch Onderzoek, SCT-GOAL, Mol, BE

Vrije Universiteit Amsterdam, Department Environment and Health, Amsterdam, NL

3 Material processing and process control

3.1 Origin of the starting material

Two fish species were employed as starting material for the production of ERM-CE102: rainbow trout (*Oncorhynchus mykiss*), locally sourced from a Belgian aquaculture farm, and wild Wels catfish (*Silurus glanis*) originating from the area of the Flix reservoir of the Ebro river, Spain. The filleted catfish, caught by angling during the period December 2010-June 2011, was already available in-house stored as deep frozen from a previous CRM project. Four different catfish tail pieces were selected upon screening for their appropriate PBDEs' content, while the purchased trout was analysed to confirm its blank PBDEs levels. The two fish species were mixed according to the ratio trout : catfish = 98 : 2 to obtain PBDEs levels in the order of hundreds ng/kg as set in the project plan. The material processing took place in December 2015 at the JRC processing facility in Geel.

3.2 Processing

About 100 kg of blank trout was sliced into fillets, removing bones and skin. Both the trout and the catfish fillets (a total of ca. 1.8 kg corresponding to four tails) were cut in cubes and thereafter shock-frozen in liquid nitrogen. The fish material was subsequently cryogenically milled using a Palla VM-KT cryogenic vibrating mill (KHD, Humboldt Wedag, Köln, DE) and stored as a paste at - 20 °C until further treatment. After controlled thawing to + 4 °C, the trout and catfish pastes were separately placed in glass jars with twist-off lids in an autoclave JBTC AR092 pilot retort for pre-cooking at 85 °C (JBT, Sint-Niklaas, BE). The lids are made by a metal sheet internally coated with PVC epoxidised soybean oil-free (used by the food industry in the packaging of fatty foodstuff).

At this point, the catfish paste was re-analysed for confirming that PBDEs' content had not changed significantly. After the pre-cooking step to reach the desired matrix texture, the catfish and trout batches were first homogenised separately in a Stephan UM12 mixer (Hameln, DE), followed by a step-wise mixing scheme using a Stephan UM200 mixer. In this way, the catfish batch was subsequently diluted with batches of blank trout until obtaining ca. 100 kg of a well homogenised fish paste with the desired PBDEs levels.

Approximately 40 g of paste was filled into 60 mL glass jars with a Unifiller (Lörrach, DE). Twist-off lids of 66 mm diameter were placed on the jars using a Lenssen twist-off machine (Sevenum, NL) inside a chamber filled with steam. The under-pressure created in the head-space over the paste after cooling down ensures that the center of the lid will remain pressed down as long as the seal is not broken. Upon opening, the lid will pop open with a click as an indication that the sample has not been compromised.

The filled jars were placed according to the filling order in baskets and sterilised in autoclave at 121 °C with the peak temperature maintained for about 10 min (JBT, Sint-Niklaas, Belgium), **Figure 1**. After labelling, each jar was placed into a pre-labelled polyethylene terephthalate / aluminium / nylon / low density polyethylene pouch which was thermo-sealed using a DAKLA sealing machine (Daklapak, Kortrijk, BE). A total of 1395 units of ERM-CE102 was produced (**Figure 2**).

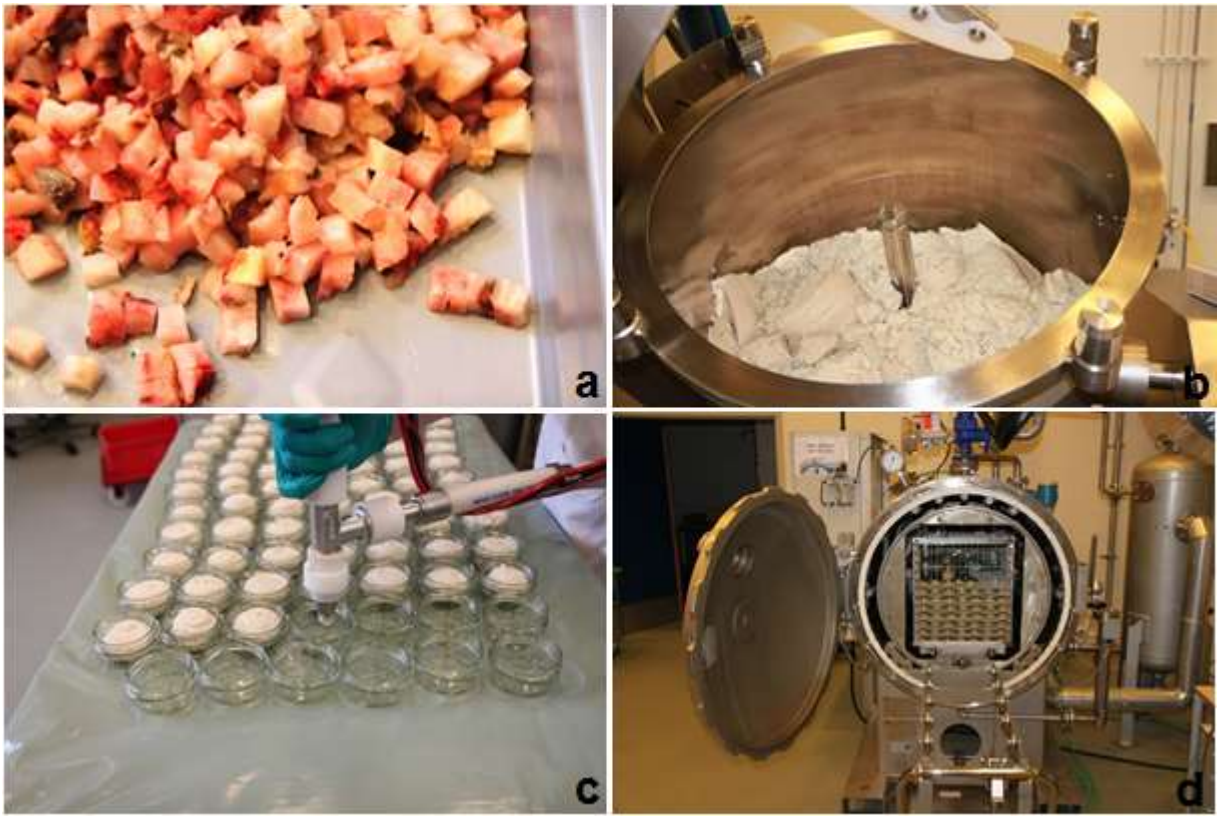

Figure 1: a) Fish material cut in cubes, b) homogenisation of the fish paste, c) filling of the jars, d) final sterilisation in the autoclave

Figure 2: Example of ERM-CE102 unit: jar filled with fish paste and its protective pouch

3.3 Process control

3.3.1 Particle size analysis

The average particle size distribution of ERM-CE102 is displayed in **Figure 3**. Ten units were measured in duplicate using a Helos laser light diffraction instrument (Sympatec, Clausthal Zellerfield, DE). Volume-weighted average particle size cumulative (Q3) and density (q3*) distributions, representative for ERM-CE102 are depicted in **Figure 3**.

As an overall assessment of comparability of the particle size distribution between the different units, the sum of the average 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. For this material, the largest difference from the mean was about 10 %, which shows that the whole batch was homogeneous with respect to particle size.

The results of the particle size distribution measurements are summarised **Table 1**.

Table 1: Particle size data for ERM-CE102 (n = 20)

Upper band limit	Average particle size [µm]	s [µm]
X_{10}	4.42	0.66
X_{50}	47.82	1.81
X_{90}	141.06	7.19

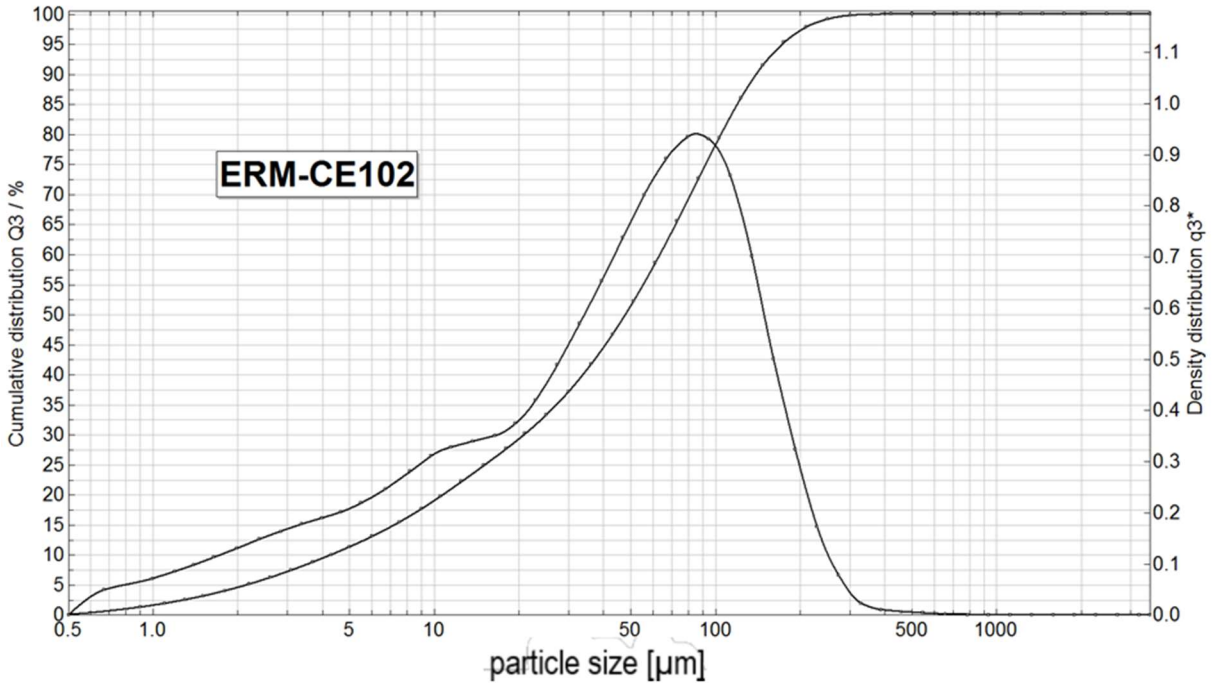

Figure 3: Average particle size distribution in ERM-CE102 using water as dispersant (n = 20)

3.3.2 Moisture content

A ventilated oven drying method at 102 °C was used for measuring the moisture content (water + other volatile components) in ERM-CE102. The volatile components are assumed to

constitute a very small fraction of the total mass loss, which is thus mainly a water loss. Ten units randomly selected over the whole batch were analysed in triplicate. An analytical balance was used to record the mass loss. No trend over the filling sequence could be observed for the moisture content in this material as shown in **Figure 4**. Each data point is displayed as the average for the CRM unit ± RSD (*n* = 3). An average water content of 74.84 % (m/m), typical of a fresh tissue material, was measured.

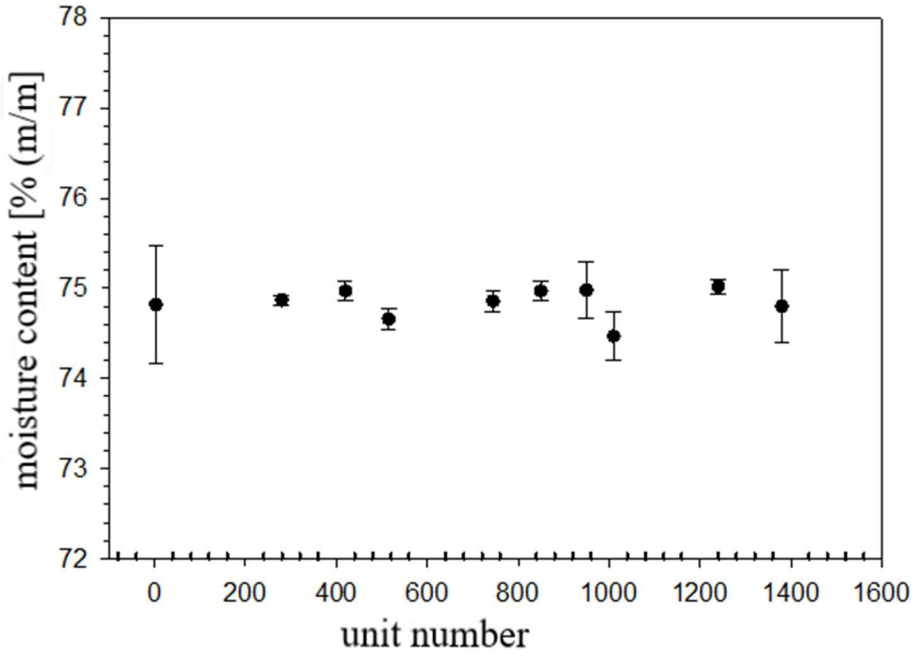

Figure 4: Moisture content in ERM-CE102 over the filling sequence. Each data point is displayed as the average for the CRM unit ± RSD (*n* = 3).

4 Homogeneity

A key requirement for any reference material aliquoted into units is 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 is significant compared to the analytical variation. Consequently, ISO 17034 [1] 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 units of the material, within the stated uncertainties.

Given the high variability of the dataset acquired for the homogeneity study, which would bring the estimation of the u_{bb} exceeding the maximum admitted value for the majority of the PBDEs, other datasets obtained during the certification process were considered for this purpose. The combined dataset of the two 1-year long-term stability studies was chosen for the homogeneity evaluation of BDE-47, -99, -100, -154, -183 and -209, while for BDE-28 and BDE-153 this was not appropriate because of trends observed in one of the study.

Alternatively, the combined dataset of the two short-term stability studies were selected for these two congeners, while the dataset of the 2-year long-term stability study was used for BDE-49 (for which the decision about the certification came only at a later stage of the project). Between eight and sixteen jars were selected using a random stratified sampling scheme covering the whole batch. Three independent samples were taken from each selected unit and analysed for the target PBDEs by GC-HRMS. The sample preparations were carried out split over 2 (4 in the case of BDE-49) days, while the measurements were performed within a single sequence, and in a randomised manner to be able to separate a potential analytical drift from a trend in the filling sequence. Day-to-day effects were looked at, given the non-repeatability conditions of the measurements results. When a significant difference between the day means was spotted (by the F-test in one-way ANOVA), normalisation of the data was carried out to detect the presence of trends and outliers. This was the case for BDE-47, -99, -100, -154 and -209, see **Table 2**. The results are shown as graphs in Annex A.

Regression analyses were performed on the original or normalised data to evaluate potential trends in the analytical sequence as well as trends in the filling sequence. A filling trend at the 95 % confidence level for BDE-153 was detected and taken into account for the estimation of the $u_{bb.}$ An analytical trend was visible at 95 % confidence level on the original data for BDE-209, pointing at a changing parameter e.g. 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 [19]. 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, the analytical trend was 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

The datasets (trend-corrected if necessary) were assessed for consistency using Grubbs outlier tests at a confidence level of 99 % on the individual results and on the unit means. One outlying individual result for BDE-154, and one outlying unit mean for BDE-49 and -209, respectively, were detected. Since no technical reason for the outliers could be found, all the data were retained for statistical analysis.

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 were representative for the whole unit. When day-to-day effects were present, two-way ANOVA was applied on the non-normalised data to calculate the pure between-unit standard deviation.

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 study

Parameter	Trends*		Outliers**		Distribution	
	Analytical sequence	Filling sequence	Individual results	Unit means	Individual results	Unit means
BDE-28	no	no	-	-	normal/ unimodal	normal/ unimodal
BDE-47	no	no	-	-	normal/ unimodal	normal/ unimodal
BDE-49	no	no	-	1-statistical (retained)	normal/ unimodal	normal/ unimodal
BDE-99	no	no	-	-	normal/ unimodal	normal/ unimodal
BDE-100	no	no	-	-	normal/ unimodal	normal/ unimodal
BDE-153	no	yes	-	-	normal/ unimodal	normal/ unimodal
BDE-154	no	no	1-statistical (retained)	-	normal/ unimodal	normal/ unimodal
BDE-183	no	no	-	-	normal/ unimodal	normal/ unimodal
BDE-209	yes***	no	-	1-statistical (retained)	normal/ unimodal	normal/ unimodal

In italics, parameters for which normalised data were used for the statistical evaluation
* 95 % confidence level
**99 % confidence level
***analytical trend on the original data, corrected before normalisation

It should be noted that $s_{bb,rel}$ and $s_{wb,rel}$ are estimates of the true standard deviations and are 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.* [20]. u^*_{bb} is comparable to the LOD 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:

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

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

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

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

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

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

n mean number of replicates per unit

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

However, a different approach was adopted for BDE-49 and BDE-209 for which one outlying unit mean was detected. In this case between-unit inhomogeneity was modelled as a rectangular distribution limited by the largest outlying unit mean, and the rectangular standard uncertainty of homogeneity was estimated by:

$$u_{rec} = \frac{\left|outlier - \overline{y}\right|}{\sqrt{3} \cdot \overline{y}} \qquad \textbf{Equation 5}$$

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

The outlying unit mean for BDE-209 comes from three high values of the three replicates conducted on the same u pointing to a real possible inhomogeneity with regard to this parameter. In any case, the uncertainty contribution for homogeneity, calculated with **Equation 5** would be estimated as 24.4 % (**Table 3**), bringing the final certified uncertainty for BDE-209 to an unacceptable large value. Additionally, outlying values of BDE-209 were detected also in other studies during the certification process of ERM-CE102 (e.g. short-term stability and 2-year long-term stability). On the basis of these findings, it was decided not to continue with the characterisation of BDE-209.

The between-unit homogeneity uncertainty was assessed differently for BDE-153, for which a significant trend (at 95 % confidence level) was detected. Here, the u_{rec} was estimated using a rectangular distribution between the highest and lowest unit mean:

$$u_{rec} = \frac{\left|highest\ result - lowest\ result\right|}{2 \cdot \sqrt{3} \cdot \overline{y}} \qquad \textbf{Equation 6}$$

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. Only in a couple of cases, the uncertainty contribution for homogeneity was determined by the method repeatability.

The homogeneity study showed no outlying unit mean or trends in the filling sequence for BDE-28, -47, -99, -100, -154 and -183. 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.

A trend in the filling sequence was evidenced for BDE-153: in this case, the inhomogeneity was quantified as u_{rec} and used as estimate of u_{bb}.

Table 3: Results of the homogeneity study

Parameter	$s_{wb,rel}$ [%]	$s_{bb,rel}$ [%]	$u^*_{bb,rel}$ [%]	$u_{rec,rel}$ [%]	$u_{bb,rel}$ [%]
BDE-28	4.0	2.1	1.2	n.a.	2.1
BDE-47	0.6	0.2	0.2	n.a.	0.2
BDE-49	8.7	1.1	3.2	7.5	7.5
BDE-99	1.0	0.5	0.3	n.a.	0.5
BDE-100	0.9	0.6	0.3	n.a.	0.6
BDE-153	1.9	2.1	0.6	2.6	2.6
BDE-154	2.1	n.c	0.6	n.a.	0.6
BDE-183	2.4	1.2	0.7	n.a.	1.2
BDE-209	8.8	16.2	2.5	24.4	24.4

n.c.: cannot be calculated as MSbetween < MSwithin
n.a.: not applicable

One outlying unit mean was found for BDE-49 and for BDE-209. However, in the case of BDE-49, taking this extreme value into account, the inhomogeneity quantified as u_{rec} was considered still acceptable. Therefore, u_{rec} was used as estimate of u_{bb}. In the case of BDE-209, the inhomogeneity quantified as u_{rec} is extremely large. In addition, some evidence of possible inhomogeneity raised during several other studies on the material, therefore it was decided not to assign any value for BDE-209 in ERM-CE102 and from now on, no further results for BDE-209 will be reported.

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.

Study of decreasing sample intakes

To estimate the minimum sample intake, a series of measurements with decreasing amounts of sample on nine randomly selected units were performed. The following sample intakes were tested: 2, 5 and 8 g (wet mass). For each sample intake, 3 units were measured in triplicate by GC-ECNI-MS. The sample preparations were carried out split over 2 days, while the measurements were performed within a single sequence, and in a randomised manner to be able to separate a potential analytical drift from a trend in the filling sequence. No day-to-day effects were detected for BDE-28, -47, -99, -100, -153, -154 and -183. The measurement method was robust over the whole range of the sample intake tested (Section 6).

The obtained datasets (all sample intakes taken together) were first tested whether it followed a normal, or at least 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). All results were normally and unimodally distributed.

