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# EURL Proficiency Test on the Determination of PCDD/Fs, PCBs and BFRs in Feed fat 2020

***EURL-PT-DPB-2003-FF***

# FEED

## Report

## PBDEs and HBCDDs

(Version 1.0)

**23 March 2021**

This report on the EURL Proficiency Test on the Determination of PCDD/Fs, PCBs and BFRs in Feed fat [EURL-PT-DPB_2003-FF] organized by the EURL for halogenated Persistent Organic Pollutants (POPs) in Feed and Food is only available as pdf-version. The forwarding and reproduction of this report is permitted only as entire document, including 11 annexes.

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## Summary


| | |
|---|---|
| **Test samples (feed)** | Palm fatty acid distillate – 2003-FFA<br>Rapeseed oil – 2003-FFB |
| **Analytes of interest** | Mandatory for NRLs:<br>- PBDEs (BDE-28, BDE-47, BDE-49, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, BDE-209)<br>- HBCDDs (α-HBCDD, β-HBCDD, γ-HBCDD) |
| **Methods** | PBDEs:<br>Any kind of method<br>HBCDDs:<br>Any kind of method |
| **Participants** | NRLs, OFLs, other official laboratories, commercial laboratories performing the analysis of samples taken by feed business operators |
| **Statistical evaluation** | ISO 13528:2015, IUPAC Protocol |
| **Report** | 23 March 2021 |

# 1. Structure of the PT, test material and analytes

This proficiency test (PT) on the determination of PCDD/Fs, PCBs, PBDEs and HBCDDs in feed fat was organized by the EURL for halogenated Persistent Organic Pollutants (POPs) in Feed and Food to be performed between June and October 2020. The objective was to assess analytical performance of laboratories and the interlaboratory comparability of results from analyses of PCDD/Fs, PCBs, PBDEs and HBCDDs in one sample of palm fatty acid distillate and one sample of rapeseed oil.

National Reference Laboratories (NRLs) for Halogenated POPs in Feed and Food from EU member states were requested to participate as part of their work programme for 2020. NRLs were invited to encourage the participation of Official Laboratories (OFLs) from their member states as part of their duties following Article 101 of regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017. Furthermore, participation of OFLs will allow the extension of the data basis for calculation of assigned values and evaluation of results.

This PT was also open for other official laboratories and commercial laboratories per-forming the analysis of samples taken by feed business operators in order to check the comparability of results not only within the EURL/NRL/OFL network, but also with official and private laboratories performing official control or self-control of feed business operators.

The evaluated results were discussed by representatives of EU Commission, NRLs and the EURL at the COM/EURL/NRL workshop in November 2020.

## 1.1 Samples and coding

The feed fat test samples were prepared of regular market feed and partly fortified with the analytes of interest using PCDD/F standards and technical mixtures of PCBs, PBDEs and HBCDDs.
The concentrations for PCDD/Fs, PCBs, PBDEs and HBCDDs in the test samples were partly in the lower concentration range reflecting also possible lowering of legal limits for PCDD/Fs and PCBs in the future.

| | |
|---|---|
| **Palm fatty acid distillate** | **Sample no. 2003-FFA-xxx** |
| **Rapeseed oil** | **Sample no. 2003-FFB-xxx** |

Each participant received about 20 g of each test sample.

## 1.2 Analytes of interest

NRLs for halogenated POPs in feed and food are requested to determine the following parameters:

PBDEs and HBCDDs:

- PBDEs: BDE-28, BDE-47, BDE-49, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, BDE-209
- Sum of 8 PBDEs (without BDE-209), sum of 9 PBDEs (with BDE-209)
- HBCDDs: α-HBCDD, β-HBCDD, γ-HBCDD
- Sum of α-, β-, γ-HBCDD or total HBCDD (using GC methods)

## 1.3 Coding of laboratories and confidentiality

The laboratory code of the participating laboratories will be kept confidential and will not be revealed to other participants.

For NRLs, the “Protocol for management of underperformance in comparative testing and/or lack of collaboration of National Reference Laboratories (NRLs) with Community reference laboratories (CRLs) activities” will be observed. The confidentiality of NRLs will be kept according to this protocol.

The identity of OFLs will be kept confidential, unless a Member State initiated a co-operation between the NRL, OFLs and the EURL.

