Comparative Study on the Determination of linear, branched, and total PFOS in Pork Liver 2023

Final report

(Version 1.0)

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Summary

Test sample	**Pork liver** [EURL-PT-POP_2201-PL]
Analytes of interest	Branched PFOS (br-PFOS) Linear PFOS (L-PFOS) Total PFOS (sum of linear + branched)
Methods	Any kind of method
Participants	Members of CWG PFAS
Shipment of standard solution and samples	**22 May 2023**
Deadline for reporting of results	31 August 2023
Draft report of final results	29 January 2024
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. Introduction

This comparative study on the determination of **linear (L-PFOS), branched (br-PFOS), and total PFOS** in pork liver was organised by the EURL for halogenated POPs in Feed and Food to be performed between June and August 2023. The objective was to assess the accuracy of different approaches for quantitation of branched PFOS. All core working group “PFAS” members were invited to participate in this comparative study.

The preliminary results were presented by representatives of the EURL at the COM/EURL/NRL workshop on 23 November 2023. The final results were discussed by members of the core working group “PFAS” and the EURL on 30 and 31 January 2024.

1.1. Test sample

The test sample was prepared from commercially available food (pork liver mixed with wild boar liver) and was naturally contaminated with all analytes of interest. The test material was originally prepared for the proficiency test organised by EURL POPs in 2022 (EURL-PT-POP_2201-PL). Each participant received about 90 g of the test sample in a can.

1.2. Standard solution

Each participant received 300 µL of a standard solution of br-PFOSK[1] at 5 µg/mL in methanol. The standard solution contained L-PFOS and branched isomers at a known composition[2]. All participants were asked to calculate the concentrations of L-PFOS and br-PFOS based on the certificate provided by themselves. The standard solution did not contain isotope labelled PFOS.

1.3. Analytes of interest

Participants were asked to determine the following parameters:

- L-PFOS
- Br-PFOS

1.4. Methods

One or more of the following **detection methods** could be applied:

- LC-HRMS
- LC-MS/MS
- GC-HRMS
- GC-MS/MS

1.5. Quantification of linear, branched and total PFOS

For quantification of L-PFOS, br-PFOS, and total PFOS a standard solution of br-PFOSK (see chapter 3) was provided. Isotope labelled PFOS had to be added to the standard solution before analysis.

[1] Analytical standard of L-PFOS with branched isomers (potassium salt) obtained from Wellington Laboratories

[2] Analytical certificate of producer provided

1.5.1. Linear and branched PFOS

L-PFOS:

All participants quantified L-PFOS against L-PFOS by using the standard solution provided.

Br- PFOS:

All participants quantified br-PFOS using the following two approaches:

1. Based on the calibration curve of L-PFOS
2. Based on the calibration curve of sum of branched isomers

Participants were asked to prepare the test sample in **triplicate** using any kind of sample preparation method. The addition of isotope labelled internal standard was carried out at the very beginning of the analytical method, e.g. prior to extraction.
For quantification, a five-point solvent calibration in the range of 0.5 – 50 µg/kg by using the solution provided was prepared.
The calibration standards and extracts were analysed by LC-MS (LC-MS/MS, LC-HRMS) or GC-MS (GC-MS/MS, GC-HRMS).

L-PFOS was quantified by integrating L-PFOS in the chromatogram of the calibration standards and samples. Participants reported two concentrations per replicate: one concentration by using the most intense MS/MS transition as quantifier (e.g. 499 → 80) and one concentration by using the second most intense MS/MS transition as quantifier (e.g. 499 → 99).

Br-PFOS was quantified by

1. integrating L-PFOS in the chromatogram of the calibration standards and the sum of branched isomers in the chromatogram of the samples. Two concentrations per replicate were reported: one concentration by using the most intense MS/MS transition as quantifier (e.g. 499 → 80) and one concentration by using the second most intense MS/MS transition as quantifier (e.g. 499 → 99).
2. integrating the sum of branched isomers in both the chromatogram of calibration standards and samples. Two concentrations per replicate were reported: one concentration by using the most intense MS/MS transition as quantifier (e.g. 499 → 80) and one concentration by using the second most intense MS/MS transition as quantifier (e.g. 499 → 99).

1.5.2. Total PFOS

Total PFOS was automatically calculated as sum of br- and L-PFOS.

