European Union Reference Laboratory
for Halogenated POPs in Feed and Food

State Institute for Chemical and Veterinary Analysis of Food, Freiburg, Germany

Chemisches und Veterinäruntersuchungsamt Freiburg
PO Box 100462 ◆ D-79123 Freiburg ◆ Germany

EURL Proficiency Test on the Determination of Perfluoroalkyl Substances in Wheat Flour 2019

EURL-PT-PF_1903-WF

FOOD

Report

(Version 1.0)

06 September 2019

This report on the EURL proficiency test on the Determination of Perfluoroalkyl Substances in Wheat Flour (EURL-PT-PF_1903-WF) 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 13 annexes.

Office Building
Bissierstrasse 5
79114 Freiburg

Phone/Fax
Phone: +49-761-8855-500
Fax: +49-761-8855-100

E-Mail
EURL: eurl-dioxin@cvuafr.bwl.de

Internet
www.eurl-dioxin-freiburg.eu

Summary

Test sample (food)	Wheat flour - 1903-WFA Wheat flour - 1903-WFB
Analytes of interest	Short and long chain perfluoroalkylcarboxylic acids Short and long chain perfluoroalkylsulfonic acids
Methods	Any kind of method
Participants	NRLs, OFLs, other official laboratories, commercial laboratories
Statistical evaluation	ISO 13528:2015, IUPAC Protocol
Report	06 September 2019

1. Structure of the PT, test material and analytes

This proficiency test (PT) on the determination of Perfluoroalkyl Substances in wheat flour was organized by the EURL for Halogenated Persistent Organic Pollutants (POPs) in Feed and Food to be performed between February and April 2019. The objective was to assess analytical performance of laboratories and the interlaboratory comparability of results from analyses of short and long chain perfluoroalkylcarboxylic acids and short and long chain perfluoroalkylsulfonic acids in two samples of wheat flour.

National Reference Laboratories (NRLs) for Halogenated POPs from EU member states were asked to participate according to their current responsibilities and capabilities. NRLs were invited to encourage the participation of Official Laboratories (OFLs) from their member states. The 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 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 feed business operators.

The evaluated results were circulated in the report of the preliminary results on April 24th, 2019 and were presented and discussed at the COM/EURL/NRL workshop on May 14th – 15th, 2019 in Riga, Latvia.

1.1 Samples and coding

The wheat flour test samples were prepared of wheat from a contamination site in Germany, which was naturally contaminated with short chain perfluoroalkylcarboxylic acids (PFBA, PFPeA, PFHxA, PFHpA) and of blank wheat flour from the market. Both test samples were additionally fortified with selected standard solutions of PFOS, PFOA and PFDA.

Wheat flour A	**Sample no. 1903-WFA-xxx**
Wheat flour B	**Sample no. 1903-WFB-xxx**

Each participant received about 50 g of each test sample.

1.2 Analytes of interest

Participants were requested to determine the following compounds:

Short chain perfluoroalkylcarboxylic acids[1]	Long chain perfluoroalkylcarboxylic acids[1] $C_nF_{2n+1}COOH$ (n≥7)
• Perfluorobutanoic acid (PFBA) • Perfluoropentanoic acid (PFPeA) • Perfluorohexanoic acid (PFHxA) • Perfluoroheptanoic acid (PFHpA)	• Perfluorooctanoic acid (PFOA) • Perfluorononanoic acid (PFNA) • Perfluorodecanoic acid (PFDA)

Short chain perfluoroalkylsulfonic acids[1]	Long chain perfluoroalkylsulfonic acids[1] $C_nF_{2n+1}SO_3H$ (n≥6)
• Perfluorobutanesulfonic acid (PFBS) • Perfluoropentanesulfonic acid (PFPeS)	• Perfluorohexanesulfonic acid (PFHxS) • Perfluoroheptanesulfonic acid (PFHpS) • Perfluorooctanesulfonic acid (PFOS) • Perfluorononanesulfonic acid (PFNS) • Perfluorodecanesulfonic acid (PFDS)

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 Reporting of Results

Laboratories should:

- use their own reference standards for identification and quantification,
- report results for each analyte in µg/kg,
- 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)*

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

29 laboratories reported results for at least one of the requested parameters.

