



European Union Reference Laboratory
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

PFASs

(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 6 annexes.

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Summary

Test samples (food)	Fish fillet (fresh water fish) - 2001-FI
Analytes of interest	<u>Mandatory for NRLs:</u> - PFASs (PFOS, PFOA) <u>Optional for NRLs:</u> - Other PFASs (perfluoroalkylcarboxylic acids and perfluoroalkylsulfonic acids)
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	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:

PFASs

- Perfluorooctanesulfonic acid (PFOS)
- Perfluorooctanoic acid (PFOA)

The following optional parameters may additionally be reported:

PFASs

- Perfluoroalkylsulfonic acids (PFASAs):
Perfluorobutanesulfonic acid (PFBS), perfluoropentanesulfonic acid (PFPeS), perfluorohexanesulfonic acid (PFHxS), perfluoroheptanesulfonic acid (PFHpS)
- Perfluoroalkylcarboxylic acids (PFCAs):
Perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorononanoic acid (PFNA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA)

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 cooperation between the NRL, OFLs and the EURL.

1.4 Results of PFASs

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,



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- report results for PFAS calculated on the anion-basis
- 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 have to be reported in in **µg/kg wet weight** for PFASs.

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 PFASs

Reported results	Fish fillet (2001-FI)
PFOS	37
PFOA	35
PFBS	27
PFPeS	14
PFHxS	29
PFHpS	19
PFBA	24
PFPeA	24
PFHxA	27
PFHpA	27
PFNA	29
PFDA	28
PFAUnDA	28
PFDODA	28
PFTTrDA	22
PFTeDA	23

2.2 Accreditation

Table 3: Reported accreditation according to ISO/IEC 17025 by participants for PFASs

Accreditation according to ISO/IEC 17025	PFASs
yes	21
no	15

3. Detection methods

The following detection methods were applied:

- LC-MS/MS
- LC-HRMS



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, PCBs and PBDEs. The test materials showed sufficient homogeneity for these analytes in the 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, PCBs and PBDEs. The test materials showed sufficient stability for this proficiency test. The sufficient homogeneity and stability can then also be concluded for other halogenated contaminants, which were also naturally contaminated and not spiked.

5. Determination of the assigned values

Statistical evaluation of the PT results was 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 was performed according to [1] by estimating of the assigned value as the consensus of participants' results. The Huber robust mean was taken as assigned value after excluding extreme outliers (outside the range of $\pm 50\%$ 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 was only calculated for each compound individually 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 $\pm 50\%$ of the median of all reported results. Levels for individual compounds were only taken for evaluation, if these levels are equal to or above the LOQ; otherwise the LOQ will be taken.

Assigned values were calculated for PFDA, PUnDA, PDoDA and PFOS. Additionally the median of all values was calculated.

Assigned values were not calculated for other PFASs due to the limited number of reported results above the LOQ or no 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 PFASs. No significant differences between the assigned values calculated for both data sets were observed.



Table 4: Comparison of assigned values for all participants and participants with reported accreditation according to ISO/IEC 17025 for PFASs

Test sample Fish fillet (2001-FI)	Assigned value	Assigned value	Deviation
	All participants	ISO/IEC 17025 accreditation	
	µg/kg wet weight	µg/kg wet weight	%
PFDA	1.36	1.37	1
PFUnDA	0.315	0.292	7
PFDoDA	0.580	0.592	2
PFOS	10.3	10.3	0

5.1 Assigned values for PFASs

The assigned values for the test sample 2001-FI were calculated as consensus of participants' results for PFASs.

Table 5: Assigned values for PFASs (rounded to three significant figures)

Test sample	PFDA	PFUnDA	PFDoDA	PFOS
	µg/kg wet weight	µg/kg wet weight	µg/kg wet weight	µg/kg wet weight
Fish fillet (2001-FI)	1.36	0.315	0.580	10.3