1.6. Reporting

Laboratories

- used the reference standard provided (br-PFOSK) for identification and quantification,
- used two different approaches to quantify br-PFOS,
- reported the levels of L-PFOS and sum of br-PFOS in calibration standards,
- reported results for each analyte,
- reported the limit of quantification (LOQ),
- reported the measurement uncertainty (if possible),
- gave instrument information,
- gave information on the mass transitions used to quantify br-PFOS (e.g. 499 → 80, 499 → 99, etc.).

Results were reported in µg/kg wet weight (w. w.).

Laboratories were allowed to submit multiple datasets if different detection methods/systems from different manufacturers were used.

2. Participating laboratories

This comparative study was open for participation of members of the core working group "PFAS", only.

12 laboratories registered for this study. However, only 11 laboratories submitted results. One laboratory submitted two datasets.

Table 1: Participating laboratories

Country	Name of laboratory
NRL Belgium	Sciensano
NRL Denmark	Technical University of Denmark
NRL Finland	Finnish Institute for Health and Welfare
NRL France	LABERCA

NRL Germany	German Federal Institute for Risk Assessment
NRL Greece	Mass Spectrometry and Dioxin Analysis Lab. NCSR Demokritos
NRL Ireland	State Laboratory Ireland
NRL Latvia	Institute of Food Safety, Animal Health and Environment "BIOR"
NRL Norway	Institute of Marine Research
NRL The Netherlands	Wageningen Food Safety Research
EURL POPs	CVUA Freiburg

3. Results

In total, 12 datasets were submitted to EURL POPs. The individual results of the participating laboratories are given in **Table 2**.

Note: Dataset 7 and 8 were submitted by the same laboratory using two different types of MS instruments.

Table 2: Individual results of participating laboratories (rounded to two significant figures)

Data-set		L-PFOS	Br-PFOS based on the calibration curve of			MS/MS transition quantifier	MS/MS transition qualifier	MS instrument
			L-PFOS	sum of branched isomers	L-PFOS using the average of both MS transitions*			
		µg/kg wet weight						
1	Analysis 1 (using the quantifier for quantification)	25	0.57	1.2	1.3	499/99		EVOQ Elite triple quadrupole (QqQ) MS/MS (Bruker Corp)
	Analysis 2 (using the quantifier for quantification)	27	0.66	1.5	1.3	499/99		
	Analysis 3 (using the quantifier for quantification)	27	0.63	1.4	1.4	499/99		
	Analysis 1 (using the qualifier for quantification)	32	2.0	1.3			499/80 #	
	Analysis 2 (using the qualifier for quantification)	33	2.0	1.4			499/80 #	
	Analysis 3 (using the qualifier for quantification)	34	2.2	1.5			499/80 #	
2	Analysis 1 (using the quantifier for quantification)	24	0.64	0.91	1.0	499/99		Waters Xevo TQ-S
	Analysis 2 (using the quantifier for quantification)	25	0.63	0.87	1.1	499/99		
	Analysis 3 (using the quantifier for quantification)	25	0.64	0.93	1.1	499/99		
	Analysis 1 (using the qualifier for quantification)	25	1.4	0.93			499/80	
	Analysis 2 (using the qualifier for quantification)	26	1.6	0.93			499/80	
	Analysis 3 (using the qualifier for quantification)	27	1.6	0.92			499/80	
3	Analysis 1 (using the quantifier for quantification)	25	0.57	1.0	1.1	499/99		Sciex 7500 QQQ
	Analysis 2 (using the quantifier for quantification)	20	0.48	0.9	0.90	499/99		
	Analysis 3 (using the quantifier for quantification)	26	0.61	1.1	1.2	499/99		

Data-set		L-PFOS	Br-PFOS based on the calibration curve of			MS/MS transition quantifier	MS/MS transition qualifier	MS instrument
			L-PFOS	sum of branched isomers	L-PFOS using the average of both MS transitions*			
		µg/kg wet weight						
	Analysis 1 (using the qualifier for quantification)	25	1.6	1.2			499/80	
	Analysis 2 (using the qualifier for quantification)	21	1.3	1.0			499/80	
	Analysis 3 (using the qualifier for quantification)	28	1.8	1.4			499/80	
4	Analysis 1 (using the quantifier for quantification)	31	3.0	1.3	1.9	499/80		Orbitrap
	Analysis 2 (using the quantifier for quantification)	32	3.0	1.4	2.0	499/80		
	Analysis 3 (using the quantifier for quantification)	32	3.0	1.3	2.0	499/80		
	Analysis 1 (using the qualifier for quantification)	30	0.93	1.0			499/99	
	Analysis 2 (using the qualifier for quantification)	30	0.92	1.0			499/99	
	Analysis 3 (using the qualifier for quantification)	30	0.91	1.0			499/99	
5	Analysis 1 (using the quantifier for quantification)	24	1.4	1.3	0.9	499/80		Agilent 6470
	Analysis 2 (using the quantifier for quantification)	22	1.2	1.2	0.8	499/80		
	Analysis 3 (using the quantifier for quantification)	23	1.3	1.2	0.8	499/80		
	Analysis 1 (using the qualifier for quantification)	24	0.38	1.1			499/99	
	Analysis 2 (using the qualifier for quantification)	23	0.29	0.84			499/99	
	Analysis 3 (using the qualifier for quantification)	24	0.34	0.93			499/99	