Table 1: Participating laboratories

Participating laboratories	Region	No. of participants
National Reference Laboratories	European Union	10
	Other Countries	1
Official Laboratories	European Union	11
	Other European Countries	0
	Africa	0
	Americas	0
	Asia	0
	Oceania	0
Commercial Laboratories	European Union	5
	Other European Countries	0
	Africa	0
	Americas	2
	Asia	0
	Oceania	1
	Total	**29**

2.1 Accreditation

Table 2: Reported accreditation according to ISO/IEC 17025 by participants for PFAS analysis

Accreditation according to ISO/IEC 17025	PFASs
yes	13
no	16

2.2 Number of reported results

Table 3: Reported results for individual PFAS substances

Reported results	PFBA	PFPeA	PFHxA	PFHpA	PFOA	PFDA	PFNA	PFOS
Wheat flour (1903-WFA)	22	23	23	11	28	23	2	27
Wheat flour (1903-WFB)	22	24	24	12	29	26	4	29

3. Methods

Any kind of chromatographic separation and detection methods could be applied for analysis. All 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 five laboratories, which applied high resolution mass spectrometry (HRMS) as detection method, whereas four laboratories used *Orbitrap* technology and one *time-of-flight* technology (TOF).

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 each test sample - 1903-WFA and 1903-WFB - were analyzed in duplicate for PFASs. The test for sufficient homogeneity was performed for each detected individual substance (PFBA, PFPeA, PFHxA, PFOA, PFDA and PFOS). Both test materials proved to be adequately homogeneous for this proficiency test.

The stability check of the analytes of interest applying room temperature storage was performed according to ISO 13528:2015 [2]. Both test materials proved to be adequately stable at room temperature 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 13528:2015 [3] and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [2].

The determination of the assigned value was performed according to [2] 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 ± 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 ± 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 PFBA, PFPeA, PFHxA, PFOA, PFDA and PFOS in test sample "wheat flour A" and for PFOA, PFDA and PFOS in test sample "wheat flour B" (including limits of quantification (LOQs)). Additionally, the median of all values was calculated.

Assigned values could not be calculated for PFBA, PFPeA and PFHxA in test sample "Wheat flour B" due to the high variation of participants' results leading to a bimodal distribution.

Assigned values were not calculated for PFHpA, PFNA, PFBS, PFPeS, PFHxS, PFHpS, PFNS and PFDS in either of the two samples 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.

5.1 Assigned values

The assigned values for the test samples 1903-WFA and 1903-WFB for individual PFAS substances were calculated as consensus of participants' results taken into account the criteria for calculation as described above.

For all individual substances without a calculated assigned value the median was calculated informatively.

Table 4: Assigned values for individual PFAS substances with number of results from participants contributing to the calculation of the respective assigned value (rounded to three significant figures)

Test sample	PFBA	PFPeA	PFHxA	PFOA	PFDA	PFOS
	µg/kg	µg/kg	µg/kg	µg/kg		
Wheat flour (1903-WFA)	4.40	4.94	2.67	2.07	1.93	1.78
No. of results contributing to assigned value	14	20	19	26	22	25
Wheat flour (1903-WFB)	-	-	-	9.45	9.54	8.76
No. of results contributing to assigned value				26	23	25

