



**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

PFASs

(Report Version 1.0)

21 November 2022



Summary

Test sample	FOOD: Pork Liver [2201-PL]
Analytes of interest	<p>PFASs (L-PFOS, PFOA, PFNA, PFHxS, Sum of L-PFOS, PFOA, PFNA, PFHxS)</p> <p>Mandatory for NRLs:</p> <p>Other PFASs (perfluoroalkylcarboxylic acids, perfluoroalkylsulfonic acids, perfluoroalkane sulfonamides)</p> <p>Optional for NRLs:</p>
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	21 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 ILS, 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
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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:

- Linear perfluorooctanesulfonic acid (L-PFOS)
- Perfluorooctanoic acid (PFOA)
- Perfluorononanoic acid (PFNA)
- Perfluorohexanesulfonic acid (PFHxS)
- Sum of L-PFOS, PFOA, PFNA, PFHxS

Participants were encouraged to determine the additional parameters:

- **Perfluoroalkylsulfonic acids (PFSA)s**: perfluorobutanesulfonic acid (PFBS), perfluoropentanesulfonic acid (PFPeS), perfluoroheptanesulfonic acid (PFHpS), branched perfluorooctanesulfonic acids (br-PFOS), perfluorononanesulfonic acid (PFNS), perfluorodecanesulfonic acid (PFDS), perfluoroundecane sulfonic acid (PFUnDS), perfluorododecane sulfonic acid (PFDoDS), perfluorotridecane sulfonic acid (PFTrDS)
- **Perfluoroalkylcarboxylic acids (PFCAs)**: perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA)
- Sum of total PFOS, PFOA, PFNA, PFHxS
- Perfluorooctane sulphonamide (FOSA)

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 PFAS

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 all PFAS compounds.

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. 34 laboratories reported results for PFASs; of the 34, two laboratories submitted two sets of data for PFASs.

Table 1: Participating laboratories

Participating laboratories	Region	No. of participants
National Reference Laboratories	European Union	15
	Other Countries	1
Official Laboratories	European Union	15
	Other European Countries	-
	Africa	-
	Americas	-
	Asia	-
	Oceania	-
Commercial Laboratories	European Union	3
	Other European Countries	-
	Africa	-
	Americas	-
	Asia	-
	Oceania	-
	Total	34



2.1. Number of reported results

Table 2: Reported results for individual PFASs for pork liver (2201-PL) of all laboratories

Analyte	Abbreviation	2201-PL
Perfluorobutanoic acid	(PFBA)	23
Perfluoropentanoic acid	(PFPeA)	30
Perfluorohexanoic acid	(PFHxA)	30
Perfluoroheptanoic acid	(PFHpA)	30
Perfluorooctanoic acid	(PFOA)	35
Perfluorononanoic acid	(PFNA)	34
Perfluorodecanoic acid	(PFDA)	30
Perfluoroundecanoic acid	(PFUnDA)	29
Perfluorododecanoic acid	(PFDoDA)	29
Perfluorotridecanoic acid	(PFTrDA)	23
Perfluorotetradecanoic acid	(PFTeDA)	22
Perfluorobutanesulfonic acid	(PFBS)	29
Perfluoropentanesulfonic acid	(PFPeS)	24
Perfluorohexanesulfonic acid	(PFHxS)	34
Perfluoroheptanesulfonic acid	(PFHpS)	27
Linear Perfluorooctanesulfonic acid	(L-PFOS)	33
Perfluorononanesulfonic acid	(PFNS)	19
Perfluorodecanesulfonic acid	(PFDS)	24
Perfluoroundecanesulfonic acid	(PFUnDS)	7
Perfluorododecanesulfonic acid	(PFDoDS)	13
Perfluorotridecanesulfonic acid	(PFTrDS)	6
Perfluorooctane sulphonamide	(FOSA)	10

**Table 3:** Reported results for PFASs sum parameters for pork liver (2201-PL) of all laboratories

Analyte	2201-PL
Sum of branched perfluorooctanesulfonic acids (br-PFOS)	27
Sum of branched & linear perfluorooctanesulfonic acids (Total-PFOS)	29
Sum of L-PFOS, PFOA, PFNA, PFHxS (upper bound)	33
Sum of L-PFOS, PFOA, PFNA, PFHxS (lower bound)	33
Sum of Total-PFOS, PFOA, PFNA, PFHxS (upper bound)	24
Sum of Total-PFOS, PFOA, PFNA, PFHxS (lower bound)	24

2.2. Accreditation

Table 4: Reported accreditation according to ISO/IEC 17025 by participants for PFASs

Pork Liver	PFASs
Accreditation	21
No accreditation	12

2.3. Detection methods

Any kind of chromatographic separation and detection methods could be applied for analysis. Most of the participating laboratories applied ultra- or high-performance liquid chromatography (U/HPLC) as separation method combined with low resolution tandem mass spectrometry (MS/MS) as detection method, except two laboratories, which applied high resolution mass spectrometry (Orbitrap HRMS) as detection method.

