Ensuring the reliability of brominated flame retardant data on food and feed occurrence through harmonised analytical criteria and proficiency testing

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Highlights

- An approach to provide reliable data for risk assessment and potential regulation
- Harmonised analytical criteria provide guidance for experienced and new laboratories
- Established criteria are backed by excellent BFR proficiency testing results

Abstract

The volume of occurrence data on food and animal feed contaminants such as polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDDs) is slowly increasing as more laboratories develop analytical capability. This data allows an evaluation of current background levels in different countries and regions and is also useful for estimating the health risk through dietary exposure and as evidence for the formulation of future control strategies. Existing data varies in the number of analytes reported and the quality measures applied. In order to ensure reliability and comparability, guidance on analytical criteria such as precision and trueness, limits of quantitation, recovery, positive identification, etc. is provided. These parameters are based on several years of collective experience and allow validation and regular quality control of analysis of individual PBDE congeners and HBCDD stereoisomers. The criteria-based approach also allows laboratories the flexibility to use different analytical methodologies and techniques for generating data. The effectiveness of this approach has been demonstrated by a successful proficiency testing scheme that has been used for a number of years and has attracted an increasing number of participants. The majority of participating laboratories (> 80 %) have been able to demonstrate performance within the 95% confidence interval ($\left|\text{z-score}\right| \leq 2$) and a further 10 % of laboratories demonstrated performance with a z-score of ($2 < \left|\text{z-score}\right| < 3$). The combined support of these guidance criteria backed by successful proficiency testing will ensure the reliability and comparability of results, in particular, to refine risk assessments and to help the formulation of regulatory policy.

1.0 Introduction

Polybrominated diphenyl ethers (PBDEs) and hexabromocyclododecanes (HBCDDs) are brominated flame retardants (BFRs) that were manufactured in high volumes (tens of thousands of tons per year) and used globally, in a range of industrial and domestic applications, such as transport (vehicles, trains, aircraft, etc.), plastics, furnishings, insulation, paints, electronic goods, etc. As awareness of their adverse health effects grew, the use of these chemicals was restricted in Europe – PBDEs by 2008, and HBCDDs by 2015 - and other countries. Both chemicals are listed as persistent organic pollutants (POPs) by the Stockholm convention with a call for global elimination of production (Stockholm Convention).

Although both chemicals have been replaced by other flame retardants, the inherent properties of environmental and biological persistence coupled with the nature of their additive incorporation in materials, has resulted in a legacy of continuing occurrence in the environment and in animal and human tissues. Some products in which these chemicals are incorporated, such as plastics, vehicles, building materials, etc. are long-lived and will continue to be a latent emissive source, even after end-of-use. Newer products may also contain these chemicals, either through imports from areas where restriction are lax or unenforced, or because they may contain recycled material from earlier periods. There are also recent reports that despite restrictions, production of PBDEs continues in some places (Wang et al., 2019). These observations indicate that the currently observed environmental and food contamination with PBDEs and HBCDDs will continue into the future.

As part of its efforts to define the extent of this contamination, the European Commission (EC) issued a recommendation in 2014 (EC, 2014), for member states to investigate the occurrence of a range of BFRs in food. The investigations were a snapshot of occurrence in member states during 2014 and 2015 and revealed widespread contamination of PBDEs and to a somewhat lesser extent, HBCDDs, in food and feed (Eljarrat et al., 2014; Fernandes et al., 2016; Garcia-Lopez et al., 2018; Poma et al., 2018; Venisseau et al., 2018). Toxicologically, there have been relatively fewer studies (in comparison to other similar contaminants such as polychlorinated biphenyls - PCBs), and these report a range of endocrine and neurodevelopmental effects for PBDEs (Dishaw et al., 2014; Lam et al., 2017), and neurodevelopmental, reproductive and immunosuppressive effects for HBCDDs (EFSA, 2021). Other effects have been reported, but as many of these are single studies, confirmation is dependent on further investigation.

From a food safety point of view, the outcome of these observations on occurrence and toxicology is obvious – the minimisation of human exposure. Although other pathways (e.g. ingestion of house dust) are recognised, and may be significant for occupationally exposed populations, dietary intake is the principal exposure route (Sjödin et al., 2003; Lyche et al., 2105; Martellini et al., 2016; Pietroń et al., 2019) for most populations. Further and sustained monitoring of the food and animal feed supply would therefore be a prudent first step to establish the current status of contamination, along with support for toxicological studies to confirm effects. As part of the effort to further explore the issue and potentially to exert control on exposure, the EC established a European Union Reference Laboratory (EURL) on halogenated persistent organic pollutants (POPs) including PBDEs and HBCDDs, with a direction to establish a validated and harmonised mechanism to allow monitoring across the national reference laboratory (NRL) and official control laboratory (OFL) network. Historically, a small number of NRLs and other laboratories already had the capability of analysing these contaminants in food (Fernandes et al., 2004; Driffield et al., 2008; van Leeuwen and de Boer, 2008), but the selection of PBDE analytes as well as the parameters used to define individual methodologies, varied across laboratories. In order to maintain the flexibility of different methodologies and measurement techniques, and also ensure a harmonised, validated approach to the determination of these contaminants, analytical criteria were established to allow reliable determination. These criteria include characteristic analytical validation parameters of unambiguous analyte identification, precision and accuracy of reported data, limits of quantitation as well as supporting parameters such as analyte recovery, linearity of measurement, etc.

The provision of a suitable mechanism to externally validate the newly developed capabilities of the networks laboratories is a logical and supportive following step to the approach. The European Union Reference Laboratory for halogenated POPs in Feed and Food (EURL POPs), already had a long and successful record of conducting proficiency testing (PT) for polychlorinated dibenzo-*p*-dioxins and dibenzofurans (PCDD/Fs) and PCBs over several years, with participant numbers sometimes exceeding 100. Thus the capability for the production of food and animal feed test materials, the testing of homogeneity of these materials, the test material distribution process and evaluation of results was already in place. The capability has been expanded to include the testing of laboratory proficiency in PBDE and HBCDD analysis and will also extend to other brominated contaminants in the future.

This document describes the criteria established in order to allow validated and reliable determination of PBDEs and HBCDDs in food and animal feed for a set of defined analytes. The validity of the approach has been evaluated using PT for both analytes on different food and feed matrices, and the outcome is also described. The analytical criteria will form the basis for the ongoing provision of occurrence data on defined PBDE and HBCDD analytes, using an approach that promotes reliability and can thus support risk assessment and potentially future control measures. Other purposes could include studies on time trends and patterns in order to identify the source(s) of possible contamination particularly during incidents involving such contamination.