An analytical trend was visible at 95 % confidence level on the original data for BDE-183 and it was corrected as shown in **Equation 1**. The results (all sample intakes taken together) were scrutinised for outliers using the single Grubbs test at a confidence level of 99 %. One

outlying individual result for BDE-47 and BDE-99 each, and one outlying unit mean for BDE-99 were detected. Since no technical reason for the outliers could be found, the results were retained. In any case, their removal would not affect the overall result of the minimum sample intake determination for ERM-CE102.

The minimum sample intake was established by comparison of variances obtained for 2 and 5 g sample intakes with the variance obtained for 8 g sample intake, via the F-test for equality of two samples for variances with 8 degrees of freedom and a confidence level of 95 %.

The obtained results are presented in Annex B, Table B1 and summarised in **Table 4**.

The minimum sample intake commonly valid for all analytes is 8 g. In addition, a sample intake of 8 g was used in the study for the homogeneity assessment giving an acceptable repeatability and demonstrating that the within-unit inhomogeneity does not longer contribute to the analytical variation at this sample intake.

Table 4: Results of the minimum sample intake determination

Parameter	Mass [g]
BDE-28, -99	5
BDE-47	8
BDE-100, -153, -154, -183	2

5 Stability

Time, temperature, light (including ultraviolet radiation) and microbial growth were regarded as the most relevant influences on the stability of the material. The influence of ultraviolet or visible light was minimised by packing the material's jars in pouches which prevent light exposure (see **Figure 2**). Materials are stored in the dark and dispatched in boxes, thus removing any possibility of degradation by light. Additionally the material was sterilised by heat treatment (in an autoclave) to eliminate microbial growth. Therefore, only the influences of time and temperature needed to be investigated.

Stability testing is necessary to establish the conditions for storage (long-term stability) as well as the conditions for dispatch of the material to the customers (short-term stability). During transport, especially in summer time, temperatures up to 60 °C can 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 [21]. 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. 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 at 4 °C. Two units per storage time were selected using a random stratified sampling scheme. From each unit, three samples were measured by GC-HRMS. The sample preparations were carried out split over 2 days, while the measurements were performed within a single randomised sequence, used to differentiate any potential analytical drift from a trend over storage time. Day-to-day effects were looked at, given the non-repeatability conditions of the measurement results. When a significant difference between the day means was spotted (by the F-test in one-way ANOVA), normalisation of the data was carried out to detect the presence of trends and outliers. This was the case for the datasets of BDE-154 at 18 °C and at 60 °C.

At the time of the study, the BDE-49 was not included as a target analyte, therefore no data are available.

The 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 %. One outlying individual result was found for BDE-99 and BDE-183 each (**Table 5**). As no technical reason for the outliers could be found, all data were retained for statistical analysis.

In addition, the data were evaluated against storage time, and regression lines of mass fraction versus time were calculated, to test for potential increase/decrease of the PBDEs mass fraction due to shipping conditions. The slope of the regression lines was tested for statistical significance. None of the trends was statistically significant at a 95 % confidence level at 18 °C. On the other hand, a positive trend, statistically significant at a 95 % confidence level, was observed for BDE-154 at 60 °C. As the analyte cannot be created in the sample, a positive trend could only be due to degradation of the matrix. This, however, should be seen for all PBDEs, which is not the case. The observed trend was therefore regarded as statistical artefact.

The results of the measurements are shown 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*		Significance of the trend **	
	18 ºC	60 ºC	18 ºC	60 ºC
BDE-28	none	none	no	no
BDE-47	none	none	no	no
BDE-99	1	none	no	no
BDE-100	none	none	no	no
BDE-153	none	none	no	no
BDE-154	none	none	no	yes
BDE-183	1	none	no	no

* 99 % confidence level
** 95 % confidence level

One statistical outlier was detected for BDE-99 and BDE-183 each, and these outliers were retained for the estimation of u_{sts}. None of the trends was statistically significant on a 95 % confidence level at 18 °C. Even though the trend observed for BDE-154 was regarded as a

statistical artefact, a conservative approach was chosen and the material shall be shipped under cooled conditions.

5.2 Repeated use

During the minimum sample intake study, three units of ERM-CE102 were analysed in triplicate during two consecutive days. After opening and taking the aliquots on the first day, the units were immediately re-closed with the lid, stored at 4 °C in the dark overnight and re-analysed the following day. A one-way ANOVA statistical analysis performed on the results for BDE-28, -47, -99, -100, -153, -154, and -183 did not detect any significant difference between the mean of day 1 and the mean of day 2. Therefore it can be concluded that the certified values of ERM-CE102 are still valid within 1 day from the opening of a CRM unit, provided it is carefully closed and stored at 4 °C in the dark. The results of the characterisation study, in which replicates from the same CRM unit had to be analysed over 2 days (i.e. intermediate precision conditions), also confirm this conclusion.

5.3 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 for the 4 °C study and at 4 °C for the 18 °C study, respectively. Two units per storage time were selected using a random stratified sampling scheme. From each unit, three samples were measured by GC-HRMS. The sample preparations were carried out split over 4 days, while the measurements were performed within a single randomised sequence, to be able to separate any potential analytical drift from a trend over storage time. Day-to-day effects were looked at, given the non-repeatability conditions of the measurements results. When a significant difference between the day means was spotted (by the F-test in one-way ANOVA), normalisation of the data was carried out to detect the presence of trends and outliers. This was the case for the dataset of BDE-154 at 4 °C and BDE-28, -47, -49 and -183 at 18 °C.

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 %. Outlying individual results were found for BDE-28, -99, -100, -153 and -154, while outlier unit means were found for BDE-49 and BDE-183 (**Table 6**). As no technical reasons for the outliers could be found, all data were retained for statistical analysis.

In addition, 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 at 4 °C, while at 18 °C a significant trend was detected for BDE-49 and BDE-183.

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**.

The trend observed at 18 °C for BDE-49 and BDE-183 was positive. As the analyte cannot be created in the sample, a positive trend could only be due to 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. Anyway, the material appeared to be stable at 4 °C and it was decided to store it at this temperature.

Table 6: Results of the long-term stability tests

Parameter	Number of individual outlying results*		Significance of the trend**	
	4 ºC	18 ºC	4 ºC	18 ºC
BDE-28	1	none	no	no
BDE-47	none	none	no	no
BDE-49	1 (unit mean)	none	no	yes
BDE-99	2	1	no	no
BDE-100	none	1	no	no
BDE-153	1	none	no	no
BDE-154	1	none	no	no
BDE-183	2 (unit means)	none	no	yes

* 99 % confidence level
** 95 % confidence level

5.4 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 or intermediate precision, 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 [22] for each PBDE congener. 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:

$$u_{sts,rel} = \frac{s_{rel}}{\sqrt{\sum\left(t_i - \bar{t}\right)^2}} \cdot t_{tt} \qquad \textbf{Equation 7}$$

$$u_{lts,rel} = \frac{s_{rel}}{\sqrt{\sum\left(t_i - \bar{t}\right)^2}} \cdot t_{sl} \qquad \textbf{Equation 8}$$

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 t_i

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

t_{sl} chosen shelf life (24 months at 4 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 for one week.
- $u_{lts,rel}$, the stability during storage. This uncertainty contribution was estimated from the 4 °C studies. The uncertainty contribution describes the possible degradation during 24 months storage at 4 °C.

The results of these evaluations are summarised in **Table 7**.

The material showed no significant degradation for transport below 18 °C. Cooled shipment is therefore necessary.

The material can be stored at 4 °C.

After the certification study, ERM-CE102 will be included in the JRC's regular stability monitoring programme, to control its further stability.

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 4 °C and 24 months.

Parameter	$u_{sts,rel}$ [%]	$u_{lts,rel}$ [%]
BDE28	0.6	3.8
BDE47	0.2	2.8
BDE49	not available	5.3
BDE99	0.3	4.1
BDE100	0.7	2.7
BDE153	0.3	4.2
BDE154	0.3	2.5
BDE183	0.7	4.0

6 Characterisation

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

This 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. Due to the nature of the analytes however, all participants used methods based on gas chromatography coupled to mass spectrometry for the measurements. This approach aims at randomisation of laboratory bias, which reduces the combined uncertainty.

6.1 Selection of participants

Twelve 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

measurement of PBDEs in biota matrices by submitting results of attended intercomparison exercises and/or method validation reports. 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 or three units of ERM-CE102 and was requested to provide six independent results. Accordingly, and depending on the sample intake, three or two replicates per unit were performed. The units for material characterisation were selected using a random stratified sampling scheme and covered the whole batch. The sample preparations and measurements had to be spread over at least two days to ensure intermediate precision conditions. Fresh calibration solutions had to be prepared for each day of measurement. Results had to be reported relative to wet weight.

Each participant received a sample of NIST SRM 1946 Lake Superior Fish Tissue as a blinded method quality control (MQC) sample. The results for the MQC 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 methods [accelerated solvent extraction (ASE), Soxhlet, organic solvent(s) extraction] and clean-up [multilayer (acidic, basic and neutral) silica gel, carbon and alumina columns, liquid-liquid extraction (LLE), gel permeation chromatography (GPC) and a combination thereof] with different quantification steps [GC-MS both in electron ionisation (EI) and electron capture negative ionisation (ECNI) modes, GC-HRMS and GC-MS/MS in EI mode] were used to characterise the material. The combination of results from methods based on completely different principles mitigates undetected method bias.

All methods used during the characterisation study are summarised in Annex E, Table E1. 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. L00) and abbreviation of the measurement method used (e.g. GC-MS).

6.4 Evaluation of results

The characterisation study resulted in fourteen datasets for PBDEs 28, 47, 99, 100, 153, 154 and 183 and twelve datasets for BDE-49 (L00 and L01 did not analyse this congener), respectively. All individual results of the participants, grouped per analyte are displayed in tabular and graphical form in Annex F, Table F1-F8 and Figure F1-F8.

6.4.1 Technical evaluation

The obtained data 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

- scrutiny and assessment of values given as below limit of detection or below limit of quantification
- method performance:

  agreement of the measurement results with the assigned values of the MQC sample (corresponding to certified and reference values for the relevant PBDEs in NIST SRM 1946) applying the ERM Application Note 1 [23],

  N.B.: in SRM 1946, a certified value is assigned to the sum of BDE-28 and BDE-33. While the laboratories were requested to report a value for BDE-28 alone, most of them used a chromatographic column which is known not to be able to separate these two congeners. Therefore, the evaluation was carried out considering the sum of BDE-28+BDE-33.

  coherence between method repeatability values as provided by the laboratory *a priori* (based on method validation data) and extrapolated from the characterisation measurement dataset.

The presence of BDE-33 in ERM-CE102 was checked and found to be about the LOD level, so its level could only be tentatively indicated as possibly being between 10 and 15 % of BDE-28, which is present already at a very low level. Another laboratory analysed ERM-CE102 twice, first with a column not able to separate BDE-28 from BDE-33 and then with a column able to separate these two congeners: the results were not significantly different. Considering the above, it was decided to assign an indicative value to BDE-28 in ERM-CE102.

Based on the above criteria, the following datasets were rejected as not technically valid (**Table 8**). The datasets L06 and L07 correspond to a newly measured set of results, as interference on the signal of the internal standard used for quantifying BDE-47, -49, -99, -100, -153, -154 and -183 in the first submitted set of results was discovered.

Table 8: Datasets that showed non-compliances and action taken

Analyte	Lab code	Description of problem	Action taken
BDE-28 (+BDE-33)	L02, L08, L09, L10, L13 L05 L01 L03, L07	no agreement with MQC interference reported by the lab "less than" values reported for MQC "less than" values reported for ERM-CE102	not used for statistical evaluation
BDE-47	L02, L03, L04, L05, L06, L07	no agreement with MQC	not used for statistical evaluation
BDE-49	L02, L05, L06, L07 L03	no agreement with MQC "less than" values reported for ERM-CE102	not used for statistical evaluation
BDE-99	L03, L06, L07	no agreement with MQC	not used for statistical evaluation
BDE-100	L02, L03, L06, L07	no agreement with MQC	not used for statistical evaluation
BDE-153	L02, L03, L06, L07	no agreement with MQC	not used for statistical evaluation
BDE-154	L02, L03, L05, L06, L07	no agreement with MQC	not used for statistical evaluation
BDE-183	L00, L01, L02, L06, L08, L09, L10, L11 L03 L07	no agreement with MQC "less than" values reported for MQC and ERM-CE102 "less than" values reported for ERM-CE102	not used for statistical evaluation

6.4.2 Statistical evaluation

The datasets accepted based on technical reasons were tested for normality of dataset means using kurtosis/skewness tests 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 9**.

The laboratory means follow normal distributions. None of the data contains outlying means. The statistical evaluation flags laboratory L01 as outlying variance for BDE-47, -99, -100 and -153; laboratory L02 as outlying variance for BDE-99; laboratory L10 as outlying variance for BDE-49 and Laboratory L12 as outlying variance for BDE-28. This merely reflects the fact that different methods have different intrinsic variability. As all measurement methods were found technically sound, all results were retained.

Table 9: Statistical evaluation of the technically accepted datasets for ERM-CE102

p: number of technically valid datasets

Analyte	*p*	Outliers		Normally distributed	Statistical parameters			
		Means	Variances		Mean [µg/kg]	*s* [µg/kg]	$s_{between}$ [µg/kg]	s_{within} [µg/kg]
BDE-28	5	--	L12	insuff. data	0.00774	0.00083	0.00079	0.00061
BDE-47	8	--	L01	yes	0.2272	0.0199	0.0195	0.0101
BDE-49	7	--	L10	yes	0.0327	0.0037	0.0035	0.0028
BDE-99	11	--	L01, L02	yes	0.1234	0.0135	0.0124	0.0136
BDE-100	10	--	L01	yes	0.0600	0.0068	0.0065	0.0044
BDE-153	10	--	L01	yes	0.0692	0.0055	0.0053	0.0041
BDE-154	9	--	--	yes	0.1094	0.0085	0.0083	0.0043
BDE-183	4	--	--	insuff. data	0.0136	0.0024	0.0025	0.0014

The datasets are consistent and the mean of laboratory means is a good estimate of the true value.
The methods used in the characterisation are methods routinely applied for measuring PBDEs in biota matrices (i.e. fish). The agreement of results from different methods demonstrates that the processing did not affect any properties relevant for these methods and that ERM-CE102 behaves like a real sample.

The uncertainty related to the characterisation is estimated as the standard error of the mean of laboratory means (**Table 10**).

Table 10: Uncertainty of characterisation for ERM-CE102

Analyte	*p*	Mean [µg/kg]	*s* [µg/kg]	u_{char} [µg/kg]
BDE-28	5	0.00774	0.00083	0.00037
BDE-47	8	0.2272	0.0199	0.007
BDE-49	7	0.0327	0.0037	0.0014
BDE-99	11	0.1234	0.0135	0.0040
BDE-100	10	0.0600	0.0068	0.0021
BDE-153	10	0.0692	0.0055	0.0017
BDE-154	9	0.1094	0.0085	0.0029
BDE-183	4	0.0136	0.0024	0.0012

7 Value Assignment

Certified and indicative values were assigned.

Certified values are values that fulfil the highest standards of accuracy. Procedures at the JRC, Directorate F 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.

Indicative values are values where either the uncertainty is deemed too large or where too few independent datasets were available to allow certification. Uncertainties are evaluated according to the same rules as for certified values.

7.1 Certified values and their uncertainties

The unweighted mean of the means of the accepted datasets as shown in **Table 9** was assigned as certified value for each parameter.

The assigned uncertainty consists of uncertainties relating 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). The uncertainty related to degradation during transport was found to be negligible for all PBDEs investigated, therefore it was assumed the u_{sts} to be negligible also for BDE-49 (for which no short-term stability study is available). u_{sts} was accordingly not taken into account in the estimation of the certified uncertainty. These different contributions were combined to estimate the relative expanded uncertainty of the certified value ($U_{CRM, rel}$) with a coverage factor k given as:

$$U_{CRM,rel} = \mathbf{k} \cdot \sqrt{u^2_{bb,rel} + u^2_{lts,rel} + u^2_{char,rel}} \qquad \textbf{Equation 9}$$

- u_{char} was estimated as described in Section 6
- u_{bb} was estimated as described in Section 4.1.
- u_{lts} was 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 of 2 was applied to all PBDEs except BDE-183, to obtain the expanded uncertainties. The effective number of degrees of freedom was calculated for BDE-28 and BDE-183 using the Welch-Sattertwaithe equation [1], because of the low number of datasets accepted for the characterisation, i.e. 5 and 4, respectively. The number of degrees of freedom was found to be 10 for BDE-28, thus still sufficiently high to apply a coverage factor k of 2, but only 5 for BDE-183. Therefore, a coverage factor of 2.571 (corresponding to 5 degrees of freedom) was applied for BDE-183 to obtain the expanded uncertainty.

The certified values and their uncertainties are summarised in **Table 11**.

Table 11: Certified values and their uncertainties for ERM-CE102

	Certified value[1)] [µg/kg]	$u_{char,rel}$ [%]	$u_{bb,rel}$ [%]	$u_{lts,rel}$ [%]	$U_{CRM,rel}$ [2)] [%]	U_{CRM} [2)] [µg/kg]
BDE-47	0.227	3.1	0.2	2.8	8.4	0.019
BDE-49	0.033	4.3	7.5	5.3	20.2	0.007
BDE-99	0.123	3.3	0.5	4.1	10.6	0.013
BDE-100	0.060	3.6	0.6	2.7	9.0	0.006
BDE-153	0.069	2.5	2.6	4.2	11.1	0.008
BDE-154	0.109	2.6	0.6	2.5	7.3	0.008

1) expressed on wet weight basis
2) expanded (k = 2) and rounded uncertainty

7.2 Indicative values and their uncertainties

Indicative values were assigned for BDE-28 and BDE-183. Only five and four datasets were accepted, respectively, for these congeners after the technical scrutiny. However, the results are obtained by different sample preparations and/or detection methods, and were regarded as sufficiently trustworthy to assign indicative values. An indicative value may not be used as a certified value. The uncertainty budgets were set up as for the certified values and are listed together with the assigned values in **Table 12**.

Table 12: Indicative values and their uncertainties for ERM-CE102

Analyte	Indicative value[1)] [µg/kg]	$u_{char,\ rel}$ [%]	$u_{bb,\ rel}$ [%]	$u_{lts,\ rel}$ [%]	$U_{CRM,rel}$ [2)] [%]	U_{CRM} [2)] [µg/kg]
BDE-28	0.0077	4.8	2.1	3.8	12.9	0.0010
BDE-183	0.014	8.8	1.2	4.0	25.1	0.004

1) expressed on wet weight basis
2) expanded (k = 2 for BDE-28 and k = 2.571 for BDE-183) and rounded uncertainty.

7.3 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.

An additional material information value was assigned for the fat content of ERM-CE102. Two units were randomly selected over the whole batch and from each unit two aliquots of 1 g were analysed in-house according to the following procedure [24]: after ASE, the extract was concentrated under a gentle stream of nitrogen, placed in the oven for 3 h at 60 °C and finally dried at 105 °C until constant mass was reached. The extractable fat content of ERM-CE102, expressed as the mean of three replicates and given as mass fraction % (equivalent to g/100g) relative to wet weight was determined as 6.9 %.

8 Metrological traceability and commutability

8.1 Metrological traceability

Identity

PBDEs 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 absence of measurement bias. Nevertheless, since all participants used methods based on gas chromatography and mass spectrometry for their determination, the measurands are operationally defined as obtained by gas chromatography coupled to mass spectrometry.

Quantity value

Only validated methods were used for the determination of the assigned values. Different calibrants and 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 SI, as it 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 International System of Units (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 select specific (or specific groups of) analytes from the sample for the subsequent 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 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 that define this concept. For instance, the CLSI Guideline C53-A [25] 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 is therefore a crucial characteristic when applying different measurement methods. When the commutability of a CRM is not established, 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-CE102 was produced from naturally contaminated wild fish and farmed blank fish by cryogenic milling, mixing and cooking to produce a sterilised paste, with the goal of enhancing the commutability of the material by avoiding any freeze drying process. The analytical behaviour is expected to be the same as for routine biota samples as also confirmed by the agreement of results from the different analytical methods used in the characterisation, validated for analysing PBDEs in biota matrices.

9 Instructions for use

9.1 Safety information

The usual laboratory safety measures apply.

9.2 Storage conditions

The materials should be stored at 4 ± 3 °C in the dark.

