EURL Proficiency Test on the Determination of Chlorinated Paraffins in Infant formula 2021

EURL-PT-CP_2103-IF

Final report

30 March 2022

EURL for halogenated POPs in Feed and Food
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Summary

test sample	Infant formula (sample no. 2103-IFA) Secondary exercise: Fortified infant formula (sample no. 2103-IFB)
analytes of interest	Chlorinated paraffins (short and medium chained)
methods	Any kind of method
participants	All interested laboratories
statistical evaluation	ISO 13528:2020, IUPAC Protocol, combined z-scores
participation fee	Participation fee for OFLs, other official and commercial laboratories, universities and academic research institutes
preliminary results	01 December 2021
publication	EURL reserves all rights to publish and present the anonymised results of the proficiency test and secondary exercise in scientific journals and/or during conferences

1. Structure of the exercise, test material and analytes

This proficiency test (PT) on the determination of chlorinated paraffins (CPs) **in infant formula** was organized by the EURL for halogenated POPs in Feed and Food to be performed between June and October 2021. The objective was to assess the interlaboratory comparability of results from chlorinated paraffins in a **naturally contaminated food sample**, investigating the influence of different extraction methods on the derived CP amount. A **secondary exercise** was designed to compare derived CP amounts in a **fortified infant formula** with and without reconstitution of the powdered sample.

Official laboratories, research laboratories and commercial laboratories were invited to participate in this proficiency test. First results have been discussed among members of the Core Working Group CP and by representatives of the European Commission, NRLs and the EURL at the COM/EURL/NRL workshop in November 2021.

1.1. Test samples

The **PT test sample** was prepared from commercially available (baby) food. The fat content of the test sample was declared by the producer as 20.1 g/100 g dry sample.

The **secondary exercise sample** was prepared from commercially available (baby) food and fortified with commercially available SCCP and MCCP quantification standard mixtures. The fat content of this test sample was declared by the producer as 21.2 g/100 g dry sample.

Infant formula	**Sample no. 2103-IFA-xxx**
Fortified infant formula	**Sample no. 2103-IFB-xxx**

Each participant received about 25 g of dry PT test sample (2103-IFA) and about 70 g of dry secondary exercise test sample (2103-IFB). Standard reconstitution of both products uses 14.6 g dry sample with 90 mL water to receive 100 mL ready-to-drink product.

1.2. Analytes of interest

Participants were asked to determine:

- SCCPs
- MCCPs
- sum of CPs

as sum parameters and if possible giving homologue patterns. Additionally reporting CP-chain-specific concentrations was welcomed. Laboratories were further asked to report the fat content determined for the samples.

1.3. Methods

All kinds of detection and quantification methods could be applied. Participants were asked to indicate if results were reported for the dry sample or for the sample after reconstitution. **For the secondary exercise, reporting results for both types of sample treatment was highly encouraged.**

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. 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.

1.5. Reporting of results

Laboratories should:

- use their own reference standards for identification and quantification,
- report results for each analyte group,
- clearly indicate if the results were obtained for the dry or reconstituted sample,
- report the limit of quantification (LOQ), at least for each non-quantified analyte group,
- report the determined lipid content of each sample and
- give method information.

Results had to be reported in **ng/g lipid**.

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
- Academic research laboratories

Of twelve registered participants, ten laboratories reported results for at least one of the requested parameters for CPs.

3. Test for sufficient homogeneity

The test for sufficient homogeneity was performed according to ISO 13528:2020 [1] and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [2].

Therefore, five duplicate portions of the test sample 2103-IFA and 2103-IFB were analyzed for CPs. The test for sufficient homogeneity was performed for sum concentrations (ΣSCCP, ΣMCCP, ΣCPs). Based on the results of these samples, the test materials showed sufficient homogeneity for this proficiency test.

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 ISO 13528:2020, Statistical methods for use in proficiency testing by interlaboratory comparisons, International Organization for Standardization, and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [1].

The determination of the assigned value was performed according to “The international harmonized protocol for the proficiency testing of analytical chemistry laboratories" [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 ± 50% of the median of all reported results) and examination of the distribution of the remaining results using histogram and kernel density estimation. Assigned values based on an insufficient number of results are given as provisional only.

The assigned value was calculated for sum of SCCPs, sum of MCCPs and sum of CPs (excluding limits of quantification (LOQs)). Additionally, the median of all values was calculated. Due to the very low number of results being available for evaluation, assigned values for sample 2103-IFB and for sum of SCCPs in sample 2103-IFA are provisional only.

5. Scoring of results

5.1. Participants' results

5.1.1. Z-scores

For evaluation of 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

For CP sum parameters, the standard deviation for proficiency assessment σ_p is 25%.

Interpretation of z-scores:

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

5.1.2. Combined z-score AZ^2

For evaluation of the overall performance of laboratories concerning the determination of chlorinated paraffins, the average of the squared z-score (AZ^2) was used [4]. The AZ^2 is calculated as follows:

$$AZ^2 = \frac{\sum_{i=1}^{n} z_i^2}{n}$$

Where n is the number of z-scores to be considered in the calculation.