2.0 Materials and Methods

2.1 Setting of Analytical Criteria

In 2017, a core working group (CWG) on brominated contaminants was set up by the EURL POPs. The group was composed of experts on the analysis of brominated contaminants including PBDEs, HBCDDs, other emerging BFRs and polybrominated dibenzo-*p*-dioxins and furans (PBDD/Fs), from a number of EU states. The group had two foci: firstly, facilitating reliable and valid analyses of PBDEs and HBCDDs among the National Reference Laboratory (NRL) and subsequently, Official Laboratory (OFL) network of EU member states, and secondly, prioritising emerging brominated contaminants occurrence in food and animal feed for further investigation. Some laboratories within the CWG had prior experience of PBDE and HBCDD analysis and the EC's request for occurrence data (EC, 2014) for risk assessment, provided further impetus to the objective of producing robust and harmonised analytical criteria that would allow the provision of reliable occurrence data. These criteria would also be evaluated by the outcome of PT exercises. The main parameters identified and discussed from the different methodologies used to generate occurrence data were the specificity of the analytical methods,

the working range and limits of quantitation (LOQ), the trueness of the methods based on reference materials as well as proficiency testing, the interim precision of the methods and the measurement uncertainty (U) associated with the obtained results. The criteria relate to the determination of nine PBDE congeners, three HBCDD diastereomers and the corresponding summed concentrations of these analytes (Table 1) and are discussed in the next section. The selection of the main analytes to be monitored was based on their occurrence in food and animal feed, on their abundance in commercial BFR products, and also on their previous selection for monitoring in food and feed within the EU (EC, 2014). Although a wider selection of PBDEs has been investigated in earlier studies on different foods (Houde et al., 2014; Fernandes et al., 2016, 2018; Garcia-Lopez et al., 2018), the analytes listed in Table 1 were seen to be the most prominently occurring PBDEs, and showed only minor differences in summed total concentrations compared to the sum of a wider range of measured PBDE congeners (Fernandes et al., 2018).

Insert Table 1

2.2 Proficiency Testing Exercises (or Inter-laboratory Comparison Exercises)

The EURL POPs regularly organises interlaboratory studies (ILSs) and PTs on the determination of various halogenated POPs, e.g. PCDD/Fs, PCBs, PBDEs and HBCDDs in different feed and food matrices. The objective of these ILSs and PTs is the assessment of the analytical performance of laboratories and the inter-laboratory comparability of results from the analyses of these compounds. The ILSs and PTs are organized for the National Reference Laboratories (NRLs) of EU member states and certain other countries, but are also open to other official control and commercial laboratories that perform the analysis of these contaminants.

For determination of PBDEs and HBCDDs, the PT/ILS test samples are prepared using market available feed and food and are sometimes, partly fortified with the analytes of interest using technical mixtures of PBDEs and HBCDDs. As environmental weathering and metabolism cause changes to the original pattern of HBCDD occurrence in commercial products, food samples of animal origin are partially fortified with HBCDD technical mixtures after thermal isomerisation. Participating laboratories in these ILSs and PTs are requested to determine the individual congeners, diastereomers and sum parameters listed in Table 1. Additionally, the results of screening total HBCDD using GC methods can also be reported.

2.2.1 Reporting of results

Participants are asked to use their own analytical methods and reference standards for identification and quantification, report results for each analyte, and provide the limit of quantitation (LOQ) for each non-quantified analyte. Additionally, method information and laboratory accreditation status to the ISO/IEC 17025 (ISO, 2017A) standard is also requested. Collectively, this information provides metrological traceability of each laboratory's data, allowing consensus values to be determined, which are then used as the assigned values for a particular exercise.

2.2.2 Test material

The test materials used for PTs are generally composed of commonly consumed foods and animal feeds. The homogeneity and stability of these materials is of utmost importance for the reliability of the evaluation of individual laboratory performance. The tests for sufficient homogeneity were performed according to ISO 13528:2015 guidelines (ISO, 2015), in combination with the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories (Thompson et al., 2006). In brief, 10 portions of the test samples were analysed in duplicate for the individual congeners or diastereomers. If naturally contaminated test materials shows sufficient homogeneity for one analyte group, the homogeneity also can be extrapolated to the other analyte groups in this study, due to the similar physico-chemical properties and matrix distribution of non-polar halogenated POPs. The same can also be concluded when the test material is fortified, provided that a single spiking solution containing all the analytes of interest is used.

The stability check on the analytes of interest applying respective storage conditions was performed according to ISO 13528:2015 (ISO, 2015). As concluded for homogeneity testing, once checked, the stability of the test material and the analytes of interest can also be extrapolated to other halogenated POPs due to similar physico-chemical properties.

2.2.3 Statistical evaluation

Statistical evaluation of ILS/PT results was performed according to ISO 13528:2015 (ISO, 2015) guidelines and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories (Thompson et al., 2006). In brief, the determination of the assigned value was performed by estimating the assigned value as the consensus of participants' results. The Huber robust mean was taken as the assigned value after excluding extreme outliers outside the range of ± 50 % of the median of all reported results and examination of the distribution of the remaining results using a histogram and kernel density estimation (Thompson et al., 2006).

The assigned values were calculated for individual PBDE congeners, HBCDD diastereomers and sum parameters including limits of quantification (LOQs) if possible, and additionally for information, the medians of the values reported for all analytes, were also calculated.

For individual congeners including LOQs, assigned values were only calculated according to the above mentioned procedure, if more than 2/3 of all results were above the LOQ and less than 1/3 of all results including LOQs were outside the range of ± 50 % of the median of all reported results. Levels for individual congeners were only taken for evaluation and calculation, if these levels were equal to or above the LOQ; otherwise the LOQ value was taken.

Since there are no traceable reference values available, the assigned values were calculated on the basis of the Huber robust mean of the participants´ results. Therefore, the assigned values were only traceable to the participants´ results. Additionally, all reported results and the results of participants having accreditation according to ISO/IEC 17025 were compared for PBDE and HBCDD sum parameters.

For evaluation of results the z-scores were calculated according to the following formula:

$z = (x - x_a) / \sigma_p$, where

x_a: assigned value

x: participant's result

σ_p: fitness-for-purpose-based standard deviation for proficiency assessment

For PBDE congeners, HBCDD diastereomers and PBDE and HBCDD sum parameters, the standard deviation for proficiency assessment σ_p, was defined as 20 %.

Z-scores for individual congeners and diastereomers were only calculated and reported if the reported concentrations were equal to or above the LOQ.

The Z-scores may be interpreted as follows:

$\lvert$ z-score $\rvert \leq 2$	satisfactory performance
$2 < \lvert$ z-score $\rvert < 3$	questionable performance (warning signal)
$\lvert$ z-score $\rvert \geq 3$	unsatisfactory performance (action signal)

The evaluations of the PT or interlaboratory studies are summarized in preliminary and final reports for the participants. Additionally, participants receive a certificate of analysis with further information on the test and on individual performance.

3.0 Analytical criteria

3.1 General recommendations

In order to achieve and sustain reliable performance for the determination of BFRs, a laboratory should establish the procedures required for successful analysis, validate these procedures using fortified food and feed samples and set-up an ongoing quality control plan for regular sample analysis. The procedures should be supported by performance data collected during validation and during subsequent routine analysis, and externally validated by demonstrating successful participation in inter-laboratory comparisons or PT exercises. The procedures should be documented in detail, e.g. as a standard operating procedure (SOP), so that all laboratory staff can follow these in detail, and any subsequent improvements or changes should be similarly recorded. As an additional measure, laboratories should be accredited, e.g. to the EN ISO/IEC 17025 standard by a recognised body operating in accordance with ISO/IEC 17011:2017 (ISO, 2017B) guidelines, in order to ensure that they are applying routine analytical quality assurance.