## 1.4 Results of PBDEs and HBCDDs

Laboratories should:

- use their own reference standards for identification and quantification,
- report results for each analyte,
- report the limit of quantification (LOQ), at least for each non-quantified analyte,
- give method information and
- give information about the accreditation of the laboratory according to ISO/IEC 17025 *(for metrological traceability of consensus values of participants used as assigned values)*.

Results had to be reported in µg/kg, relative to a feed with a moisture content of 12 %, for PBDEs and HBCDDs.

## 2. Participating laboratories

This proficiency test was open for participation of:

- National Reference Laboratories (NRLs) of EU member states
- National Reference Laboratories of other European countries
- Official laboratories
- Commercial laboratories

Table 1: Participating laboratories

| Participating laboratories | Region | No. of participants |
|---|---|---|
| National Reference Laboratories | European Union | 16 |
| | Other Countries | 0 |
| Official Laboratories | European Union | 7 |
| | Other European Countries | 0 |
| | Africa | 0 |
| | Americas | 0 |
| | Asia | 0 |
| | Oceania | 0 |
| Commercial Laboratories | European Union | 2 |
| | Other European Countries | 0 |
| | Africa | 0 |
| | Americas | 0 |
| | Asia | 0 |
| | Oceania | 0 |
| | **Total** | **25** |

## 2.1 Number of reported results

Table 2: Reported results for PBDEs

| Reported results | Palm fatty acid distillate (2003-FFA) | Rapeseed oil (2003-FFB) |
|---|---|---|
| **BDE-28** | 25 | 25 |
| **BDE-47** | 25 | 25 |
| **BDE-49** | 21 | 21 |
| **BDE-99** | 25 | 25 |
| **BDE-100** | 24 | 25 |
| **BDE-153** | 25 | 25 |
| **BDE-154** | 24 | 24 |
| **BDE-183** | 25 | 25 |
| **BDE-209** | 19 | 19 |
| **Sum of 8 PBDEs (without BDE-209)** | 25 | 25 |
| **Sum of 9 PBDEs (with BDE-209)** | 19 | 19 |

Table 3: Reported results for HBCDDs

| Reported results | Palm fatty acid distillate (2003-FFA) | Rapeseed oil (2003-FFB) |
|---|---|---|
| **α-HBCDD** | 15 | 16 |
| **β-HBCDD** | 15 | 16 |
| **γ-HBCDD** | 15 | 16 |
| **Sum of α-, β-, γ-HBCDD** | 14 | 15 |
| **Total HBCDD (using GC methods)** | 2 | 2 |

## 2.2 Accreditation

Table 4: Reported accreditation according to ISO/IEC 17025 by participants for PBDEs and HBCDDs

| **Palm fatty acid distillate (2003-FFA)** | **PBDEs** | **HBCDDs** |
|---|---|---|
| yes | 16 | 4 |
| no | 8 | 12 |

| **Rapeseed oil (2003-FFB)** | **PBDEs** | **HBCDDs** |
|---|---|---|
| yes | 16 | 4 |
| no | 8 | 12 |

# 3. Detection methods

The following detection methods were applied:

- GC-HRMS-, GC-MS/MS-methods for PBDEs
- GC-HRMS-, GC-MS/MS-, LC-MS/MS-, LC-HRMS-methods for HBCDDs

Table 5: Overview of chromatographic separation and detection methods for PBDEs and in palm fatty acid distillate (2003-FFA) and rapeseed oil (2003-FFB)

| **Detection methods** | **PBDEs** | **HBCDDs** |
|---|---|---|
| GC-HRMS | 16 | 2 |
| GC-MS/MS | 9 | - |
| GC-LRMS | - | - |
| LC-MS/MS | - | 14 |
| LC-HRMS | - | 2 |

## 4. Homogeneity and stability of the test material

The test for sufficient homogeneity was performed according to ISO 13528:2015 [2] and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [1].

Therefore, 10 portions of the test samples 2003-FFA were analyzed in duplicate for PBDEs. The test for sufficient homogeneity was performed for the individual congeners. The test materials showed sufficient homogeneity for PBDEs in this proficiency test. This can also be concluded for HBCDDs. Homogeneity for the test sample 2003-FFB for PBDEs and HBCDDs can be concluded from the homogeneity of PCDD/Fs and PCBs due to the use of the same spiking solutions for all analytes of interest.

