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for Halogenated POPs in Feed and Food

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EURL Proficiency Test on the Determination of PCDD/Fs, PCBs, PBDEs and HBCDDs in Baby Food 2021

EURL-PT-DPB_2101-BF

FOOD

Report

PBDEs and HBCDDs

(Version 1.0)

05 August 2021

This report on the EURL Proficiency Test on the Determination of PCDD/Fs, PCBs, PBDEs and HBCDDs in baby food 2021 [EURL-PT-DPB-2101-BF] 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 7 annexes.

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Summary

Test sample (food)	Baby food – 2101-BF
Analytes of interest	Mandatory for NRLs: - **PBDEs** (BDE-28, BDE-47, BDE-49, BDE-99, BDE-100, BDE-153, BDE-154, BDE-183, BDE-209) - **HBCDDs** (α-HBCDD, β-HBCDD, γ-HBCDD)
Methods	PBDEs: Any kind of method HBCDDs: Any kind of method
Participants	NRLs, OFLs, other official laboratories, commercial laboratories performing the analysis of samples taken by food business operators
Statistical evaluation	ISO 13528:2020, IUPAC Protocol
Report	05 August 2021 (Version 1.0)

1. Structure of the PT, test material and analytes

This proficiency test (PT) on the determination of PCDD/Fs, PCBs, PBDEs and HBCDDs in baby food was organised by the EURL for halogenated persistent organic pollutants (POPs) in feed and food to be performed between February and April 2021. 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 baby food.

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 2021. 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 to other official laboratories and commercial laboratories analysing samples taken by food business operators in the performance of official control or self-control in order to check the comparability of results not only within the EURL/NRL/OFL network, but also with official and private laboratories.

The evaluated results were discussed by representatives of EU Commission, NRLs and the EURL at the COM/EURL/NRL workshop in May 2021.

1.1 Samples and coding

The baby food test sample was prepared of regular market food (pumpkin, pork, vegetable oil). The test sample was fortified with the analytes of interest using PCDD/F standards and technical mixtures of PCBs, PBDEs and HBCDDs.

Baby food	**Sample no. 2101-BF-xxx**

Each participant received about 100 g of the test sample.

1.2 Analytes of interest

NRLs for halogenated POPs in feed and food were requested to determine the following parameters, all other participants were requested to determine at least one of the following parameters:

- Polybrominated diphenyl ethers (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)
- Hexabromocyclododecanes (HBCDDs): α-HBCDD, β-HBCDD, γ-HBCDD
- Sum of α-, β-, γ-HBCDD (using HPLC methods) or total HBCDD (using GC methods)

1.3 Methods

One or more of the following detection methods could be applied:

- Any kind of method for PBDEs and HBCDDs

1.4 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.

1.5 Results of PBDEs and HBCDDs determined by physico-chemical methods

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,
- report the ion abundance ratios and applied limits for PBDEs and HBCDDs (as basis for the derivation of possible analytical criteria)
- 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 wet weight** 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

102 laboratories registered for this proficiency test and 33 laboratories reported results.

Table 1: Participating laboratories

Participating laboratories	Region	No. of participants
National Reference Laboratories	European Union	18
	Other Countries	-
Official Laboratories	European Union	10
	Other European Countries	1
	Africa	-
	Americas	-
	Asia	1
	Oceania	
Commercial Laboratories	European Union	1
	Other European Countries	-
	Africa	-
	Americas	1
	Asia	-
	Oceania	
	Total	**33**

2.1 Number of reported results

Table 2: Reported results for PBDEs

Reported results	Baby Food (2101-BF)
BDE-28	32
BDE-47	32
BDE-49	25
BDE-99	32
BDE-100	32
BDE-153	32
BDE-154	32
BDE-183	32
BDE-209	25
Sum of 8 PBDEs (without BDE-209) (ub)	32
Sum of 8 PBDEs (without BDE-209) (lb)	31
Sum of 9 PBDEs (with BDE-209) (ub)	25
Sum of 9 PBDEs (with BDE-209) (lb)	25

Table 3: Reported results for HBCDDs

Reported results	Baby Food (2101-BF)
α-HBCDD	20
β-HBCDD	20
γ-HBCDD	20
Sum of α-, β-, γ-HBCDD (ub)	19
Sum of α-, β-, γ-HBCDD (lb)	17
Total HBCDD (using GC methods)	2

2.2 Accreditation

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

Baby Food (2101-BF)	PBDEs	HBCDDs
yes	21	6
no	10	15

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 the determination of PBDEs and HBCDDs in baby food (2101-BF)

Detection methods	PBDEs	HBCDDs
GC-HRMS	23	2
GC-MS/MS	8	-
GC-LRMS	1	-
LC-MS/MS	-	18
LC-HRMS	-	2

4. Homogeneity and stability of the test material

The test for sufficient homogeneity was performed according to ISO 13528:2020 [2] and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [1].

