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 PCDD/Fs and DL-PCBs in milk fat by Bioanalytical Screening Methods 2020

EURL-PT-DP-2002-MF

FOOD

Report

PCDD/Fs and DL-PCBs

(Report Version 1.0)

12 March 2021

Office Building
Bissierstrasse 5
79114 Freiburg

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

E-Mail
EU-RL: eurl-pops@cvuafr.bwl.de

Internet
www.eurl-dioxin-freiburg.eu

Summary

Test sample (food)	5 samples of milk fat 2002-MFA, 2002-MFB, 2002-MFC, 2002-MFD, 2002-MFE
Analytes of interest	PCDD/Fs, dioxin-like PCBs
Methods	Bioanalytical screening methods
Participants	NRLs, OFLs, other official laboratories, commercial laboratories performing the analysis of samples taken by food business operators
Statistical evaluation	ISO 13528:2015, IUPAC Protocol, Positive scoring system
Report	12 March 2021 (Version 1.0)

1. Structure of the PT, test materials and analytes

This proficiency test (PT) on the determination of PCDD/Fs and dioxin-like PCBs in milk fat was organized by the EURL for halogenated persistent organic pollutants (POPs) in Feed and Food to be performed between June and September 2020.

Five samples of milk fat were fortified with PCDD/Fs and PCBs at different levels also in the range of respective action and maximum levels. The main objective of the evaluation is the comparison of the reported BEQ-levels of bioanalytical screening methods with the TEQ-levels known from physico-chemical analysis.

This PT was open for National Reference Laboratories (NRLs) for Halogenated POPs from EU member states, for OFLs and other official laboratories and commercial laboratories using bioanalytical screening methods 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 results were presented and discussed by representatives of EU Commission, NRLs and the EURL at the COM/EURL/NRL workshop on 17 and 18 November 2020.

1.1 Samples and coding

The milk fat test samples were prepared of regular market food. The test samples were fortified with PCDD/F standards and technical mixtures of PCBs. The fortified concentrations for PCDD/Fs and DL-PCBs were in the range of about 1/5th to 5 times of the respective maximum and action levels, reflecting also possible lowering of legal limits in the future.

Milk fat A	**Sample no. 2002-MFA-xxx**
Milk fat B	**Sample no. 2002-MFB-xxx**
Milk fat C	**Sample no. 2002-MFC-xxx**
Milk fat D	**Sample no. 2002-MFD-xxx**
Milk fat E	**Sample no. 2002-MFE-xxx**

Each participant received about 20 g of each test sample.

1.2 Analytes of interest and reporting of results

Participants using bioanalytical screening methods were requested to determine the following parameters:

- PCDD/F-PCB-BEQ, PCDD/F-BEQ and/or PCB-BEQ, if applicable
- report if the samples are suspected to be non-compliant with EU legal limits and confirmation is required
- report the reporting limit, maximum / action level, which the evaluation is based on, and the bioassay cut-off, if applicable

Results had to be reported in pg BEQ/g fat.

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.

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

Table 1: Participating laboratories

Participating laboratories	Region	No. of participants
National Reference Laboratories	European Union	5
	Other Countries	0
Official Laboratories	European Union	3
	Other European Countries	0
	Africa	0
	Americas	0
	Asia	0
	Oceania	0
Commercial Laboratories	European Union	1
	Other European Countries	0
	Africa	0
	Americas	0
	Asia	0
	Oceania	0
	Total	**9**

Table 2: Number of reported results for PCDD/F-PCB-BEQ, PCDD/F-BEQ and/or PCB-BEQ

No. of reported results	PCDD/F-PCB-BEQ	PCDD/F- BEQ	PCB-BEQ
Milk fat A – E (2002-MF)	10	4	4

3. Detection methods

The following detection methods were applied:

- Bioanalytical screening methods for PCDD/Fs and dioxin-like PCBs
- Cell lines:
  - rat hepatoma cell line H4IIE pGudLuc 1.1
  - fat hepatoma cell line H4IIE, wild type
  - mouse hepatoma cell line H1L6.1c3

4. Test for sufficient homogeneity

The test for sufficient homogeneity and stability was performed according to ISO 13528:2015 [2] and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [1].

