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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, BFRs, PFASs and CPs in Fish fillet 2020

EURL-PT-POP-2001-FI

FOOD

Report

PBDEs and HBCDDs

(Version 1.0)

25 September 2020

This report on the EURL Proficiency Test on the Determination of PCDD/Fs, PCBs, BFRs, PFASs and CPs in Fish fillet 2020 [EURL-PT-POP_2001-FI] 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 samples (food)	Fish fillet (fresh water fish) - 2001-FI
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 13528:2015, IUPAC Protocol, Positive scoring system
Report	25 September 2020

1. Structure of the PT, test material and analytes

This proficiency test (PT) on the determination of PCDD/Fs, PCBs, BFRs, PFASs and CPs in fish fillet (fresh water fish) was organized by the EURL for Halogenated Persistent Organic Pollutants (POPs) in Feed and Food to be performed between February and September 2020. The objective was to assess analytical performance of laboratories and the interlaboratory comparability of results from analyses of PCDD/Fs, PCBs, BFRs, PFASs and CPs in one sample of fish fillet.

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 performing the analysis of samples taken by food 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 food business operators.

The evaluated results will be 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 fish fillet test sample was prepared of regular market food. The test sample was not fortified with analytes of interest.

Fish fillet (fresh water fish)	**Sample no. 2001-FI-xxx**

Each participant received about 125 g of the 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 ng/g 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

Table 1: Participating laboratories

Participating laboratories	Region	No. of participants
National Reference Laboratories	European Union	27
	Other Countries	2
Official Laboratories	European Union	60
	Other European Countries	0
	Africa	0
	Americas	2
	Asia	1
	Oceania	0
Commercial Laboratories	European Union	16
	Other European Countries	1
	Africa	0
	Americas	2
	Asia	2
	Oceania	0
	Total	**113**

2.1 Number of reported results

Table 2: Reported results for PBDEs

Reported results	Fish fillet (2001-FI)
BDE-28	37
BDE-47	37
BDE-49	27
BDE-99	37
BDE-100	37
BDE-153	37
BDE-154	37
BDE-183	37
BDE-209	30
Sum of 8 PBDEs (without BDE-209)	36
Sum of 9 PBDEs (with BDE-209)	30

Table 3: Reported results for HBCDDs

Reported results	Fish fillet (2001-FI)
α-HBCDD	20
β-HBCDD	20
γ-HBCDD	20
Sum of α-, β-, γ-HBCDD	18
Total HBCDD (using GC methods)	5

2.2 Accreditation

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

Accreditation according to ISO/IEC 17025	PBDEs	HBCDDs
yes	17	8
no	11	11

3. Detection methods

The following detection methods were applied:

- GC-HRMS-, GC-MS/MS-, GC-LRMS-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 HBCDDs in fish fillet (2001-FI)

Detection methods	PBDEs	HBCDDs
GC-HRMS	22	3
GC-MS/MS	9	1
GC-LRMS	3	-
LC-MS/MS	-	14
LC-HRMS	-	3

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 sample 2001-FI were analyzed in duplicate for PCDD/Fs and PCBs. The test for sufficient homogeneity was performed for the sum parameters WHO-PCDD/F-PCB-TEQ, WHO-PCDD/F-TEQ, WHO-PCB-TEQ, the sum of six indicator PCBs and PCDD/F, PCB and PBDE congeners. The test materials showed sufficient homogeneity for this proficiency test.

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 this proficiency test.

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 to [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 WHO-PCDD/F-PCB-TEQ, WHO-PCDD/F-TEQ, WHO-PCB-TEQ, the sum of six indicator PCBs and individual PCDD/F and PCB (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, if these levels are equal to or above the LOQ; otherwise the LOQ will be taken.

Assigned values could not be calculated for BDE-183 and BDE-209, β-, γ-HBCDD and total HBCDD due to the high variation of participants' results or the limited number of reported results above the LOQ.

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

Test sample **Fish fillet (2001-FI)**	**Assigned value** **All participants**	**Assigned value** **ISO/IEC 17025 accreditation**	**Deviation**
	pg/g, ng/g wet weight	pg/g, ng/g wet weight	%
Sum of PBDE without BDE-209 ub	0.91	0.888	2
Sum of PBDE including BDE-209 ub	0.96	0.946	1
Sum of α-, β-, γ-HBCDD ub	0.892	* (median: 0.765)	-

* Not enough results for calculation of assigned value available

5.1 Sum parameters and individual congeners/diastereomers

The assigned values for the test sample 2001-FI 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)
	ng/g wet weight	ng/g wet weight	ng/g wet weight	ng/g wet weight
Fish fillet (2001-FI)	0.888	0.946	0.892	-

