EURL Proficiency Test on the Determination of Perfluoroalkyl Substances in Liquid Whole Egg and Honey 2021

EURL-PT-PF_2102-LWE

FOOD

Final Results – Honey

(Report Version 1.0)

27 September 2022

EURL for halogenated POPs in Feed and Food
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Summary

Test sample (food)	Honey – 2102-HO
Analytes of interest	**Mandatory for NRLs:** ▪ linear PFOS (please report also branched PFOS and total PFOS, if possible) ▪ PFOA ▪ PFNA ▪ PFHxS ▪ Sum of PFOS, PFOA, PFNA, PFHxS **Optional for NRLs:** ▪ Other short and long chain perfluoroalkyl carboxylic acids (C4 – C14) ▪ Other short and long chain perfluoroalkyl sulfonic acids (C4 – C10)
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	27 September 2022
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 **perfluoroalkyl substances (PFAS)** in **liquid whole egg** (mandatory) and **honey** (voluntary) was organised by the EURL for halogenated Persistent Organic Pollutants (POPs) in Feed and Food to be performed between August and December 2021 for honey. The objective was to assess analytical performance of laboratories and the interlaboratory comparability of results from analyses of short and long chain perfluoroalkyl carboxylic acids and short and long chain perfluoroalkyl sulfonic acids in **food samples**. Final results for liquid whole egg were already published on 23 September 2022.

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 allowed 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 food business operators. The evaluated final results will be discussed by representatives of European Commission, NRLs and the EURL at the EURL/NRL workshop on 29 and 30 November 2022.

1.1. Samples and coding

The honey test sample were prepared of honey from a contaminated site and was not fortified with analytes of interest.

Honey	**Sample no. 2102-HO-xxx**

Each participant received about **30 g** of the test sample in a HDPE bottle.

1.2. Analytes of interest

NRLs for halogenated POPs in feed and food were requested to determine the following parameters:

- PFOS **(at least linear PFOS should be reported, if possible also branched and total PFOS)**
- PFOA
- PFNA
- PFHxS
- Sum of PFOS, PFOA, PFNA, PFHxS

The following optional parameters could be reported additionally:

- Perfluoroalkylsulfonic acids (PFSAs):
  Perfluorobutanesulfonic acid (PFBS), perfluoropentanesulfonic acid (PFPeS), perfluoroheptanesulfonic acid (PFHpS), perfluorononanesulfonic acid (PFNS), perfluorodecanesulfonic acid (PFDS)
- Perfluoroalkylcarboxylic acids (PFCAs):
  Perfluorobutanoic acid (PFBA), perfluoropentanoic acid (PFPeA), perfluorohexanoic acid (PFHxA), perfluoroheptanoic acid (PFHpA), perfluorodecanoic acid (PFDA), perfluoroundecanoic acid (PFUnDA), perfluorododecanoic acid (PFDoDA), perfluorotridecanoic acid (PFTrDA), perfluorotetradecanoic acid (PFTeDA)

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.

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.**

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

43 laboratories registered for this proficiency test and **22** reported results for honey.

Table 1: Participating laboratories

Participating laboratories	Region	No. of participants
National Reference Laboratories	European Union Other Countries	15 -
Official Laboratories	European Union Other European Countries Africa Americas Asia Oceania	11 1 - - - 1
Commercial Laboratories	European Union Other European Countries Africa Americas Asia Oceania	9 1 - 2 - -
University Research Institutes	European Union Other Countries	3 -
	Total	**43**

2.1. Number of reported results

Table 2: Reported results for PFASs for honey (2102-HO) of all laboratories

Analyte	Abbreviation	2102-HO
Perfluorobutanoic acid	(PFBA)	21
Perfluoropentanoic acid	(PFPeA)	22
Perfluorohexanoic acid	(PFHxA)	22
Perfluoroheptanoic acid	(PFHpA)	22
Perfluorooctanoic acid	(PFOA)	22
Perfluorononanoic acid	(PFNA)	21
Perfluorodecanoic acid	(PFDA)	21
Perfluoroundecanoic acid	(PFUnDA)	21
Perfluorododecanoic acid	(PFDoDA)	21
Perfluorotridecanoic acid	(PFTrDA)	17
Perfluorotetradecanoic acid	(PFTeDA)	17
Perfluorobutanesulfonic acid	(PFBS)	20
Perfluoropentanesulfonic acid	(PFPeS)	16
Perfluorohexanesulfonic acid	(PFHxS)	21
Perfluoroheptanesulfonic acid	(PFHpS)	20
Perfluorononanesulfonic acid	(PFNS)	15
Perfluorodecanesulfonic acid	(PFDS)	18
Linear Perfluorooctanesulfonic acid	(L-PFOS)	19
Sum of branched Perfluorooctanesulfonic acid	(Sum br-PFOS)	14
Sum of branched and linear Perfluorooctanesulfonic acid	(Total PFOS)	15

2.2. Accreditation

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

Honey	PFASs
Accreditation	11
No accreditation	13

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 one laboratory, which applied high resolution mass spectrometry (Orbitrap) as detection method.