For the purpose of calculating the AZ^2, z-scores higher than |5| were classified as |5|. Z-scores derived based on reported LOQs were not included. Based on the AZ^2 achieved, the overall performance of the laboratories was considered as follows:

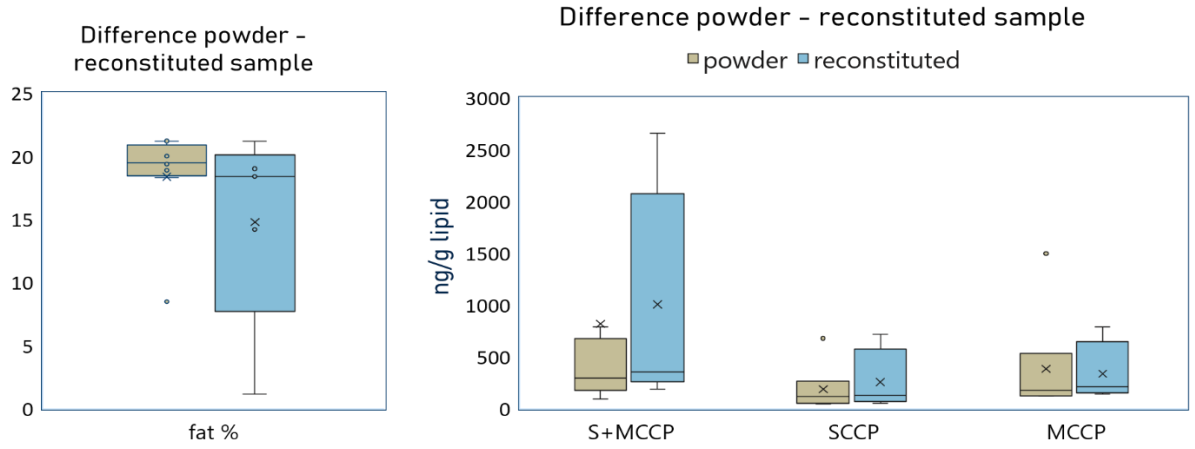
$AZ^2 \leq 2$	satisfactory overall performance
$2 < AZ^2 < 3$	questionable overall performance (warning signal)
$AZ^2 \geq 3$	unsatisfactory overall performance (action signal)

Combined z-scores are considered to be of lesser importance than the individual z-scores. Results reported for powdered and reconstituted samples were evaluated as one dataset.

6. Comparison between powder and reconstituted sample

As for the naturally contaminated sample (2103-IFA), only two participants reported results for the reconstituted sample, so no comparisons were possible. Results for the secondary exercise sample allowed for more evaluation.

Reported lipid content in the reconstituted sample had a much higher variety than results reported for the powder. The very low results might indicate difficulties with lipid extraction from the liquid sample, which should be investigated further. Consequently, the lower reported lipid content in the reconstituted sample lead to higher CP results expressed on lipid base. One participant commented that results expressed on product base were comparable, while on lipid base notably differing.



Incomplete lipid extraction as main reason for differences between powder and reconstituted sample could also be confirmed by comparing the obtained homologue patterns. Specifically, there seemed to be a slight shift of pattern towards higher chlorinated homologue groups, with some of the lower chlorinated homologue groups being completely missing in some cases. Again, this pointed towards issues with extraction efficiency from the reconstituted sample in contrast to a previously theorized increased CP amount from encapsulated ingredients in the powder. No correlations between methodology and results were observed.

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 chlorinated paraffin results for the PT test sample infant formula (2103-IFA) and the secondary exercise sample (2103-IFB) are given in the following annexes. Laboratories are coded according to the laboratory codes sent after registration.

9. Citation

This final report has been made publicly available on the EURL POPs website (www.eurl-pops.eu). The recommended citation for this report is as follows:

EURL for halogenated POPs in feed and food (2022): EURL Proficiency Test on the Determination of Chlorinated Paraffins in Infant formula [EURL-PT-CP_2103-CP]. Final Report of 30 March 2022. Available online under https://eurl-pops.eu/news/download-eurl-pt-infant-formula-2021-report.

Literature

[1] ISO 13528:2020, Statistical methods for use in proficiency testing by interlaboratory comparisons, International Organization for Standardization

[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] EU Reference Laboratories for Residues of Pesticides, General Protocol for EU Proficiency Tests on Pesticide Residues in Food and Feed, 9th Edition, Released on 15 November 2019

Annexes

Infant formula – 2103-IF		
1	Assigned values	
2	Participants' results – Tables	
3	Participants' z-scores – Tables	
4	Participants' z-scores – Charts	
5	Participants' results – homologue group patterns	
6	Comparison between powder and reconstituted sample	
7	Test for sufficient homogeneity	
8	Participants' methods	
9	Participants' feedback	

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