Therefore, four portions of the test sample 2002-MFA were analyzed in duplicate for PCDD/Fs and PCBs. The test for sufficient homogeneity was performed for the sum parameters WHO-PCDD/F-PCB-TEQ, WHO-PCDD/F-TEQ and WHO-PCB-TEQ and individual congeners. This test material showed sufficient homogeneity and stability for this proficiency test. Additionally two portions of the other test samples - prepared in exactly the same way as 2002-MFA - were analyzed in duplicate.

5. Determination of the assigned value

Statistical evaluation of the PT results was performed by the EURL for Halogenated POPs in Feed and Food partly according to ISO 13528:2015 [2] and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories [1].

The estimation of the assigned values for PCDD/Fs and DL-PCBs was based on the GC-MS results of the analysis of the five samples, analyzed at the EURL for halogenated POPs in Feed and Food. Therefore, two portions were analyzed in duplicate and the mean values of the results was taken as assigned value. TEQ-based results were calculated using the WHO-TEFs of 2005 [3].

For additionally comparison of results, a consensus value of participants' BEQ results is calculated. The median of reported results without exclusion of outliers is taken as consensus value.

5.1 Comparison of assigned values and consensus values with legal limits

Maximum levels for food are defined in Commission Regulation (EC) No 1881/2006 of 19 December 2006 setting maximum levels for certain contaminants in foodstuff. Action levels are defined in Commission Recommendation of 3 December 2013 on the reduction of the presence of dioxins, furans and PCBs in feed and food (2013/711/EU).

Table 3: Maximum and action levels for raw milk and dairy products, including butter fat

Raw milk and dairy products, including butter fat		**Maximum level**	**Action level**
WHO-PCDD/F-PCB-TEQ	pg/g fat	5.5	-
WHO-PCDD/F-TEQ	pg/g fat	2.5	1.75
WHO-PCB-TEQ	pg/g fat	-	2.00
Sum of 6 Indicator PCBs	ng/g fat	40	-

The assigned values for the test samples were based on GC-HRMS TEQ-results, consensus values were based on participants' BEQ results.