3. Test for sufficient homogeneity and stability

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 analysed in duplicate for PFOA, PFNA, PFDA, PFUnDA, PFDoDA and L-PFOS. The test materials showed sufficient homogeneity for PFASs in this proficiency test. The stability check of the analytes of interest applying room temperature storage was performed according to DIN ISO 13528:2020 [2]. The test material showed sufficient stability for PFASs.



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 $\pm 50\%$ of the median of all reported results) and examination of the distribution of the remaining results using histogram and Kernel density estimation, if necessary.

Assigned values were calculated for individual PFAS compounds, and sum parameters (including limits of quantification (LOQs)), if possible. Additionally the median of all values was calculated.

For individual substances (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 $\pm 50\%$ 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.

Assigned values were calculated for the perfluoroalkylcarboxylic acids PFOA, PFNA, PFDA, PFUnDA and PFDoDA, for the perfluoroalkylsulfonic acids L-PFOS and sum of branched and linear Perfluorooctanesulfonic acids and for the sum parameters sum of L-PFOS, PFOA, PFNA, PFHxS and sum of total-PFOS, PFOA, PFNA, PFHxS in test sample "pork liver" (2201-PL), including limits of quantification (LOQs). Assigned values could not be calculated for all other perfluoroalkylcarboxylic acids, perfluoroalkylsulfonic acids and perfluorooctane sulphonamide (FOSA) due to the limited number of reported results above the LOQ, but median values of participants' results are given in this report.

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 PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFOA, L-PFOS and sum parameters (sum of branched and linear Perfluorooctanesulfonic acids, Sum of L-PFOS, PFOA, PFNA, PFHxS and Sum of Total-PFOS, PFOA, PFNA, PFHxS). 21 of 34 reporting laboratories were accredited according to ISO/IEC 17025 for PFAS. After eliminating outliers, 12 to 20 results contributed to the calculation of the assigned values from the ISO/IEC 17025 group. No significant differences ($<1 - 4\%$) between the assigned values calculated for both data sets for PFASs were observed (Table 5).



Table 5: Comparison of assigned values for 2201-PL for all participants and participants with reported accreditation according to ISO/IEC 17025 for PFAS

Sum parameters	Assigned value	Assigned value	Deviation
	All participants	ISO/IEC 17025 accreditation	
	µg/kg w.w.		%
PFOA	0.847	0.810	4
PFNA	1.46	1.44	1
PFDA	0.884	0.860	3
PFUnDA	0.567	0.555	2
PFDoDA	0.486	0.491	1
PFOA	0.847	0.810	4
L-PFOS	27.8	27.7	<1
Total PFOS	29.8	29.7	<1
Sum of L-PFOS, PFOA, PFNA, PFHxS (ub)	31.0	31.1	<1
Sum of L-PFOS, PFOA, PFNA, PFHxS (lb)	31.0	31.3	1
Sum of total PFOS, PFOA, PFNA, PFHxS (ub)	31.0	31.4	1
Sum of total PFOS, PFOA, PFNA, PFHxS (lb)	31.0	31.4	1

4.1 PFASs – individual substances and sum parameter

The assigned values for the test sample 2201-PL were calculated as consensus of participants' results for individual PFASs and sum parameters, taking into account the calculation criteria described above (Table 6 - 8 ; tabular summary see annex 1; Figure 1-3).

