EURL Proficiency Test on the Determination of PCDD/Fs, PCBs, PBDEs, HBCDDs, PFASs and CPs in Pork Liver 2022

EURL-PT-POP_2201-PL

FOOD

Report

PBDEs and HBCDDs

(Report Version 1.0)

17 November 2022

EURL for halogenated POPs in Feed and Food
c/o State Institute for Chemical and
Veterinary Analysis Freiburg

Bissierstraße 5
79114 Freiburg
D-Germany

contact@eurl-pops.eu
+49 761 8855 500

eurl-pops.eu

Summary

Test sample	**FOOD:** Pork Liver [2201-PL]
Analytes of interest **Mandatory** for NRLs:	**PBDEs** (BDE-28, -47, -49, -99, -100, -153, -154, -183, -209) **HBCDDs** (α-HBCDD, β-HBCDD, γ-HBCDD or total HBCDD)
Methods	Any kind of method
Participants	NRLs, OFLs, other official laboratories, commercial laboratories performing the analysis of samples taken by food business operators
Statistical evaluation	DIN ISO 13528:2020, IUPAC Protocol
Report of final results	17 November 2022 (Version 1.0)
Publication	EURL POPs reserves all rights to publish and present the anonymised results of the interlaboratory study in scientific journals and/or during conferences.

1. Structure of the PT, test material and analytes

This proficiency test (PT) on the determination of **PCDD/Fs, PCBs, PBDEs**, **HBCDDs, PFASs** and **CPs** in **pork liver** was organized by the European Union Reference Laboratory (EURL) for halogenated persistent organic pollutants (POPs) in Feed and Food to be performed between February and April 2022. The objective was to assess analytical performance of laboratories and the interlaboratory comparability of results from analyses of PCDD/Fs, PCBs, PBDEs, HBCDDs, PFASs and CPs in one sample of **pork liver.**

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 2022. 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 allowed the extension of the data basis for calculation of assigned values and evaluation of results.

Other official laboratories and **commercial laboratories** performing the analysis of samples taken by food business operators were invited to participate in this proficiency test. The evaluated results were discussed by representatives of European Commission, NRLs and the EURL at the EURL/NRL workshop on 18 and 19 May 2022.

1.1. Samples and coding

The test sample was prepared from commercially available food (pork liver mixed with wild boar liver). The test sample was not fortified with analytes of interest. The production of the fully preserved cans was subcontracted.

Pork liver	**Sample no. 2201-PL-xxx**

Each participant received about **90 g** of the test sample in a HDPE bottle.

1.2. Analytes of interest

Participants were requested to determine the following parameters:

Polybrominated diphenyl ethers (PBDEs)

- Individual congeners: BDE-28, -47, -49, -99, -100, -153, -154, -183, -209
- Sum of 8 PBDEs (without BDE-209)
- Sum of 9 PBDEs (with BDE-209)

Hexabromocyclododecanes (HBCDDs)

- α-HBCDD, β-HBCDD, γ-HBCDD stereoisomers
- Sum of α-, β-, γ-HBCDD (using HPLC methods)
- Total HBCDD (using GC methods)

1.3. Methods

All kinds of detection and quantification methods could be applied.

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. The confidentiality between NRLs and their OFLs will be kept unless a Member State initiated a cooperation between the NRL, OFLs and the EURL.
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.

1.5. 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 wet weight (w. w.)** 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

124 laboratories registered for this proficiency test.

Table 1: Participating laboratories

Participating laboratories	Region	No. of participants
National Reference Laboratories	European Union Other Countries	18 2
Official Laboratories	European Union Other European Countries Africa/Americas/Asia/Oceania	9 - -
Commercial Laboratories	European Union Other European Countries Africa/Americas/Asia/Oceania	3 - -
	Total	**32**

2.1. Number of reported results

Table 2: Reported results for PBDEs and lipid content for pork liver (2201-PL)

Reported results (2201-PL)	All laboratories
BDE-28, -47, -99, -100, -153, -154	29
BDE-49 / BDE-183 / BDE-209	23 / 28 / 24
Sum of 8 PBDEs (without BDE-209) (ub)	27
Sum of 8 PBDEs (without BDE-209) (lb)	22
Sum of 9 PBDEs (with BDE-209) (ub)	23
Sum of 9 PBDEs (with BDE-209) (lb)	18
Lipid content	27