The stability check of the analytes of interest applying room temperature storage was performed according to ISO 13528:2015 [2] for PCDD/Fs and PCBs. The test materials showed sufficient stability for PCDD/Fs and PCBs and can be concluded also for PBDEs and HBCDDs due to similar physico-chemical properties.

## 5. Determination of the assigned values

Statistical evaluation of the PT results is performed by the EURL for Halogenated POPs in Feed and Food according to ISO 13528:2015 [2] and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [1].

The determination of the assigned value is performed according [1] by estimating of the assigned value as the consensus of participants' results (using only results of physico-chemical methods). The Huber robust mean is taken as 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 histogram and kernel density estimation, if necessary.

The assigned value is calculated for individual PBDE congeners and HBCDD diastereomers (including limits of quantification (LOQs)), if possible. Additionally the median of all values is calculated.

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

Assigned values could not be calculated for total HBCDD in sample 2003-FFA and BDE-28, BDE-49, BDE-209 and γ- HBCDD in sample 2003-FFB due to the high variation of participants' results and/or the high number of reported LOQs.

Since there are no traceable reference values available, the assigned values in this PT were calculated on the basis of the Huber robust mean of the results of the participants. Therefore, the assigned values are only traceable to the results of the participants. Additionally the results of all participants reporting results and the results of participants having accreditation according ISO/IEC 17025 were compared for PBDE and HBCDD sum parameters. No significant differences between the assigned values calculated for both data sets for PBDEs were observed.

Table 6: Comparison of assigned values for all participants and participants with reported accreditation according to ISO/IEC 17025 for PBDE and HBCDD sum parameters

| **Palm fatty acid distillate (2003-FFA)** | **Assigned value** **All participants** | **Assigned value** **ISO/IEC 17025 accreditation** | **Deviation** |
|---|---|---|---|
| | µg/kg product* | µg/kg product* | % |
| Sum of PBDE without BDE-209 ub | 2.62 | 2.56 | 2 |
| Sum of PBDE including BDE-209 ub | 4.39 | 4.31 | 2 |
| Sum of α-, β-, γ-HBCDD ub | 3.71 | 3.69 | 1 |

* relative to a feed with a moisture content of 12 %

| **Rapeseed oil (2003-FFB)** | **Assigned value** **All participants** | **Assigned value** **ISO/IEC 17025 accreditation** | **Deviation** |
|---|---|---|---|
| | µg/kg product* | µg/kg product* | % |
| Sum of PBDE without BDE-209 ub | 0.438 | 0.420 | 4 |
| Sum of PBDE including BDE-209 ub | 0.868 | 0.963 | 11 |
| Sum of α-, β-, γ-HBCDD ub | 1.28 | 1.25 | 2 |

* relative to a feed with a moisture content of 12 %

## 5.1 Sum parameters and individual congeners/diastereomers

The assigned values for the test samples 2003-FFA and 2003-FFB were calculated as consensus of participants' results for the PBDE and HBCDD sum parameters.

Table 7: Assigned values for physico-chemical methods for PBDE and HBCDD sum parameters (rounded to three significant figures)

| Test sample | Sum of PBDE without BDE-209 ub | Sum of PBDE including BDE-209 ub | Sum of α-, β-, γ-HBCDD upper bound | Total HBCDD (using GC-methods) |
|---|---|---|---|---|
| | µg/kg product* | µg/kg product* | µg/kg product* | µg/kg product* |
| Palm fatty acid distillate (2003-FFA) | 2.62 | 4.39 | 3.71 | - |
| Rapeseed oil (2003-FFB) | 0.438 | 0.868 | 1.28 | - |

* relative to a feed with a moisture content of 12 %

Polybrominated diphenyl ethers (PBDEs)