In order to maintain their integrity, test samples should be stored and transported under UV-protected conditions (e.g. using amber glassware) in containers that can be demonstrated to be free from PBDEs and HBCDDs. The moisture content should be reduced by drying, taking care to minimise analyte losses during this step and samples should be thoroughly mixed by fine grinding, blending, etc. in order to achieve complete homogenisation and to allow efficient analyte extraction (e.g. solid samples should be reduced to 1 mm particle size).

3.2 Analytical Standards

Analytical reference standards are commercially available for all of the PBDE congeners and HBCDD diastereomers listed in Table 1, and these should be used for calibration and measurement. ^{13}C-isotope labelled analogues are also available for the listed analytes (except BDE-49, at the time of writing), and ideally, all should be used as internal standards (ISs) for quantitation or recovery standards (RSs/syringe standards) for quality control. Internal standards should be introduced to the sample aliquot at the start of analysis. Otherwise, at least one labelled internal standard should be used for each homologue group. Additional ^{13}C-labelled PBDEs and deuterated HBCDDs are also commercially available for use as recovery standards added to the purified sample extracts just before gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry (LC-MS) measurement. In order to compensate for the higher levels of adsorption that are observed for the higher brominated PBDEs in GC-MS systems, two or more temporally spaced recovery standards would provide a more realistic measure of the analytical recovery.

3.3 Procedural Blanks and Control samples

As part of method validation, laboratories should investigate the range of PBDE and HBCDD background arising from reagents, glassware and other sources used in the analytical procedure. Procedural blanks should be included and evaluated during routine analysis, and used to establish the limit of quantitation. This involves carrying out the entire analytical procedure omitting only the sample matrix. Similarly, control samples should also be included as internal quality control measures during routine analysis. The control sample could be a reference material, or in the absence of these, materials from successful and accredited PT exercises or in-house reference materials. The data from these control samples provide a useful indication of the accuracy of the analytical procedure. Quality control (QC) charts for procedural blanks and control samples provide a record and also an indication of ongoing performance of the analytical method and the resulting trends can alert the analyst to any deviations from acceptable performance.

3.4 Limit of quantification

The limit of quantification (LOQ) is a key characteristic of the capability of an analytical method. For the analysis of PBDEs and HBCDDs in food and feed, the LOQs are currently indicated by EC recommendation (EC, 2014), on the monitoring of trace levels of brominated flame retardants in food. However, much of the food occurrence for PBDEs and HBCDDs is below the specified LOQ of

0.01 µg/kg wet weight (w.w.) and this value may be revised in the future according to evaluations resulting from new toxicological studies and hazard assessments (Dishaw et al., 2014; EFSA, 2021). Additionally, data reported above the LOQ, are associated with a lower level of uncertainty in risk assessment in comparison to the ambiguity associated with occurrence levels that are below the LOQ. Thus, for PBDE congeners, although the recommended LOQ value is 0.01 µg/kg w.w. for individual congeners (EC, 2014), a lower LOQ value of 0.001 µg/kg w.w. (for all congeners except BDE-209, as procedural blank levels and chromatographic adsorption makes this currently difficult to achieve) is desirable. For individual HBCDD diastereomers, the indicated LOQs of 0.01 µg/kg w.w. specified for fish and other seafood, meat and meat products, milk and dairy products, eggs and egg products, as well as infant and follow-up formula (EC, 2014), appear to have proven adequate for the most recent evaluation of HBCDD exposure and risk (EFSA, 2021). Practically, the estimation of LOQs requires consideration of the procedural blanks, and contributions from the blank, of levels ≥ 20% compared to the batch sample levels, requires the inclusion of blank levels in the estimation of LOQs. Subtraction of blank concentrations may be performed, if levels remain relatively constant over time. Guidance on estimation of LOQ is given in the guidance document on the estimation of LOD and LOQ for measurements in the field of contaminants in feed and food (EC, 2017A).

3.5 Positive analyte identification

The reliability of the data produced for PBDE and HBCDD analysis depends on unambiguous identification of the GC-MS (PBDEs) or HPLC-MS (HBCDDs) signals during measurement, so it is necessary to specify the requirements for positive identification. The separation of targeted PBDE congeners and HBCDD stereoisomers from interfering matrix and other halogenated compounds, should be carried out by suitable adsorption chromatography techniques (suggested, effective adsorbents are alumina, Florisil™, etc.). Exclusion of matrix interference is highly recommended in order to reduce adsorption during GC-MS measurement of PBDEs and to reduce suppression effects during LC-MS measurement of HBCDDs, as this may lead to incorrect quantitation.

Requirements for positive identification are provided in Table 2 and are given separately for the most common instrument configurations that are currently used.

Insert Table 2

3.6 Precision and trueness of measured analytes

The accuracy of the determined values for PBDEs and HBCDDs may be practically assessed by estimating the precision and trueness of the adopted methods. An initial measure of the precision of the analytical methodology can be obtained from the validation experiments that are used to set up the methodology for PBDE and HBCDD analysis, including the results generated under within-laboratory repeatability and reproducibility conditions. When routine analysis of feed and food samples commences, laboratories are expected to progress to the recording of intermediate precision, obtained e.g. by different analysts, different standards or instrument calibrations over a longer time frame. The coefficient of variation for within-laboratory reproducibility should not be greater than 20 % for all PBDE congeners, HBCDD diastereomers and summed parameters except for BDE-209 where it may be more practical to extend this range to 40 %. Trueness can be estimated from regular analysis of certified reference materials, fortification experiments or through participation in inter-laboratory studies. Given the dearth of CRMs or even RMs, inter-laboratory studies or proficiency testing is a more practical external measure. The trueness for all specified analytes should be within ± 30 %.

3.7 Other measures

The control of analytical recovery is essential for reliable analysis. The recovery of the added internal standards may conveniently be measured, relative to the recovery standards. For both, PBDE congeners as well as HBCDD diastereomers, the recoveries of the individual ISs should be in the range of 40 to 120 % (although for some analytes such as BDE-209, the range may be extended from 30 to 140 %, as a practical interim measure).

The linear range of the measurement process should be established during validation. The lower range of the calibration curve is indicated by the LOQ – 0.01 µg/kg (or targeted LOQ of 0.001 µg/kg for PBDEs). This should extend to between 5.0 and 10 µg/kg w.w., at the higher end of range, reflecting the concentrations for BDE-47 and BDE-209 and in some cases α-HBCDD, that are reported in the current literature (Fernandes et al., 2016).