Table 3: Reported results for HBCDDs for pork liver (2201-PL)

Reported results (2201-PL)	All laboratories
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

Pork Liver	PBDEs	HBCDDs
yes	21	10
no	7	12

2.3. 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 pork liver (2201-PL)

Detection methods	PBDEs	HBCDDs
GC-HRMS	18	1
GC-MS/MS	7	-
GC-LRMS	2	1
LC-MS/MS	-	17
LC-HRMS	-	2

3. Homogeneity and stability of the test material

The test for sufficient homogeneity was performed according to DIN ISO 13528:2020 [2] and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [1].
Therefore, 10 portions of the test samples 2201-PL were analyzed in duplicate for PCDD/Fs and PCBs. The test for sufficient homogeneity was performed for the sum parameters WHOPCDD/F-PCB-TEQ, WHO-PCDD/F-TEQ, WHO-PCB-TEQ, the sum of six non-dioxin-like PCBs and individual congeners. The stability check of the analytes of interest applying room temperature storage was performed according to ISO 13528:2020 [2]. The test materials showed sufficient homogeneity and stability for PDCC/Fs and PCBs for this proficiency test. Homogeneity and stability can be concluded also for the PBDEs and HBCDDs, due to similar physico-chemical properties.

4. Determination of the assigned value

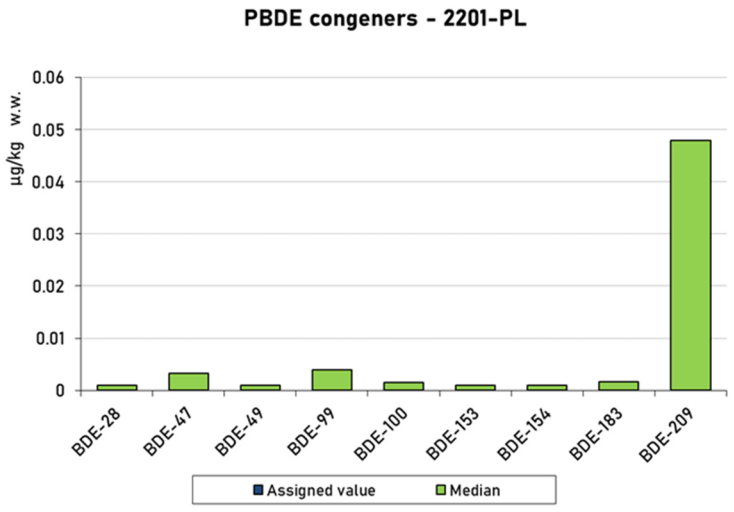
Statistical evaluation of the PT results was performed by the EURL for halogenated POPs in feed and food according to DIN ISO 13528:2020 [2] and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [1].
The determination of the assigned value was performed 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 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.
Assigned values were calculated for individual PBDE congeners, sum of 8 (without BDE-209) and sum of 9 (with BDE-209) PBDEs, for individual HBCDD diastereomers, sum of α-, β- and γ-HBCDD and total HBCDD (including limits of quantification (LOQs)), if possible. Additionally the median of all values was 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 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 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 all PBDE and all HBCDD parameters.

4.1. PBDEs – individual congeners and sum parameter

The median values for individual PBDE congeners and sum parameters for the test sample 2201-PL of all participants' results are summarized in Table 6 (tabular summary see annex 1; Figure 1).

Table 6: Median values for PBDEs (rounded to three significant figures)

Pork Liver (2201-PL)	Median value µg/kg (wet weight)
BDE-28	0.00100
BDE-47	0.00329
BDE-49	0.00100
BDE-99	0.00400
BDE-100	0.00150
BDE-153	0.00100
BDE-154	0.000970
BDE-183	0.00165
BDE-209	0.0480
Sum of 8 PBDEs (without BDE-209) (ub)	0.0299
Sum of 8 PBDEs (without BDE-209) (lb)	0.00655
Sum of 9 PBDEs (with BDE-209) (ub)	0.0811
Sum of 9 PBDEs (with BDE-209) (lb)	0.0264