3. Test for sufficient homogeneity

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 2102-HO were analysed in duplicate for PFBA, PFHpA, PFHxA and PFPeA. The test for sufficient homogeneity was performed for the individual substances. 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 per-formed according to DIN ISO 13528:2020 [2] for PFBA, PFHpA, PFHxA and PFPeA. 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 ± 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 ± 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 PFBA, PFPeA, PFHxA and PFHpA in test sample "honey" (2102-HO), including limits of quantification (LOQs).
Assigned values could not be calculated for PFOA, PFNA, PFDA, PFUnDA, PFDoDA, PFTrDA, PFTeDA, all PFSAs mentioned above and sum parameters, due to the limited number of reported results above the LOQ.
Additionally the median of all values was calculated for each substance.

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 PFBA, PFPeA, PFHxA and PFHpA. 11 of 24 reporting laboratories were accredited according to ISO/IEC 17025 for PFAS. After eliminating outliers, 10 to 11 results contributed to the calculation of the assigned values from the ISO/IEC 17025 group. No significant differences (3-8 %) between the assigned values calculated for both data sets for PFASs were observed (Table 4).

Table 4: Comparison of assigned values for 2102-HO for all participants and participants with reported accreditation according to ISO/IEC 17025 for PFAS

Sum parameters	Assigned value All participants	Assigned value ISO/IEC 17025 accreditation	Deviation
	µg/kg w.w.		%
PFBA	20.9	21.6	3
PFPeA	155	161	4
PFHxA	66.9	70.1	5
PFHpA	0.265	0.246	8

4.1 PFASs – individual substances and sum parameter

The assigned values for the test sample 2102-HO were calculated as consensus of participants' results for individual PFASs and sum parameters, taking into account the calculation criteria described above (Table 5 - 7 ; tabular summary see annex 1; Figure 1-3).

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

Honey (2102-HO)	Assigned value PFCA µg/kg w.w.
PFBA	20.9
PFPeA	155
PFHxA	66.9
PFHpA	0.265

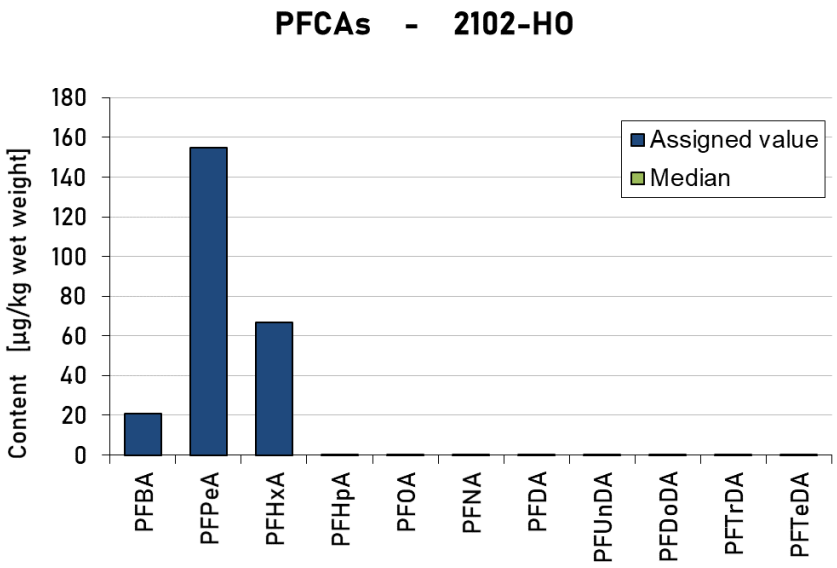

Figure 1: Assigned values (blue) and median values (green) for PFCAs individual substances for honey (2102-HO) [µg/kg w.w.]

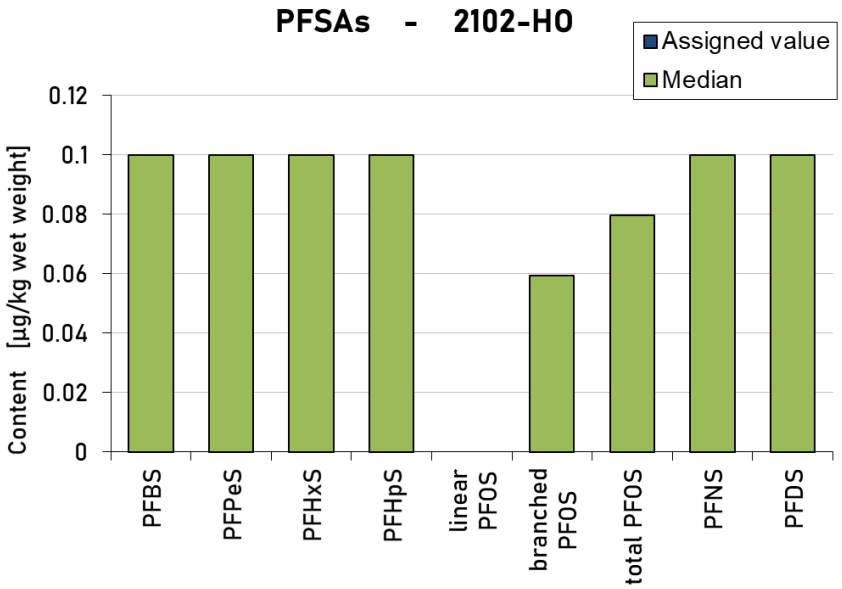

Figure 2: Assigned values (blue) and median values (green) for PFSAs individual substances for honey (2102-HO) [µg/kg w.w.]