Therefore, 10 portions of the test sample 2101-BF were analyzed in duplicate for PBDEs. The test for sufficient homogeneity was performed for the individual congeners. The test material was sufficiently homogeneous for PBDEs in this proficiency test. This can also be concluded for HBCDDs due to similar physico-chemical properties for this proficiency test.

The stability check of the analytes of interest applying room temperature storage was performed according to ISO 13528:2020 [2] for PBDEs. The test material showed sufficient stability for PBDEs and can be concluded also for HBCDDs due to similar physico-chemical properties for this proficiency test.

5. Determination of the assigned value

Statistical evaluation of the PT results was performed by the EURL for halogenated POPs in feed and food according to ISO 13528:2020 [2] and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [1].

The determination of the assigned value was 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 was 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.

Assigned values are calculated for individual PBDE congeners, sum of 8 PBDEs (without BDE-209, ub, lb) and sum of 9 PBDEs (with BDE-209, ub, lb) as well as for individual HBCDD diastereomers, sum of α-, β- and γ-HBCDD (ub, lb) and total HBCDD (including limits of quantification (LOQs)), if possible. Additionally the median of all values is 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 are equal to or above the LOQ; otherwise the LOQ will be taken instead.

Due to high variation of participants' results in the range of the respective LOQ or too few results, no assigned values could be calculated for:

- (+/–)-β- HBCDD
- (+/–)-γ- HBCDD
- Sum of α-, β-, γ-HBCDD upper bound
- Total HBCDD (using GC-methods)

Since there are no traceable reference values available, the assigned values in this PT were calculated based on the Huber robust mean of the participants' results. 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 to ISO/IEC 17025 were compared for PBDE and HBCDD sum parameters. No significant differences (<1 %) between the assigned values calculated for both data sets for PBDEs were observed. Only six out of 21 participating laboratories were accredited according to ISO/IEC 17025 for HBCDDs. After eliminating outliers, only 4 results contributed to the calculation of the assigned values. Therefore, no assigned values for HBCDDs could be calculated for this group of participants and the median values were

used for comparison instead. No significant differences (<1 %) between the assigned values and median values calculated for both data sets for HBCDDs 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

Baby Food (2101-BF)	**Assigned value**	**Assigned value**	**Median**	**Deviation**
	All participants	**ISO/IEC 17025 accreditation**		
	µg/kg wet weight			%
Sum of PBDE without BDE-209 (ub)	0.659	0.656		<1
Sum of PBDE without BDE-209 (lb)	0.654	0.656		<1
Sum of PBDE including BDE-209 (ub)	0.991	0.996		<1
Sum of PBDE including BDE-209 (lb)	0.946	0.944		<1
α-HBCDD	0.0904	-*	0.0900	<1
Sum of α-, β-, γ-HBCDD (lb)	0.102	-*	0.101	<1

*calculation of assigned values was not possible, therefore the median values were used for comparison

5.1 PBDEs – individual congeners and sum parameter

The assigned values for the test sample 2101-BF were calculated as consensus of participants' results for individual PBDEs and sum parameters.

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

Baby Food (2101-BF)	**Sum of PBDE without BDE-209 ub**
	µg/kg wet weight
BDE-28	0.00239
BDE-47	0.212
BDE-49	0.00511
BDE-99	0.285
BDE-100	0.0615
BDE-153	0.0322
BDE-154	0.0238
BDE-183	0.0348
BDE-209	0.345
Sum of 8 PBDEs (without BDE-209) (ub)	0.659
Sum of 8 PBDEs (without BDE-209) (lb)	0.654
Sum of 9 PBDEs (with BDE-209) (ub)	0.991
Sum of 9 PBDEs (with BDE-209) (lb)	0.946