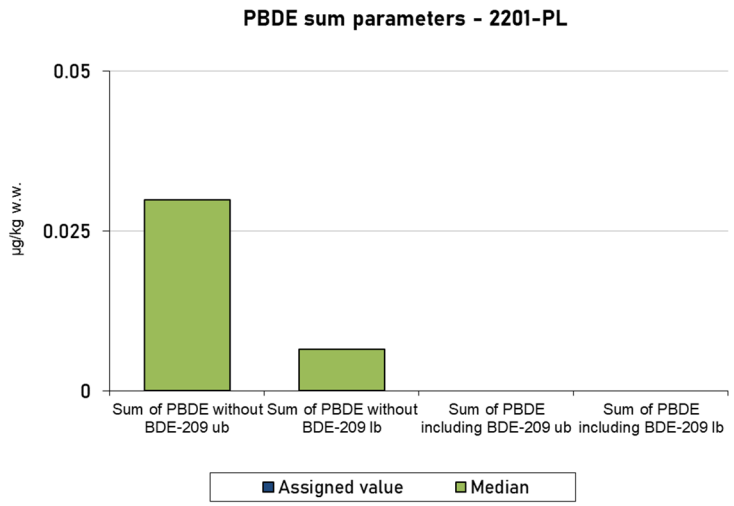


Figure 1: Median values (green) for PBDE individual congeners and sum parameters for pork liver (2201-PL) [µg/kg w.w.]

4.2. HBCDDs – individual stereoisomers and sum parameter

The median values for individual HBCDD stereoisomers and sum parameters for the test sample 2201-PL of all participants' results for HBCDDs are summarized in Table 7 (tabular summary see annex 1; Figure 2).

Table 7: Median values for HBCDDs (rounded to three significant figures)

Pork Liver (2201-PL)	Median value µg/kg (wet weight)
(+/–)-α-HBCDD	0.0183
(+/–)-β- HBCDD	0.0113
(+/–)-γ- HBCDD	0.0235
Sum of α-, β-, γ-HBCDD (ub)	0.0900
Sum of α-, β-, γ-HBCDD (lb)	0
Total HBCDD	2.07*

*two reported values (4.41 and 0.009)