Table 6: Assigned values for Perfluoroalkylcarboxylic acids (rounded to three significant figures)

Pork Liver (2201-PL)	Assigned value PFCAs µg/kg w.w.
PFOA	0.847
PFNA	1.46
PFDA	0.884
PFUnDA	0.567
PFDoDA	0.486

Table 7: Assigned values for Perfluoroalkylsulfonic acids (rounded to three significant figures)

Pork Liver (2201-PL)	Assigned value PFASs µg/kg w.w.
L-PFOS	27.8
Total-PFOS	29.8

Table 8: Assigned values for sum of PFOS, PFOA, PFNA, PFHxS (rounded to three significant figures)

Pork Liver (2201-PL)	Assigned value µg/kg w.w.
Sum of L-PFOS, PFOA, PFNA, PFHxS (ub)	31.0
Sum of L-PFOS, PFOA, PFNA, PFHxS (lb)	31.0
Sum of Total PFOS, PFOA, PFNA, PFHxS (ub)	31.0
Sum of Total PFOS, PFOA, PFNA, PFHxS (lb)	31.0

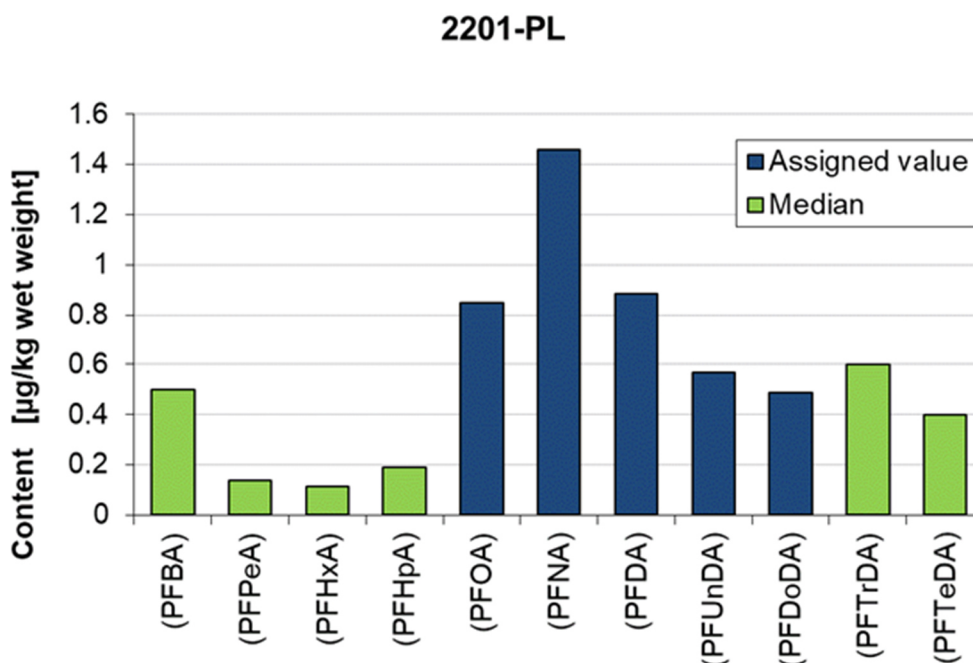


Figure 1: Assigned values (blue) and median values (green) for PFCAs individual substances for pork liver (2201-PL) [µg/kg w.w.]