All determined values in test samples should be expressed as µg/kg wet w.w., rounded to two significant figures, or µg/kg product (optionally, relative to a feed with a moisture content of 12 %) for feed. In order to facilitate comparison and harmonise reporting, PBDE congeners should be listed in the order of increasing IUPAC number (PBDE-28, PBDE-47, PBDE-49, PBDE-99, PBDE-100, PBDE-153, PBDE-154, PBDE-183 and PBDE-209), followed by the summed concentration. Similarly, HBCDD diastereomers should be listed in the conventional order i.e. α-HBCDD, β-HBCDD and γ-HBCDD, followed by the sum. The "<LOQ value" should be included for those congeners/diastereomers that were below the LOQ. In order to maximise the reported information and also allow interpretation of the results according to specific requirements, the upper bound sums (summed concentration of all congeners including the LOQ value for undetected congeners) and lower bound sum (summed concentration of all congeners that were ≥ LOQ), should also be included. If some congeners/diastereomers were detected, but the measured concentrations were below the corresponding LOQ, this additional information could optionally be included in the report, provided that there was evidence that these were not introduced by the analytical procedure.

The uncertainty of measurement should also be reported as an aid to the interpretation of the data. The analytical results shall be reported as x +/– U whereby x is the analytical result and U is the expanded measurement uncertainty using a coverage factor of 2 which gives a level of confidence of approximately 95 %. Measurement uncertainty can be estimated using the guidance provided for PCDD/F and PCB analysis, since this procedure (European Commission, 2017B), can be extended to other contaminants using isotope dilution analysis as has been demonstrated in other studies (Falandysz et al., 2019; Fernandes et al., 2018, 2019; Srebočan et al ., 2019).

A summary of the main analytical criteria for the determination of PBDEs and HBCDD are given in Table 3.

Insert Table 3

4.0 Results of inter-laboratory studies and proficiency tests

During 2019 and 2020, EURL-POPs conducted four PTs on the determination of PBDEs and HBCDDs, analysing 5 different food and feed matrices. Grass (1901-GR, feed) and egg yolk powder (1902-EY, food) were covered in 2019; fish fillet (2001-FI, food), palm fatty acid distillate (2003-FFA, feed) and rapeseed oil (2003-FFB, feed) were used as test materials in 2020. 1901-GR, 1902-EY, 2003-FFA and 2003-FFB were fortified with PBDE and HBCDD technical mixtures, whereas 2001-FI was tested as a naturally contaminated material.

Between 23 and 37 laboratories participated in the PBDE testing, with the highest participation rate being achieved for the fish fillet sample in 2020. In most cases, all laboratories reported results for at least seven of the nine relevant PBDE congeners (Table 1). A lower reporting rate of about 75 % was observed for BDE-49 and BDE-209 in all of the PTs. Over the same period, up to 20 laboratories reported results for HBCDDs in the different matrices tested, with the fish matrix once again seeing the highest participation rate. In addition to α-, β- and γ-HBCDD and the sum of these three diastereomers, a small number of laboratories also reported total HBCDD using GC-methods. An overview of the number of participants for the respective PTs is given in Figure 1.

Insert Figure 1

The quality of the reported data was good enough for assigned values to be calculated for the 9 PBDE congeners (approx. 90%, 40 out of 45 cases) and for the sum parameter (in all cases) in all of the five PTs. The concentrations of the assigned values covered PBDE occurrence over three orders of

magnitude ranging from 0.005 to 6.5 µg/kg for individual congeners. As seen in figure 2, the highest concentrations were associated with BDE-209 and ranged down to the lowest values for BDE-28 and BDE-49.

Insert Figure 2

The quality of the data reported for α-HBCDD allowed calculation of assigned values for 80% (4 out of 5) of the data (Figure 3), but a lower level (<50%) of satisfactory data was reported for the other two diastereomers, β-HBCDD and γ-HBCDD which tended to occur at concentrations that were close to the LOQ. The assigned values ranged from 0.1 to 3 µg/kg. The data also allowed calculation of an assigned value for the sum of α-HBCDD, β-HBCDD and γ-HBCDD in three of the five cases. Due to the low number of reported results for total HBCDD screening by GC-based methods, assigned values could not be calculated for this parameter. Figure 3 summarizes the assigned values for the individual diastereomers.

Insert Figure 3

As described in section 2.2.3, z-scores were calculated for individual analytes and sum parameters. These are an important tool for evaluation and comparison of the overall results submitted to each PT exercise, and also allows participants to evaluate the performance of their individual methodology. For the overall data reported for PBDEs and HBCDDs, a fitness-for-purpose-based standard deviation for proficiency assessment of 20 % was allowed, which results in an acceptable deviation of ± 40% of the assigned value.

Over the five PT exercises run between 2019 and 2020, a total of 1264 z-scores were calculated for individual PBDE congeners and sum parameters. A summary of the overall performance in these exercises expressed as percentages of z-scores achieved by the participants for individual PBDE congeners is illustrated in Figure 4. More than 80% of the reported results show a z-score within the range of ± 2, and this covers all matrix types and concentration ranges. The results reported outside this acceptable range showed a slight tendency by participants to overestimate reported data (a higher percentage was found for z-scores above +2), possibly due to elevated method LOQs or procedural blank levels.

Insert Figure 4

Due to the lower number of participants in the five HBCDD exercises, assigned values and resulting z-scores could only be calculated for a total of 144 results. In the cases where this was possible, more than 90% of results for individual diastereomers were in the range of ± 2 z-scores as summarised in Figure 5. It was not possible to calculate either an assigned values or z-scores for the results of total HBCDD screening by GC-based methods. However, a comparison of the results for total HBCDD with the assigned values for the sum of α-, β-and γ-HBCDD showed very good agreement between both parameters. A provisional estimation of the data for total HBCDDs showed a narrow distribution range that would have corresponded to z-scores of between ± 2 for the nine reported sets.

Insert Figure 5

5.0 Discussion

It was noted, at the time when the EC initiated the process to set-up an EURL for halogenated POPs, that a number of laboratories within the NRL network already had the capability to conduct validated

analyses for PBDEs and HBCDDs in food and animal feed (Fernandes et al., 2004; Pirard et al., 2005; van Leeuven and de Boer, 2008, Antignac et al., 2009). Some of these had been in use for more than a decade and involved different analytical methods, instrumentation and reported different sets of analytes. In recent years, there has been a diversification in GC-MS instrumentation from the classical high resolution dual sector mass spectrometers, to multiple quadrupoles-, ion-trap-, time-of-flight- etc. detectors, and also a greater emphasis on automated methods for sample extraction and purification. It was therefore logical that a specific methodology, instrumentation and conditions of analysis should not be imposed on the network's laboratories. Instead it was decided that a framework of analytical criteria with specific performance outcomes would be defined that would ensure that any extraction methodology and instrument technique would be able to initially demonstrate adequate performance through a validation procedure and subsequently maintain this performance during routine analysis. The quality of this performance could be verified by an ongoing series of PT. In addition to allowing the network's laboratories the flexibility of using available instrumentation and adapting or developing their own analytical methods, the approach also accommodates continuous improvements and efficiencies in the different methodologies as newer instrumentation and techniques emerge. The specification of a common set of analytes and criteria for satisfactory performance, also allows harmonisation of the data that is generated by different laboratories, streamlining the evaluation of occurrence levels during risk assessment.