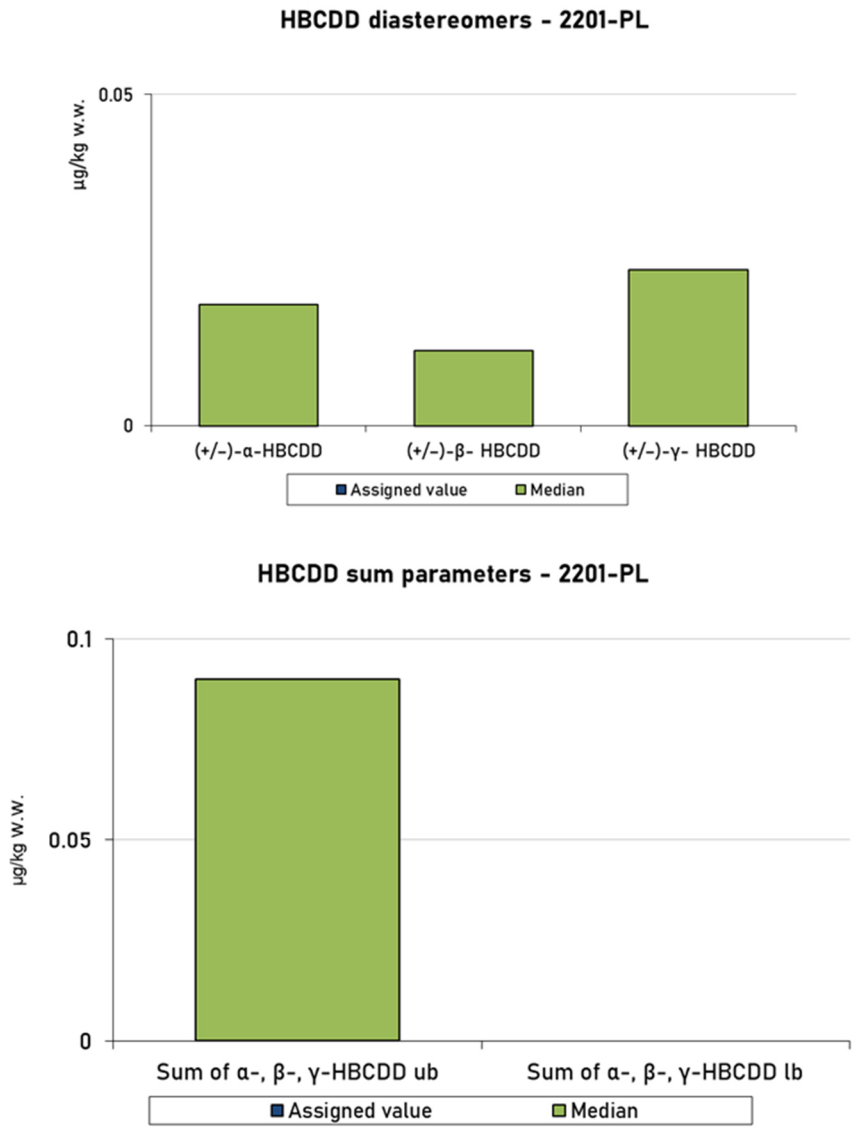


Figure 2: Median values (green) for HBCDD individual congeners and sum parameters for pork liver (2201-PL) [µg/kg w.w.]

4.3. Lipid content

For the lipid content an assigned value of 4.33 % for the test sample 2201-PL was calculated as a consensus of the participants' results, taking into account the calculation criteria described above (tabular summary see annex 1).

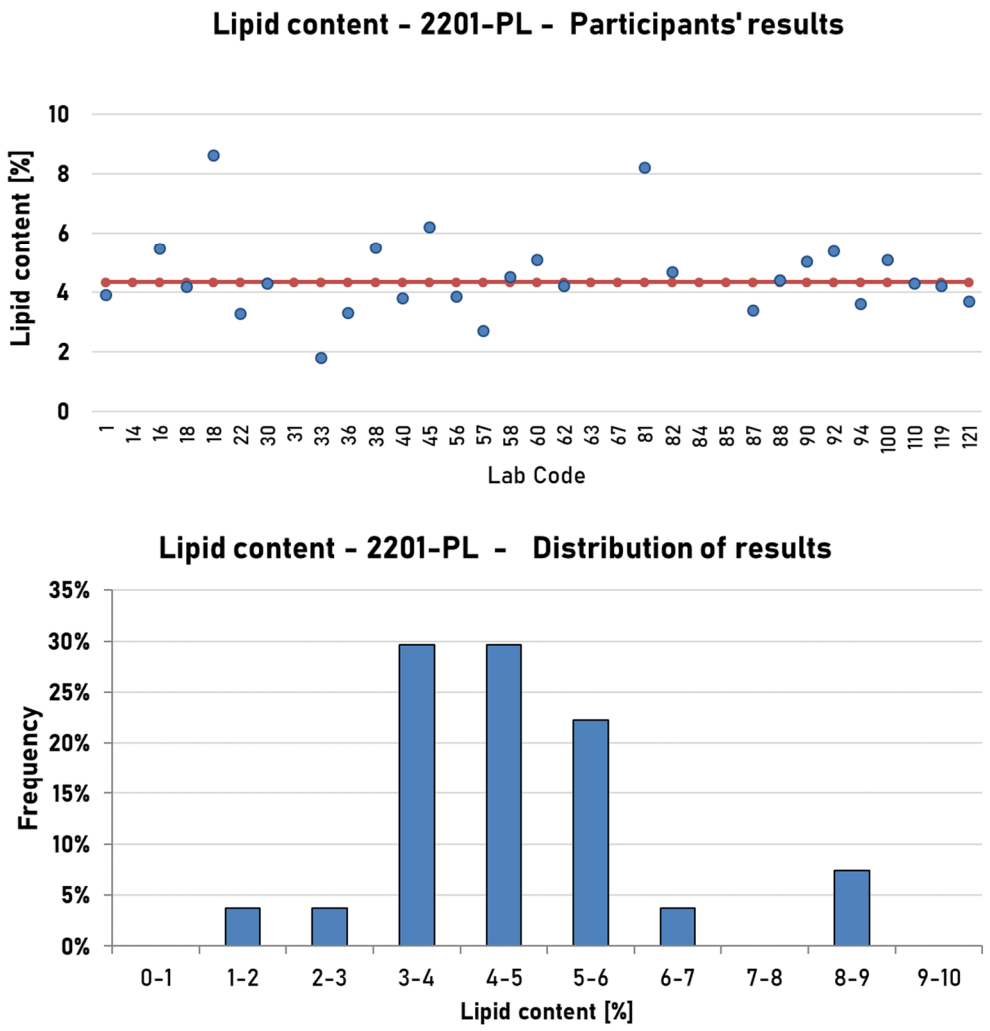


Figure 3: Participant's results (red line assigned value) and distribution of participant's results (blue dots) of the lipid content in % for pork liver (2201-PL)