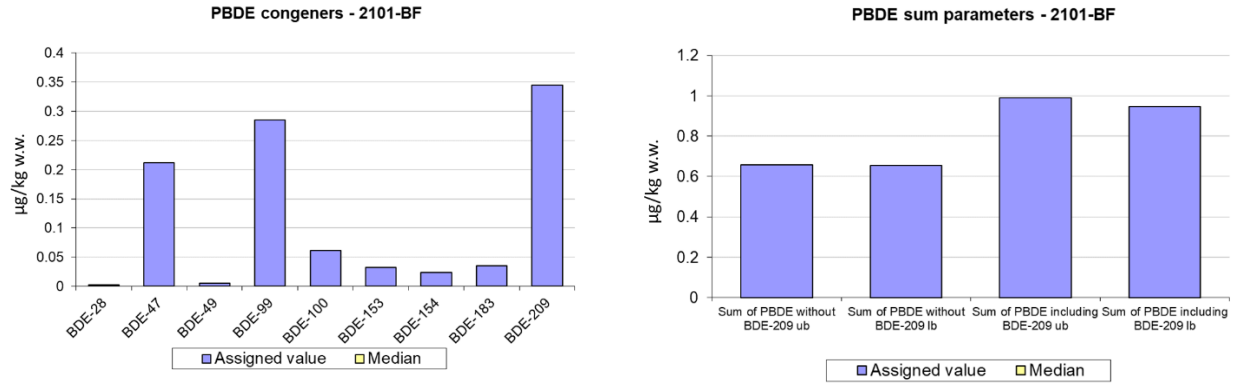

Figure 1: Assigned values for PBDE congeners and sum parameters for baby food (2101-BF) [µg/kg w.w.]

5.2 HBCDDs – individual stereoisomers and sum parameter

The assigned values for the test sample 2101-BF were calculated as consensus of participants' results for individual HBCDDs and sum parameters.

Table 8: Assigned values for physico-chemical methods for HBCDD stereoisomers and sum parameters (rounded to three significant figures)

Baby Food (2101-BF)	**Sum of α-, β-, γ-HBCDD upper bound**
	µg/kg wet weight
α- HBCDD	0.0904
Sum of α-, β-, γ-HBCDD upper bound	0.102

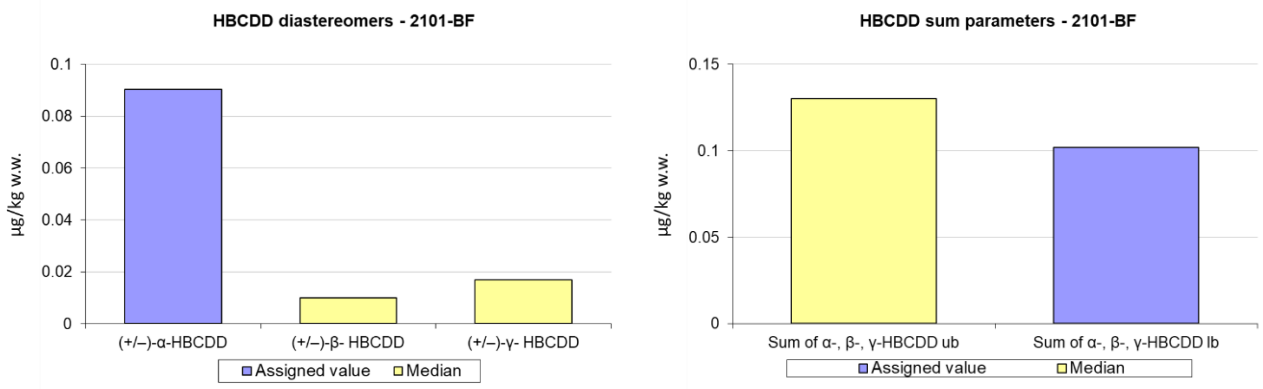

Figure 2: Assigned and median values for HBCDD diastereomers and sum parameters for baby food (2101-BF) [µg/kg w.w.]

5.3 Lipid content

For the lipid content an assigned value of 12.0 % for the test sample 2101-BF was calculated as a consensus of the participants' results, taking into account the calculation criteria described above.

5.4 Comparison of assigned values with recommended LOQs

The limits of quantification are currently based on the values specified in Commission Recommendation of 3 March 2014, on the monitoring of trace levels of brominated flame retardants in food (2014/118/EU). For PBDEs the recommended LOQ value is 0.01 µg/kg w.w. for individual congeners (Table 9). However, it was discussed in the meetings of the core working group "Brominated Contaminants and PCNs" of the EURL/NRL network that a lower LOQ value of 0.001 µg/kg w.w. is preferable for all congeners except BDE-209, given that some foods show concentrations below this level (Table 10). Valid data on the background contamination of foodstuffs with BFRs is particularly important for a reliable risk assessment. For HBCDDs the recommended LOQ value is 0.01 µg/kg w.w. for α-, β- and γ-stereoisomers (Table 9). For total HBCDD measured by GC-MS, the corresponding LOQ value is 0.003 µg/kg (as cumulative response of all possible HBCDD diastereomers, Table 10).