Much of the good performance and competence seen in the PTs on PBDEs can be attributed to the experience of the network's laboratories with ultra-trace analysis. Almost all of the laboratories were able to draw on their substantial analytical experiences with PCDD/F analysis (the EURL POPs network was originally drawn up as a PCDD/F and PCB group, more than a decade earlier) and extend this to PBDEs. Indeed many of the methods currently used for PBDEs have been adapted from the original PCDD/F and PCB methods. They thus have an inherent level of quality control already built-in, which could be extended to PBDE analysis with relative ease.

With the publication of the Commission Recommendation on the monitoring of traces of brominated flame retardants in food (EC, 2014) in 2014, which included target LOQs for the different groups of brominated contaminants, the external quality control of analytical results within the EU became more important. In the same year, the EURL POPs initiated its first PT (Schächtele et al., 2015) on brominated flame retardants using cod liver and fish liver oil as the test matrices, and including most of the BFRs listed in the Commission Recommendation. In this initial PT on BFRs, up to 27 laboratories reported results for PBDEs and 11 laboratories responded for HBCDDs. Encouragingly, most of the participant's z-scores were within the range of +/- 2 for both test materials and both analyte groups, indicating that a number of the networks laboratories were already using well-established and validated methods at this time. Depending on the type of test material used, the number of participants for these two groups increased over the following years, especially for HBCDDs.

This increase was seen despite the fact that speciated analysis for the HBCDD stereoisomers, required the use of LC-based instrumentation, which is not commonly (or not at all) used for PCDD/F and PCB analysis. HBCDD methods for food and feed analysis were therefore required to be independently established and validated. However some of the networks laboratories (Fernandes et al., 2008, 2016; van Leeuven et al., 2008; Törnkvist et al., 2011; Fournier et al., 2012) had already developed the capability for food and feed analysis of HBCDDs – a collective experience that was used to establish parameters for reliable analysis. The requirement for independent methods has meant that the establishment of analytical capability for HBCDDs and participation in PTs has been at a lower rate than for PBDEs. Additionally, while assigned values for individual HBCDD diastereomers in these PTs, could be established at higher concentrations (above 0.1 µg/kg), this was not possible when test material concentrations were close to the target LOQ of 0.01 µg/kg. Laboratory performance in some cases could therefore not be formally assessed. This outcome contrasted with the PT data reported for PBDEs where assigned values could be calculated over a wide range of concentrations including the LOQ. In terms of analytical methods for HBCDDs, further improvement of the analytical sensitivity in the very low concentration range around the target LOQ appears to be indicated for some laboratories.

The update on the HBCDD risk assessment in foods (EFSA, 2021) identified a requirement for more data on HBCDD occurrence in foods. Just under 50% of the networks laboratories currently have analytical capability for HBCDDs (which explains the lower number of participants in the HBCDD PTs) but a good proportion of the remainder are currently in the process of setting-up this capability.

The analytical criteria and proficiency testing for PBDEs and HBCDD described here are a first step in ensuring that reliable data of good quality will be available for risk assessment and potentially for future regulation of food and feed levels. The toxicology that supports the future direction of both these functions continues to grow and although the use of both PBDEs and HBCDDs is now restricted in Europe, imported and recycled items continue to be a source of exposure (Straková et al., 2018; Fatunsin et al., 2020), along with long lived domestic and commercial furnishing, as well as synthetic recycled materials. Continued monitoring of the food and animal feed supply for the presence of these BFRs is therefore prudent.

6.0 Conclusions

The guidance provided in these criteria combined with the confirmation of satisfactory performance in the PTs (as an external assessment) on PBDE and HBCDD analysis will allow the provision of reliable data for risk assessment and would also provide a strong basis for any future regulatory actions. In a wider sense, this combined approach to provide more reliable data for as yet unregulated contaminants is a measure of the proactivity of the EURL POPs in tackling newer contaminants like these endocrine disrupting chemicals. It is also supportive of the EU's chemicals strategy for sustainability towards a toxic-free environment (European Commission, 2020).

The data produced following this guidance would allow easy comparability of PBDE and HBCDD occurrence among different EU member state NRLs, OFLs and other laboratories that use the guidance. Additionally it would provide a reliable comparison of regional variations in PBDE and HBCDD occurrence in food and feed.

The criteria given for PBDEs and HBCDD also serve as a template for the inclusion of other brominated contaminants, many of them currently in use or those that are inadvertently produced such as PBDD/Fs, which are currently under consideration by the working group on brominated contaminants. Occurrence data reported for some of these contaminants in food (Fernandes and Falandysz, 2021; Venisseau et al., 2018; Zacs et al., 2021) is supported by quality assurance measures but these would benefit from a similar measure of harmonisation as described here.

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Tables 1 - 3

Table 1: Selected PBDE and HBCDD analytes to be monitored, and sum parameters.

Analyte	Description	CAS Number
BDE - 28	2,2′,4-tribromodiphenyl ether	41318-75-6
BDE - 47	2,2′,4,4′-tetrabromodiphenyl ether	5436-43-1
BDE - 49	2,2′,4,5′-tetrabromodiphenyl ether	243982-82-3
BDE - 99	2,2′,4,4′,5-pentabromodiphenyl ether	60348-60-9
BDE - 100	2,2′,4,4′,6-pentabromodiphenyl ether	189084-64-8
BDE - 153	2,2′,4,4′,5,5′-hexabromodiphenyl ether	68631-49-2
BDE - 154	2,2′,4,4′,5,6′-hexabromodiphenyl ether	207122-15-4
BDE - 183	2,2′,3,4,4′,5′,6-heptabromodiphenyl ether	207122-16-5
BDE - 209	2,2′,3,3′,4,4′,5,5′,6,6′-decabromodiphenyl ether	1163-19-5
[A]Σ_9-PBDEs	**Summed concentration of the above nine PBDE congeners**	
α-HBCDD	(1,2,5,6,9,10-hexabromo-(1R,2R,5S,6R,9R, 10S)-rel-cyclododecane	134237-50-6
β-HBCDD	(1,2,5,6,9,10-hexabromo-(1R,2S,5R,6R,9R,10S)- rel-cyclododecane	134237-51-7
γ-HBCDD	(1,2,5,6,9,10-hexabromo-(1R,2R,5R,6S,9S, 10R)- rel-	134237-52-8
Σ-HBCDD	**Summed concentration of α-, β- and γ-HBCDD**	
Total HBCDD	**Cumulative response of all HBCDD diastereomers, measured using GC-based detection methods (screening method)**	

[A]As an interim measure, the sum of the 8 PBDEs (**Σ_8-PBDEs,** excluding BDE-209) may also be reported by those laboratories that are currently developing the capability to measure BDE-209.