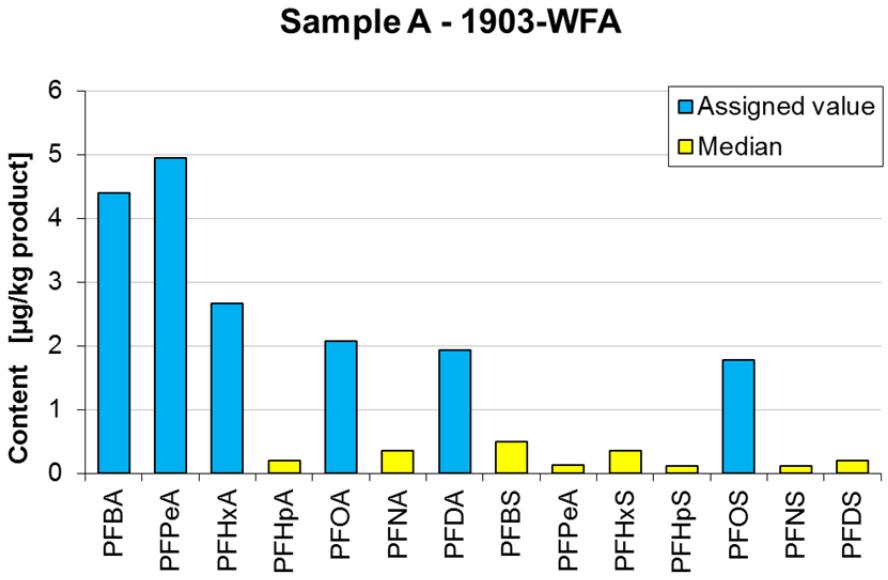

Figure 1: Assigned (blue) and median (yellow) values for individual PFAS substances for test sample 1903-WFA [µg/kg product]

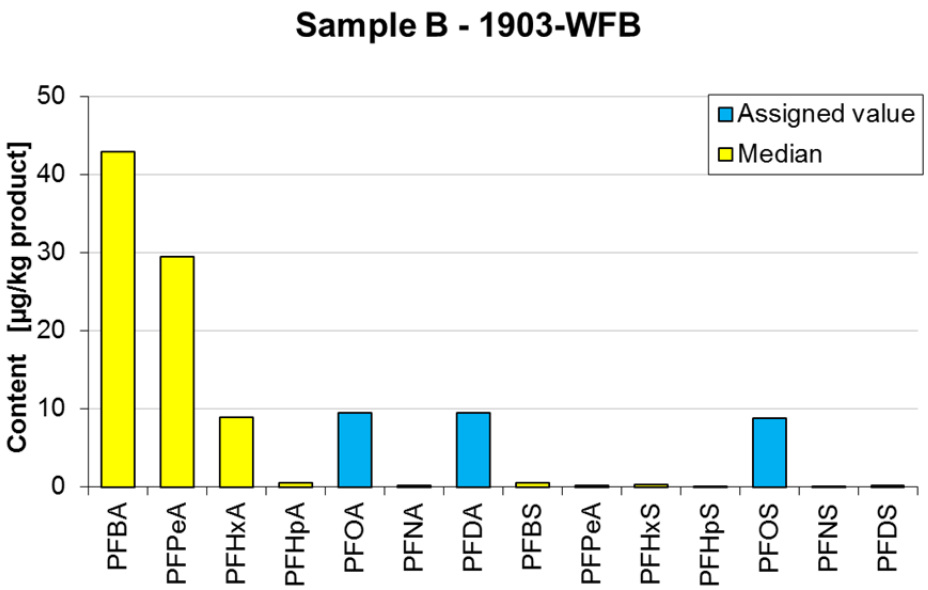

Figure 2: Assigned (blue) and median (yellow) values for individual PFAS substances for test sample 1903-WFB [µg/kg product]

5.2 Bimodal distribution of participants' results

In test sample "Wheat flour B" (1903-WFB) assigned values could not be calculated for the naturally contaminated analytes PFBA, PFPeA and PFHxA. These substances were present in the test sample at levels in the range of the levels of the fortified analytes L-PFOS, PFOA and PFDA and up to a factor of 4 higher. In test sample "Wheat flour A" (1903-WFA) the same substances were also present as naturally contaminated analytes but with about one order of magnitude lower levels. Assigned values could be calculated for all three substances in this sample.

Participants' results for test sample B showed a bimodal distribution for PFBA, PFPeA and PFHxA. In all three cases the level of the minor mode was about 50 % lower than the major mode indicating a possible systematic error. But possible reasons for this bimodal distribution of results could not be identified.