Table 9: Recommended LOQs for PBDEs and HBCDDs from COMMISSION RECOMMENDATION of 3 March 2014 on the monitoring of traces of brominated flame retardants in food (2014/118/EU)

Food		**Limit of quantification** per congener/stereoisomer
PBDEs	*ng/g wet weight*	**≤ 0.01**
HBCDDs	*ng/g wet weight*	**≤ 0.01**

Table 10: Analytical recommendations from "Guidance document on analytical parameters for the determination of organobromine contaminants in food and feed" (CWG "BCons and PCNs")

Food		**Limit of quantification** per congener/stereoisomer
PBDEs	*µg/kg wet weight*	**0.01** **0.001** (all congeners except BDE-209)
HBCDDs	*µg/kg wet weight*	**0.01** **0.01** (sum of HBCDDs) **0.003** (total HBCDD)

PBDEs:

For individual PBDE congeners, the recommended LOQs are 0.01 µg/kg w.w. and the targeted LOQs are 0.001 µg/kg w.w., except for BDE-209 (Table 9 and 10). The calculated assigned values for BDE-28 (0.002 µg/kg) and BDE-49 (0.005 µg/kg) were in the range between the targeted and recommended LOQs for the baby food test sample (2101-BF). For the calculation of the BDE-28 assigned value, 28 out of 32 results were above the LOQs of the laboratories and for BDE-49 24 out of 25 results, showing that most participating laboratories are able to reliably achieve the recommended LOQ of 0.01 µg/kg for baby food.

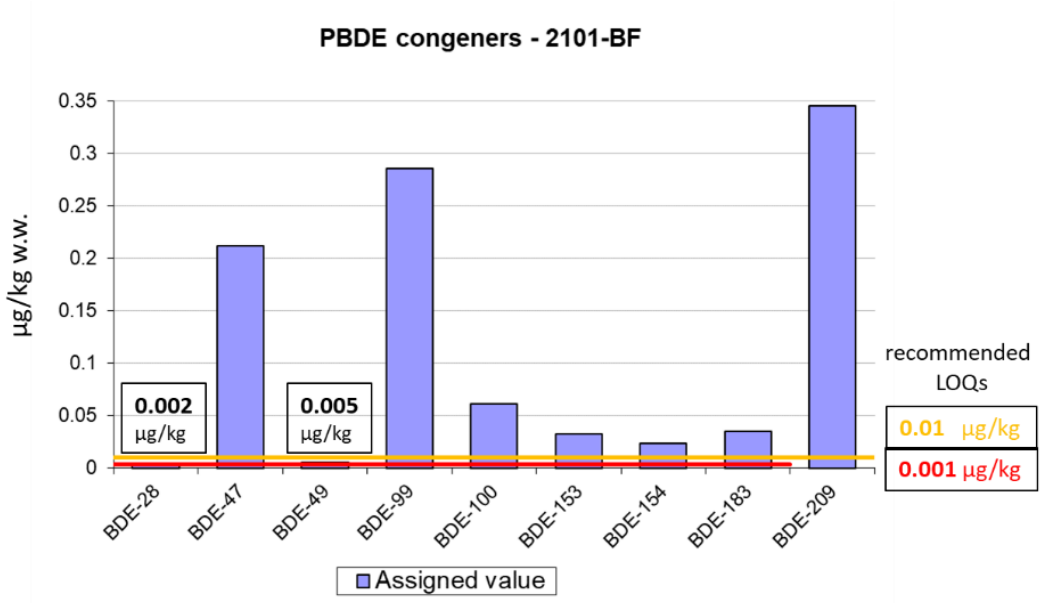

Figure 3: Comparison of assigned values for PBDE congeners with recommended LOQs (yellow and red lines) in one sample of baby food (2101-BF) [µg/kg w.w.]

HBCDDs:

For individual α-HBCDD, β-HBCDD, γ-HBCDD stereoisomers the recommended LOQs are 0.01 µg/kg (Table 9 and 10). For β- and γ-HBCDD no assigned values could be calculated, because less than 2/3 of all 20 reported results were above the LOQs (see calculation criteria section 5). Therefore, the median values were taken for comparison with the recommended LOQs. For β- and γ-HBCDD both median values of 20 participants were in the range of the recommended LOQs.