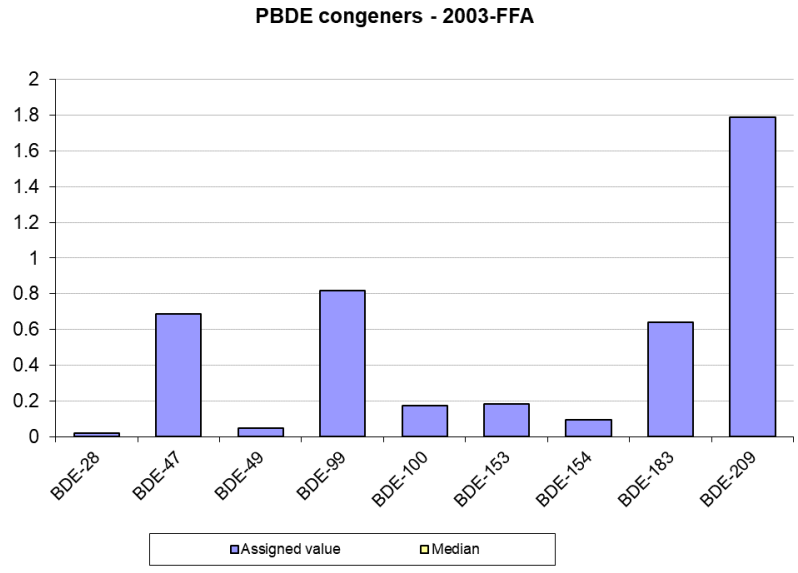


Figure 1: Assigned and median values for PBDE congeners for palm fatty acid distillate (2003-FFA) [µg/kg product, relative to a feed with a moisture content of 12 %]

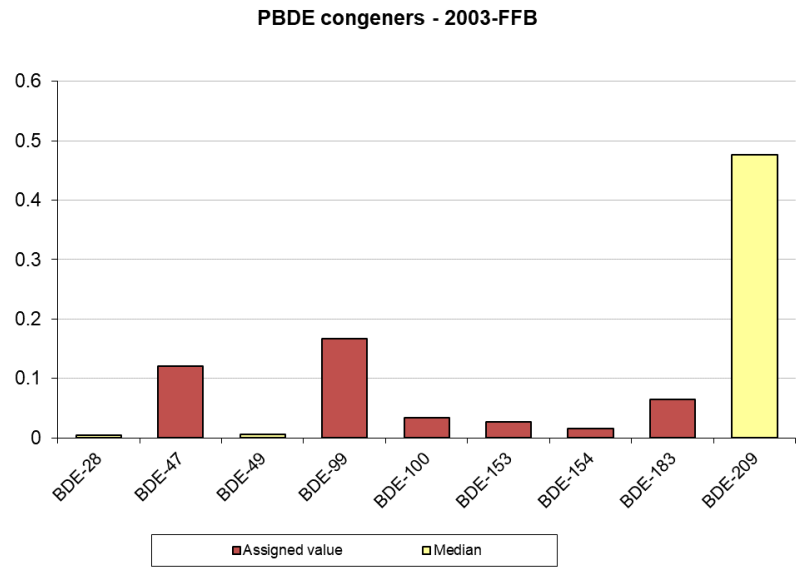


Figure 2: Assigned and median values for PBDE congeners for rapeseed oil (2003-FFB) [µg/kg product, relative to a feed with a moisture content of 12 %]

## Hexabromocyclododecanes (HBCDDs)

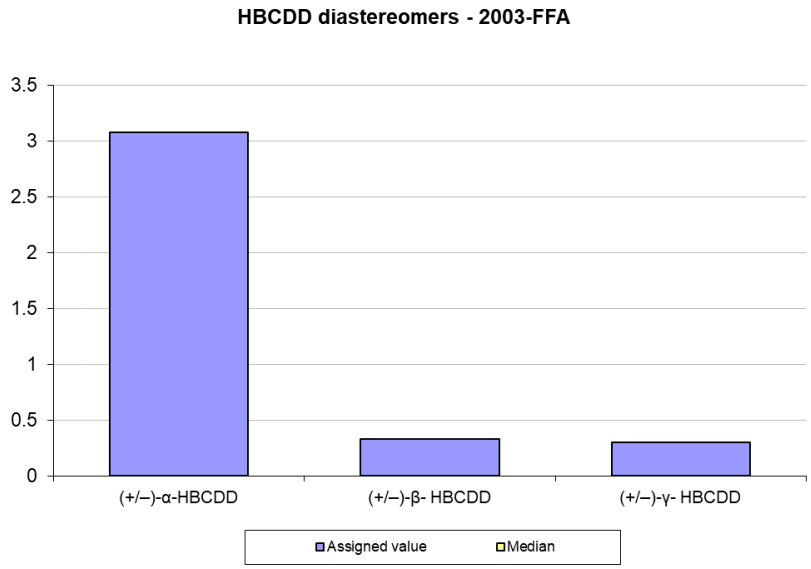


Figure 3: Assigned and median values for HBCDD diastereomers for palm fatty acid distillate (2003-FFA) [µg/kg product, relative to a feed with a moisture content of 12 %]