Data-set		L-PFOS	Br-PFOS based on the calibration curve of			MS/MS transition quantifier	MS/MS transition qualifier	MS instrument
			L-PFOS	sum of branched isomers	L-PFOS using the average of both MS transitions*			
		µg/kg wet weight						
6	Analysis 1 (using the quantifier for quantification)	23	1.3	1.0	1.4	499/80		Agilent 6495C
	Analysis 2 (using the quantifier for quantification)	25	1.2	0.93	1.3	499/80		
	Analysis 3 (using the quantifier for quantification)	24	1.2	0.94	1.3	499/80		
	Analysis 1 (using the qualifier for quantification)	25	1.4	0.81			499/99	
	Analysis 2 (using the qualifier for quantification)	26	1.5	0.78			499/99	
	Analysis 3 (using the qualifier for quantification)	26	1.3	0.72			499/99	
7	Analysis 1 (using the quantifier for quantification)	28	1.9	1.5	1.3	499/80		Agilent 6495 B
	Analysis 2 (using the quantifier for quantification)	26	2.1	1.7	1.5	499/80		
	Analysis 3 (using the quantifier for quantification)	27	2.1	1.7	1.5	499/80		
	Analysis 1 (using the qualifier for quantification)	28	0.76	1.3			499/99	
	Analysis 2 (using the qualifier for quantification)	30	0.85	1.5			499/99	
	Analysis 3 (using the qualifier for quantification)	30	0.89	1.6			499/99	
8	Analysis 1 (using the quantifier for quantification)	24	1.9	1.3	1.2	499/80		AB Sciex Q-Trap 6500
	Analysis 2 (using the quantifier for quantification)	25	2.1	1.4	1.4	499/80		
	Analysis 3 (using the quantifier for quantification)	25	2.1	1.4	1.5	499/80		

Data-set		L-PFOS	Br-PFOS based on the calibration curve of			MS/MS transition quantifier	MS/MS transition qualifier	MS instrument
			L-PFOS	sum of branched isomers	L-PFOS using the average of both MS transitions*			
		µg/kg wet weight						
	Analysis 1 (using the qualifier for quantification)	23	0.57	0.80			499/99	
	Analysis 2 (using the qualifier for quantification)	23	0.79	1.1			499/99	
	Analysis 3 (using the qualifier for quantification)	28	0.94	1.3			499/99	
9	Analysis 1 (using the quantifier for quantification)	28	1.7	1.5	1.3	499/80		Q-Exactive Orbitrap
	Analysis 2 (using the quantifier for quantification)	28	2.0	1.7	1.5	499/80		
	Analysis 3 (using the quantifier for quantification)	31	1.8	1.5	1.3	499/80		
	Analysis 1 (using the qualifier for quantification)	24	0.83	1.1			499/99	
	Analysis 2 (using the qualifier for quantification)	24	0.95	1.3			499/99	
	Analysis 3 (using the qualifier for quantification)	26	0.86	1.2			499/99	
10	Analysis 1 (using the quantifier for quantification)	27	1.5	0.97	1.0	499/80		Waters Xevo TQ-S
	Analysis 2 (using the quantifier for quantification)	27	1.6	1.0	1.1	499/80		
	Analysis 3 (using the quantifier for quantification)	27	1.5	0.99	1.1	499/80		
	Analysis 1 (using the qualifier for quantification)	26	0.55	0.89			499/99	
	Analysis 2 (using the qualifier for quantification)	25	0.58	0.92			499/99	
	Analysis 3 (using the qualifier for quantification)	25	0.56	0.90			499/99	