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 for opened units.

Nevertheless, the repeated use study (Section 5.2) results indicate that the certified values of ERM-CE102 are still valid within 1 day from the opening of a CRM unit, if the jar is immediately closed and stored at 4 °C in the dark.

9.3 Preparation and use of the material

Before analysis, ERM-CE102 units should be left to equilibrate to room temperature. To make it ready for use and before taking aliquots, the material must be manually and thoroughly re-homogenised with the help of a spatula. In case that small quantities of material are stuck to the lid, it is advisable not to include them.

9.4 Minimum sample intake

The minimum sample intake representative for all PBDEs is 8 g (wet weight).

9.5 Use of the certified value

The main purpose of this material is to assess method performance, i.e. for checking accuracy of analytical results.

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 [23].

When assessing the method performance, the measured values of the CRMs are compared with the certified values. The procedure is summarised here:

- Calculate the absolute difference between mean measured value and the certified value (Δ_{meas}).
- Combine the 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 $\Delta_{meas} \leq U_{\Delta}$ then no significant difference exists between the measurement result and the certified value, at a confidence level of approximately 95 %.

Use in quality control charts

The materials can be used for quality control charts. Using CRMs for quality control charts has the added value that a trueness assessment is built into the chart.

10 Acknowledgments

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The authors would finally like to thank S. Choquette from NIST (USA) for the provision of SRM 1946 units.

11 Reference

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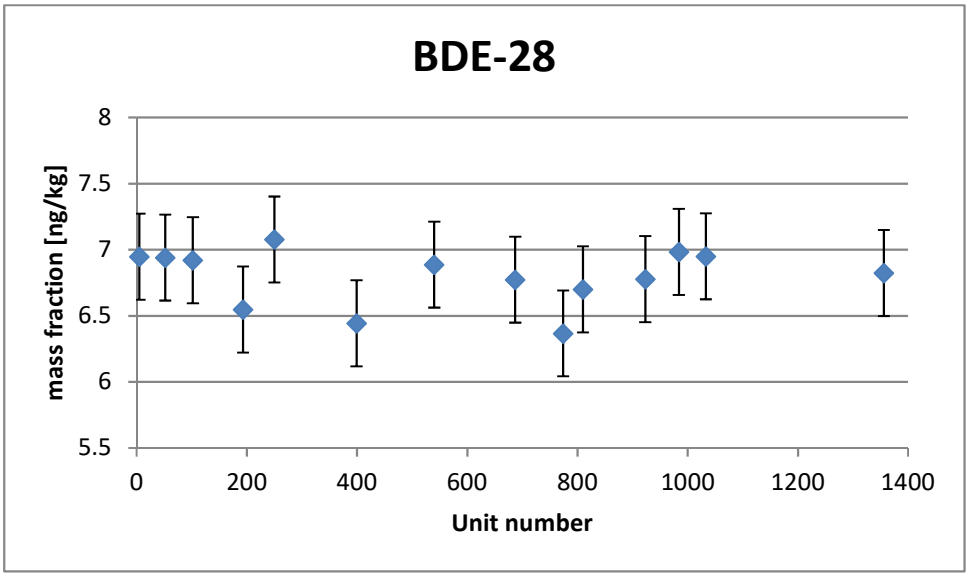
Annexes

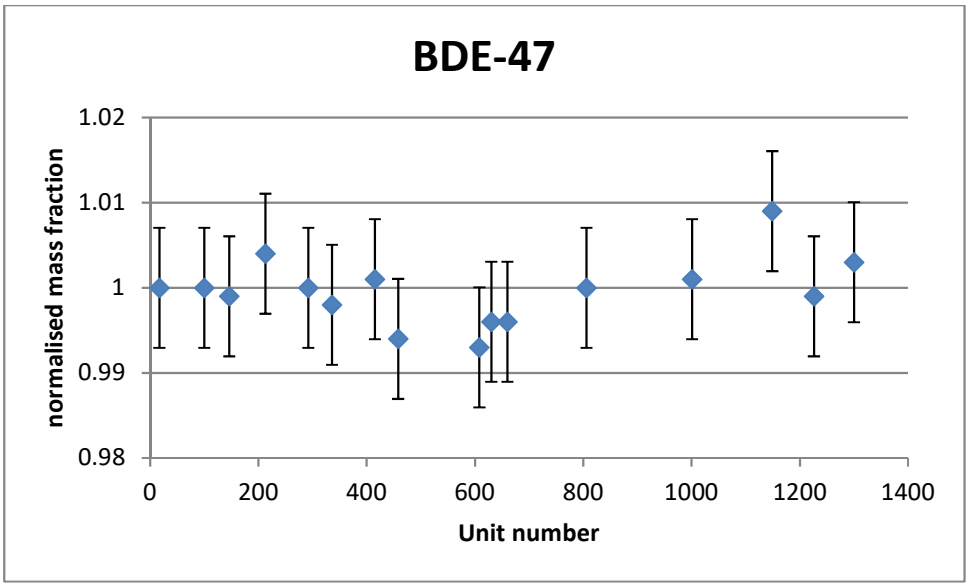
Annex A: Results of the homogeneity measurements

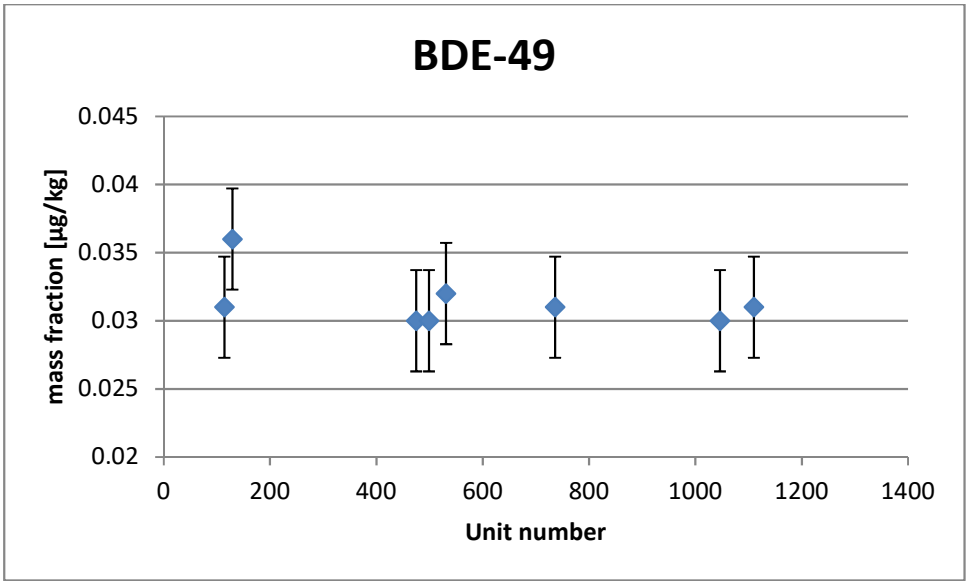
Analytical method applied for BDE-28, -47, -99, -100, -153, -154, -183 and -209: GC-HRMS after extraction with ethyl acetate, pre-cleaning by dispersive acidified silica and final clean-up by acidified silica column chromatography. Quantification by ^{13}C-labelled internal standards.

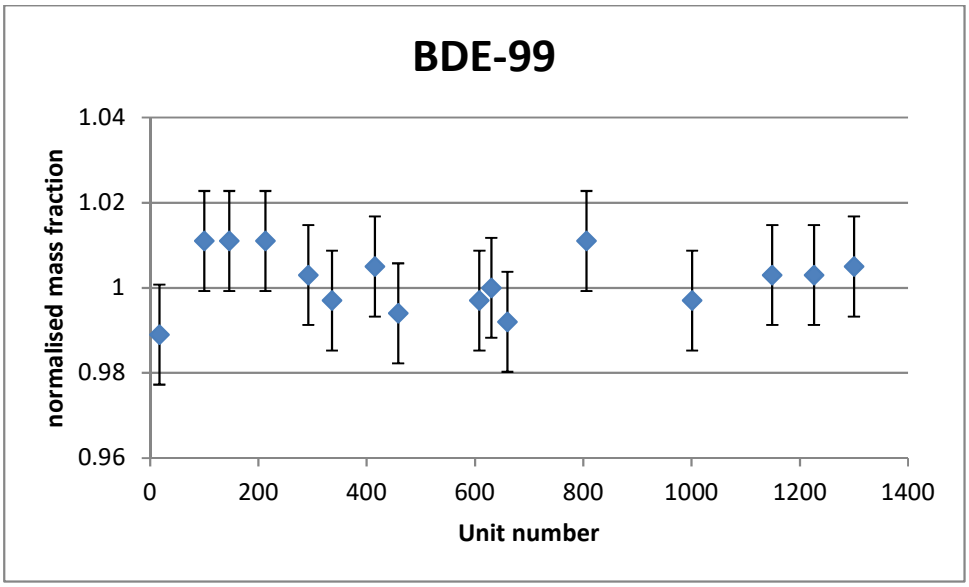
Analytical method applied for BDE-49: GC-HRMS after Soxhlet extraction and clean-up by an automatic system (silvernitrate silica, sulfuric acidic silica, carbon and alumina columns). Quantification by ^{13}C-labelled internal standards.

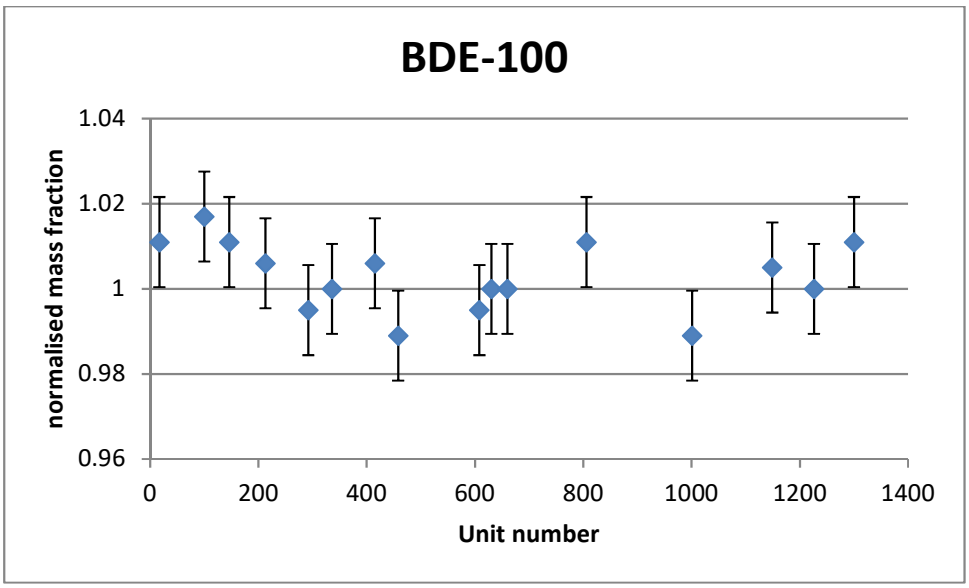
- The graphs report unit means ± confidence interval of the means (same CI calculated from s_{wb} from ANOVA for all units) expressed as (normalised) mass fraction.

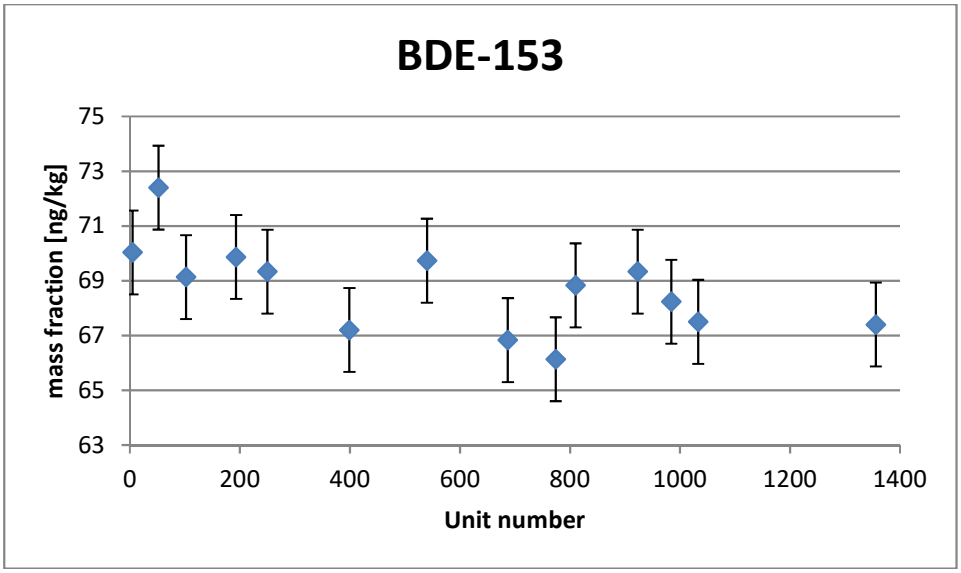


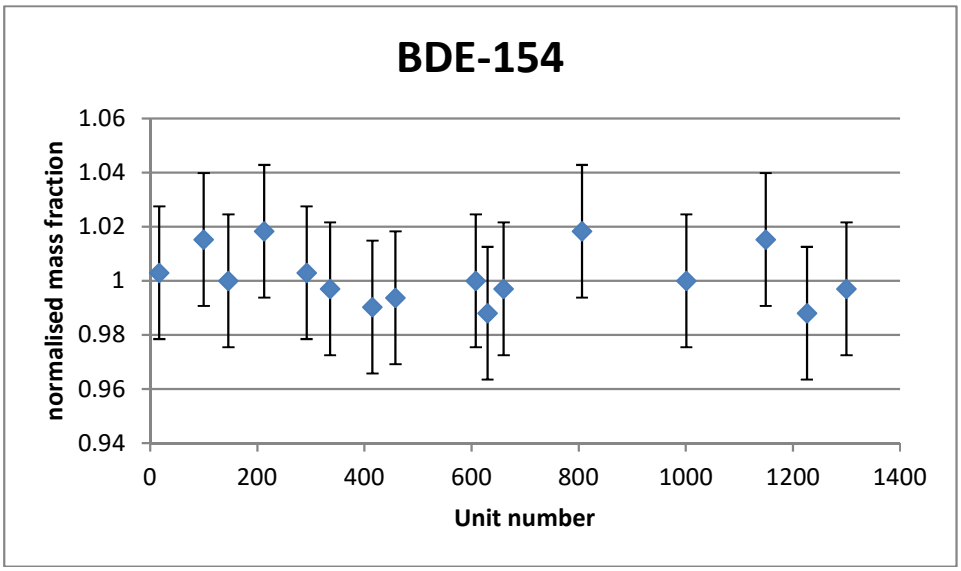


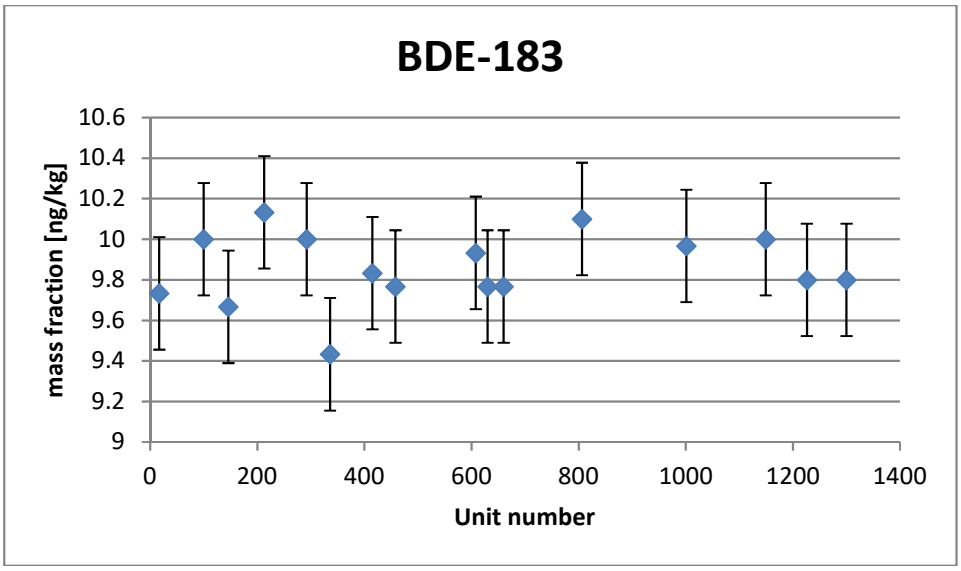

BDE-49
mass fraction [µg/kg]
0.045
0.04
0.035
0.03
0.025
0.02
0
200
400
600
800
1000
1200
1400
Unit number

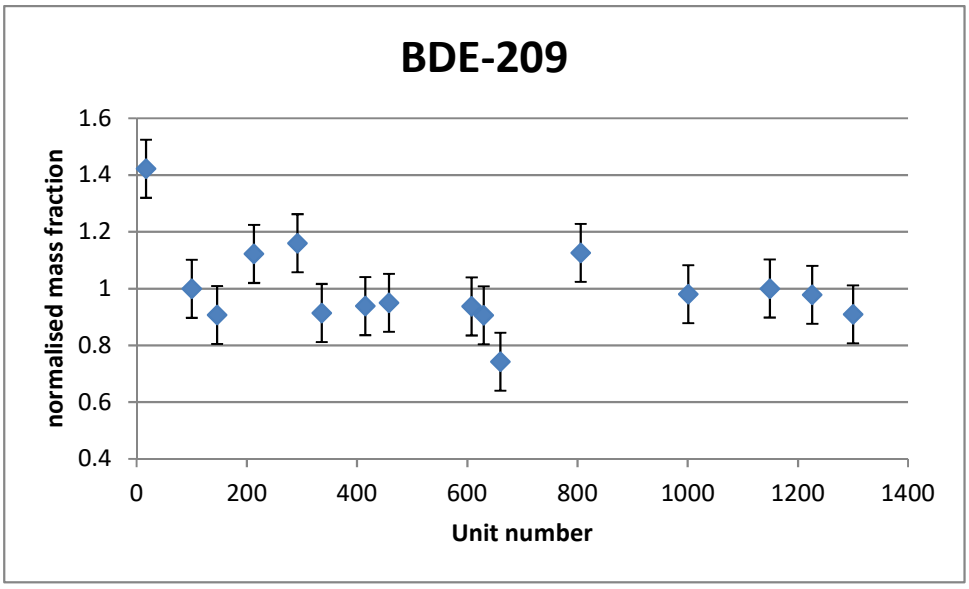

BDE-99
normalised mass fraction
1.04
1.02
1
0.98
0.96
0
200
400
600
800
1000
1200
1400
Unit number


BDE-100
normalised mass fraction
1.04
1.02
1
0.98
0.96
0
200
400
600
800
1000
1200
1400
Unit number


BDE-153
mass fraction [ng/kg]
75
73
71
69
67
65
63
0
200
400
600
800
1000
1200
1400
Unit number


BDE-154
normalised mass fraction
1.06
1.04
1.02
1
0.98
0.96
0.94
0
200
400
600
800
1000
1200
1400
Unit number


BDE-183
mass fraction [ng/kg]
10.6
10.4
10.2
10
9.8
9.6
9.4
9.2
9
0
200
400
600
800
1000
1200
1400
Unit number



N.B. the graph for BDE-209 is reported for information only, as it was decided not to assign any value for this congener in ERM-CE102

Annex B: Results of the minimum sample intake measurements

Analytical method applied: GC-ECNI-MS after mixing with distilled water and extraction with ethyl acetate and clean-up by silica column chromatography. Quantification by BDE-37, BDE-77 and ^{13}C-BDE-209 internal standards.