4.4. Comparison of median 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 8). In the EURL "Guidance document on analytical parameters for the determination of organobromine contaminants in food and feed", a lower LOQ value of 0.001 µg/kg w.w. is targeted for all congeners except BDE-209, since some foods have concentrations below this value (Table 9; [3]). 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 8). 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 9).

Table 8: 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 µg/kg (wet weight)
PBDEs	≤ 0.01
HBCDDs	≤ 0.01

Table 9: Analytical recommendations from “Guidance document on analytical parameters for the determination of organobromine contaminants in food and feed” [3]

Undesirable Substances in Feed	Limit of quantification per congener/stereoisomer µg/kg (wet weight)
PBDEs	0.01 and 0.001 (all congeners except BDE-209)
HBCDDs	0.01 (sum of HBCDDs) and 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 8 and 9). All calculated median values, except for BDE-209 are in the range of the targeted LOQ. For BDE-209 the calculated median value is 0.0264 µg/kg w.w. No assigned value for BDE-209 in the range of the recommended LOQ could be calculated from 18 participants’ reported values.

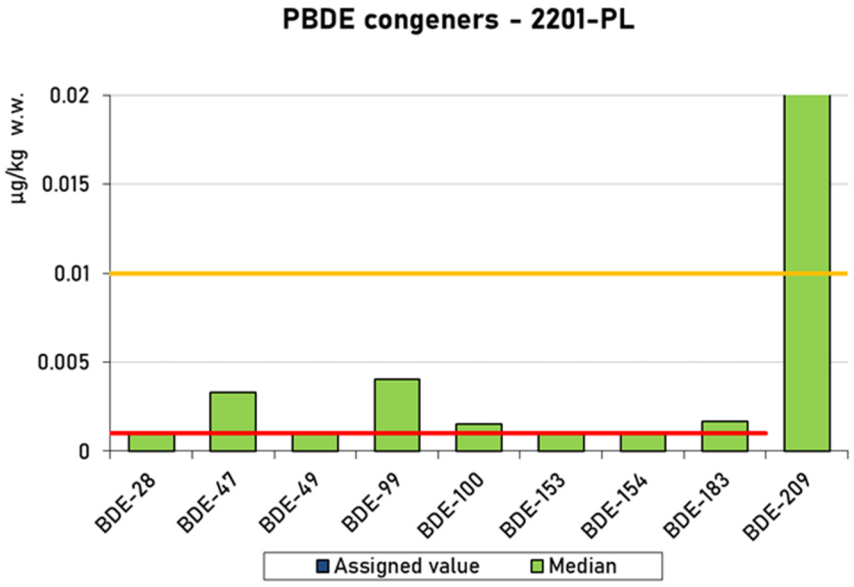


Figure 4: Comparison of median values for PBDE congeners with recommended LOQs (yellow line at 0.01 µg/kg w.w. and red line at 0.001 µg/kg w.w.) in pork liver (2201-PL)

HBCDDs:

For individual α-HBCDD, β-HBCDD, γ-HBCDD stereoisomers the recommended LOQs are 0.01 µg/kg (Table 8 and 9). All calculated median values from 20 participants' reported values for individual HBCDD stereoisomers were at the recommended LOQ, but no assigned values could be calculated.