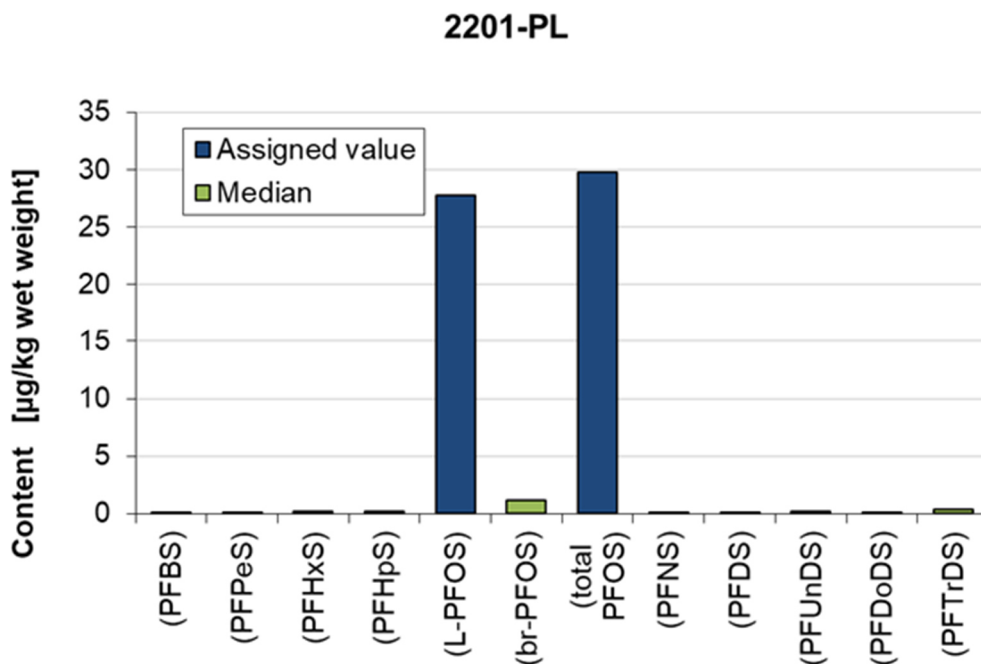


Figure 2: Assigned values (blue) and median values (green) for PFSA's individual substances for pork liver (2201-PL) [µg/kg w.w.]

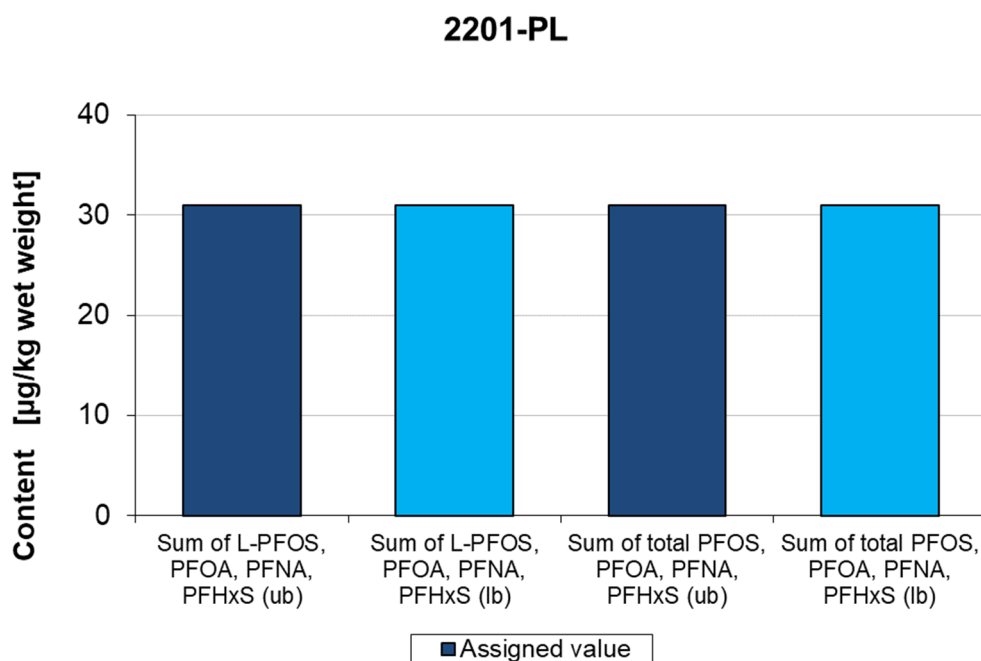


Figure 3: Assigned values (dark blue upper bound and light blue lower bound values) for sum parameters of L-PFOS, PFOA, PFNA and PFHxS and total-PFOS, PFOA, PFNA and PFHxS for pork liver (2201-PL) [µg/kg w.w.]



5. Evaluation of results

5.1. Z-scores calculation

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

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

Z-scores for individual substances and sum parameters were 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:

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

5.2. PFASs - Participants' z-scores

Z-scores for individual substances and sum parameters were within the range of ± 2 for 82% of all participants (Table 9 - 11; tabular summary see annex 3; Figure 4-6).

Table 9: Distribution of participants' z-scores for PFCAs for pork liver (2201-PL)

Percentage of participants' results PFCAs	$ z\text{-score} \leq 2$	$2 < z\text{-score} < 3$	$ z\text{-score} \geq 3$
PFOA	86%	8%	6%
PFNA	82%	6%	12%
PFDA	90%	-	10%
PFUnDA	93%	-	7%
PFDoDA	92%	-	8%

Table 10: Distribution of participants' z-scores for PFSA for pork liver (2201-PL)

Percentage of participants' results PFSA	$ z\text{-score} \leq 2$	$2 < z\text{-score} < 3$	$ z\text{-score} \geq 3$
L-PFOS	94%	3%	3%
Total PFOS	93%	7%	-

Table 11: Distribution of participants' z-scores for sum parameters for pork liver (2201-PL)