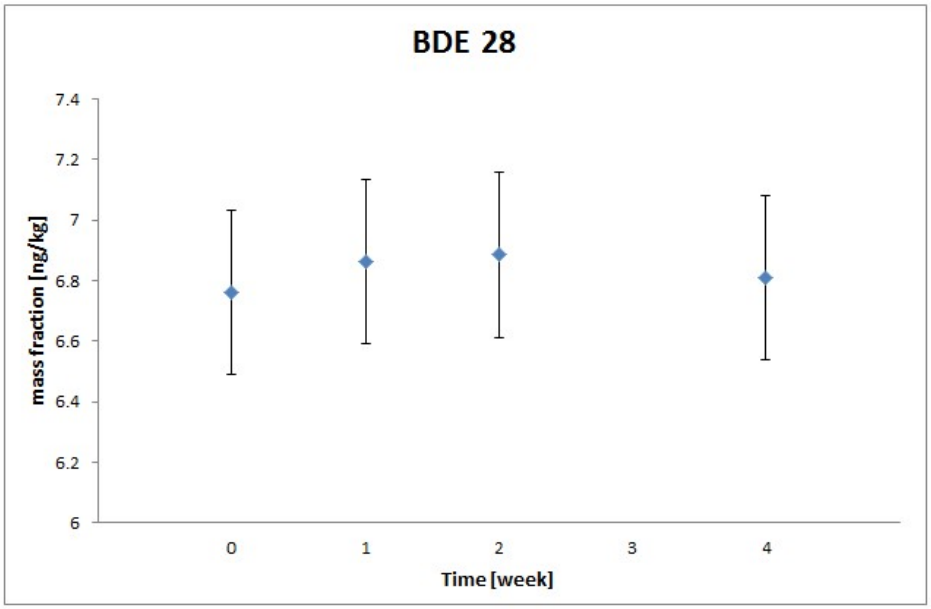
Table B1: Relative standard deviation (RSD) of measurement results for different sample intakes (9 independent replicates per sample intake)

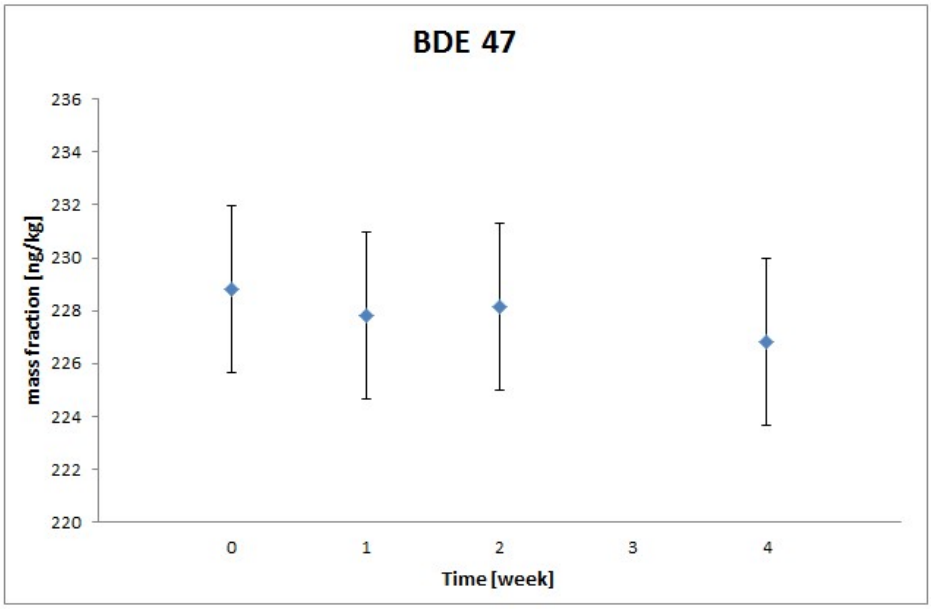
	RSD [%]		
	8 g	**5 g**	**2 g**
BDE-28	7.6	9.2	13.3
BDE-47	8.7	14.5	7.8
BDE-99	5.7	7.3	22.2
BDE-100	10.3	8.7	18.3
BDE-153	10.3	9.7	9.1
BDE-154	12.5	7.8	7.5
BDE-183	19.9	23.0	10.6

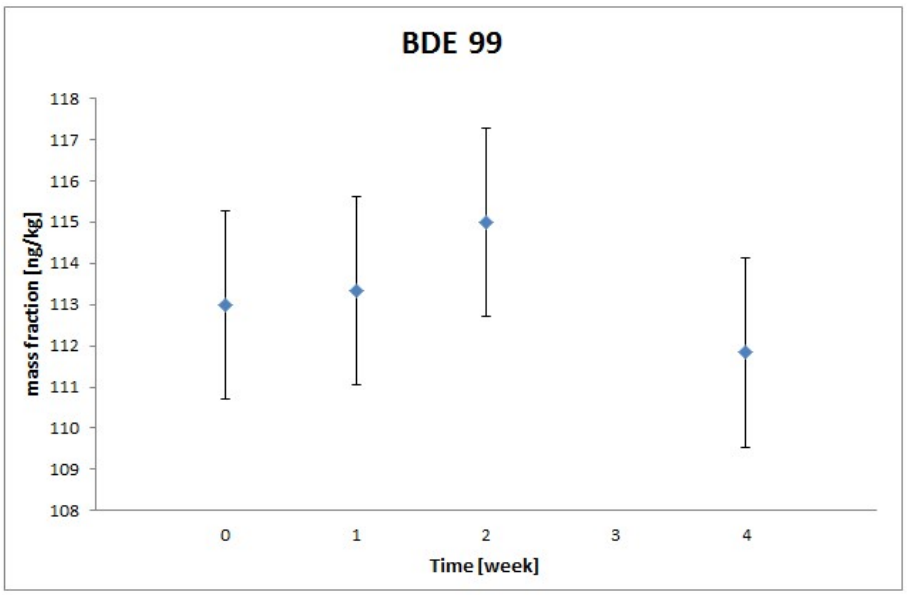
Annex C: Results of the short-term stability measurements

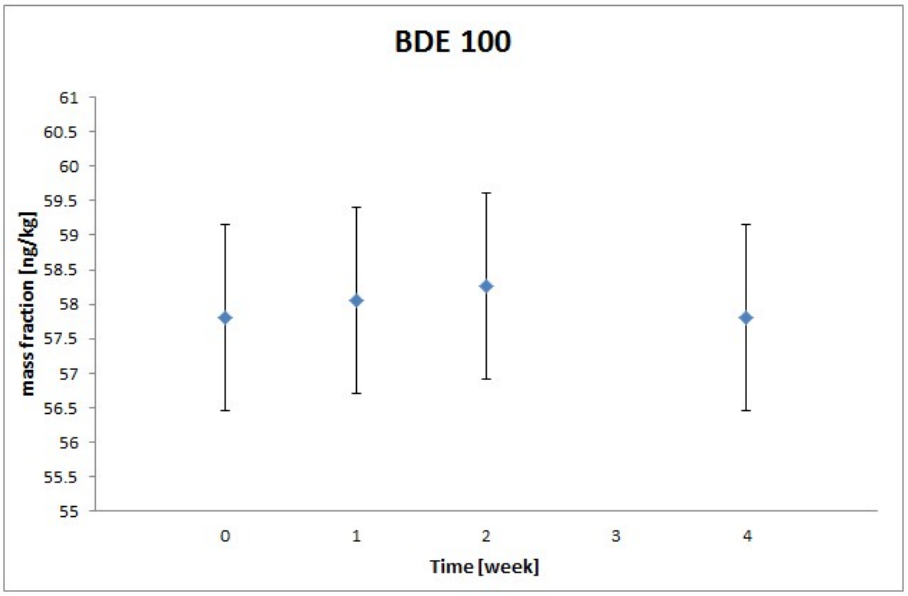
Analytical method applied: GC-HRMS after extraction with ethyl acetate, pre-cleaning by dispersive acidified silica and final clean-up by acidified silica column chromatography. Quantification by ^{13}C-labelled internal standards.

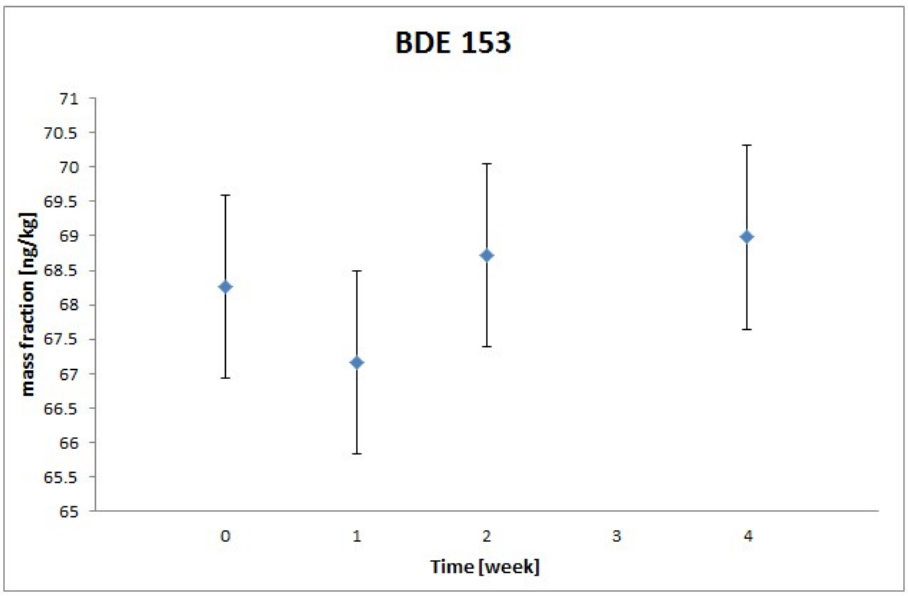
- Data of the short-term stability study at **18 °C**. The graphs report the means per time point ± confidence interval of the means (same CI calculated from ANOVA for all times) expressed as mass fraction.

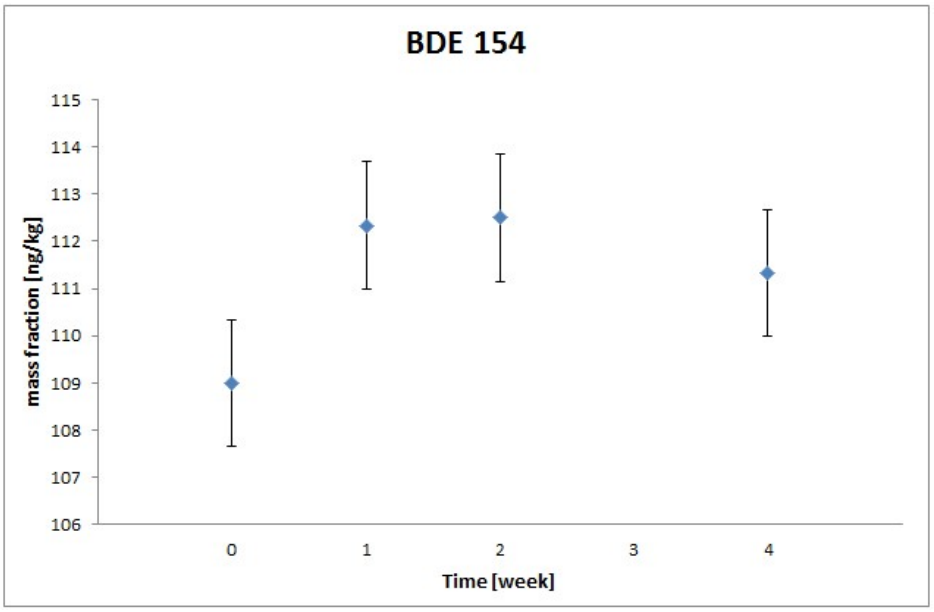


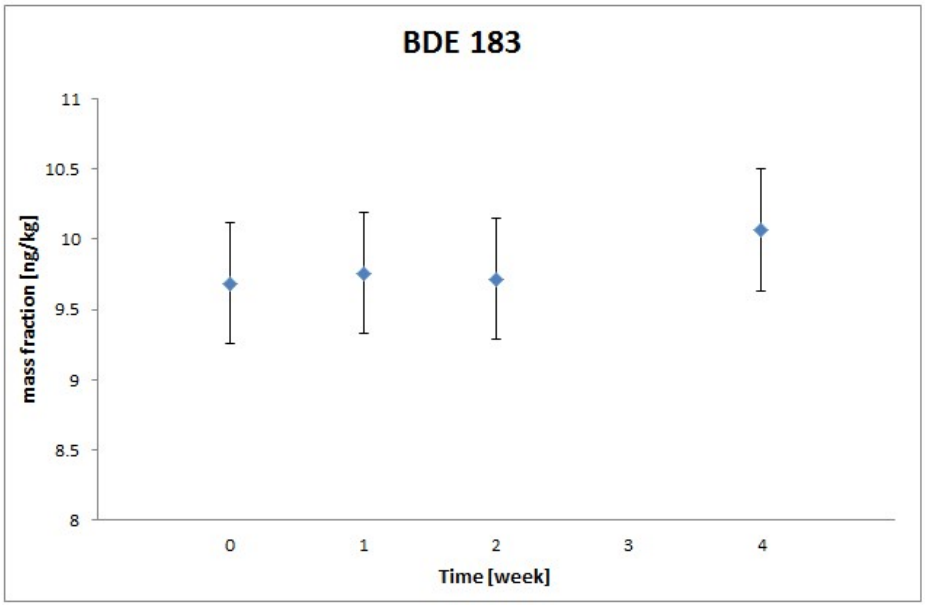



BDE 99
mass fraction [ng/kg]
118
117
116
115
114
113
112
111
110
109
108
0
1
2
3
4
Time [week]


BDE 100
mass fraction [ng/kg]
61
60.5
60
59.5
59
58.5
58
57.5
57
56.5
56
55.5
55
0
1
2
3
4
Time [week]

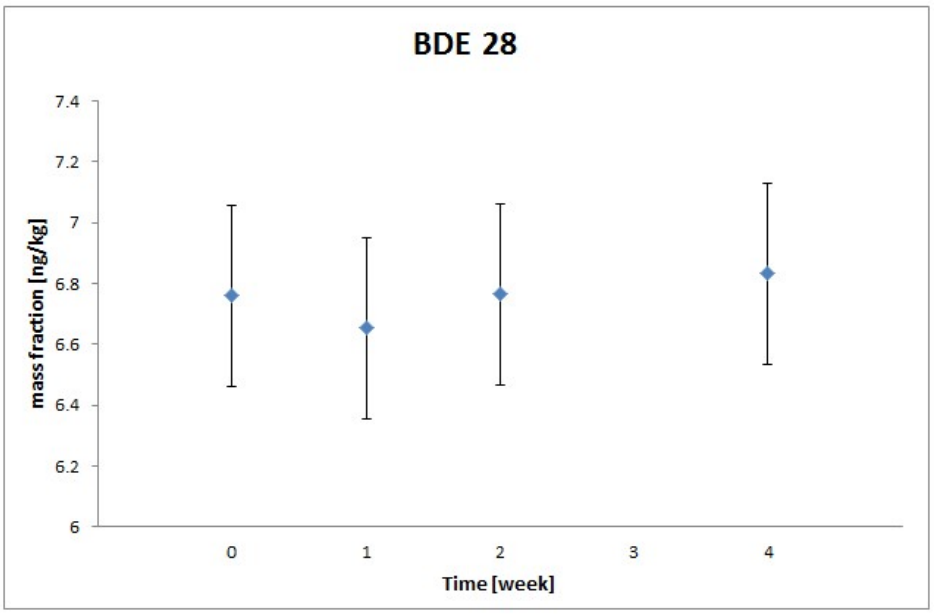

BDE 153
mass fraction [ng/kg]
71
70.5
70
69.5
69
68.5
68
67.5
67
66.5
66
65.5
65
0
1
2
3
4
Time [week]

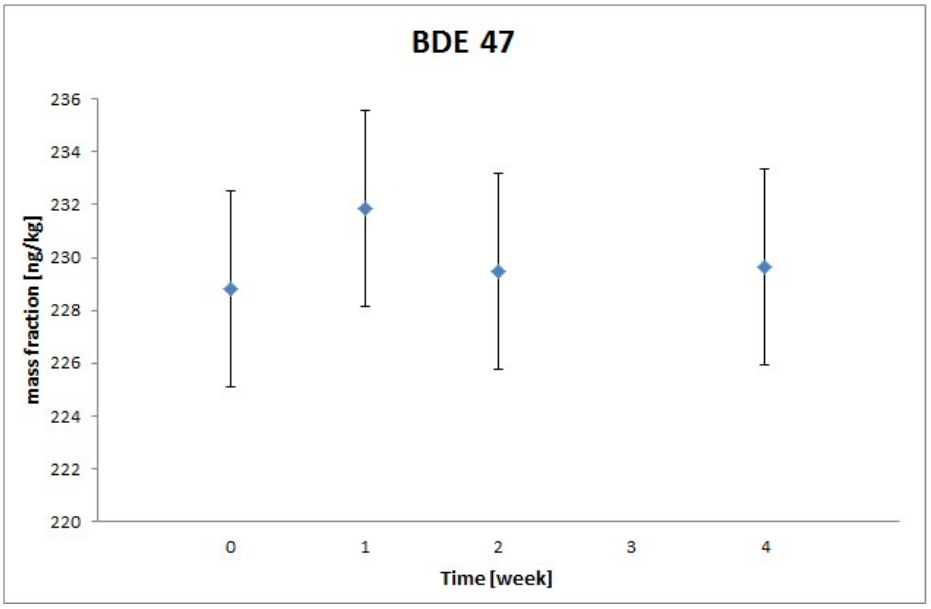

BDE 154
mass fraction [ng/kg]
115
114
113
112
111
110
109
108
107
106
0
1
2
3
4
Time [week]

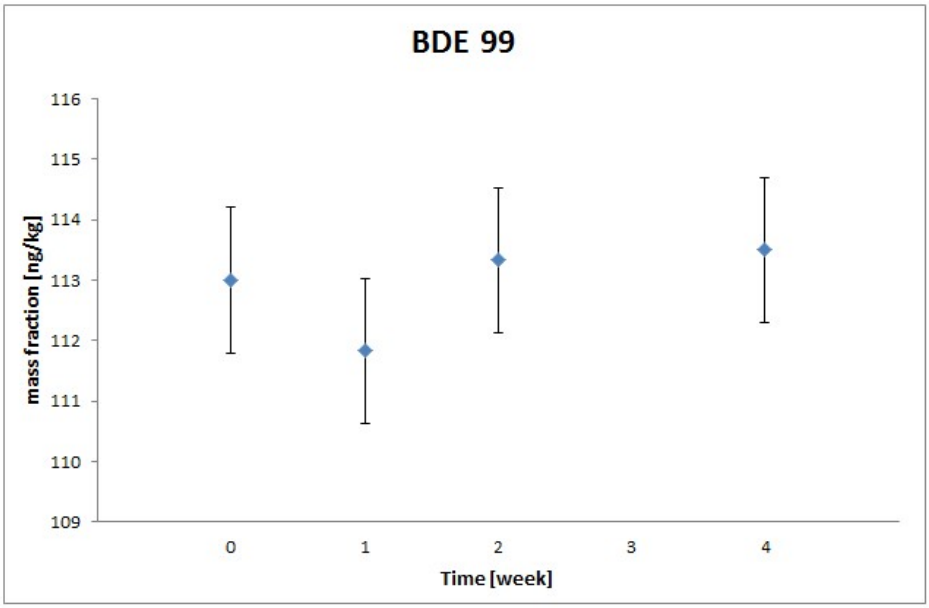

BDE 183
mass fraction [ng/kg]
11
10.5
10
9.5
9
8.5
8
0
1
2
3
4
Time [week]

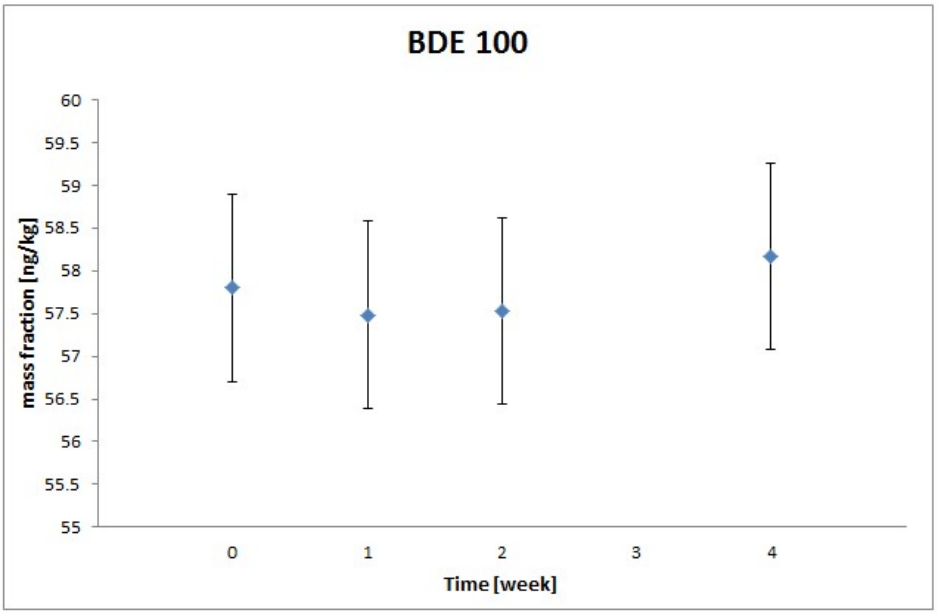
- Data of the short-term stability study at **60 °C**. The graphs report the means per time point ± confidence interval of the means (same CI calculated from ANOVA for all times) expressed as mass fraction.