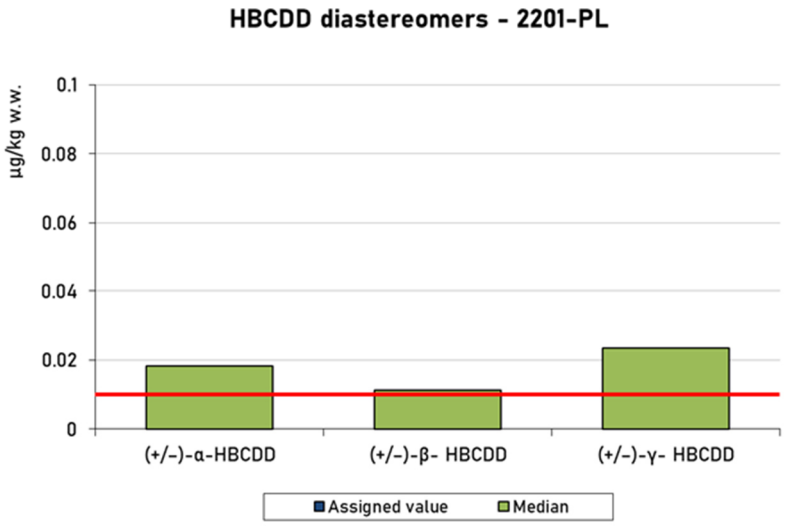


Figure 4: Comparison of median values for HBCDD stereoisomers with recommended LOQs (red line at 0.01 µg/kg wet weight) in pork liver (2201-PL)

5. Scoring of results – Z-scores

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

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

x: *participant's result*

x_a: *assigned value*

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

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

Z-scores for individual congeners / substances 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:

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

6. Participants' feedback

A questionnaire for feedback from participants of this EURL proficiency test was available as online survey between 16 May 2021 and 07 June 2022. The survey was anonymous, but participants could also give their laboratory name. The identity of the laboratories is kept confidential. The survey included several questions related to different topics (participants' information, organization of the proficiency test, PT test samples and evaluation of results an d summary of data) and a possibility to include comments and further suggestions. In total, 7 laboratories (22 % of all PT participants) replied to this survey.

Participants

Type of laboratory	Answers
National Reference Laboratory (NRL)	3
Official Laboratory (OFL)	2
Commercial laboratory	2
Other (e.g. research and development)	0
No Answer	0

General aspects

How satisfied are you with the organization of this proficiency test in general? Please rate the parts below according to your experience, with 0 stars meaning "no opinion" and 5 stars meaning "full satisfaction".

Announcement 

Instructions 

Sample shipment 

Reporting of results 

Preliminary report 

Specific aspects of this proficiency test

We would like to know a bit more about specific aspects of this proficiency test. Please rate the aspects below according to your experience, with 0 stars meaning "no opinion" and 5 stars meaning "full satisfaction".

Was all necessary information for participation and performance of the PT provided in an understandable way?	
Was the time frame acceptable?	
Was the handling of EUSurvey as webtool for reporting and source of instructions manageable?	
Was the evaluation of participant's results and the information in the preliminary report clear and comprehensible?	

Additional comments:

- report was very comprehensive and good; convoluted structure of the document does make it difficult to read
- it is easier with the webtool than sending email with an excel file
- the delay to give the preliminary results was very short

Was the selected sample adequate for the goal to assess analytical performance of laboratories in relevant matrices?

Choice of matrix	
Level of contamination	

Additional comments: spike levels are very low (too low ?) regarding regulation levels (PCB)
[Remark EURL POPs: The matrix was naturally contaminated and not spiked at all]

7. 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).

8. Results of participants

An overview of the PBDE and HBCDD results for the PT test sample pork liver (2201-PL) are given in the following annexes. Laboratories are coded according to the laboratory codes sent after registration.

9. 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] DIN ISO 13528:2020, Statistical methods for use in proficiency testing by interlaboratory comparisons, International Organization for Standardization

[3] EURL for halogenated POPs in feed and food (2022): Guidance Document on the Determination of Organobromine Contaminants, Analytical Parameters in food and feed, version 1.1 of 20 April 2022

10. Annex

(Please download the report and open it with a common pdf reader. After that you can open the annexes by double clicking the pdf icons.)

Pork liver – 2201-PL		
1	Assigned and Median values – Lipid content and PBDEs, HBCDDs	
2	Participants' results – Tables – Lipid content and PBDEs, HBCDDs	
3	Participants' z-scores – Tables – Lipid content	
4	Homogeneity and stability test	
5	Participants' methods – PBDE, HBCDD	

EURL for halogenated POPs in Feed and Food
c/o State Institute for Chemical and Veterinary Analysis of Food Freiburg

Theresa Zwickel
Phone: +49 761 8855 500 E-Mail: pt@eurl-pops.eu