Table 2: Criteria for positive identification of PBDE congeners and HBCDD diastereomers

Analyte	Instrumentation	Identification/Confirmation Criteria
PBDEs	**GC-HRMS**	
	Mass resolution	≥ 10 000 at 10 % valley (entire mass range)
	Identification Confirmation	Simultaneity of analyte retention relative to IS; i.e. -1/+2 sec.
		Simultaneity of response of monitored ions for each PBDE; 2 ions should be monitored from the same isotopic cluster
	Isotope ratio	± 20 % of the theoretical value or of the corresponding reference standard
	GC-MS/MS	
	Mass resolution	Unit mass (both quadrupoles) or wider resolution for Q1 and unit mass for Q3 as established during validation
	Identification Confirmation	Simultaneity of analyte retention, relative to IS; i.e. -1/+2 sec.
		Simultaneous response for both analyte transitions; 2 transitions (including one precursor ion and one product ion each) e.g. from the same isotopic cluster should be monitored
	Transition response ratio	± 20 % relative to mean ratio of calibration standards
HBCDD	**HPLC-HRMS**	
	Mass resolution	> 10 000 at 50% FWHM (full width at half maximum height)
	Identification Confirmation	Simultaneity of analyte retention relative to IS; i.e. not greater than ± 1 %
		Simultaneity of response of monitored ions for each HBCDD 2 isotopic ions should be monitored from the same isotopic pattern
	Isotope ratio	± 20 % of the theoretical value or of the corresponding reference standard
	HPLC-MS/MS	
	Mass Resolution	Unit resolution. Quadrupoles set to unit mass or wider resolution for Q1 and unit mass for Q3 as established during validation
	Identification Confirmation	Simultaneity of analyte retention relative to IS; i.e. not greater than ± 1 %

		Simultaneous response for both analyte transitions 2 transitions (including one precursor ion and one product ion each) e.g. from the same isotopic pattern should be monitored
	Transition response ratio	± 20% relative to mean ratio of calibration standards

Table 3. Summary of the main analytical criteria for PBDE and HBCDD analysis

Parameter	**PBDE congeners**	**BDE-209**	**HBCDD diastereomers**
Trueness	≤ 30 %	≤ 30 %	≤ 30 %
Reproducibility (precision)	≤ 20 %	≤ 40 %	≤ 20 %
Limit of quantification (wet weight basis)	0.01 µg/kg Target LOQ: 0.001 µg/kg	0.01 µg/kg	[A]0.01 µg/kg
Internal Standard Recovery	40 – 120 %	30 – 140 %	40 – 120 %

[A] for total HBCDD screening by GC-MS techniques, the corresponding LOQ is 0.003 µg/kg

Figures 1-5

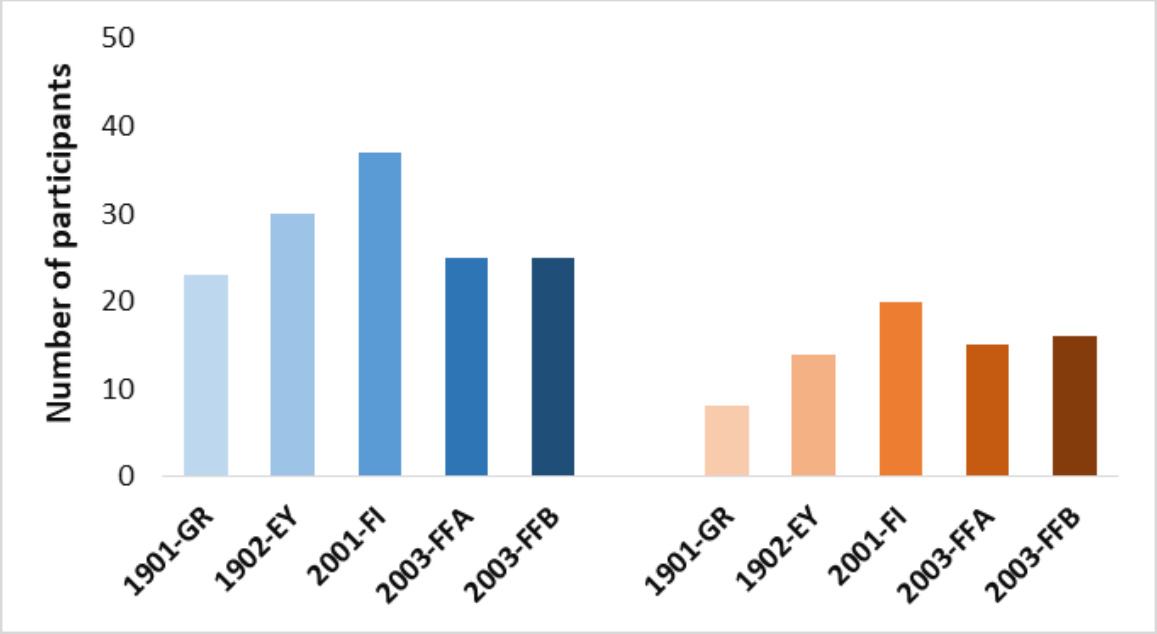

Figure 1: Number of participants for PBDEs and HBCDDs in EURL proficiency tests in 2019 and 2020 (1901-GR: grass, 1902-EY: egg yolk powder, 2001-FI: fish fillet, 2003-FFA: palm fatty acid distillate, 2003-FFB: rapeseed oil)