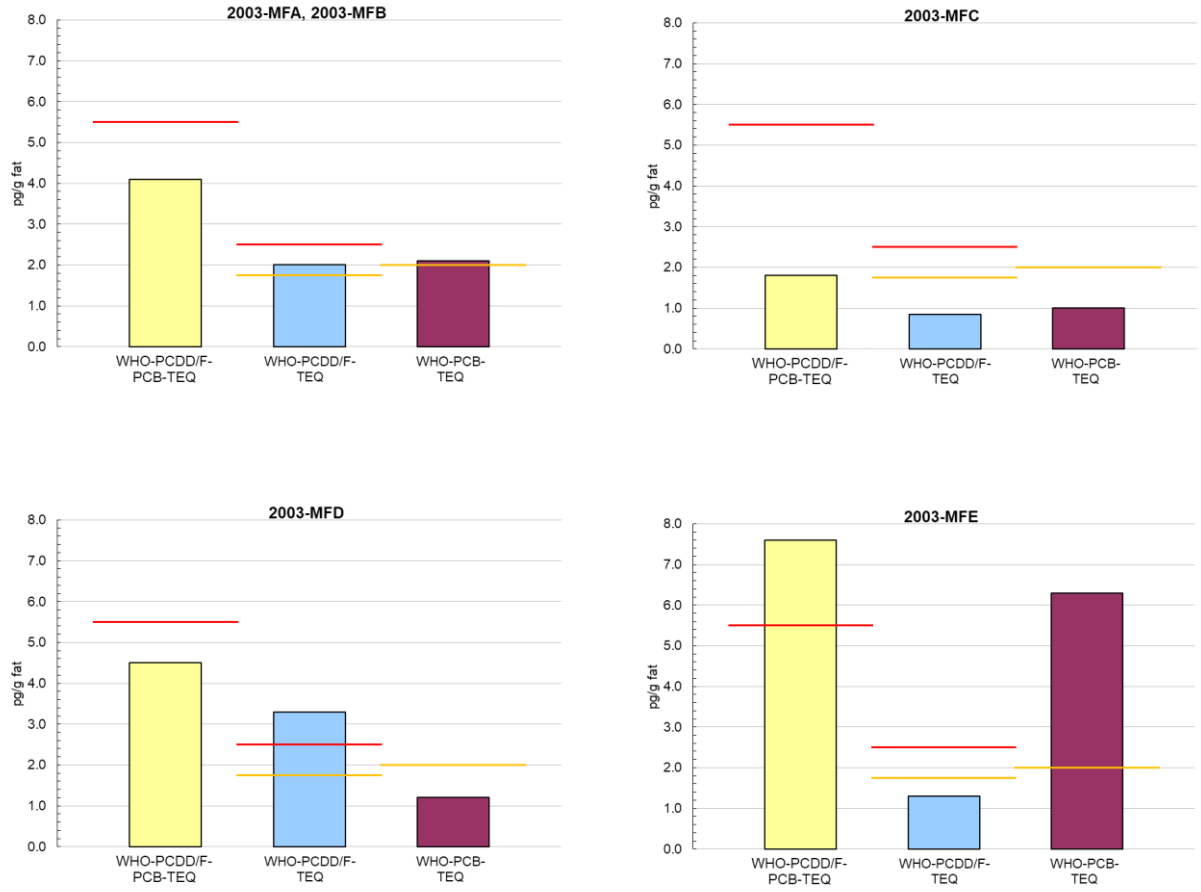

Figure 1: Assigned values in WHO-TEQ for PCDD/Fs+PCBs, PCDD/Fs and PCBs based on GC-HRMS results for test samples 2003-MFA to 2003-MFE, comparison with maximum levels (red line) and action levels (yellow line) [pg/g fat]