Percentage of participants' results Sum parameters	$ z\text{-score} \leq 2$	$2 < z\text{-score} < 3$	$ z\text{-score} \geq 3$
Sum of L-PFOS, PFOA, PFNA, PFHxS (ub)	88%	6%	6%
Sum of L-PFOS, PFOA, PFNA, PFHxS (lb)	85%	6%	9%
Sum of total-PFOS, PFOA, PFNA, PFHxS (ub)	92%	4%	4%
Sum of total-PFOS, PFOA, PFNA, PFHxS (lb)	88%	4%	8%

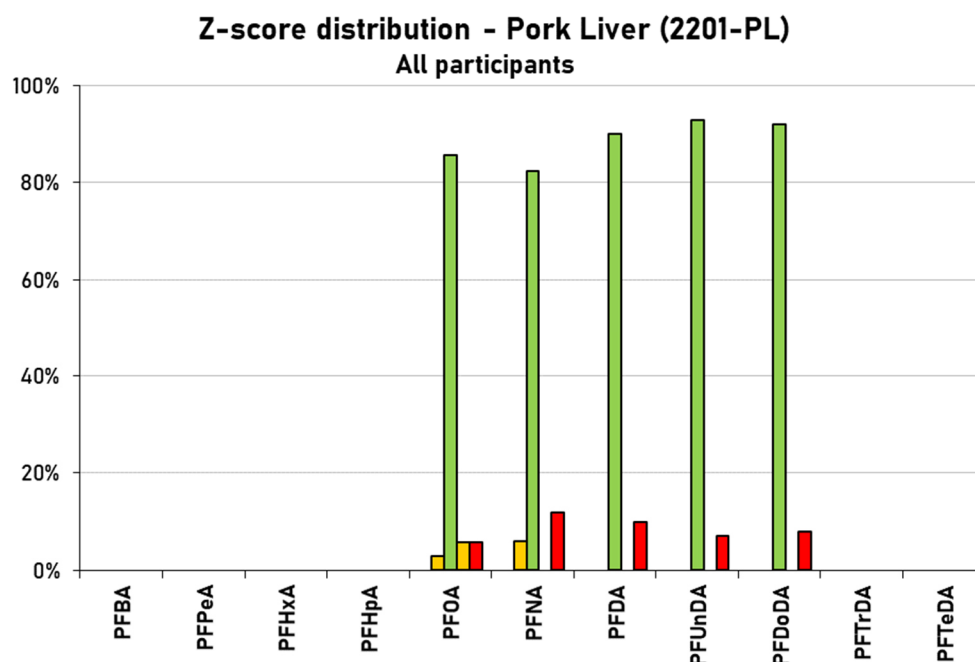


Figure 4: Distribution of participants' z-scores for individual PFCAs for pork liver (2201-PL) [Green bars: $-2 \leq z\text{-score} \leq 2$, orange bars: $-3 < z\text{-score} < -2$, $2 < z\text{-score} < 3$, red bars: $z\text{-score} \leq -3$, $z\text{-score} \geq 3$]