Data-set		L-PFOS	Br-PFOS based on the calibration curve of			MS/MS transition quantifier	MS/MS transition qualifier	MS instrument
			L-PFOS	sum of branched isomers	L-PFOS using the average of both MS transitions*			
		µg/kg wet weight						
11	Analysis 1 (using the quantifier for quantification)	32	0.66	1.2	1.1	499/99		Agilent 6495 B
	Analysis 2 (using the quantifier for quantification)	31	0.62	1.3	1.1	499/99		
	Analysis 3 (using the quantifier for quantification)	29	0.56	1.3	1.0	499/99		
	Analysis 1 (using the qualifier for quantification)	33	1.4	1.6			499/80	
	Analysis 2 (using the qualifier for quantification)	31	1.5	1.6			499/80	
	Analysis 3 (using the qualifier for quantification)	30	1.5	1.6			499/80	
12	Analysis 1 (using the quantifier for quantification)	132	5.3	-	3.6	499/80		Waters QQQ
	Analysis 2 (using the quantifier for quantification)	95	3.6	-	2.3	499/80		
	Analysis 3 (using the quantifier for quantification)	101	4.2	-	2.9	499/80		
	Analysis 1 (using the qualifier for quantification)	49	1.9	-			499/99	
	Analysis 2 (using the qualifier for quantification)	39	1.0	-			499/99	
	Analysis 3 (using the qualifier for quantification)	42	1.5	-			499/99	

* calculated by the organiser
# remark participant: contains an interference

Evaluation of results

A statistical evaluation by one-way ANOVA was performed to determine if there is a significant difference as regards the concentration of br-PFOS between the different approaches. In total, six different approaches can be differentiated. An overview of the abbreviations used for the different approaches is given in **Table 3**. The suitability of the approaches to quantify br-PFOS is summarised in **Table 4**.

Note: For statistical evaluation dataset 12 was not taken into account.

Table 3: Abbreviations used for the six different approaches to quantify br-PFOS

	Approach
L80	Br-PFOS based on the calibration curve of L-PFOS using the MS transition *m/z* 499 → 80
L99	Br-PFOS based on the calibration curve of L-PFOS using the MS transition *m/z* 499 → 99
Br80	Br-PFOS based on the calibration curve of sum of branched isomers using the MS transition *m/z* 499 → 80
Br99	Br-PFOS based on the calibration curve of sum of branched isomers using the MS transition *m/z* 499 → 99
Av L	Br-PFOS based on the calibration curve of L-PFOS using the average of both MS transitions (*m/z* 499 → 80 and *m/z* 499 → 99)
Av br	Br-PFOS based on the calibration curve of sum of branched isomers using the average of both MS transitions (*m/z* 499 → 80 and *m/z* 499 → 99)

An overview of the evaluation in excel format can be downloaded here by double clicking on the icon:

In summary, the statistical evaluation has shown that

- no significant difference between the individual results within one laboratory can be observed;
- the variance of the results does not significantly differ among the laboratories;
- when using the "**L80**" and "**L99**" approaches the comparability of results is significantly lower compared to other approaches;
- the approaches "**L80**" and "**L99**" are affected by a systematic error;
- the mean values of the laboratories differ significantly more from each other when using the "**L80**" approach;
- the approach "**Av L**" leads to comparable results as "**Br80**", "**Br99**" and "**Av br**".

Table 4: Suitability of the different approaches to quantify br-PFOS

Approach	Suitability
L80	No
L99	No
Br80	Yes
Br99	Yes
Av L	Yes
Av br	Yes

Comparison of results with EURL-PT-POP_2201-PL results

The test material of the comparative study was the same as for the EURL proficiency test EURL-PT-POP_2201-PL. **Table 5** gives an overview of the results obtained for L-PFOS and br-PFOS in both studies. In the proficiency test, only a median value could be calculated for br-PFOS due to the high distributions of results submitted, whereas for L-PFOS an assigned value was calculated. The result (mean of all participants) for L-PFOS of the comparative study is in good agreement with the assigned value of the proficiency test. In addition, the results for br-PFOS using the Br80, Br99, Av L, Av br approaches are in good agreement with the median value.

Table 5: Assigned value (n = 33), median value (n = 27) and mean values (n = 33) obtained for L-PFOS and br-PFOS in two different studies analysing the same test material (pork liver)

Analyte	Quantification Approach	Study	Result [µg/kg wet weight]
L-PFOS	Assigned value	EURL-PT-POP_2201-PL	27.7
br-PFOS	Median value		1.16
L-PFOS	using the quantifier	Comparative study on linear, branched and total PFOS 2023	26.5
br-PFOS	L80		1.78
br-PFOS	L99		0.74
br-PFOS	Br80		1.28
br-PFOS	Br99		1.08
br-PFOS	Av L		1.26
br-PFOS	Av br		1.18

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