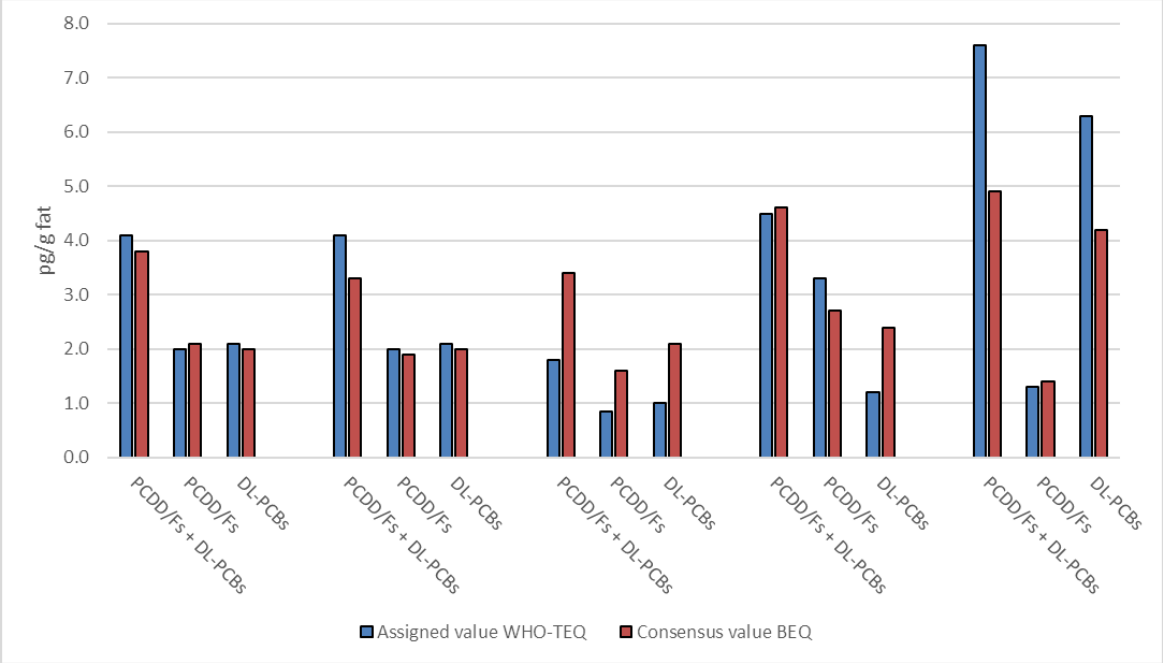

Figure 2: Comparison of assigned values in WHO-TEQ for PCDD/Fs+PCBs, PCDD/Fs and PCBs based on GC-HRMS results and consensus values in BEQ for PCDD/Fs+PCBs, PCDD/Fs and PCBs based on participants' results for test samples 2003-MFA to 2003-MFE [pg/g fat]

6. Scoring of results

6.1 Participants' results for bioanalytical screening methods

According to Commission Regulation (EU) 2017/644, "a screening method in principle classifies a sample as compliant or suspected to be non-compliant. For this, the calculated BEQ level is compared to the cut-off value […]. Samples below the cut-off value are declared compliant, samples equal or above the cut-off value as suspected to be non-compliant, requiring analysis by a confirmatory method."

Therefore, the main criterion for evaluation of results from bioanalytical screening methods is their ability to reliably identify compliant samples and samples suspected to be non-compliant with established legal limits.

For further evaluation of the performance of bioanalytical screening methods, bioassay-scores are applied: The reported BEQ-values derived from bioanalytical screening methods are compared with the WHO-TEQ assigned values calculated on basis of the results of physical-chemical methods for the concentration range of 0.5 to 2 times the level of interest. Because bioanalytical screening methods focus mainly on distinguishing between compliant and potentially non-compliant samples, a direct comparison of bioassay-scores and z-scores is not possible. However, bioassay scores may serve as a tool to assess method performance within the scope of external quality control measures of the respective laboratory.

For this proficiency test bioassay-scores were calculated based on the assigned values from the GC-HRMS analysis and based on the consensus values from participants' results.

Bioassay-scores are calculated according to the following formula:

bioassay-score =(x - x_a) / $\sigma_{bioassay}$

x_a: assigned value (physical-chemical methods)

x: participant's result (BEQ from bioanalytical screening method)

$\sigma_{bioassay}$: bioassay target deviation

For PCDD/F-BEQ, PCB-BEQ and PCDD/F-PCB-BEQ the bioassay target deviation $\sigma_{Bioassay}$ is defined as 20 %.

6.1.1 Assessment of analytical results

For evaluation of the assessment of the analytical results using bioanalytical screening methods, the assigned values for WHO-PCDD/F-PCB-TEQ, WHO-PCDD/F-TEQ and WHO-PCB-TEQ were compared with the respective maximum and action levels.

Table 4: Assessment of samples according to assigned values for WHO-PCDD/F-PCB-TEQ, WHO-PCDD/F-TEQ and WHO-PCB-TEQ with/without taking into account the measurement uncertainty (maximum level: ML, action level: AL)

	2002-MFA	**2002-MFB**	**2002-MFC**	**2002-MFD**	**2002-MFE**
WHO-PCDD/F-PCB-TEQ	< ML	< ML	< ML	< ML	**> ML**
WHO-PCDD/F-TEQ	**> AL***	**> AL***	< AL	**> ML**	< AL
WHO-PCB-TEQ	**> AL***	**> AL***	< AL	< AL	**> AL**

* no exceedance of ML/AL taking into account an expanded measurement uncertainty of 20% of the confirmatory method

Table 5: Participants' assessment of analytical results for WHO-PCDD/F-PCB-TEQ, WHO-PCDD/F-TEQ and WHO-PCB-TEQ (maximum level: ML, action level: AL)