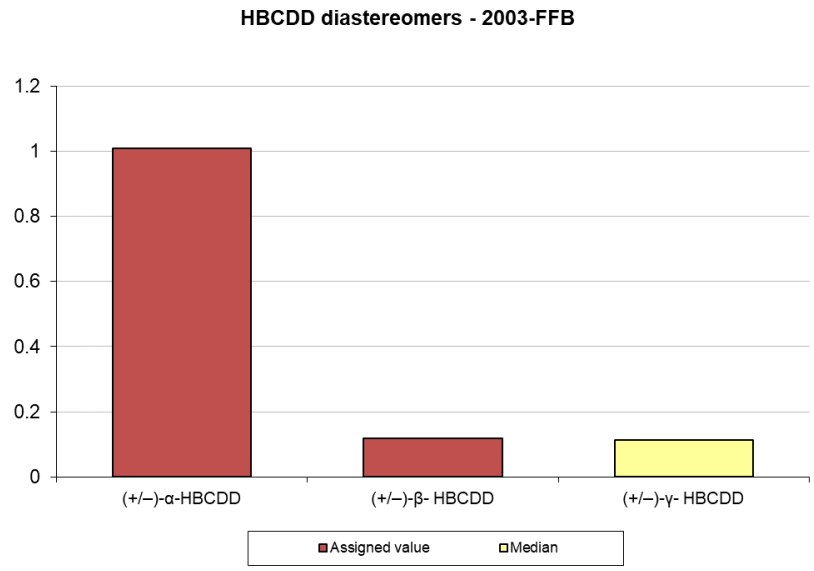


Figure 4: Assigned and median values for HBCDD diastereomers for rapeseed oil (2003-FFB) [µg/kg product, relative to a feed with a moisture content of 12 %]

# 6. Evaluation of results

## 6.1 Z-score calculation

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

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

$x_a$: assigned value

x: participant' s result

$\sigma_p$: 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 $\sigma_p$ is defined as 20 %.

Z-scores for individual congeners and diastereomers are only calculated and reported if levels for these congeners are equal to or above the LOQ. Otherwise no z-scores will be given.

Interpretation of z-scores:

| | |
|---|---|
| $\lvert$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) |

## 6.2 PBDEs and HBCDDs - Participants' z-scores

Polybrominated diphenyl ether (PBDE)

Table 8: Distribution of participants' z-scores for PBDE sum parameters for palm fatty acid distillate (2003-FFA)

| Percentage of participants' results | \|z-score\| ≤ 2 | 2 < \|z-score\| < 3 | \|z-score\| ≥ 3 |
|---|---|---|---|
| **Sum of PBDE without BDE-209** | 88 % | 4 % | 8 % |
| **Sum of PBDE including BDE-209** | 83 % | 6 % | 11 % |

Table 9: Distribution of participants' z-scores for PBDE sum parameters for rapeseed oil (2003-FFB)

| Percentage of participants' results | \|z-score\| ≤ 2 | 2 < \|z-score\| < 3 | \|z-score\| ≥ 3 |
|---|---|---|---|
| **Sum of PBDE without BDE-209** | 82 % | 0 % | 18 % |
| **Sum of PBDE including BDE-209** | 63 % | 11 % | 26 % |