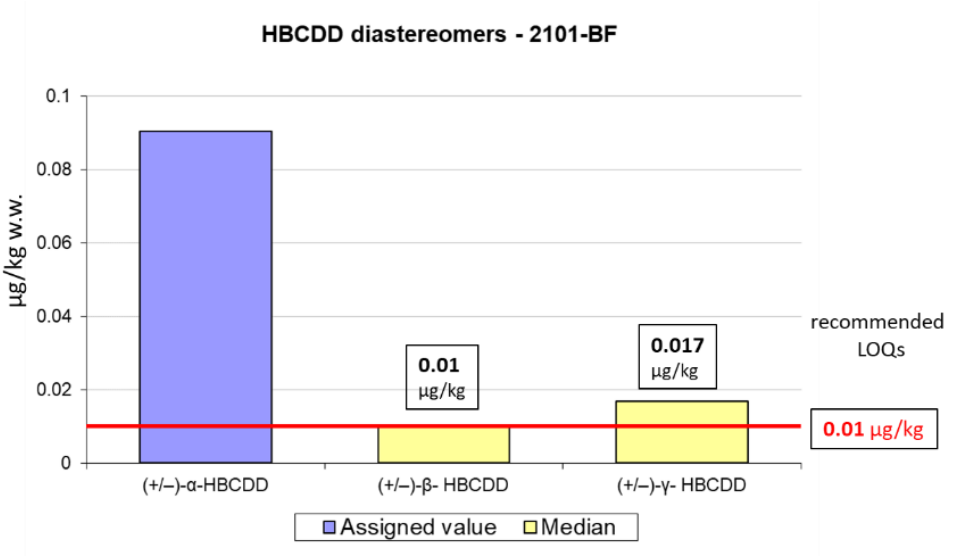

Figure 4: Comparison of assigned values for HBCDD stereoisomers with recommended LOQs (red line) in one sample of baby food (2101-BF) [µg/kg w.w.]

5.5 Comparison of reported ion abundance ratios

Participants were asked to submit ion ratios for PBDEs and HBCDDs determined with either HRMS or MS/MS to compare the deviation of the acquired ratios from the theoretical or measured (of the corresponding reference standard) ion ratios. During the meetings of the core working group “Brominated Contaminants and PCNs” of the EURL/NRL network it was discussed, that guidance on the essential analytical parameters should be given as recommendation criteria for a reliable BFR analysis. Here the relative deviation of the theoretical and acquired ion abundance ratios was set to be not greater than 15 to 20%. By querying the participants’ ion ratios in this PT, it should be clarified whether this range can be achieved.

The results showed, that most of the laboratories’ reported ion abundance ratios for PBDEs were within the range of ±15 % deviation of the theoretical ratio. Only four ratios were within ±20 % deviation and two outliers greater than -20 % were detected. For HBCDDs same can be stated, with three ratios within ±20 % deviation and two outliers (Figure 4). It can therefore be assumed that as analytical criteria a deviation not greater than ± 20 % of the theoretical value or of the corresponding reference standard can be set for recommendation.