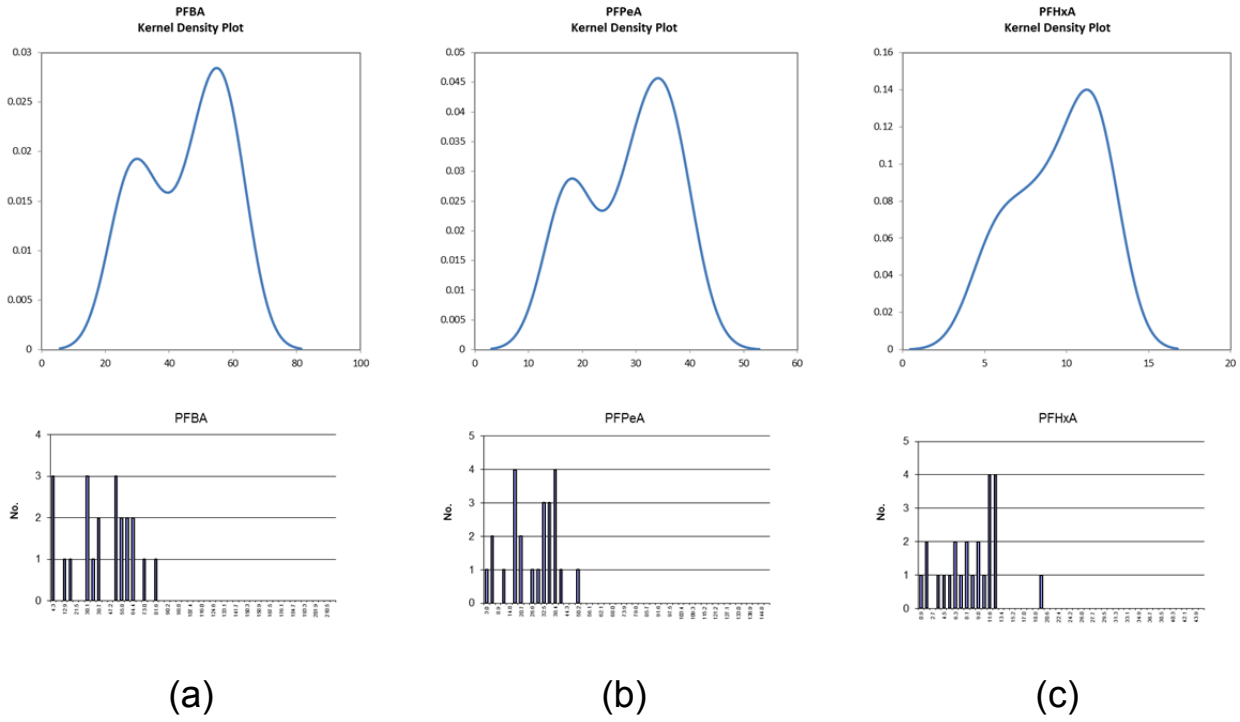

Figure 3: Kernel density plots and Histograms of individual PFAS substances PFBA (a), PFPeA (b) and PFHxA (c) in wheat flour sample B (1903-WFB)

6. Evaluation of results using z-scores

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-score| ≤ 2	satisfactory performance
2 < |z-score| < 3	questionable performance (warning signal)
|z-score| ≥ 3	unsatisfactory performance (action signal)

Table 5: Distribution of participants' z-scores for individual PFAS substances in wheat flour sample A (1903-WFA)

Percentage of participants' results	**PFBA**	**PFPeA**	**PFHxA**	**PFOA**	**PFDA**	**PFOS**
|z-score| ≤ 2	14 / 22 (63%)	21 / 23 (91%)	20 / 23 (87%)	25 / 28 (89%)	18 / 23 (78%)	24 / 27 (89%)
2 < |z-score| < 3	4 / 22 (18%)	0 / 23	0 / 23	2 / 28 (7%)	2 / 23 (9%)	1 / 27 (4%)
|z-score| ≥ 3	4 / 22 (18%)	2 / 23 (9%)	3 / 23 (13%)	1 / 28 (4%)	3 / 23 (13%)	2 / 27 (7%)

Table 6: Distribution of participants' z-scores for individual PFAS substances in wheat flour sample B (1903-WFB)