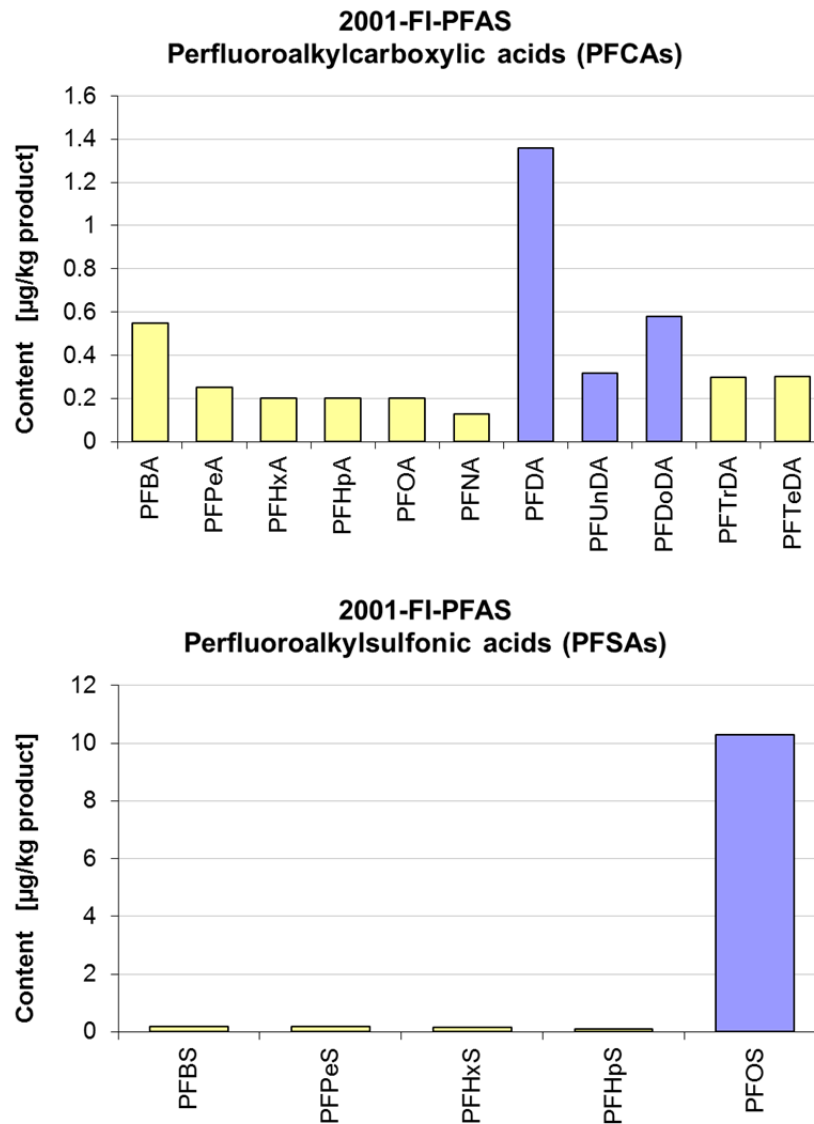


Figure 1: Assigned (blue) and median (yellow) values for PFASs for fish fillet (2001-FI) [$\mu\text{g}/\text{kg}$ wet weight]



6. Evaluation of results

6.1 Z-score calculation

The criteria for a successful participation of laboratories were based on the evaluation of the results of individual compounds.

For the evaluation of the results the **z-scores** were 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

The standard deviation for proficiency assessment σ_p was defined as 20 %.

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

Interpretation of z-scores:

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

6.2 PFASs - Participants' z-scores

Assigned values for PFDA, PFUnDA and PFDoDA were in the range of 1 µg/kg wet weight whereas the assigned value for PFOS was with around 10 µg/kg wet weight one order of magnitude higher.

Table 6: Distribution of participants' z-scores for PFASs for fish fillet (2001-FI)

Percentage of participants' results	PFDA	PFUnDA	PFDoDA	PFOS
$ z\text{-score} \leq 2$	88 %	90 %	94 %	79 %
$2 < z\text{-score} < 3$	8 %	0 %	6 %	9 %
$ z\text{-score} \geq 3$	4 %	10 %	0 %	12 %

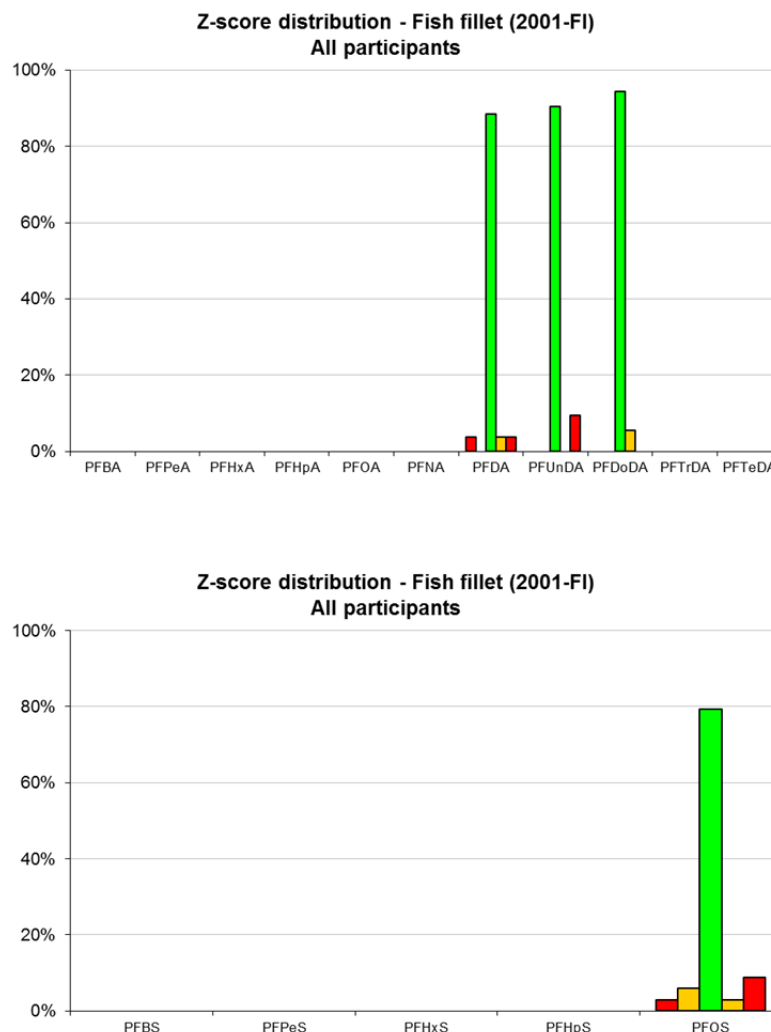


Figure 2: Distribution of participants' z-scores for PFASs for fish fillet (2001-FI)
[Green bars: $-2 \leq z\text{-score} \leq 2$, yellow bars: $-3 < z\text{-score} < -2$, $2 < z\text{-score} < 3$, red bars: $z\text{-score} \leq -3$, $z\text{-score} \geq 3$]

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 8.



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 PCDD/F and PCB results for the PT test sample fish fillet (2001-FI) and the evaluation of the results are given in the following annexes 1 - 8. 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 – PFAS	
2	Participants' results – Tables – PFAS	
3	Participants' z-scores – Tables - PFAS	
4	Participants' z-scores – Charts – PFAS	
5	Participants' methods – PFAS	

Questionnaire for feedback from participants		
6	Summary of feedback	

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