Polybrominated diphenyl ether (PBDE)

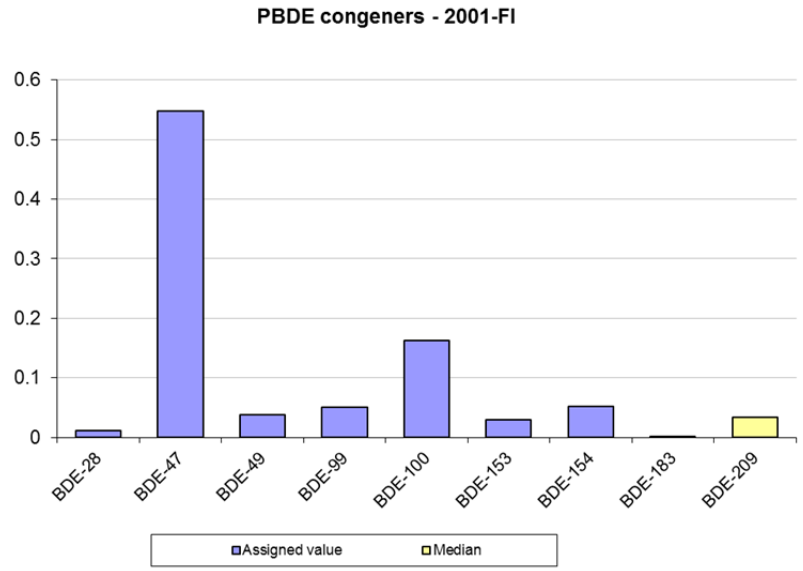

Figure 1: Assigned (blue) and median (yellow) values for PBDE congeners for fish fillet (2001-FI) [ng/g wet weight]

Hexabromocyclododecane (HBCDD)

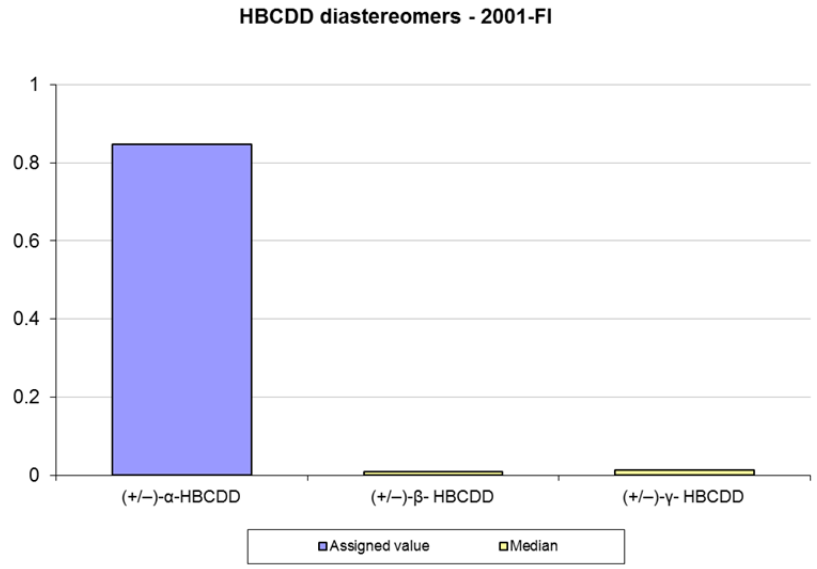

Figure 2: Assigned (blue) and median (yellow) values for HBCDD diastereomers for fish fillet (2001-FI) [ng/g wet weight]

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: participants 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 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 congeners and sum parameters for fish fillet (2001-FI)

Percentage of participants' results	|z-score| ≤ 2	2 < |z-score| < 3	|z-score| ≥ 3
BDE-28	88 %	3 %	9 %
BDE-47	95 %	0 %	5 %
BDE-49	88 %	8 %	4 %
BDE-99	97 %	0 %	3 %
BDE-100	97 %	0 %	3 %
BDE-153	92 %	5 %	3 %
BDE-154	100 %	0 %	0 %
BDE-183	-	-	-
BDE-209	-	-	-
Sum of PBDE without BDE-209	94 %	3 %	3 %
Sum of PBDE including BDE-209	94 %	3 %	3 %