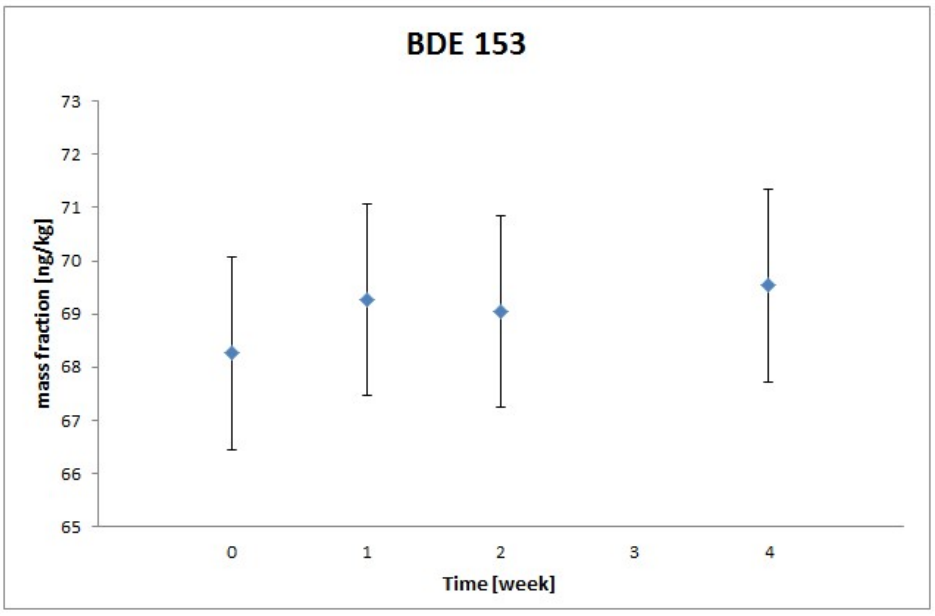
  The red line signalises a significant trend.

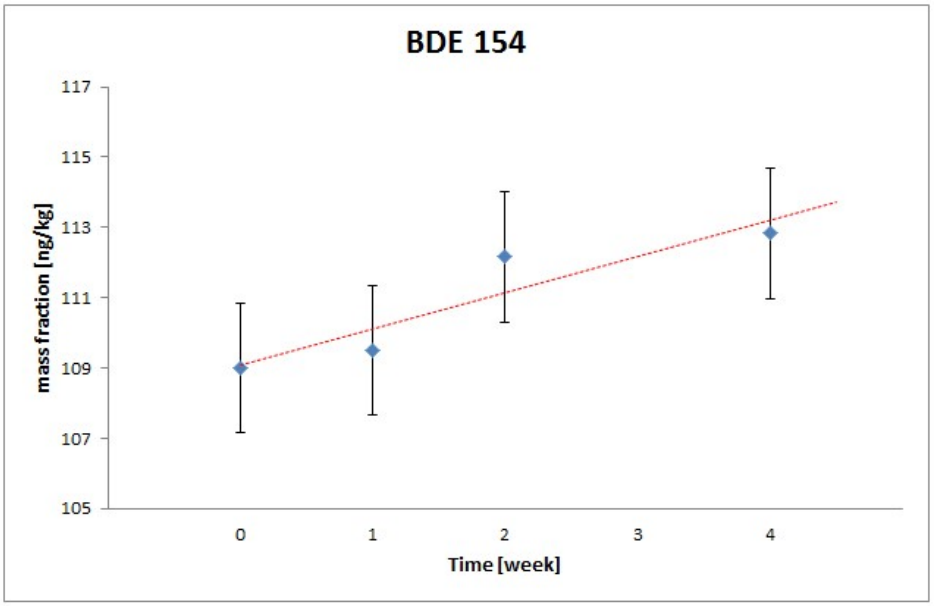


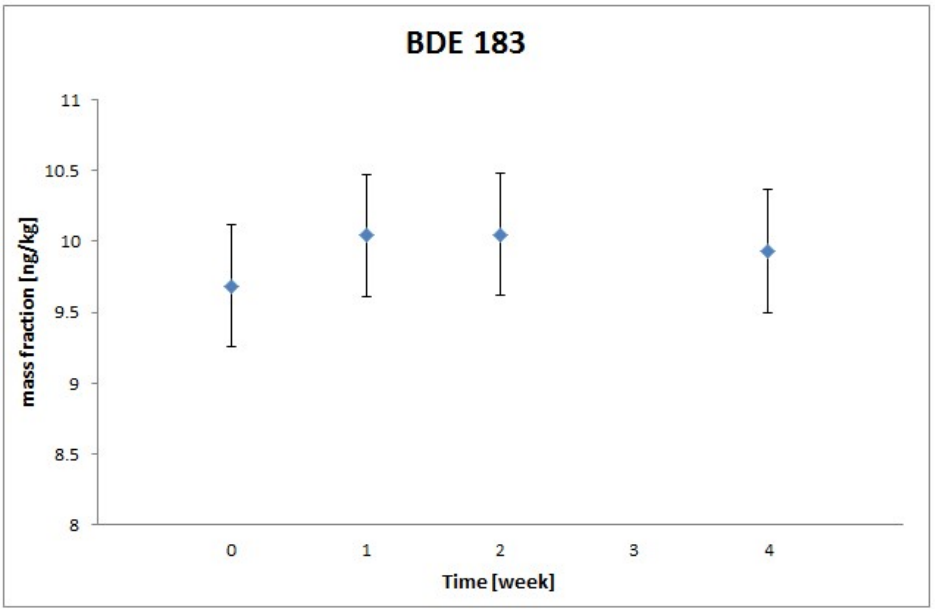



BDE 99
116
115
114
113
112
111
110
109
mass fraction [ng/kg]
0
1
2
3
4
Time [week]


BDE 100
60
59.5
59
58.5
58
57.5
57
56.5
56
55.5
55
mass fraction [ng/kg]
0
1
2
3
4
Time [week]


BDE 153
73
72
71
70
69
68
67
66
65
mass fraction [ng/kg]
0
1
2
3
4
Time [week]

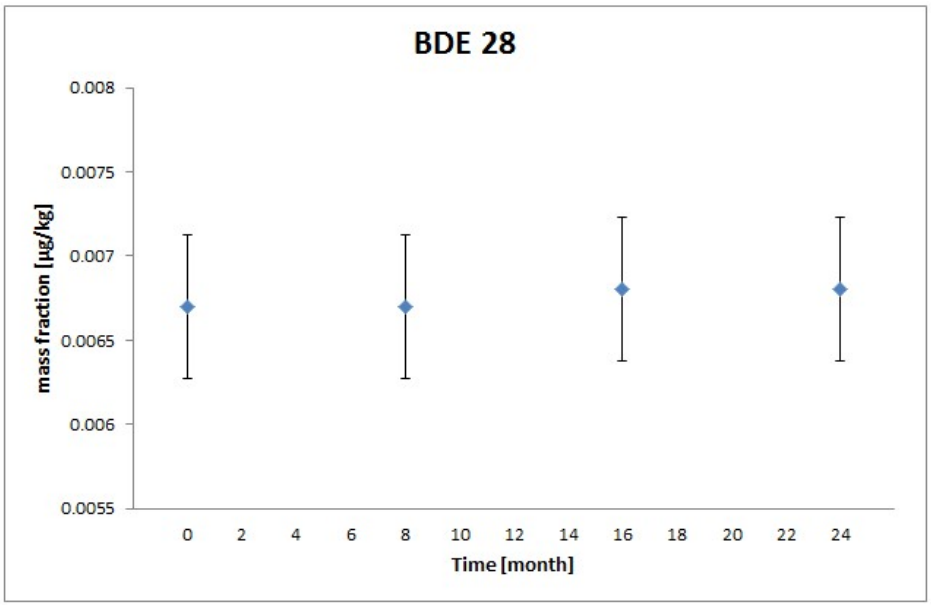

BDE 154
117
115
113
111
109
107
105
mass fraction [ng/kg]
0
1
2
3
4
Time [week]

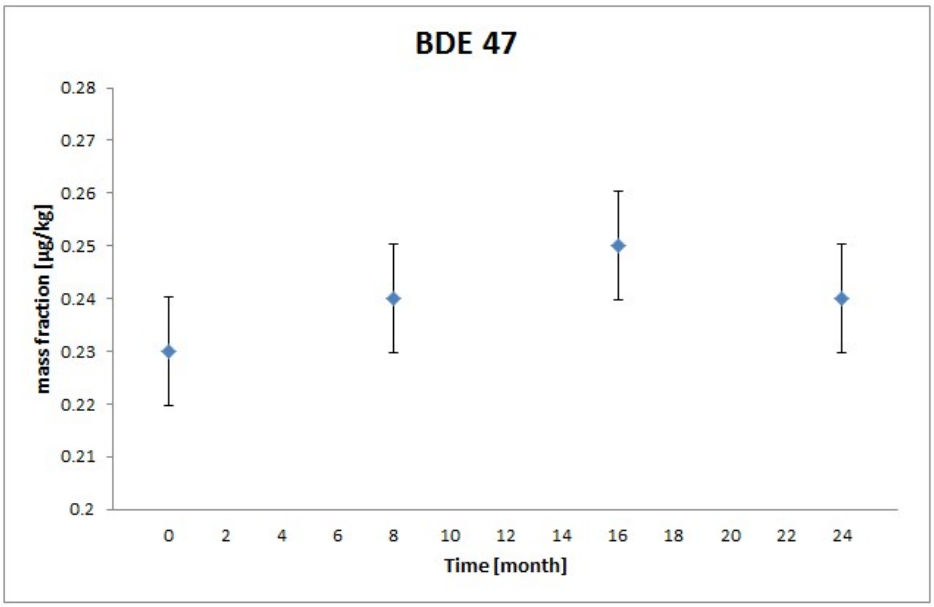

BDE 183
11
10.5
10
9.5
9
8.5
8
mass fraction [ng/kg]
0
1
2
3
4
Time [week]

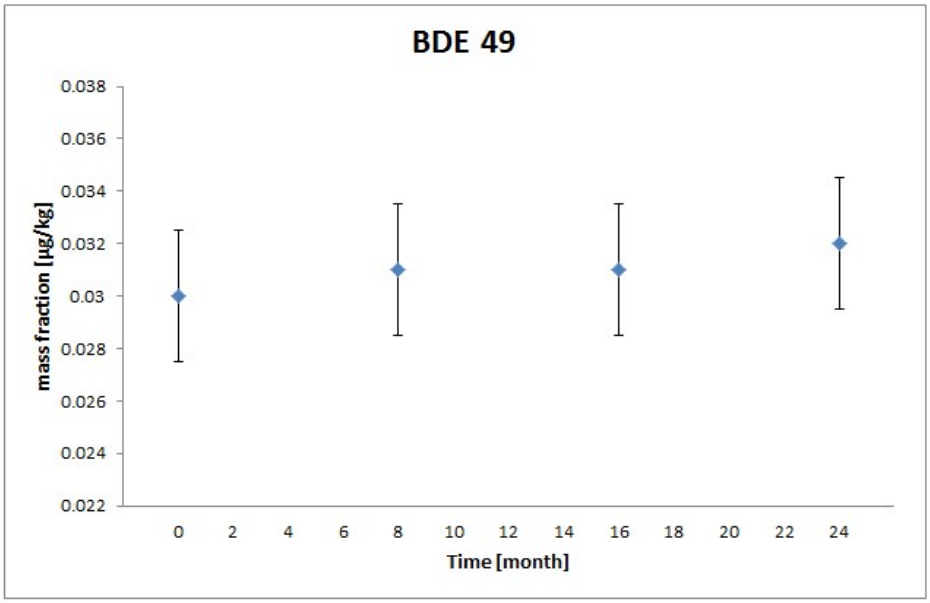
Annex D: Results of the long-term stability measurements

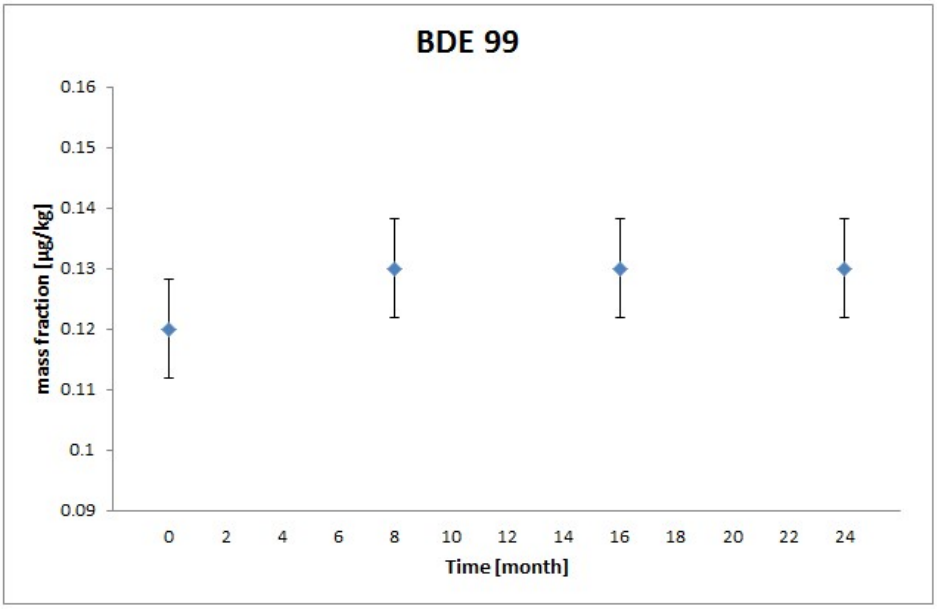
Analytical method applied: GC-HRMS after Soxhlet extraction and clean-up by an automatic system (silvernitrate silica, sulfuric acidic silica, carbon and alumina columns). Quantification by ^{13}C-labelled internal standards.

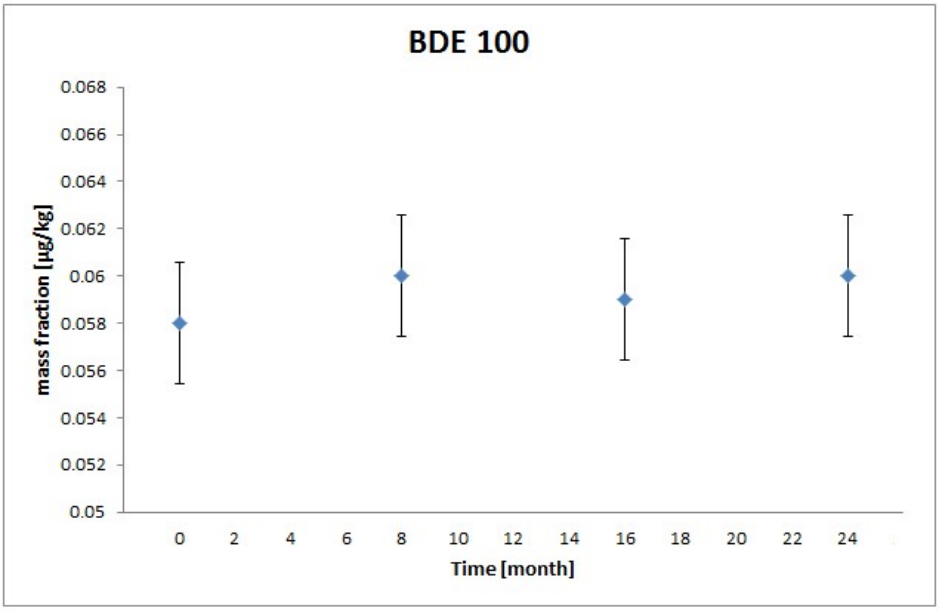
- Data of the long-term stability study at **4 °C**. The graphs report the means per time point ± confidence interval of the means (same CI calculated from ANOVA for all times) expressed as mass fraction.

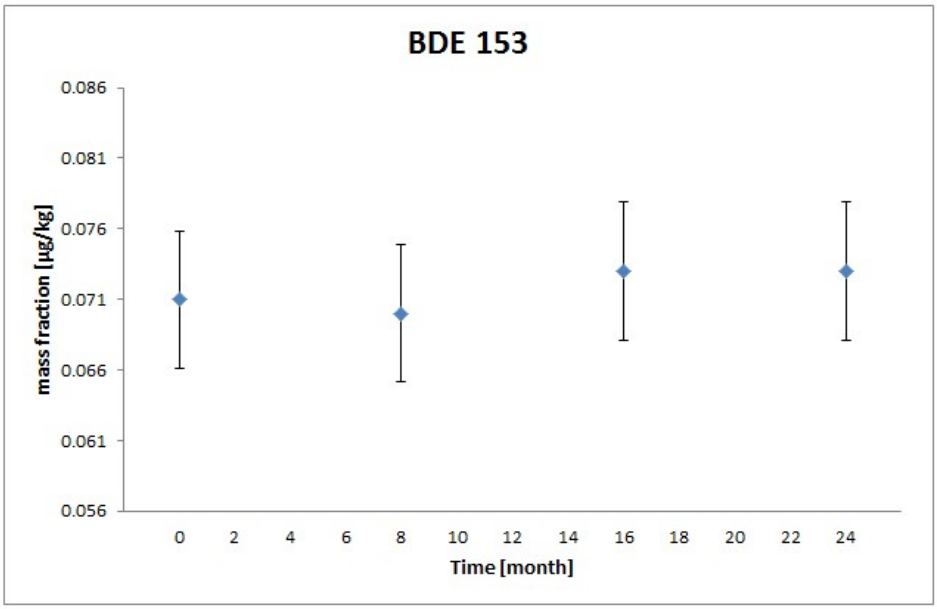


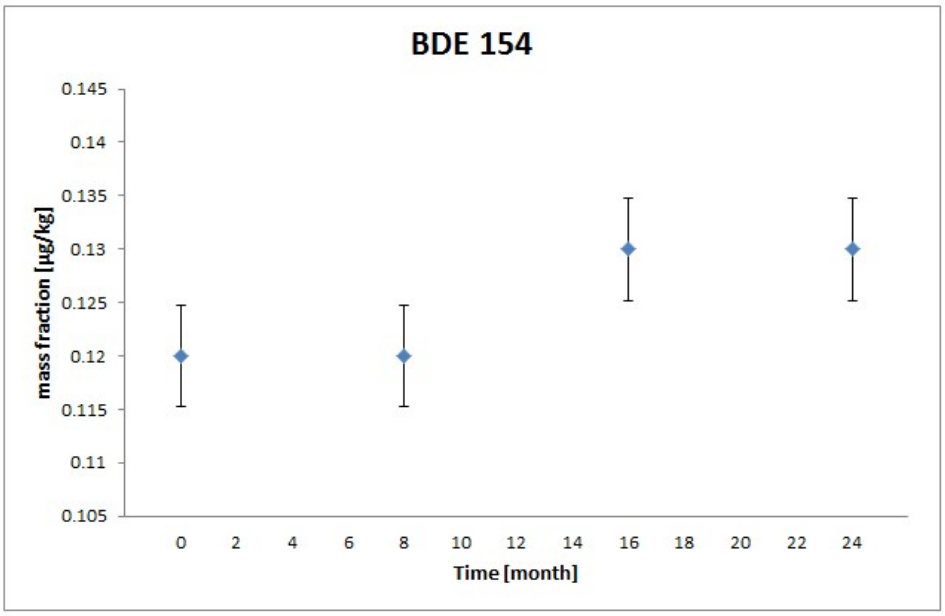


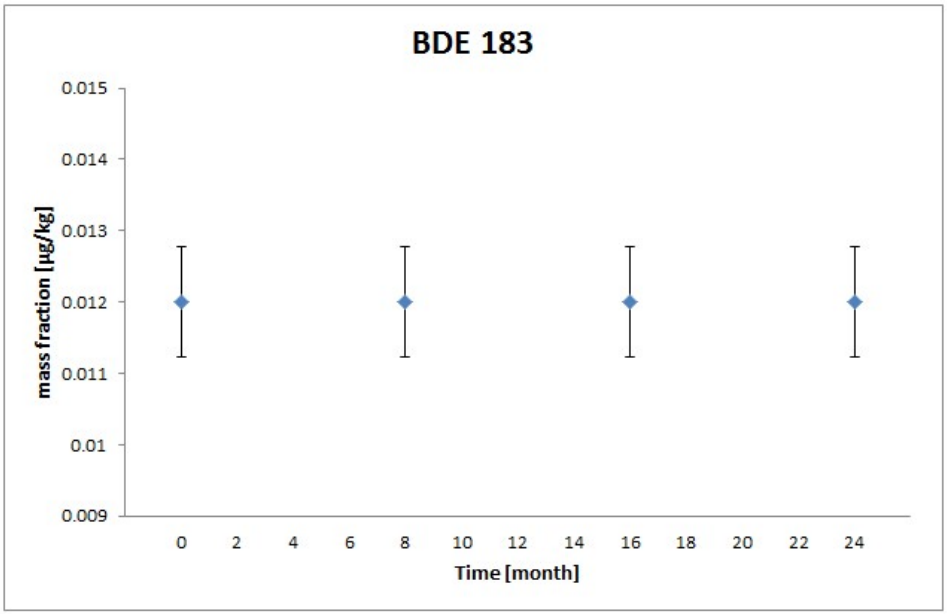

BDE 49
mass fraction [µg/kg]
0.038
0.036
0.034
0.032
0.03
0.028
0.026
0.024
0.022
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]