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:

$|z\text{-score}| \leq 2$ *satisfactory performance*

$2 < |z\text{-score}| < 3$ *questionable performance (warning signal)*

$|z\text{-score}| \geq 3$ *unsatisfactory performance (action signal)*

5.2. PFASs - Participants' z-scores

Z-scores for individual substances were within the range of ± 2 for 86% - 100% of all participants.

Table 6: Distribution of participants' z-scores for PFASs for honey (2102-HO)

Percentage of participants' results PFCAs	$\|z\text{-score}\| \leq 2$	$2 < \|z\text{-score}\| < 3$	$\|z\text{-score}\| \geq 3$
PFBA	100%	-	-
PFHpA	86%	9%	5%
PFHxA	96%	4%	-
PFPeA	95%	5%	-

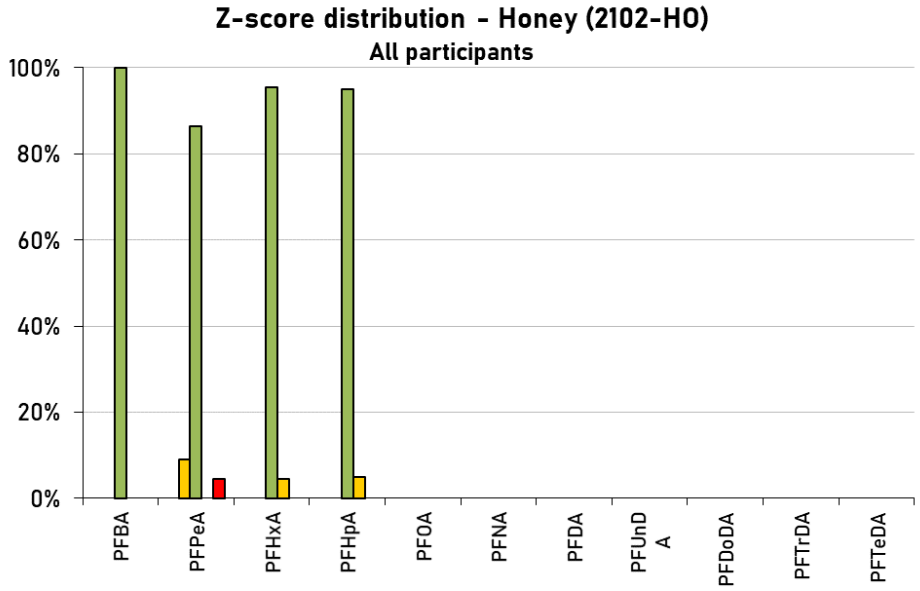

Figure 3: Distribution of participants' z-scores for individual PFCAs for honey (2102-HO) [Green bars: -2 ≤ z-score ≤ 2, orange bars: -3 < z-score < -2, 2 < z-score < 3, red bars: z-score ≤ -3, z-score ≥ 3]

6. Participants' feedback

A questionnaire for feedback from participants of this EURL proficiency test was available as online survey between 23 December 2021 and 28 January 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, only 3 laboratories (8 % of all PT participants) replied to this survey.

Table 7: Participating laboratories in the feedback survey

Type of laboratory	Answers
National Reference Laboratory (NRL)	2
Official Laboratory (OFL)	1
Commercial laboratory / Other (e.g. research and development) / no answer	0

General aspect

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	★★★★½
Preliminary report	★★★★★

Did the proficiency test meet expectations?

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?	★★★★½
Was the time frame acceptable?	★★★★★
Was the handling of EUSurvey as webtool for reporting and source of instructions manageable?	★★★★½
Was the evaluation of participant's results and the information in the preliminary report clear and comprehensible?	★★★★★

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

Choice of matrix (honey)	★★★☆☆
Level of contamination (honey)	★★½☆☆

The following comments or suggestions for improvements were submitted:

- Reporting: sum PFAS (Br included or not) was not so clear
- What would make life easier for us, is to know the concentration range in advance. For the egg, the concentrations were as expected. In case of the honey we were surprised by the high content of some of the analytes and therefore had to do several work-up procedures. This could be avoided by giving a rough concentration range.
- Matrices with high food consumption and therefore very low PFAS levels will lead to reaching the EFSA TWI are of interest, as well as matrices where an enrichment is known or foods which are often consumed.

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 samples honey (2102-HO) is 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

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.)

Honey – 2102-HO		
1	Assigned values – PFCAs	
2	Participants' results – Tables – PFCAs, PFSAs and sum parameters	
3	Participants' z-scores – Tables – PFCAs	
4	Participants' z-scores – Charts – PFCAs	
5	Test for sufficient homogeneity and stability – PFCAs	
Methods		
6	Overview participants' methods	

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