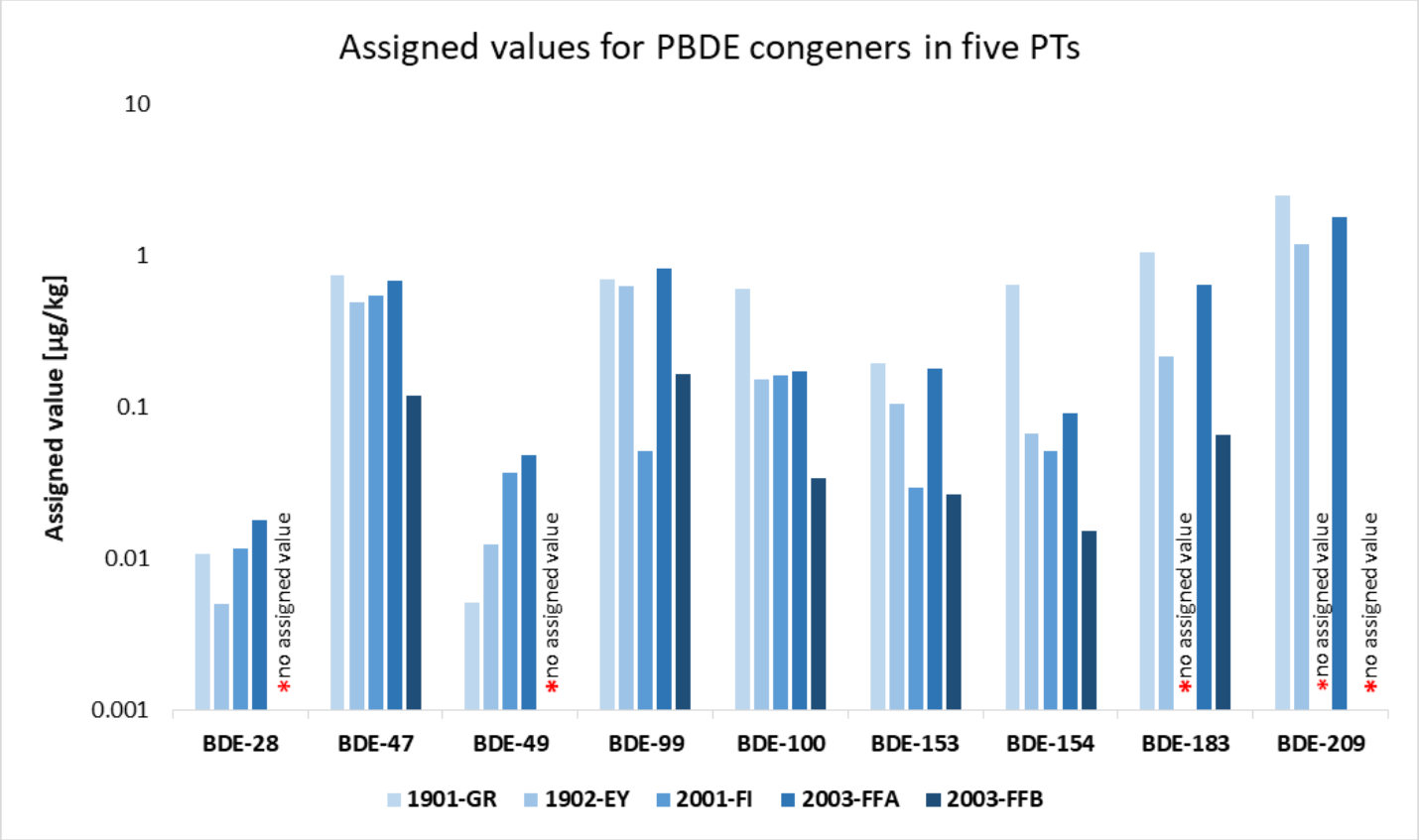

Figure 2: Assigned values (µg/kg) for individual PBDE congeners in 5 EURL PT materials (1901-GR: grass, 1902-EY: egg yolk powder, 2001-FI: fish fillet, 2003-FFA: palm fatty acid distillate, 2003-FFB: rapeseed oil)

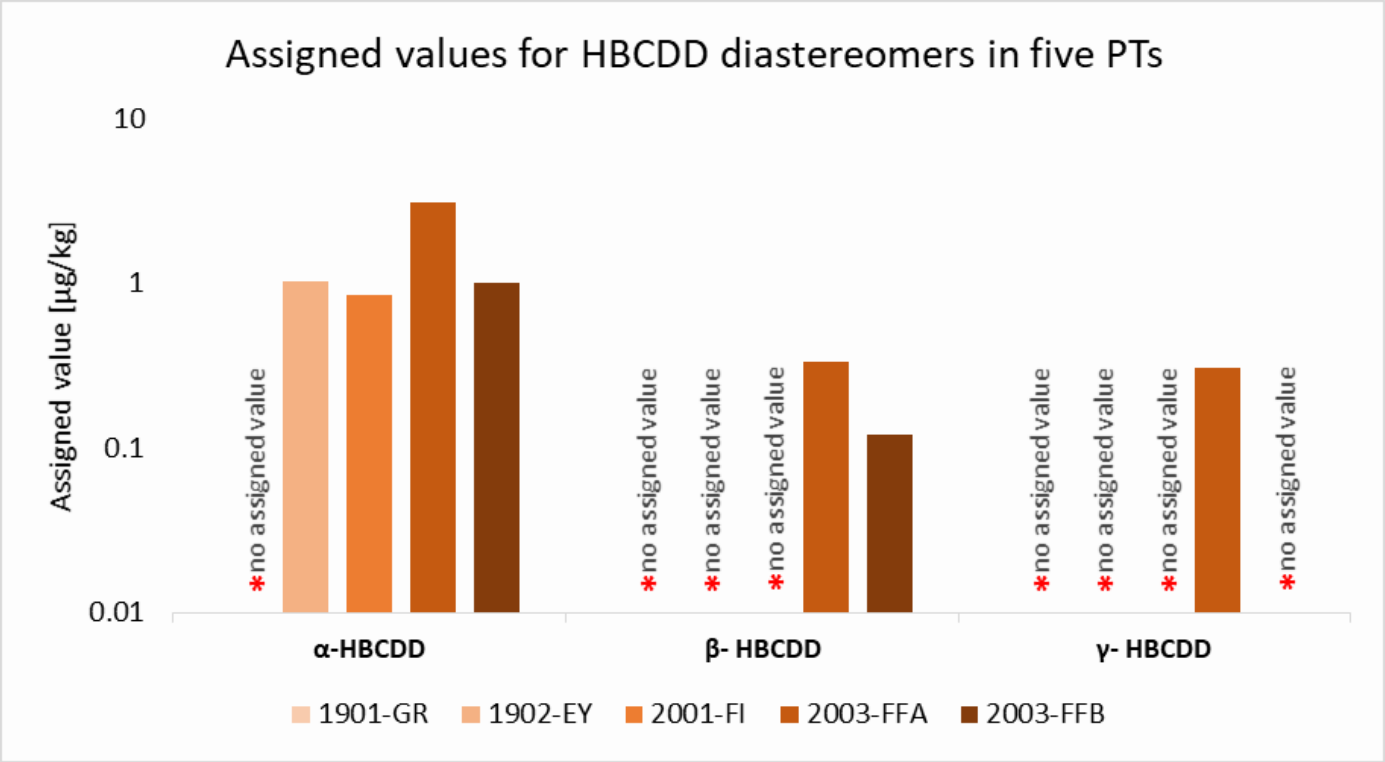

Figure 3: Assigned values (µg/kg) for individual HBCDD diastereomers in 5 EURL PT materials (1901-GR: grass, 1902-EY: egg yolk powder, 2001-FI: fish fillet, 2003-FFA: palm fatty acid distillate, 2003-FFB: rapeseed oil)