BDE 99
mass fraction [µg/kg]
0.16
0.15
0.14
0.13
0.12
0.11
0.1
0.09
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]

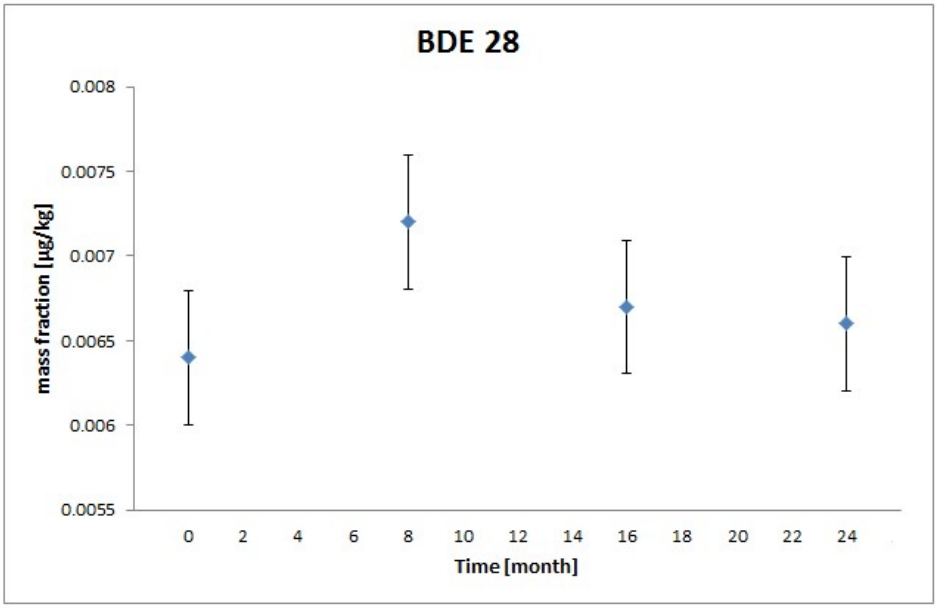

BDE 100
mass fraction [µg/kg]
0.068
0.066
0.064
0.062
0.06
0.058
0.056
0.054
0.052
0.05
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]

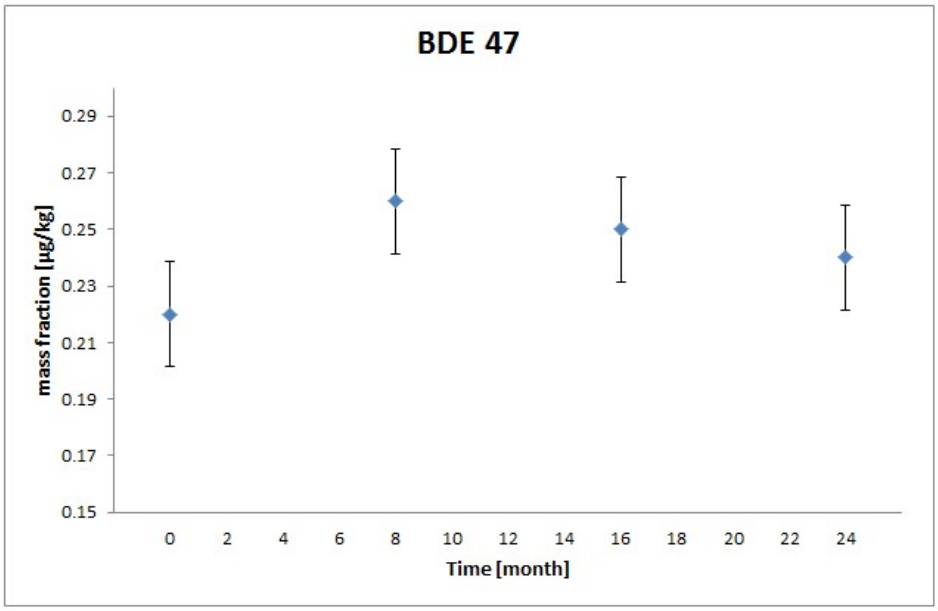

BDE 153
mass fraction [µg/kg]
0.086
0.081
0.076
0.071
0.066
0.061
0.056
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]

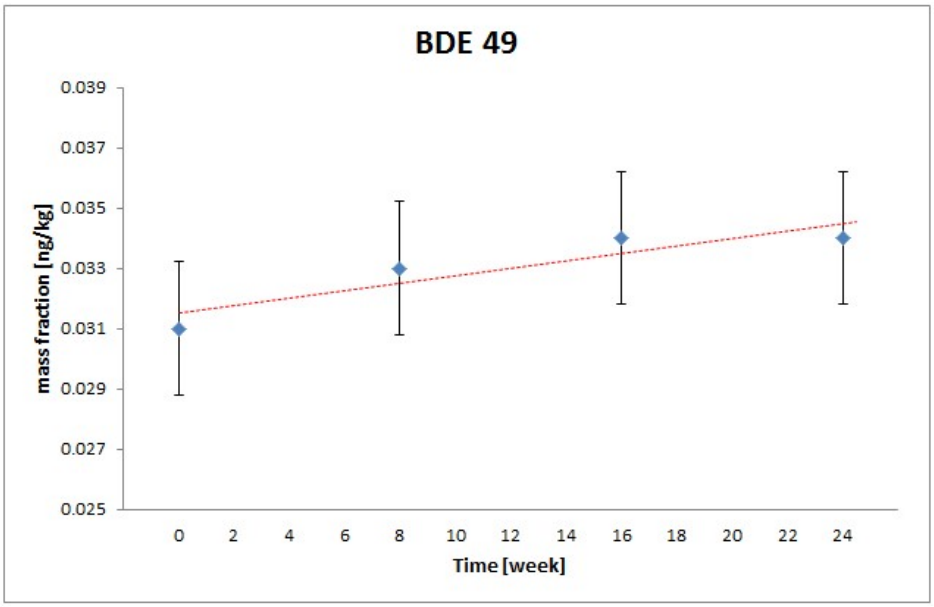

BDE 154
mass fraction [µg/kg]
0.145
0.14
0.135
0.13
0.125
0.12
0.115
0.11
0.105
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]

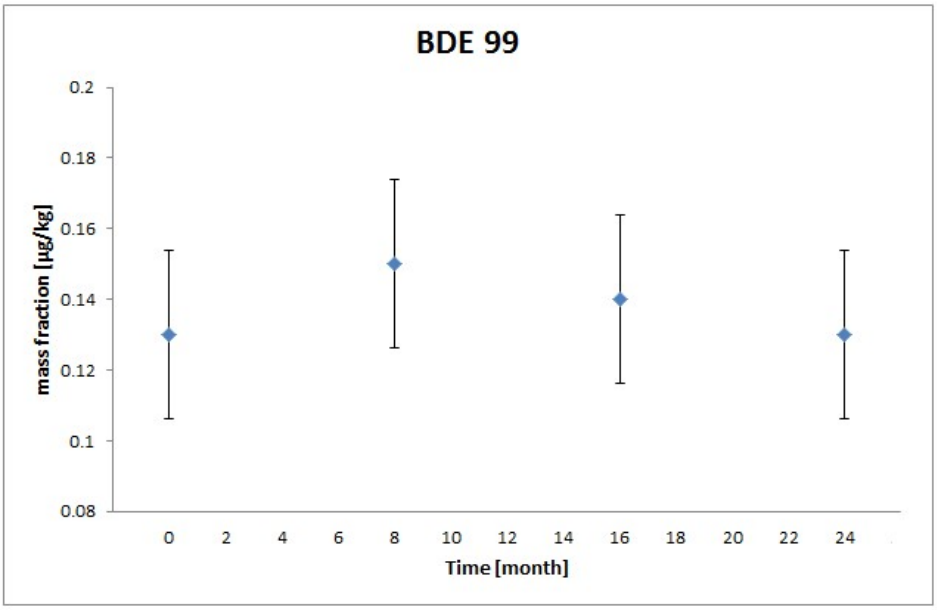

BDE 183
mass fraction [µg/kg]
0.015
0.014
0.013
0.012
0.011
0.01
0.009
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]

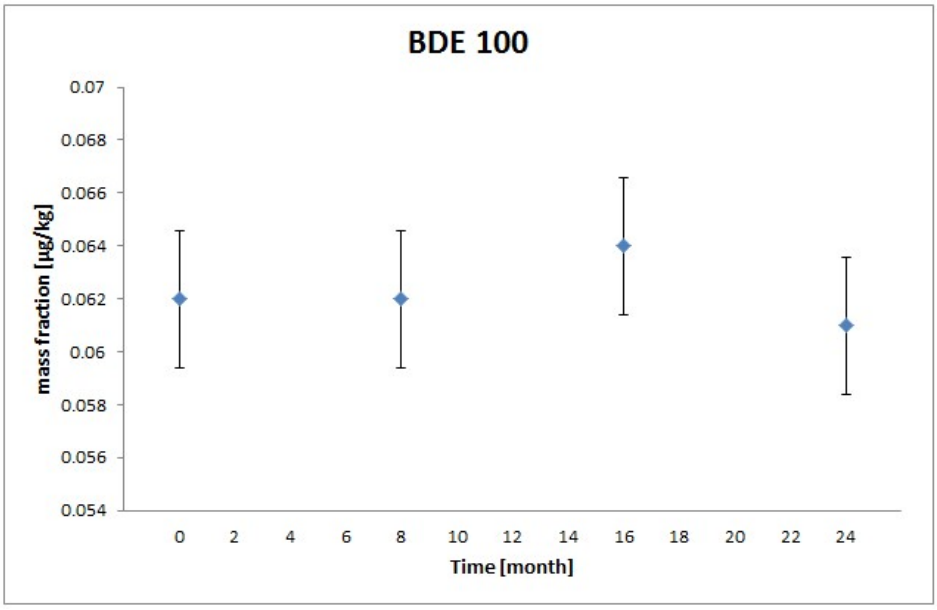
- Data of the long-term stability study at **18 °C**. The graphs report the means per time point ± confidence interval of the means (same CI calculated from ANOVA for all times) expressed as mass fraction.

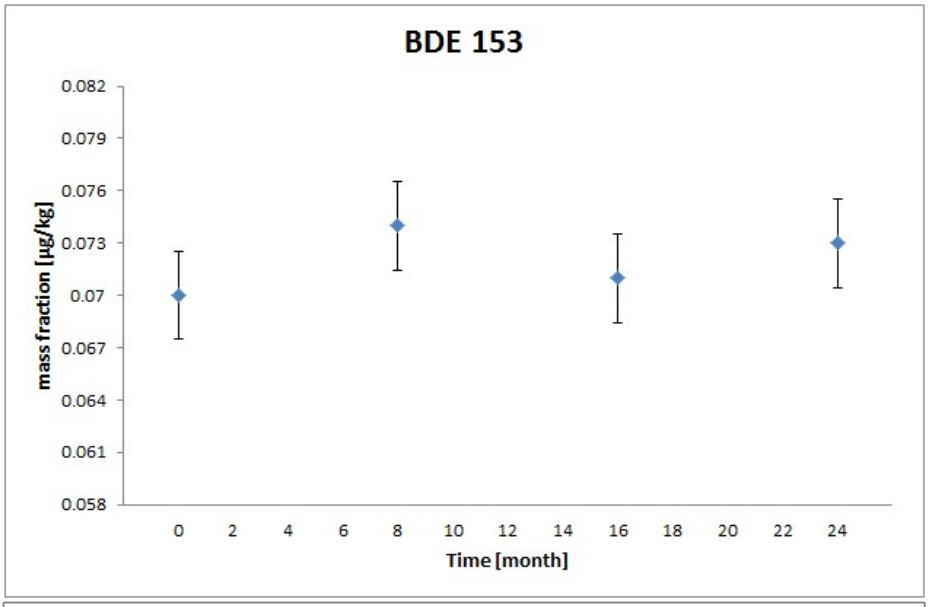


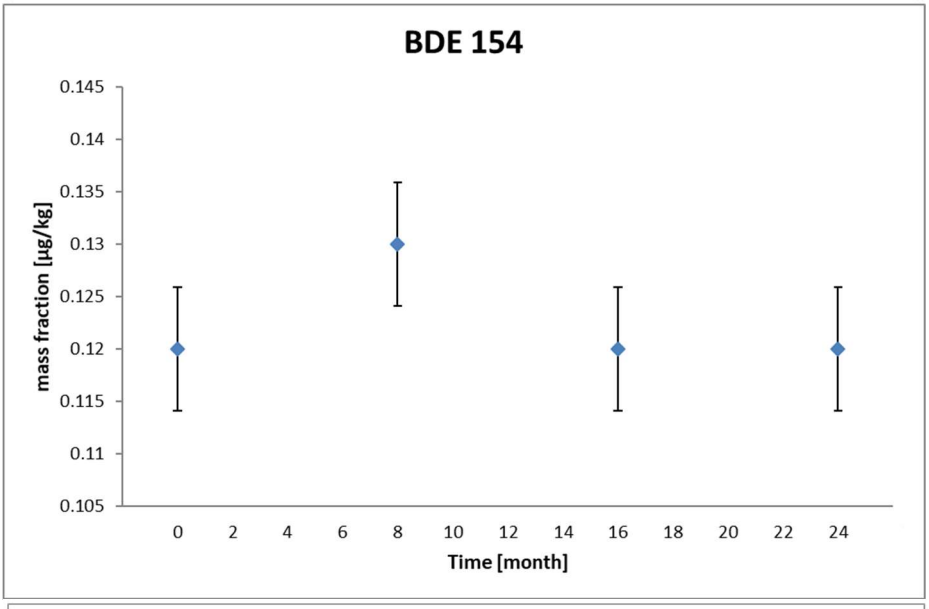


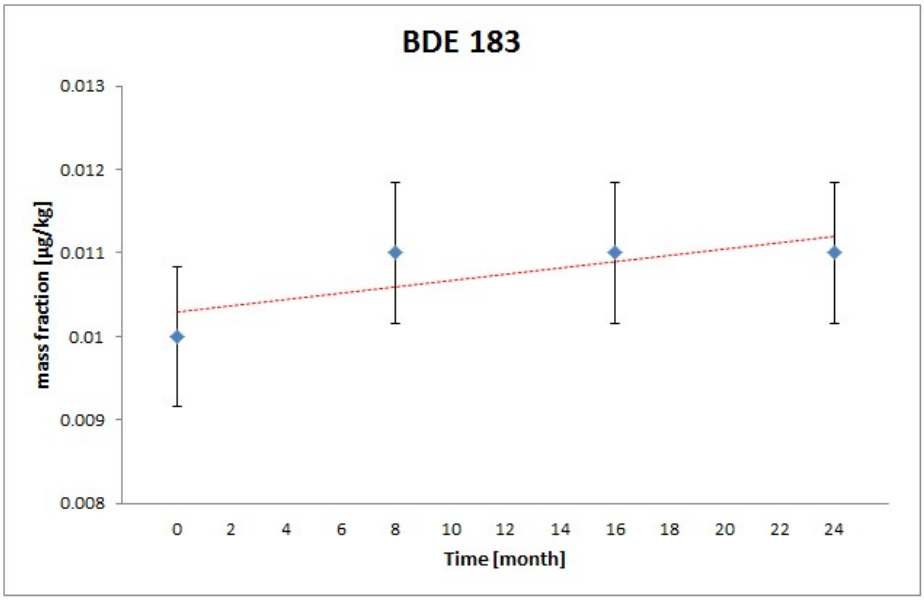

BDE 49
mass fraction [ng/kg]
0.039
0.037
0.035
0.033
0.031
0.029
0.027
0.025
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [week]


BDE 99
mass fraction [µg/kg]
0.2
0.18
0.16
0.14
0.12
0.1
0.08
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]


BDE 100
mass fraction [µg/kg]
0.07
0.068
0.066
0.064
0.062
0.06
0.058
0.056
0.054
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]


BDE 153
mass fraction [µg/kg]
0.082
0.079
0.076
0.073
0.07
0.067
0.064
0.061
0.058
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]


BDE 154
mass fraction [µg/kg]
0.145
0.14
0.135
0.13
0.125
0.12
0.115
0.11
0.105
0 2 4 6 8 10 12 14 16 18 20 22 24
Time [month]


BDE 183
mass fraction [µg/kg]
0.013
0.012
0.011
0.01
0.009
0.008
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

Table E1: Analytical method details as reported by the laboratories

Laboratory code–method	Sample pre-treatment	Quantification method GC column Internal standard(s)	Calibration and calibrants' details (purity)	LOQs [ng/kg wet weight]
L00-GC-HRMS	Sample added with water, labelled internal standards (i.s.) and EtOH. Consecutive extractions with diethylether and *n*-pentane, after separation the organic phase was treated with conc. H_2SO_4, re-extracted with *n*-hexane and cleaned-up on a multilayer silica gel column containing acidic, basic and neutral silica.	GC-EI-IDHRMS RTX-5 Sil MS 30 m x 0.25 mm x 0.25 µm MBDE-MXE (^{13}C labelled PBDE solution) by Wellington Laboratories (WL)	8 points from 0.5 to 500 pg for Tri–PeBDE 8 points from 1 to 2500 pg for Hx–HpBDE BDE-MXE, WL (> 98 %)	Tri-BDE <0.5 Te-BDE <1 Pe-BDE 1 - 2 Hx-BDE 2 - 3 Hp-BDE 2- 3
L01-GC-HRMS	After spiking with labelled i.s and adding Na_2SO_4, Soxhlet extraction with hexane/CH_2Cl_2, clean-up by liquid-liquid extraction with conc. H_2SO_4 and multilayer silica gel column (EPA Method 1614A)	GC-EI-IDHRMS STX 500, 11 m, 0.25 mm ID, 0.15 µm MBDE-MXFS MXE (13C labelled PBDE solution) by WL	5 points, 1-400 ng/ml; 5-2000 ng/ml BDE-CSV-G by WL (≥ 98 %)	5-10
L02-GC-HRMS	After spiking with labelled i.s., extraction with 10 % CH_2Cl_2/hexane on Automatic Pressurized Extraction Unit, clean-up via Power Prep System - 3 columns - silica, alumina and carbon (Method E3481-MECP)	GC-EI-IDHRMS J&W DB-5HT, 15 m x 0.25 mm x 0.1 µm $^{13}C_{12}$ - Labelled BDE Standard, BFR-LCS by WL	4 points BFR-BDE-CVS, WL	BDE-28 : 5.2 BDE-47: 311 BDE-49: 6.1 BDE-99: 217 BDE-100: 40.6 BDE-153: 10.4 BDE-154: 8.7 BDE-183: 9.1