Percentage of participants' results	**PFBA**	**PFPeA**	**PFHxA**	**PFOA**	**PFDA**	**PFOS**
|z-score| ≤ 2	-	-	-	27 / 29 (93%)	23 / 26 (88%)	24 / 29 (83%)
2 < |z-score| < 3	-	-	-	0 / 29	1 / 26 (4%)	2 / 29 (7%)
|z-score| ≥ 3	-	-	-	2 / 29 (7%)	2 / 26 (8%)	3 / 29 (10%)

7. Participants' feedback

A questionnaire for feedback from participants of this EURL proficiency test was available as online survey between 17th and 26th of May 2019. 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 (participant's 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, 15 laboratories (42 % of all participants) participated in this survey. A summary of the results is also given in annex 13.

7.1 Overview of questions and answers of participants

Participant's information:

National Reference Laboratory (NRL)	Official Laboratory (OFL)	Commercial laboratory	Other
33 %	27 %	20 %	20 %

Organization of proficiency test:

	Fully	Largely	Partly	Not at all	No opinion
Satisfied with organization of PT	73 %	13 %	7 %	-	7 %
Meeting of expectations	47 %	33 %	13 %	7 %	-
Information understandable	53 %	40 %	-	-	7 %
Time frame acceptable	53 %	40 %	-	-	7 %

PT test samples:

	Fully	Largely	Partly	Not at all	No opinion
Selection of matrix and level of contamination adequate	47 %	33 %	13 %	7 %	-

Evaluation of results and summary of data:

	Fully	Largely	Partly	Not at all	No opinion
Evaluation of results and report clear and comprehensible	27 %	53 %	7 %	7 %	7 %

7.2 Comments and suggestions

Comments referred to the relevance of this matrix type, the too low number of (spiked) analytes of interest and the bi-modal distribution of results for test sample 1903-WFB. Future PTs should include also other matrix types like vegetables, meat or fish.

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 PFASs results for the both PT test samples wheat flour A and wheat flour B (1903-WFA and 1903-WFB) is given in the following annexes. Laboratories are coded according to the laboratory codes sent after registration.

10. References

[1] Buck, R. C.; Franklin, J.; Berger, U.; Conder, J. M.; Cousins, I. T.; De Voogt, P.; Jensen, A. A.; Kannan, K.; Mabury, S. A.; van Leeuwen, S. P. J. Perfluoroalkyl and polyfluoroalkyl substances in the environment: terminology, classification, and origins. Integr Environ Assess Manag 7, 513–541, 2011.

[2] 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.

[3] ISO 13528:2015, 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.)

Wheat flour A (1903-WFA)		
1	Assigned values – PFCAs and PFSAs – wheat flour sample A	
2	Participants' results – Tables – PFCAs and PFSAs – wheat flour sample A	
3	Participants' z-scores – Tables – PFCAs and PFSAs – wheat flour sample A	
4	Participants' z-scores – Charts – PFCAs and PFSAs – wheat flour sample A	
5	Test for sufficient homogeneity and stability – PFCAs and PFSAs – wheat flour sample A	
6	Participants' methods for PFAS – wheat flour sample A	
Wheat flour B (1903-WFB)		
7	Assigned values – PFCAs and PFSAs – wheat flour sample B	
8	Participants' results – Tables – PFCAs and PFSAs – wheat flour sample B	
9	Participants' z-scores – Tables – PFCAs and PFSAs – wheat flour sample B	
10	Participants' z-scores – Charts – PFCAs and PFSAs – wheat flour sample B	
11	Test for sufficient homogeneity and stability – PFCAs and PFSAs – wheat flour sample B	
12	Participants' methods for PFAS – wheat flour sample B	
Questionnaire for feedback from participants		
13	Summary of feedback	

EURL for Halogenated POPs in Feed and Food
c/o State Institute for Chemical and Veterinary Analysis of Food Freiburg
Theresa Zwickel and Alexander Schächtele

Phone: +49 761 8855 500
E-Mail: eurl-dioxin@cvuafr.bwl.de