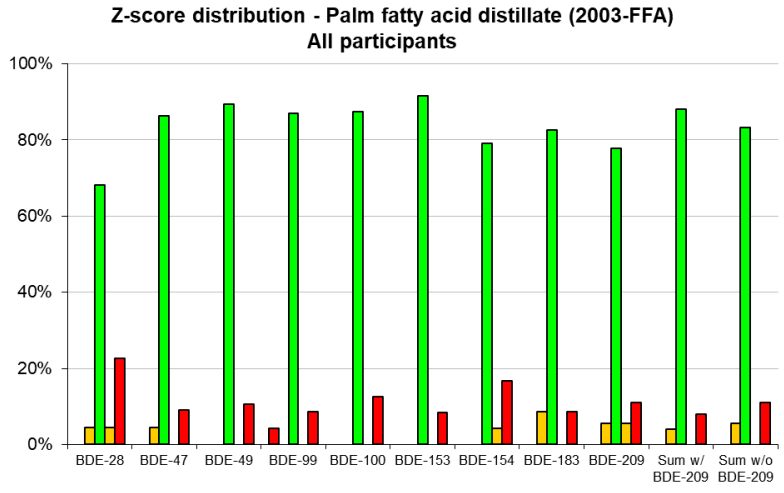


Figure 5: Distribution of participants' z-scores for PBDE congeners / sum parameters for palm fatty acid distillate (2003-FFA)
[Green bars: -2 ≤ z-score ≤ 2, yellow bars: -3 < z-score < -2, 2 < z-score < 3, red bars: z-score ≤ -3, z-score ≥ 3]

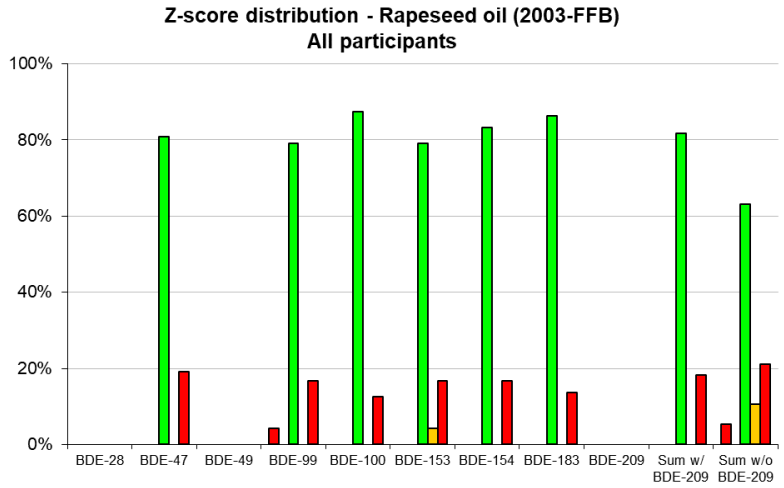


Figure 6: Distribution of participants' z-scores for PBDE congeners / sum parameters for rapeseed oil (2003-FFB)
[Green bars: -2 ≤ z-score ≤ 2, yellow bars: -3 < z-score < -2, 2 < z-score < 3, red bars: z-score ≤ -3, z-score ≥ 3]

## Hexabromocyclododecanes (HBCDDs)

HBCDD diastereomers undergo thermal isomerization at temperatures above 160 °C. With GC elution temperature of these compounds of normally above 160 °C a separation of HBCDD diastereomers using GC analysis is not possible. Only one unresolved peak is obtained. Additional thermal decomposition of HBCDDs is reported for temperatures above 240 °C. Therefore, in case of use of GC-MS methods for HBCDD analysis only total HBCDD (as sum of all originally present HBCDD diastereomers is possible).

In biota samples α-HBCDD generally dominates over β- and γ-HBCDD and other HBCDDs are only found in traces. As a consequence, the sum of α-, β-, γ-HBCDD (using LC separation) and total HBCDD can be compared.

Table 10: Distribution of participants' z-scores for HBCDD sum parameters for palm fatty acid distillate (2003-FFA)

| **Percentage of participants' results** | **\|z-score\| ≤ 2** | **2 < \|z-score\| < 3** | **\|z-score\| ≥ 3** |
|---|---|---|---|
| **Sum of α-, β-, γ-HBCDD** | 92 % | 0 % | 8 % |
| **Total HBCDD*** | 100 % | 0 % | 0 % |

*Comparison of participants' results for total HBCDD with assigned value for sum of α-, β-, γ-HBCDD

Table 11: Distribution of participants' z-scores for HBCDD sum parameters for rapeseed oil (2003-FFB)

| Percentage of participants' results | \|z-score\| ≤ 2 | 2 < \|z-score\| < 3 | \|z-score\| ≥ 3 |
|---|---|---|---|
| **Sum of α-, β-, γ-HBCDD** | 93 % | 0 % | 7 % |
| **Total HBCDD*** | 100 % | 0 % | 0 % |