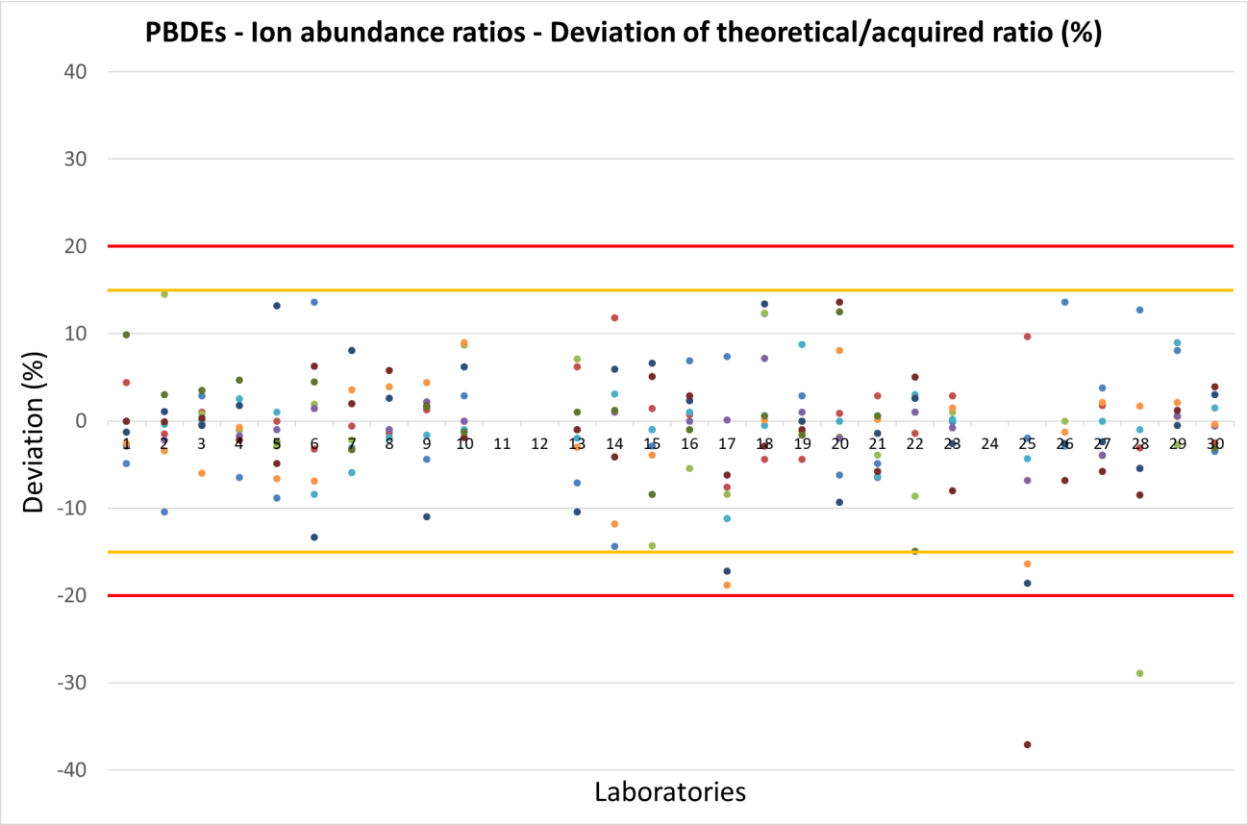

Figure 5: Deviation of measured participants' ion abundance ratios of the theoretical value or of the corresponding reference standard for PBDE congeners [%]

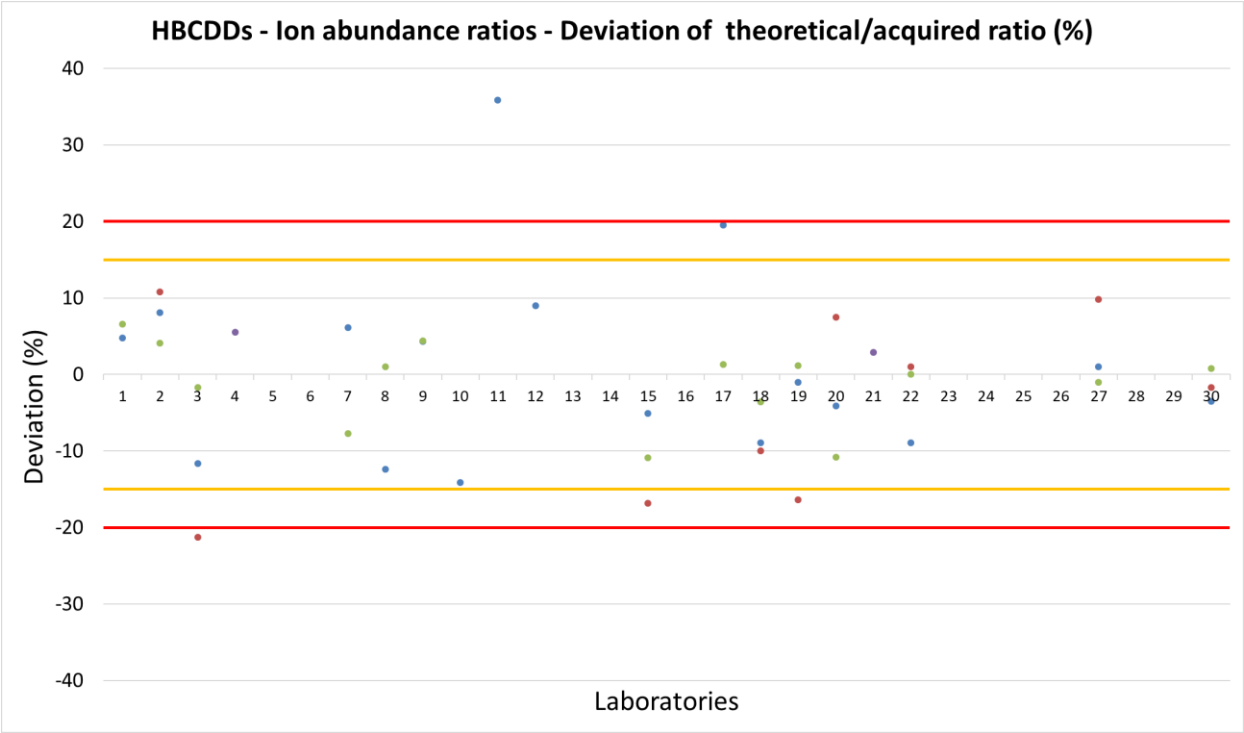

Figure 6: Deviation of measured participants' ion abundance ratios of the theoretical value or of the corresponding reference standard for HBCDD stereoisomers [%]