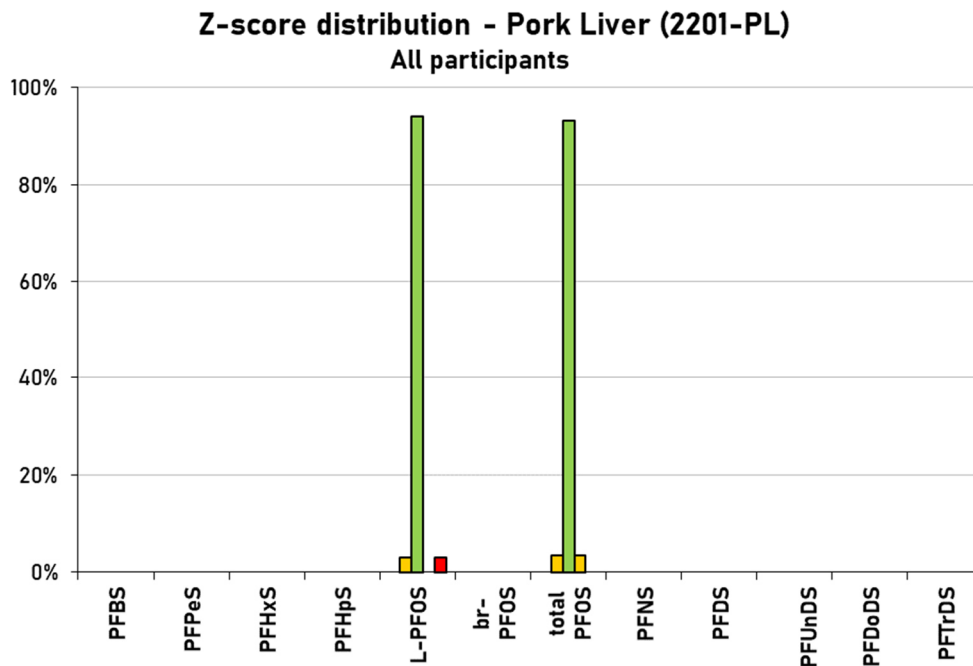


Figure 5: Distribution of participants' z-scores for individual PFASs for pork liver (2201-PL) [Green bars: $-2 \leq z\text{-score} \leq 2$, orange bars: $-3 < z\text{-score} < -2$, $2 < z\text{-score} < 3$, red bars: $z\text{-score} \leq -3$, $z\text{-score} \geq 3$]

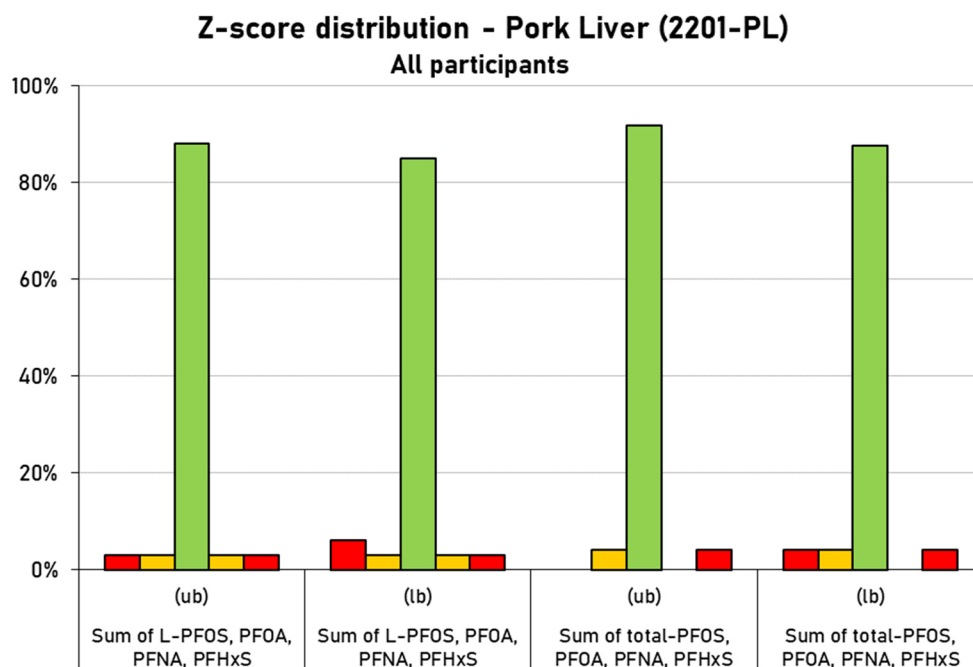


Figure 6: Distribution of participants' z-scores for sum parameters for pork liver (2201-PL) [Green bars: $-2 \leq z\text{-score} \leq 2$, orange bars: $-3 < z\text{-score} < -2$, $2 < z\text{-score} < 3$, red bars: $z\text{-score} \leq -3$, $z\text{-score} \geq 3$]

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 and summary of data) and a possibility to include comments and further suggestions. In total, 7 laboratories (21 % 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 PFASs 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] ISO 13528:2020, Statistical methods for use in proficiency testing by interlaboratory comparisons, International Organization for Standardization

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 values – PFCAs, PFSA's and sum parameters	
2	Participants' results – Tables – PFCAs, PFSA's, FOSA and sum parameters	
3	Participants' z-scores – Tables – PFCAs, PFSA's and sum parameters	
4	Participants' z-scores – Charts – PFCAs, PFSA's and sum parameters	
5	Test for sufficient homogeneity and stability – PFCAs and PFSA's	
6	Overview participants' methods – Weighed sample, internal and recovery standards and comments	
7	Overview participants' methods – Extractions, clean-up and detection	
8	Overview participants' methods – Measurement uncertainty and Limit of Quantification	

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