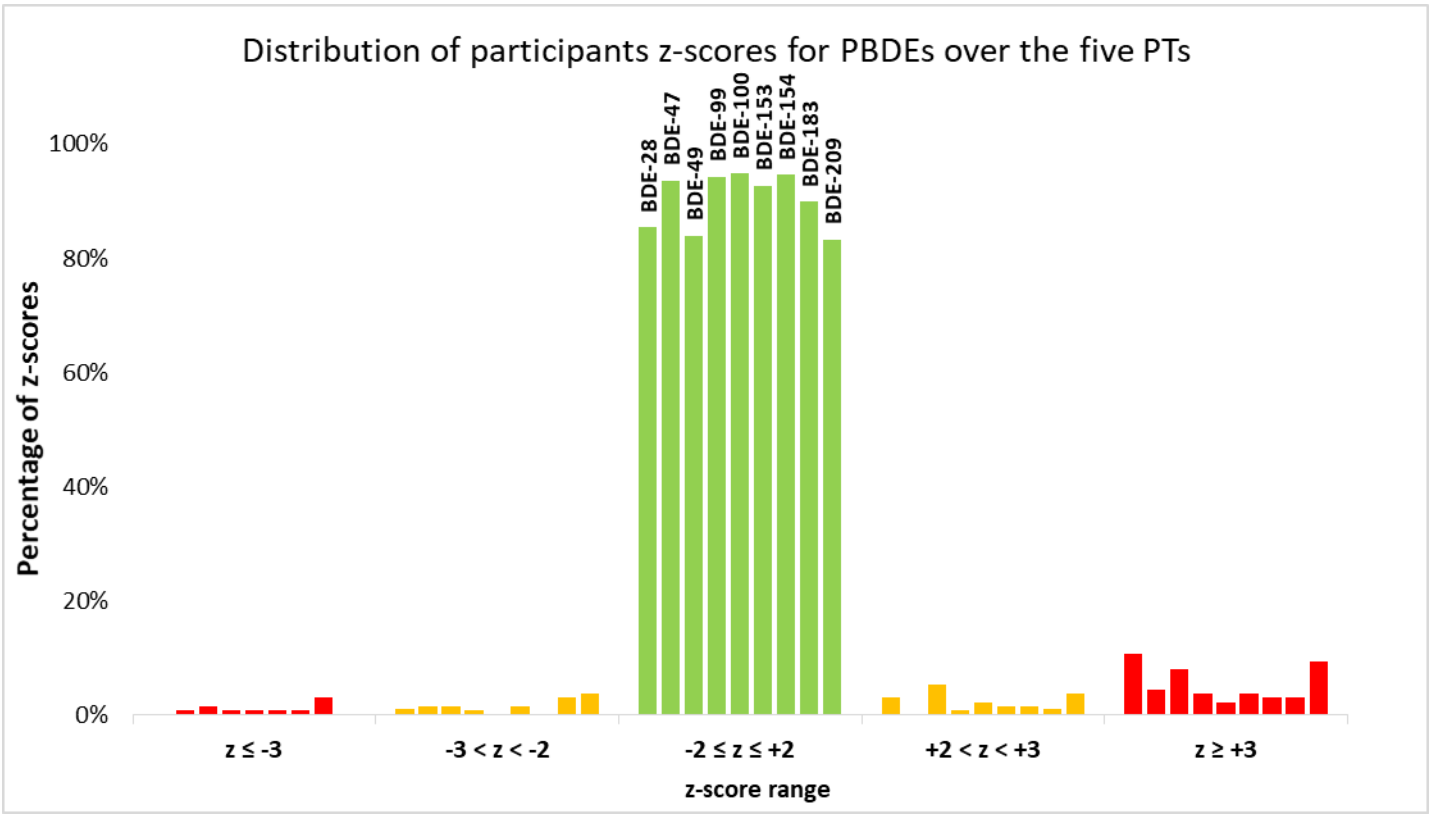

Figure 4: Percentage of z-scores for 9 individual PBDE congeners in the different ranges (z-score ≤ -3, -3 < z-score < -2, -2 ≤ z-score ≤ +2, +2 < z-score < +3, z-score ≥ +3) for a total of 1020 results.

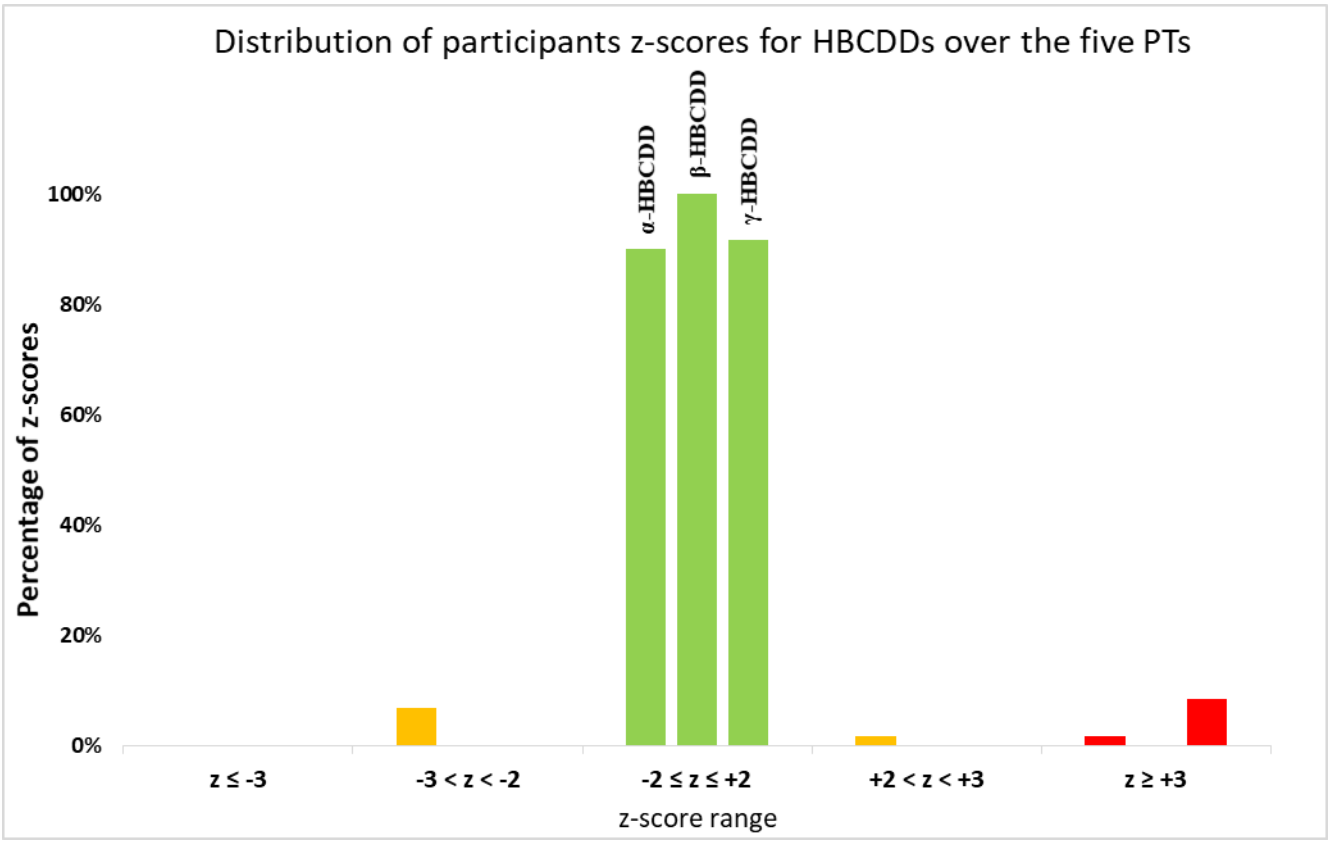

Figure 5: Percentage of z-scores for the 3 individual HBCDD diastereomers in the different ranges (z-score ≤ -3, -3 < z-score < -2, -2 ≤ z-score ≤ +2, +2 < z-score < +3, z-score ≥ +3) for a total of 97 results.