Participants' assessment	**2002-MFA**	**2002-MFB**	**2002-MFC**	**2002-MFD**	**2002-MFE**
ML for WHO-PCDD/F-PCB-TEQ	< ML	< ML	< ML	< ML	**> ML**
Suspected to be non-compliant	5	4	5	6	9
Compliant	5	5	5	4	**1**
ML for WHO-PCDD/F-TEQ	< ML	< ML	< ML	**> ML**	< ML
Suspected to be non-compliant	5	5	5	7	5
Compliant	3	3	3	**1**	3
AL for WHO-PCDD/F-TEQ	**> AL***	**> AL***	< AL	**> AL**	< AL
Suspected to be non-compliant	6	6	5	6	6
Compliant	0	0	1	0	0
AL for WHO-PCB-TEQ	**> AL***	**> AL***	< AL	< AL	**> AL**
Suspected to be non-compliant	6	5	5	6	6
Compliant	0	**1**	1	0	0

* no exceedance ML/AL taking into account an expanded measurement uncertainty of 20% of the confirmatory method

The test samples 2002-MFD and 2002-MFE were above maximum and/or action levels for WHO-PCDD/F-PCB-TEQ, WHO-PCDD/F-TEQ and/or WHO-PCB-TEQ taking into account an expanded measurement uncertainty of 20% of the confirmatory method. The test samples 2002-MFA and 2002-MFB were above the action levels for WHO-PCDD/F-TEQ and WHO-PCB-TEQ without taking into account a measurement uncertainty of the confirmatory method.

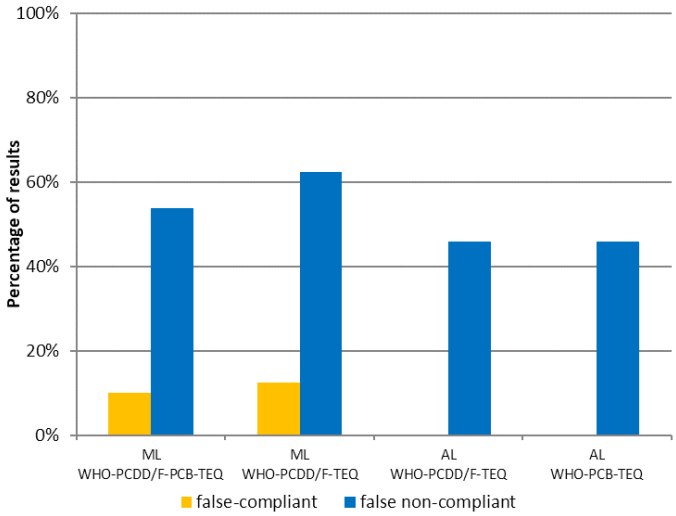

Figure 3: Percentage of false compliant and false non-compliant results based on participants' assessment of the analytical results for WHO-PCDD/F-PCB-TEQ, WHO-PCDD/F-TEQ and WHO-PCB-TEQ, taking into account an expanded measurement of 20 % for the GC-MS method

6.1.2 Participants' bioassay-scores

The assigned values for WHO-PCDD/F-PCB-TEQ, WHO-PCDD/F-TEQ and/or WHO-PCB-TEQ are in the range (about 0.5 to 2 times) of the respective maximum and action levels for test samples 2002-MFA, 2002-MFB and 2002-MFD. For test samples 2002-MFC and 2002-MFE assigned values are partly below or above this range, respectively. For PCDD/F+PCB the results of most participants are within the range of +/- 2 bioassay-scores for 4 of 5 samples for comparison with the GC-MS assigned values. Higher deviations could be observed for the lowest contaminated sample 2002-MFC. When comparing reported participants' individual results with the consensus value based on participants' results, a good agreement of the results of all participants, irrespective of the level of spiked analytes, was found.