6. Evaluation of results

6.1 Z-scores calculation

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

$$z = \frac{(x - x_{a)}}{\sigma_p}$$

x_a: *assigned value*

x: *participant's result*

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

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

Z-scores for individual congeners and stereoisomers 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 - Participants' z-scores

Table 11: Distribution of participants' z-scores for PBDE sum parameters for baby food (2101-BF)

Percentage of participants' results	|z-score| ≤ 2	2 < |z-score| < 3	|z-score| ≥ 3
BDE-28	86 %	7 %	7 %
BDE-47	91 %	-	9 %
BDE-49	83 %	4 %	13 %
BDE-99	91 %	6 %	3 %
BDE-100	91 %	3 %	6 %
BDE-153	94 %	3 %	3 %
BDE-154	84 %	6 %	9 %
BDE-183	81 %	7 %	13 %
BDE-209	86 %	5 %	9 %

Sum of 8 PBDEs without BDE-209 (ub)	88 %	3 %	9 %
Sum of 8 PBDE including BDE-209 (lb)	87%	3 %	10 %
Sum of 9 PBDE including BDE-209 (ub)	80 %	12 %	8 %
Sum of 9 PBDE including BDE-209 (lb)	80 %	8 %	12 %

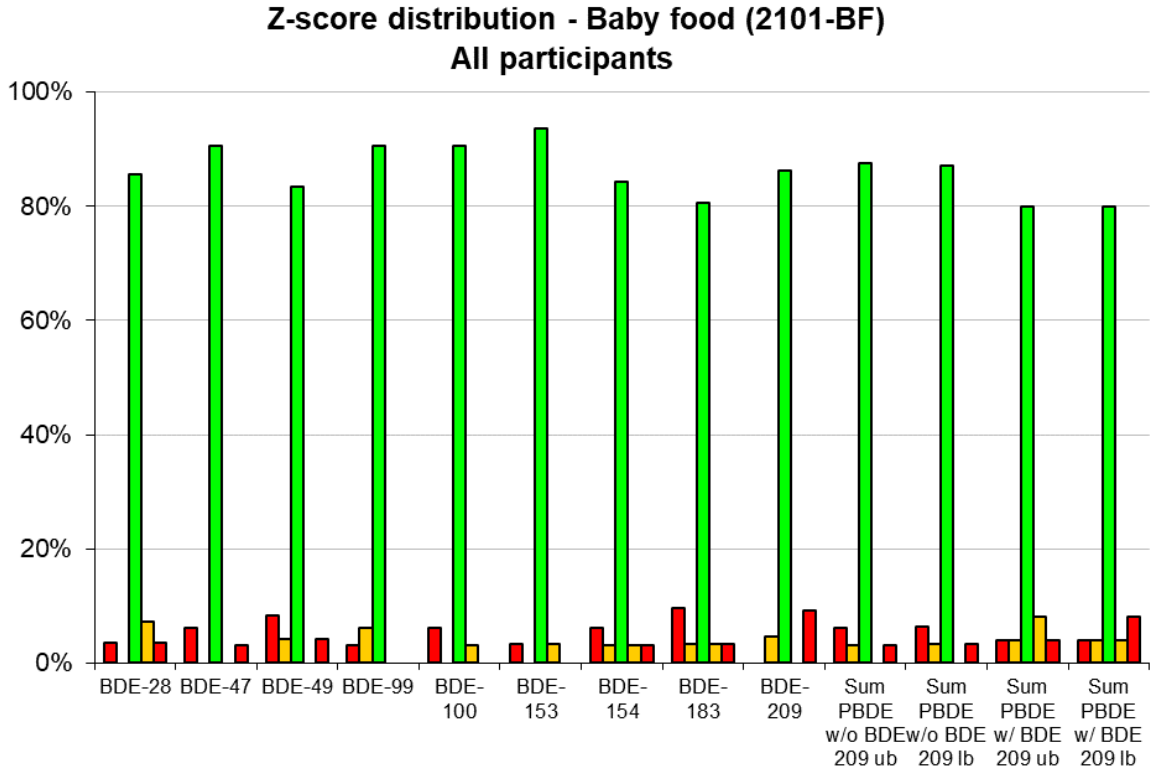

Figure 7: Distribution of participants' z-scores for PBDE congeners / sum parameters for baby food (2101-BF) [Green bars: -2 ≤ z-score ≤ 2, yellow bars: -3 < z-score < -2, 2 < z-score < 3, red bars: z-score ≤ -3, z-score ≥ 3]