L04-GC-MS/MS	After spiking with labelled i.s., Soxhlet extraction with hexane:acetone, clean-up on a multilayer silica gel column containing acidic, basic and neutral silica	GC-EI-IDMS/MS Rtx1614, 15 m x 0,25 mm ID x 0.1 µm Single congener ^{13}C labelled standard solutions by WL	2 points: 1000 and 2000 pg/L (linearity tested with 10 points) Single congener native standards by WL (> 98 %)	3 for all BDEs except BDE-183: 4
L05-GC-MS	After spiking with i.s., Soxhlet extraction with hexane:acetone, clean-up on a multilayer silica gel column containing acidic, basic and neutral silica	GC-ECNI-MS Rtx1614, 15 m x 0.25 mm ID x 0.1 µm F-BDE 28, 47, 99, 160 by Chiron	2 points: 1000 and 2000 pg/L (linearity tested with 10 points) Single congener native standards by WL (> 98 %)	BDE-28 : 3 BDE-47, -153, -154, -183: 13 BDE-49: 8 BDE-99: 7 BDE-100: 6
L08-GC-HRMS	After spiking with labelled i.s., Soxhlet extraction with toluene and water separator followed by Soxhlet extraction with toluene:EtOH 1:2, clean-up with automatic MIURA system (silvernitrate silica, sulfuric acidic silica, carbon and alumina columns)	GC-EI-IDHRMS Phenomenex SemiVolatiles, L: 20 m, ID: 0.18 mm, FT: 0.18 µm Single congener ^{13}C labelled standard solutions by Cambridge Isotope Laboratories (CIL)	6 points: 1, 5, 25,100, 500, 2500 pg/5µL Single congener native standards by CIL (> 98 %)	BDE-28 : 1 BDE-47: 20 BDE-49: 2 BDE-99: 15 BDE-100: 4 BDE-153, -154: 5 BDE-183: 3
L09-GC-HRMS	After spiking with labelled i.s., extraction by EtOAc, (purification by dispersive acidified silica clean-up for the lipid-rich samples), clean-up by silica column chromatography (1 gram activated silica and 8 gram acidified silica)	GC-EI-IDHRMS Rtx-ClPesticides, 30 m x 0.25 mm i.d. x 0.25 mm mix of ^{13}C-labelled BDEs, company not specified	9 points: 0, 50, 200, 500, 2000, 5000, 20000, 50000, 100000 pg/mL PBDE mix 10, S-4559-50-T (50 µg/ml +/- 5 %)	< 1

L10-GC-HRMS	After spiking with labelled i.s., ASE extraction with *n*-hexane/acetone 3:1, clean-up with an automated system comprising 3 chromatographic columns: multilayer (SiO_2 + SiO_2 with H_2SO_4), alumina, carbon	GC-EI-IDHRMS Rtx-1614, 15 m, ID 0.25 mm, 0.1 µm $^{13}C_{12}$ - Labelled BDE Standard, BRF-LCS by WL	Single point BFR-PAR native compounds stock solution by WL (purity relative uncertainty ± 5 %)	BDE-28: 0.8 BDE-47: 15 BDE-99: 5.5 BDE-100: 1.3 BDE-153: 5.4 BDE-154: 1.2 BDE-183: 2.0
L11-GC-HRMS	After spiking with labelled i.s., exhaustive extraction with mixed organic solvents, clean-up by adsorption chromatography and further on modified silica and alumina	GC-EI-IDHRMS Rtx-1614 30 m x 0.25 mm ID x 0.1 µm MBDE-MXE (^{13}C labelled PBDE solution) by WL and SCFB-004 by CIL	Single point ROHS PBDE Native PAR Spike by CIL (> 98 %)	2-3
L12-GC-MS	Soxhlet extraction with hexan:acetone (4:1), colum clean-up on aluminium oxide, silica and silica with sulphuric acid	GC-ECNI-MS J&W DB-5, 60 m, 0.25 mm, 0.25 µm BDE-71	8 points, range: 0.05-10 ng/mL Single congener native standards by CIL (> 98 %)	5 for all BDEs except BDE-183: 10-11
L13-GC-MS	After spiking with labelled i.s., Soxhlet extraction with of *n*-hexane:CH_2Cl_2, clean-up with H_2SO_4-Si column followed by a second alumina oxide column	GC-EI-IDMS RTX 1614 15 m, 0.10 µm film thickness, 0.25 mm ID Single congener ^{13}C labelled standard solutions by WL	5 points Single congener native standard solutions by WL (> 98 %)	BDE-28, -47, -49: 1 BDE-99, -100: 2 BDE-153, -154: 3 BDE-183: 5

Not used in the certification

L03-GC-MS	ASE extraction with hexane:acetone followed by a H_2SO_4 silica column (20 g of 40 % H_2SO_4), further clean-up with a silica column	GC-ECNI-MS Varian CPSil-8 CB (CP 7453), 50 m x 0.25 mm x 0.25 µm BDE-58	8 points: BDE 28, 47, 49, 99, 100: 0.47 - 233 ng/g *iso*-octane BDE 153,154 and 183: 0.95 - 466 ng/g *iso*-octane BDE-MXE, WL (solution > 98 %)	BDE-28, -99, -154: 65.3; BDE-47, -49: 87.1; BDE-100, -153: 81.7 BDE-183: 93.3
L06-GC-MS	Addition of water, extraction by EtOAc, clean-up on silica gel column (modified QuEChERS)	GC-ECNI-MS DB-XLB (15 m × 0.18 mm × 0.07 µm) BDE-37, BDE-77	8 points: 0.05; 0.1; 0.5; 1; 5; 10; 50; 100 ng/mL Single congener native standards by WL (> 99 %)	5
L07-GC-MS	Soxhlet extraction with a mixture of *n*-hexane:CH_2Cl_2 (1:1), clean-up by gel permeation chromatography (cyclohexane:EtOAc 1:1)	GC-ECNI-MS DB-XLB (15 m × 0.18 mm × 0.07 µm) BDE-37, BDE-77	8 points: 0.05; 0.1; 0.5; 1; 5; 10; 50; 100 ng/mL Single congener native standards by WL (> 99 %)	20

Annex F: Results of the characterisation measurements

Note: values in µg/kg (wet weight basis). Values reported as ng/kg by the laboratories were transformed accordingly.

Table F1: BDE-28

laboratory code - method	replicate 1 [µg/kg]	replicate 2 [µg/kg]	replicate 3 [µg/kg]	replicate 4 [µg/kg]	replicate 5 [µg/kg]	replicate 6 [µg/kg]	mean [µg/kg]	expanded uncertainty [µg/kg]
L00-GC-HRMS	0.00722	0.00712	0.00695	0.00759	0.00696	0.00695	0.00713	0.00143
L04-GC-MS/MS	0.00813	0.00680	0.00686	0.00695	0.00691	0.00714	0.00713	0.00086
L06-GC-MS	0.00898	0.00896	0.00893	0.00941	0.00892	0.00912	0.00905	0.00181
L11-GC-HRMS	0.007	0.007	0.007	0.008	0.007	0.008	0.007	0.004
L12-GC-MS	0.0066	0.0090	0.0074	0.0083	0.0096	0.0074	0.0081	0.0041
Results not used for the assignment of the indicative value								
L01-GC-MS	0.00817	0.00841	0.00847	0.01090	0.00925	0.00870	0.00898	0.00269
L02-GC-HRMS	0.00459	0.00464	0.00473	0.00444	0.00470	0.00471	0.00464	0.00039
L03-GC-MS	< 20	< 20	< 20	< 20	< 20	< 20	n.a.	n.a.
L05-GC-MS*	0.0143	0.0142	0.0147	0.0145	0.0127	0.0126	0.01383	0.00415
L07-GC-MS	< 20	< 20	< 20	< 20	< 20	< 20	n.a.	n.a.
L08-GC-MS	0.0068	0.0071	0.0064	0.0066	0.0067	0.0067	0.0067	0.0010
L09-GC-HRMS	0.00651	0.00648	0.00631	0.00670	0.00652	0.00654	0.00651	0.00163
L10-GC-HRMS	0.00689	0.00735	0.00781	0.00646	0.00744	0.00798	0.00732	0.00161
L13-GC-MS	0.00663	0.00580	0.00588	0.00523	0.00520	0.00555	0.00572	0.00143

*not valid results due to chromatographic interference

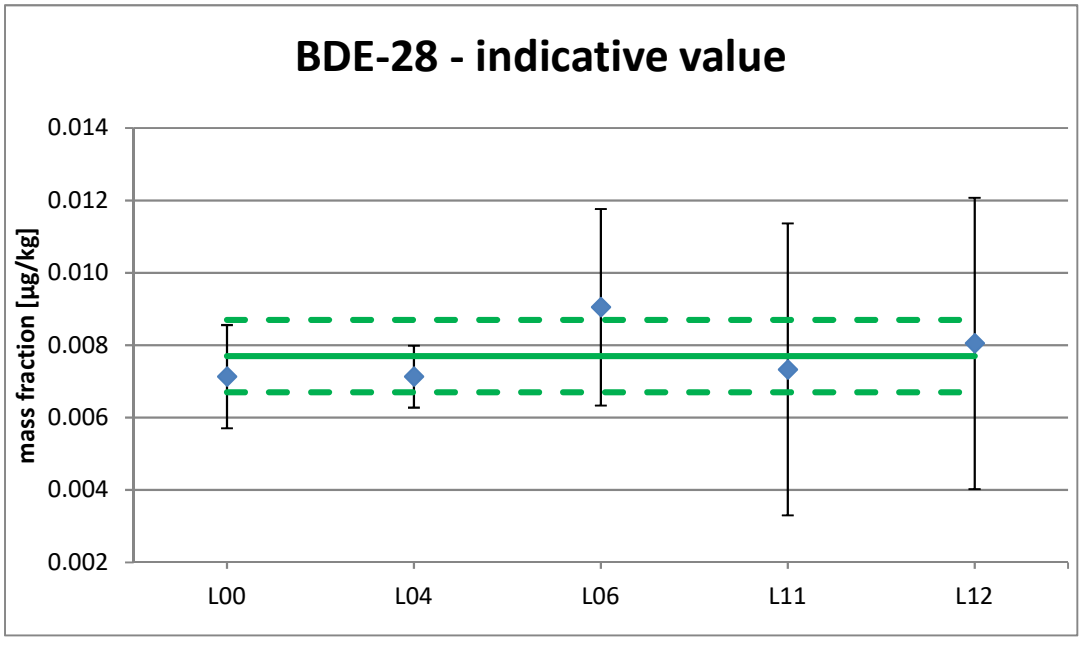


Figure F1: indicative value (solid line) ± expanded uncertainty (dashed lines) for BDE-28 in ERM-CE102, **0.0077 ± 0.0010 µg/kg**; error bars of the individual laboratory means correspond to the expanded uncertainty

Table F2: BDE-47

laboratory code - method	replicate 1 [µg/kg]	replicate 2 [µg/kg]	replicate 3 [µg/kg]	replicate 4 [µg/kg]	replicate 5 [µg/kg]	replicate 6 [µg/kg]	mean [µg/kg]	expanded uncertainty [µg/kg]
L00-GC-HRMS	0.246	0.245	0.250	0.256	0.252	0.251	0.250	0.050
L01-GC-MS	0.231	0.237	0.248	0.259	0.272	0.282	0.255	0.077
L08-GC-MS	0.23	0.24	0.23	0.22	0.24	0.23	0.23	0.03
L09-GC-HRMS	0.222	0.237	0.224	0.237	0.220	0.236	0.229	0.057
L10-GC-HRMS	0.215	0.225	0.222	0.213	0.241	0.239	0.226	0.072
L11-GC-HRMS	0.219	0.223	0.227	0.222	0.221	0.222	0.222	0.036
L12-GC-MS	0.20	0.21	0.22	0.21	0.22	0.20	0.21	0.11
L13-GC-MS	0.200	0.187	0.187	0.193	0.190	0.203	0.193	0.048
			Results not used for the certification					
L02-GC-HRMS	0.201	0.222	0.194	0.168	0.170	0.173	0.188	0.094
L03-GC-MS	0.22	0.21	0.22	0.23	0.24	0.24	0.23	0.03
L04-GC-MS/MS	0.254	0.216	0.217	0.233	0.218	0.215	0.226	0.034
L05-GC-MS	0.201	0.207	0.196	0.201	0.178	0.180	0.190	0.029
L06-GC-MS	0.157	0.151	0.164	0.183	0.164	0.208	0.171	0.051
L07-GC-MS	0.137	0.137	0.136	0.141	0.137	0.137	0.138	0.041

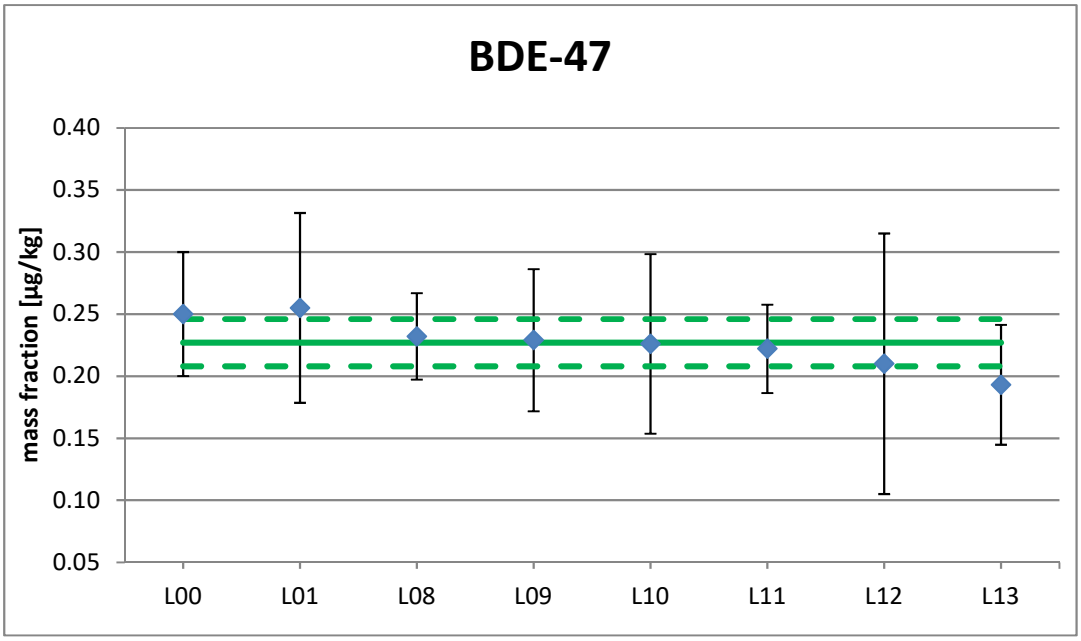


Figure F2: certified value (solid line) ± expanded uncertainty (dashed lines) for BDE-47 in ERM-CE102, **0.227 ± 0.019 µg/kg**; error bars of the individual laboratory means correspond to the expanded uncertainty

Table F3: BDE-49

laboratory code - method	replicate 1 [µg/kg]	replicate 2 [µg/kg]	replicate 3 [µg/kg]	replicate 4 [µg/kg]	replicate 5 [µg/kg]	replicate 6 [µg/kg]	mean [µg/kg]	expanded uncertainty [µg/kg]
L04-GC-MS/MS	0.0354	0.0328	0.0323	0.0347	0.0333	0.0332	0.0336	0.0020
L08-GC-MS	0.027	0.032	0.031	0.030	0.031	0.029	0.030	0.005
L09-GC-HRMS	0.0390	0.0395	0.0351	0.0399	0.0336	0.0398	0.0378	0.0095
L10-GC-HRMS	0.0324	0.0290	0.0444	0.0301	0.0350	0.0327	0.0339	0.0108
L11-GC-HRMS	0.035	0.035	0.035	0.034	0.033	0.035	0.035	0.006
L12-GC-MS	0.031	0.034	0.034	0.036	0.035	0.029	0.033	0.017
L13-GC-MS	0.0300	0.0262	0.0254	0.0243	0.0252	0.0260	0.0262	0.0066
Results not used for the certification								
L02-GC-HRMS	0.0210	0.0202	0.0213	0.0191	0.0210	0.0189	0.0203	0.0024
L03-GC-MS	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.a.	n.a.
L05-GC-MS	0.0361	0.0370	0.0346	0.0352	0.0336	0.0346	0.0352	0.0035
L06-GC-MS	0.0183	0.0193	0.0204	0.0208	0.0198	0.0199	0.0198	0.0059
L07-GC-MS	0.0186	0.0199	0.0200	0.0189	0.0210	0.0197	0.0197	0.0059

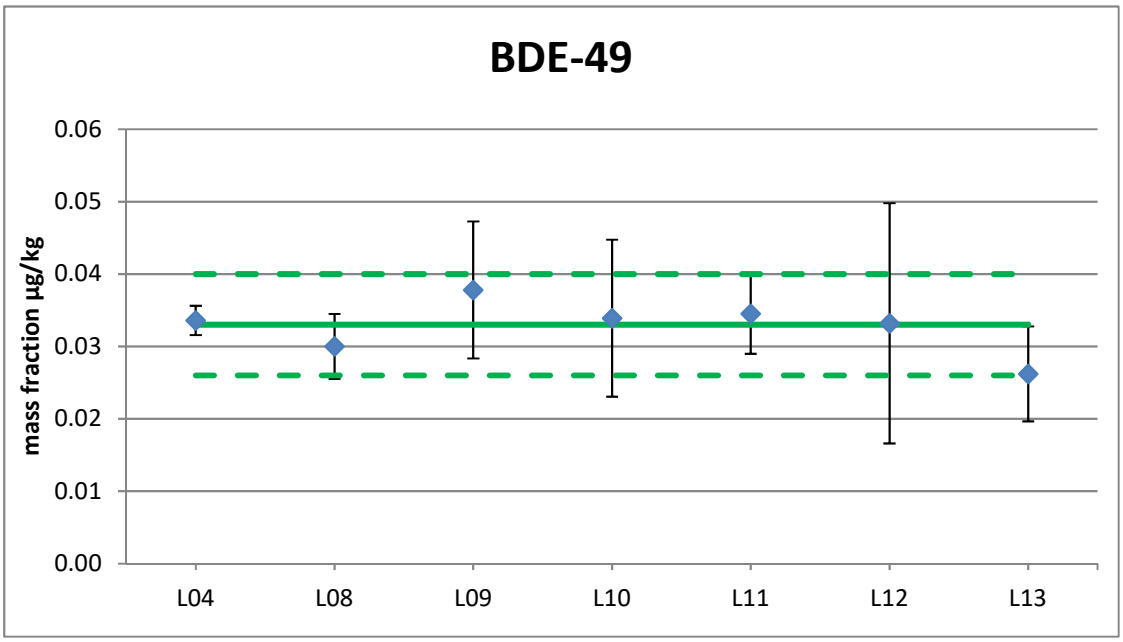


Figure F3: certified value (solid line) ± expanded uncertainty (dashed lines) for BDE-49 in ERM-CE102, **0.033 ± 0.007 µg/kg**; error bars of the individual laboratory means correspond to the expanded uncertainty

Table F4: BDE-99

laboratory code - method	replicate 1 [µg/kg]	replicate 2 [µg/kg]	replicate 3 [µg/kg]	replicate 4 [µg/kg]	replicate 5 [µg/kg]	replicate 6 [µg/kg]	mean [µg/kg]	expanded uncertainty [µg/kg]
L00-GC-HRMS	0.128	0.124	0.135	0.128	0.128	0.127	0.128	0.026
L01-GC-MS	0.122	0.121	0.133	0.148	0.154	0.166	0.141	0.042
L02-GC-HRMS	0.151	0.219	0.133	0.124	0.124	0.151	0.150	0.105
L04-GC-MS/MS	0.131	0.109	0.113	0.11	0.112	0.108	0.114	0.016
L05-GC-MS	0.116	0.124	0.121	0.115	0.118	0.118	0.119	0.011
L08-GC-MS	0.13	0.13	0.13	0.12	0.15	0.12	0.13	0.02
L09-GC-HRMS	0.116	0.124	0.115	0.121	0.114	0.124	0.119	0.030
L10-GC-HRMS	0.114	0.105	0.121	0.107	0.121	0.133	0.117	0.018
L11-GC-HRMS	0.111	0.113	0.114	0.109	0.11	0.111	0.111	0.012
L12-GC-MS	0.12	0.12	0.13	0.13	0.14	0.12	0.13	0.06
L13-GC-MS	0.105	0.102	0.102	0.103	0.1	0.105	0.103	0.026
Results not used for the certification								
L03-GC-MS	0.13	0.13	0.13	0.15	0.15	0.14	0.14	0.03
L06-GC-MS	0.0886	0.1090	0.0957	0.1200	0.0995	0.0794	0.0990	0.030
L07-GC-MS	0.0795	0.0786	0.0781	0.0748	0.0750	0.0719	0.0760	0.023