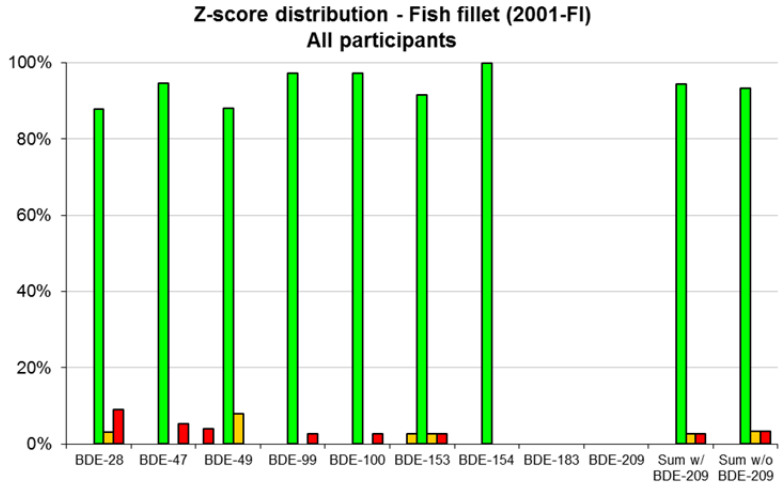

Figure 3: Distribution of participants' z-scores for PBDE congeners / sum parameters for fish fillet (2001-FI) [Green bars: -2 ≤ z-score ≤ 2, yellow bars: -3 < z-score < -2, 2 < z-score < 3, red bars: z-score ≤ -3, z-score ≥ 3]

Hexabromocyclododecane (HBCDD)

Table 9: Distribution of participants' z-scores for HBCDD diastereomers and HBCDD sum parameters for fish fillet (2001-FI)

Percentage of participants' results	|z-score| ≤ 2	2 < |z-score| < 3	|z-score| ≥ 3
(+/–)-α-HBCDD	78 %	22 %	0 %
(+/–)-β- HBCDD	-	-	-
(+/–)-γ- HBCDD	-	-	-
Sum of α-, β-, γ-HBCDD	78 %	22 %	0 %
Total HBCDD	-	-	-

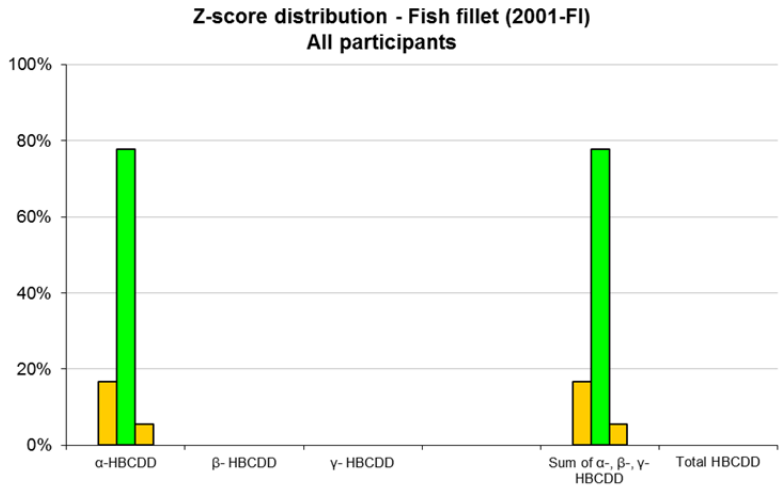

Figure 4: Distribution of participants' z-scores for HBCDD diastereomers / sum parameters for fish fillet (2001-FI) (Green bars: -2 ≤ z-score ≤ 2, yellow bars: -3 < z-score < -2, 2 < z-score < 3, red bars: z-score ≤ -3, z-score ≥ 3)

6.3 Comparison of sum of α-, β-, γ-HBCDD and total HBCDD

HBCDD diastereomers undergo thermal isomerization at temperatures above 160 °C. As the GC elution temperature of these compounds is normally above 160 °C a separation of HBCDD diastereomers is not possible using GC analysis. Only one unresolved peak is obtained. Additional thermal decomposition of HBCDD 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. Other HBCDDs are only found in traces. As a consequence, the sum of α-, β-, γ-HBCDD and total HBCDD can be compared.

Results for sum of α-, β-, γ-HBCDD and total HBCDD in the test sample fish fillet (2001-FI) are comparable. Z-scores for reported results for total HBCDD in comparison with the assigned value for the sum of α-, β-, γ-HBCDD are all in the range of +/- 2 z-scores.