6.3 HBCDDs - Participants' z-scores

HBCDD stereoisomers undergo thermal isomerization at temperatures above 160 °C. With GC elution temperature of these compounds of normally above 160 °C a separation of HBCDD stereoisomers 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 12: Distribution of participants' z-scores for HBCDD sum parameters for baby food (2101-BF)

Percentage of participants' results	|z-score| ≤ 2	2 < |z-score| < 3	|z-score| ≥ 3
α- HBCDD	88 %	6 %	6 %
Sum of α-, β-, γ-HBCDD (lb)	79 %	5 %	16 %
Total HBCDD*	100 %	0 %	0 %

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

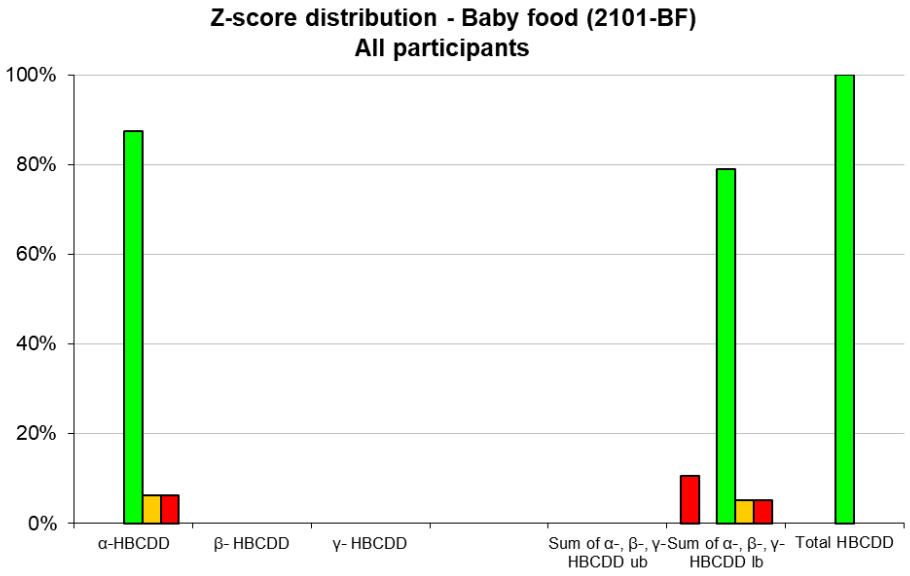

Figure 8: Distribution of participants' z-scores for PBDE congeners / sum parameters for baby food (2101-BF) [Green bars: -2 ≤ z-score ≤ 2, yellow bars: -3 < z-score < -2, 2 < z-score < 3, red bars: z-score ≤ -3, z-score ≥ 3]

7. Participants' feedback

A questionnaire for feedback from participants of this EURL proficiency test was available as online survey between 10 May 2021 and 11 June 2021. 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 (31 % 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
14	11	4	1

Rating of organization of the PT from no at all (1/5) to full satisfaction (5/5) by **29** laboratories:

	No opinion	1/5	2/5	3/5	4/5	5/5
Announcement	-	-	-	3	5	23
Instructions	-	-	1	1	4	23
Sample shipment	-	-	-	1	5	23
Reporting of results	-	-	-	1	8	20
Was all information provided in an understandable way	-	-	1	-	5	23
Was the time frame acceptable	-	-	1	1	6	21
Was the handling of EU survey as webtool mangeable	1	2	-	2	10	14

Rating of chosen PT test sample from no at all (1/5) to full satisfaction (5/5) by **29** laboratories:

	No opinion	1/5	2/5	3/5	4/5	5/5
Selection of matrix	-	1	3	4	2	19
Adequate level of contamination	-	1	3	4	10	11

Rating of evaluation of results and summary of data from no at all (1/5) to full satisfaction (5/5) by **29** laboratories:

	No opinion	1/5	2/5	3/5	4/5	5/5
Preliminary report	-	-	-	1	9	19
Evaluation of results and report clear and comprehensible	-	-	-	3	5	23
Met the PT your expectations	-	-	-	6	7	16

7.2 Comments and suggestions

Comments referred to chosen matrix and range of concentration. A more frequent matrix, higher concentrations and more discussion about levels of PCDD/Fs including LOQs would be helpful. It would be desirable if there were more opportunities to discuss some methodological aspects within the EURL/NRL network. Feedback on the experiences with the EUsurvey webtool was positive.

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. Results of participants

An overview of the PBDE and HBCDD results for the PT test sample baby food (2101-BF) are given in the following annexes 1-7. 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 13528:2020, 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.)

Baby Food – 2101-BF		
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 and stability test – PBDE, HBCDD	
6	Participants' methods – PBDE, HBCDD	
7	Participants' ion abundance ratios – PBDE, HBCDD	

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