*Comparison of participants' results for total HBCDD with assigned value for sum of α-, β-, γ-HBCDD

# 7. Participants' feedback

A questionnaire for feedback from participants of this EURL proficiency test was available as online survey between 26 October 2020 and 30 November 2020. The survey was anonymous, but participants could also give their laboratory name. The identity of the laboratories is kept confidential. The survey included seven questions related to different topics (participants' information, organization of the proficiency test, PT test samples and evaluation of results and summary of data) and a possibility to include comments and further suggestions. In total, 29 laboratories (33 % of all participants) participated in this survey

## 7.1 Overview of questions and answers of participants

Participants' information (more than one answer possible):

| National Reference Laboratory (NRL) | Official Laboratory (OFL) | Commercial laboratory | Other |
|---|---|---|---|
| 61 % | 32 % | 11 % | 0 % |

Organization of proficiency test:

| | Fully | Largely | Partly | Not at all | No opinion |
|---|---|---|---|---|---|
| **Satisfied with organization of PT** | 71 % | 29 % | - | - | - |
| **Meeting of expectations** | 39 % | 54 % | 3.5 % | - | 3.5 % |
| **Information understandable** | 64 % | 32 % | 4 % | - | - |
| **Time frame acceptable** | 57 % | 36 % | 3.5 % | - | 3.5 % |

PT test samples:

| | Fully | Largely | Partly | Not at all | No opinion |
|---|---|---|---|---|---|
| **Selection of matrix and level of contamination adequate** | 25 % | 64 % | 7 % | - | 4 % |

Evaluation of results and summary of data:

| | Fully | Largely | Partly | Not at all | No opinion |
|---|---|---|---|---|---|
| **Evaluation of results and report clear and comprehensible** | 75 % | 18 % | 3.5 % | - | 3.5 % |

### 7.2 Comments and suggestions

Comments referred to the presentation of the data in the reports. Options for filtering of results based on laboratory codes or parameters or presentation in Excel- or csv-format would be helpful for data evaluation.

## 8. Quality control

The Deutsche Akkreditierungsstelle GmbH attests that the provider of proficiency testing Chemisches und Veterinäruntersuchungsamt Freiburg, EU-Reference Laboratory (EURL) for halogenated persistent organic pollutants (POPs) in Feed and Food is competent under the terms of DIN EN ISO/IEC 17043:2010 to carry out proficiency testing in the testing field of determination of halogenated persistent organic pollutants (POPs) in food and feed (Accreditation number: D-EP-18625-01-00).

## 9. Summary of participants' results

An overview of the PBDE and HBCDD results for the PT test samples palm fatty acid distillate (2003-FFA) and rapeseed oil (2003-FFB) and the evaluation of the results are given in the following annexes 1 - 12. Laboratories are coded according to the laboratory codes sent after registration.

## 10. References

[1] M. Thompson, S.L.R. Ellison, R. Wood: The International Harmonized Protocol For The Proficiency Testing Of Analytical Chemistry Laboratories, Pure Appl. Chem., Vol. 78, No. 1, pp. 145-196, 2006.

[2] ISO 13528:2015, Statistical methods for use in proficiency testing by interlaboratory comparisons, International Organization for Standardization

## 11. Annex

(Please double click on the pdf-icons to open the annexes.)

| **Palm fatty acid distillate – 2003-FFA** | | |
|---|---|---|
| 1 | Assigned values– PBDE, HBCDD | |
| 2 | Participants' results – Tables – PBDE, HBCDD | |
| 3 | Participants' z-scores – Tables – PBDE; HBCDD | |
| 4 | Participants' z-scores – Charts – PBDE, HBCDD | |
| 5 | Homogeneity test | |
| 6 | Participants' methods – PBDE, HBCDD | |

| **Rapeseed oil – 2003-FFB** | | |
|---|---|---|
| 7 | Assigned values– PBDE, HBCDD | |
| 8 | Participants' results – Tables – PBDE, HBCDD | |
| 9 | Participants' z-scores – Tables – PBDE; HBCDD | |
| 10 | Participants' z-scores – Charts – PBDE, HBCDD | |
| 11 | Participants' methods – PBDE, HBCDD | |

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