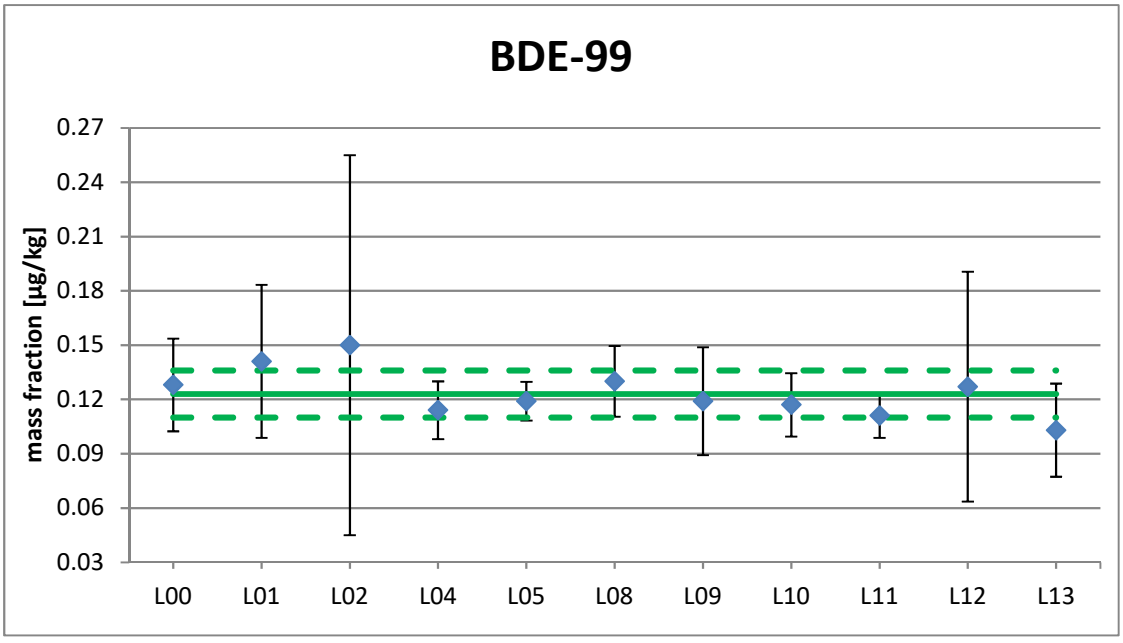


Figure F4: certified value (solid line) ± expanded uncertainty (dashed lines) for BDE-99 in ERM-CE102, **0.123 ± 0.013 µg/kg**; error bars of the individual laboratory means correspond to the expanded uncertainty

Table F5: BDE-100

laboratory code - method	replicate 1 [µg/kg]	replicate 2 [µg/kg]	replicate 3 [µg/kg]	replicate 4 [µg/kg]	replicate 5 [µg/kg]	replicate 6 [µg/kg]	mean [µg/kg]	expanded uncertainty [µg/kg]
L00-GC-HRMS	0.0494	0.0505	0.0560	0.0545	0.0543	0.0514	0.0527	0.0105
L01-GC-MS	0.0547	0.0675	0.0697	0.0746	0.0809	0.0823	0.0716	0.0215
L04-GC-MS/MS	0.0518	0.0473	0.0527	0.0475	0.0524	0.0501	0.0503	0.0050
L05-GC-MS	0.0658	0.0626	0.0555	0.0621	0.0595	0.0556	0.0602	0.0084
L08-GC-MS	0.058	0.060	0.057	0.056	0.062	0.057	0.058	0.009
L09-GC-HRMS	0.059	0.064	0.0596	0.0616	0.0587	0.0641	0.061	0.015
L10-GC-HRMS	0.0572	0.0546	0.0616	0.0585	0.0620	0.0671	0.0602	0.0157
L11-GC-HRMS	0.060	0.060	0.060	0.061	0.061	0.059	0.060	0.007
L12-GC-MS	0.065	0.070	0.073	0.069	0.078	0.065	0.070	0.035
L13-GC-MS	0.0574	0.0522	0.0543	0.0550	0.0550	0.0569	0.0551	0.0138
Results not used for the certification								
L02-GC-HRMS	0.0488	0.0583	0.0462	0.0397	0.0402	0.0404	0.0456	0.0082
L03-GC-MS	0.06	0.06	0.06	0.07	0.07	0.07	0.07	0.02
L06-GC-MS	0.0406	0.0386	0.0365	0.0477	0.0440	0.0536	0.0435	0.0131
L07-GC-MS	0.0319	0.0311	0.0358	0.0329	0.0325	0.0322	0.0327	0.0098

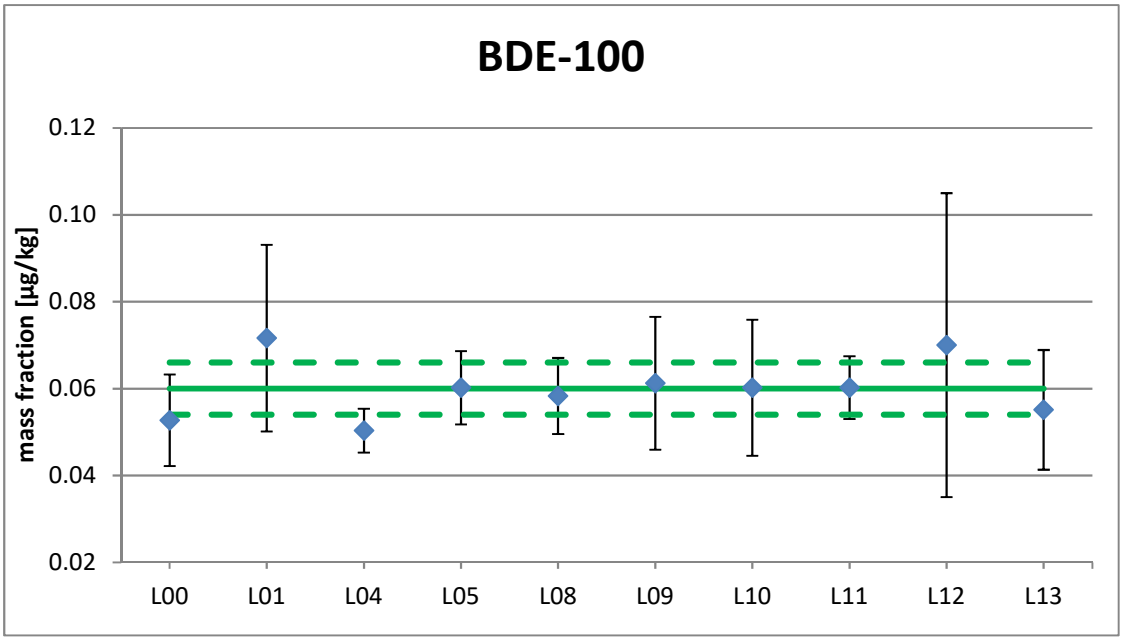


Figure F5: certified value (solid line) ± expanded uncertainty (dashed lines) for BDE-100 in ERM-CE102, **0.060 ± 0.006 µg/kg**; error bars of the individual laboratory means correspond to the expanded uncertainty

Table F6: BDE-153

laboratory code - method	replicate 1 [µg/kg]	replicate 2 [µg/kg]	replicate 3 [µg/kg]	replicate 4 [µg/kg]	replicate 5 [µg/kg]	replicate 6 [µg/kg]	mean [µg/kg]	expanded uncertainty [µg/kg]
L00-GC-HRMS	0.0786	0.0760	0.0767	0.0752	0.0712	0.0724	0.0750	0.0150
L01-GC-MS	0.0688	0.0701	0.0832	0.0879	0.0794	0.0859	0.0792	0.0238
L04-GC-MS/MS	0.0693	0.0699	0.0674	0.0665	0.0666	0.0631	0.0671	0.0047
L05-GC-MS	0.0733	0.0763	0.0713	0.0821	0.0699	0.0656	0.0731	0.0095
L08-GC-MS	0.071	0.071	0.068	0.070	0.069	0.069	0.070	0.010
L09-GC-HRMS	0.0661	0.0718	0.0691	0.0728	0.0681	0.0744	0.0704	0.0176
L10-GC-HRMS	0.0626	0.0596	0.0735	0.0653	0.0704	0.0677	0.0665	0.0100
L11-GC-HRMS	0.063	0.064	0.065	0.066	0.065	0.063	0.064	0.008
L12-GC-MS	0.065	0.062	0.066	0.071	0.068	0.061	0.066	0.033
L13-GC-MS	0.0598	0.0600	0.0606	0.0614	0.0599	0.0622	0.0607	0.0152
Results not used for the certification								
L02-GC-HRMS	0.0401	0.0409	0.0420	0.0362	0.0366	0.0403	0.0394	0.0063
L03-GC-MS	0.07	0.07	0.07	0.08	0.08	0.07	0.07	0.02
L06-GC-MS	0.0537	0.0504	0.0547	0.0666	0.0586	0.0562	0.0567	0.0170
L07-GC-MS	0.0432	0.0421	0.0391	0.0449	0.0466	0.0495	0.0442	0.0133

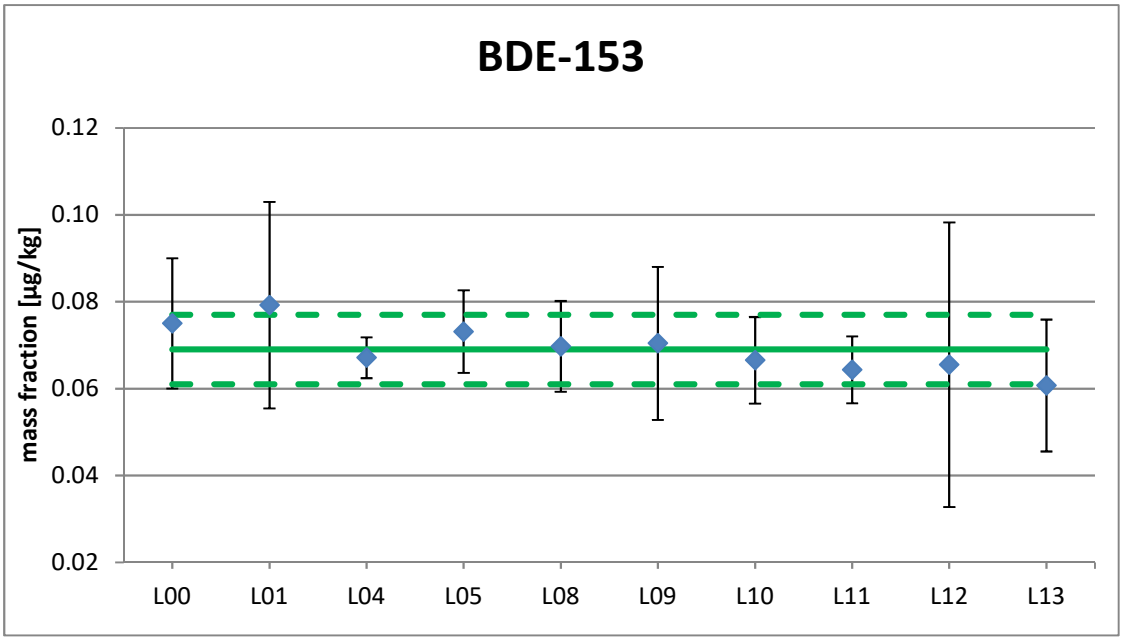


Figure F6: certified value (solid line) ± expanded uncertainty (dashed lines) for BDE-153 in ERM-CE102, **0.069 ± 0.008 µg/kg**; error bars of the individual laboratory means correspond to the expanded uncertainty

Table F7: BDE-154

laboratory code - method	replicate 1 [µg/kg]	replicate 2 [µg/kg]	replicate 3 [µg/kg]	replicate 4 [µg/kg]	replicate 5 [µg/kg]	replicate 6 [µg/kg]	mean [µg/kg]	expanded uncertainty [µg/kg]
L00-GC-HRMS	0.123	0.114	0.124	0.120	0.117	0.118	0.119	0.024
L01-GC-MS	0.115	0.114	0.122	0.127	0.119	0.129	0.121	0.036
L04-GC-MS/MS	0.113	0.105	0.104	0.104	0.104	0.101	0.105	0.006
L08-GC-MS	0.12	0.12	0.11	0.12	0.12	0.11	0.12	0.02
L09-GC-HRMS	0.104	0.111	0.103	0.110	0.101	0.106	0.106	0.026
L10-GC-HRMS	0.104	0.104	0.107	0.105	0.113	0.118	0.109	0.040
L11-GC-HRMS	0.103	0.108	0.109	0.109	0.107	0.107	0.107	0.012
L12-GC-MS	0.11	0.11	0.11	0.11	0.11	0.10	0.11	0.05
L13-GC-MS	0.0941	0.0909	0.0903	0.0927	0.0943	0.0968	0.0932	0.0233
Results not used for the certification								
L02-GC-HRMS	0.0720	0.0754	0.0732	0.0681	0.0655	0.0653	0.0699	0.084
L03-GC-MS	0.11	0.11	0.11	0.12	0.12	0.11	0.1133	0.0360
L05-GC-MS	0.124	0.118	0.109	0.132	0.107	0.101	0.1152	0.0173
L06-GC-MS	0.108	0.107	0.101	0.118	0.111	0.113	0.1097	0.0329
L07-GC-MS	0.0802	0.0815	0.0815	0.0743	0.0767	0.0671	0.0769	0.0231

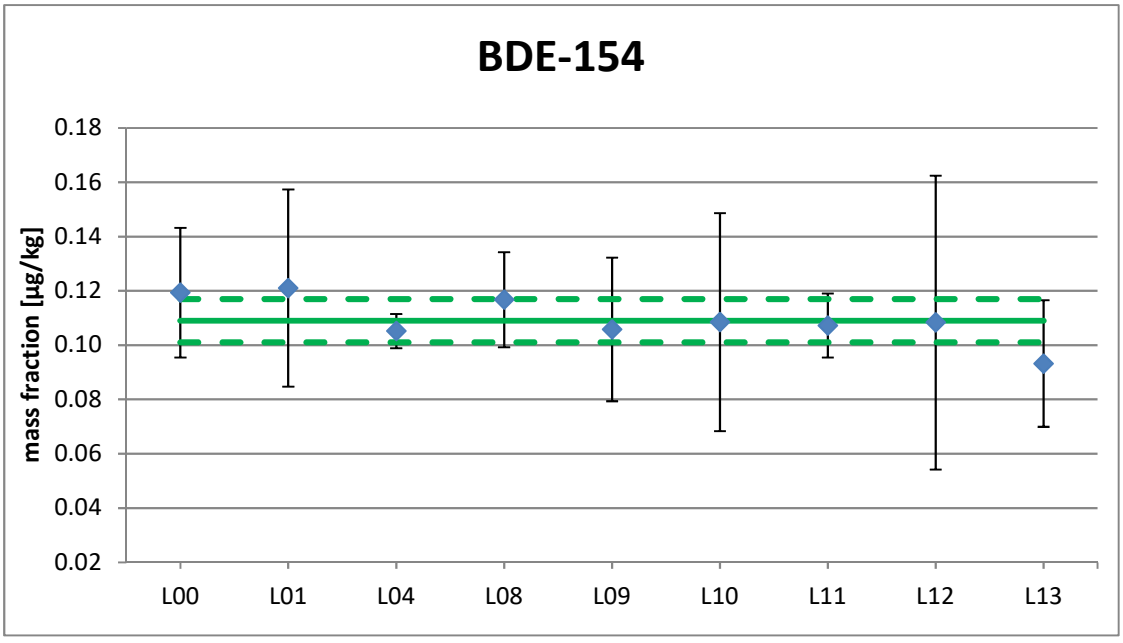


Figure F7: certified value (solid line) ± expanded uncertainty (dashed lines) for BDE-154 in ERM-CE102, **0.109 ± 0.008 µg/kg**; error bars of the individual laboratory means correspond to the expanded uncertainty

Table F8: BDE-183

laboratory code - method	replicate 1 [µg/kg]	replicate 2 [µg/kg]	replicate 3 [µg/kg]	replicate 4 [µg/kg]	replicate 5 [µg/kg]	replicate 6 [µg/kg]	mean [µg/kg]	expanded uncertainty [µg/kg]
L04-GC-MS/MS	0.0170	0.0174	0.0133	0.0143	0.0155	0.0135	0.01517	0.0049
L05-GC-MS		0.014		0.015			0.015	0.003
L12-GC-MS	0.015	0.012	0.017	0.015	0.016	0.015	0.015	0.008
L13-GC-MS	0.01150	0.00894	0.00911	0.01010	0.01010	0.01080	0.01009	0.00252
Results not used for the assignment of the indicative value								
L00-GC-HRMS	0.0142	0.0107	0.0108	0.0116	0.0115	0.0105	0.01155	0.0023
L01-GC-MS	0.0114	0.0143	0.0145	0.0113	0.0136	0.0138	0.01315	0.0039
L02-GC-HRMS	0.00781	0.00790	0.00500	0.00488	0.00506	0.00401	0.00578	0.00087
L03-GC-MS	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	<LOQ	n.a.	n.a.
L06-GC-MS	0.00827	0.00716	0.00773	0.00829	0.00815	0.00780	0.00790	0.00237
L07-GC-MS	< 20	< 20	< 20	< 20	< 20	< 20	n.a.	n.a.
L08-GC-MS	0.0110	0.0094	0.0094	0.0100	0.0094	0.0084	0.00960	0.0014
L09-GC-HRMS	0.0090	0.0099	0.0103	0.0100	0.0093	0.0100	0.00975	0.0024
L10-GC-HRMS	0.00739	0.00996	0.0119	0.00482	0.00996	0.00923	0.00888	0.00373
L11-GC-HRMS	0.009	0.010	0.010	0.010	0.009	0.009	0.010	0.004

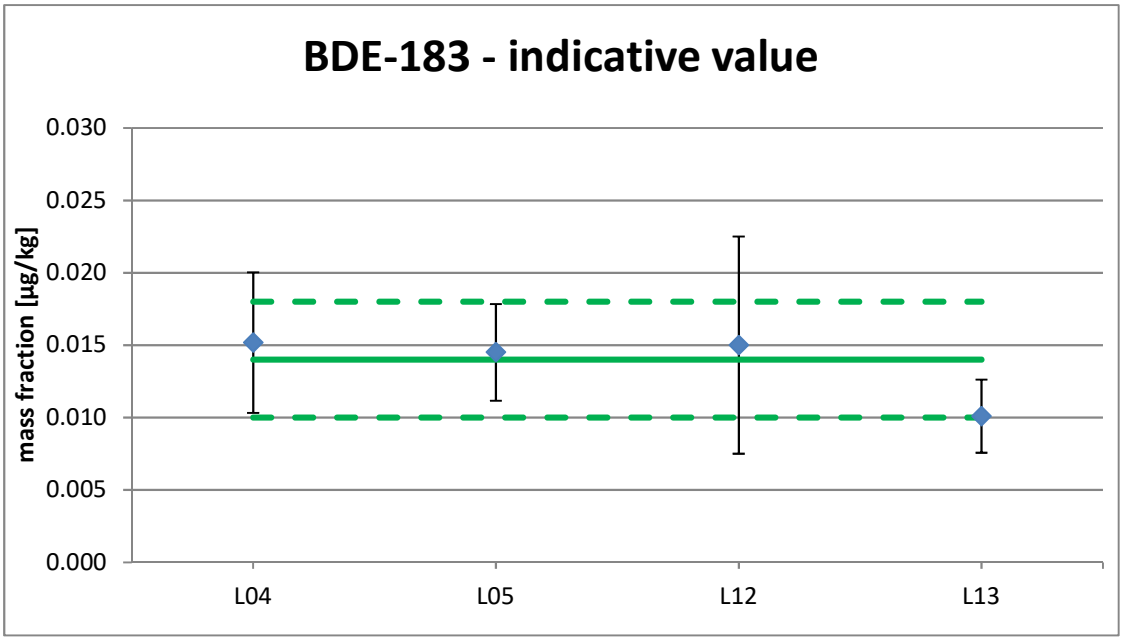


Figure F8: indicative value (solid line) ± expanded uncertainty (dashed lines) for BDE-183 in ERM-CE102, **0.014 ± 0.004 µg/kg**; error bars of the individual laboratory means correspond to the expanded uncertainty

European Commission

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