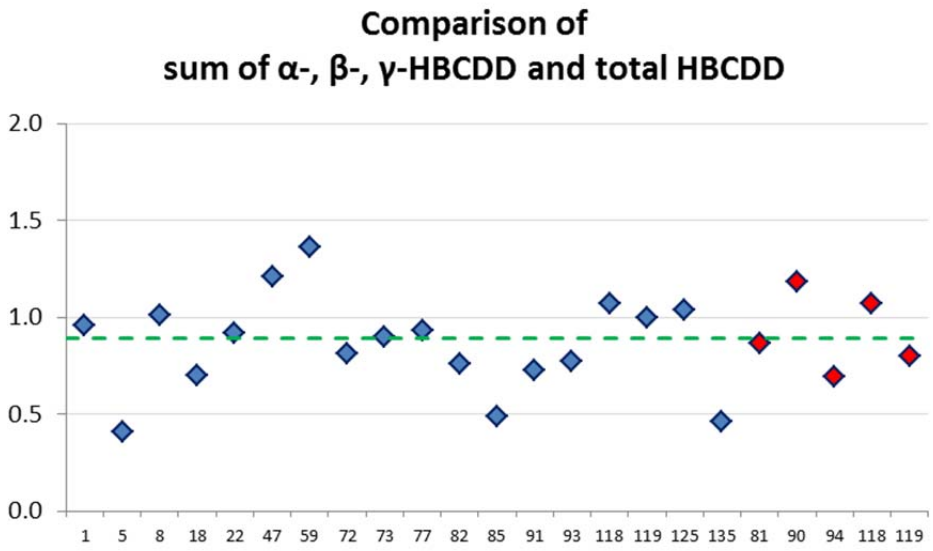

Figure 5: Participants' results for sum of α-, β-, γ-HBCDD and total HBCDD for fish fillet (2001-FI)
(Blue spots: participants' results for sum of α-, β-, γ-HBCDD, red spots: participants' results for total HBCDD)

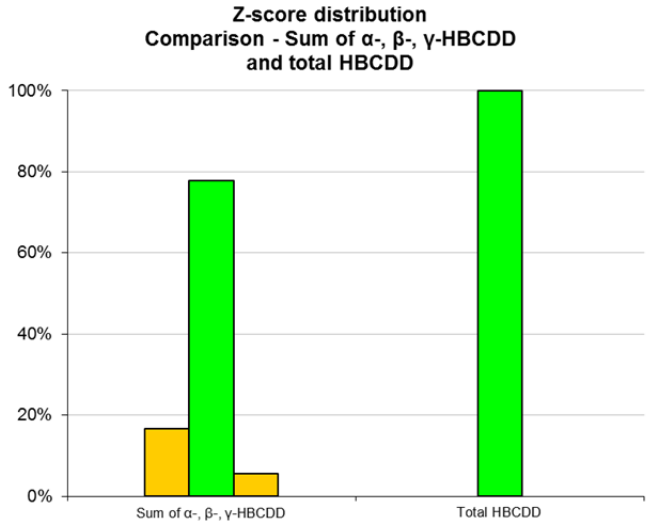

Figure 6: Distribution of participants' z-scores for sum of α-, β-, γ-HBCDD and total HBCDD for fish fillet (2001-FI) in comparison to the assigned value for sum of α-, β-, γ-HBCDD (Green bars: -2 ≤ z-score ≤ 2, yellow bars: -3 < z-score < -2, 2 < z-score < 3, red bars: z-score ≤ -3, z-score ≥ 3)

7. Participants' feedback

A questionnaire for feedback from participants of this EURL proficiency test was available as online survey between 23 July 2020 and 31 August 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, 20 laboratories (18 % of all participants) participated in this survey. A summary of the results is also given in annex 7.

7.1 Overview of questions and answers of participants

Participants' information:

National Reference Laboratory (NRL)	Official Laboratory (OFL)	Commercial laboratory	Other
63 %	5 %	26 %	11 %

Organization of proficiency test:

	Fully	Largely	Partly	Not at all	No opinion
Satisfied with organization of PT	68 %	32 %	-	-	-
Meeting of expectations	74 %	21 %	5 %	-	-
Information understandable	68 %	32 %	-	-	-
Time frame acceptable	74 %	26 %	-	-	-

PT test samples:

	Fully	Largely	Partly	Not at all	No opinion
Selection of matrix and level of contamination adequate	47 %	37 %	16 %	-	-

Evaluation of results and summary of data:

	Fully	Largely	Partly	Not at all	No opinion
Evaluation of results and report clear and comprehensible	68 %	21 %	11 %	-	-

7.2 Comments and suggestions

Comments referred to the too low concentrations for PCDD/Fs and the too complex tables for the overview of the results. Additionally also the long time between preliminary and final report was mentioned.

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 sample fish fillet (2001-FI) and the evaluation of the results 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 13528:2015, Statistical methods for use in proficiency testing by interlaboratory comparisons, International Organization for Standardization

[3] M. van den Berg et al., The 2005 World Health Organization Re-evaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-like Compounds. Toxicological Sciences 93(2), 223-241 (2006)

11. Annex

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

Fish fillet (2001-FI)		
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	
6	Participants' methods – PBDE, HBCDD	

Questionnaire for feedback from participants		
7	Summary of feedback	

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