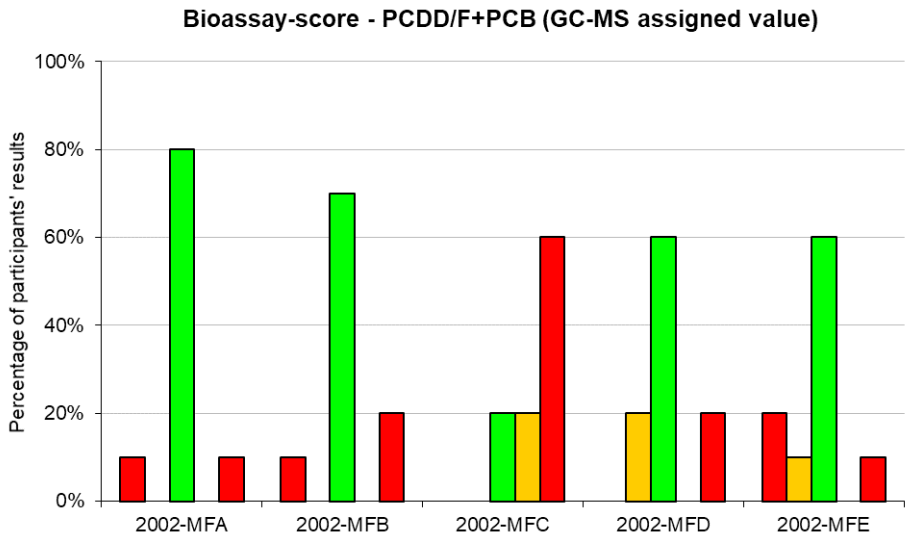

Figure 4: Distribution of participants' bioassay-scores for PCDD/F+PCB in comparison with the GC-MS assigned values

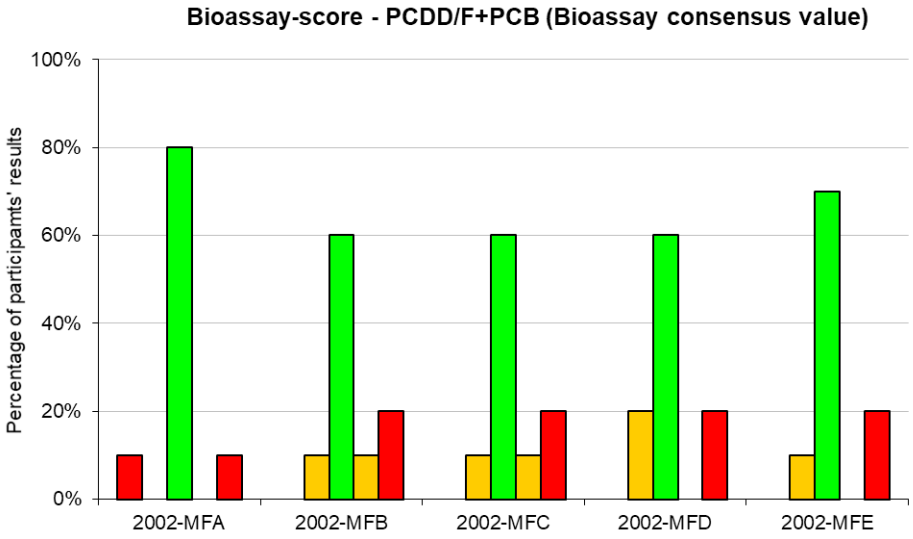

Figure 5: Distribution of participants' bioassay-scores for PCDD/F+PCB in comparison with the bioassay consensus values based on participants' results

7. Participants' feedback

A questionnaire for feedback from participants of this EURL proficiency test was available as online survey between 26 October 2020 and 30 November 2020. 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 (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 1 laboratory (11 % of all participants) participated in this survey. Therefore, further evaluation of the questionnaire was dispensed.

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 results for the PT test samples 2002-MFA to 2002-MFB is given in the following annexes. Laboratories are coded according to the laboratory codes sent after registration.

10. 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 13528:2015, Statistical methods for use in proficiency testing by interlaboratory comparisons, International Organization for Standardization

[3] M. van den Berg et al., The 2005 World Health Organization Re-evaluation of Human and Mammalian Toxic Equivalency Factors for Dioxins and Dioxin-like Compounds. Toxicological Sciences 93(2), 223-241 (2006)

11. Annex

(Please double click on the pdf-icons to open the annexes.)

Milk fat – 2002-MF		
1	Assigned values and consensus values in TEQ/BEQ	
2	Participants' results – Tables	
3	Participants' bioassay-scores (comparison with GC-MS assigned values) – Tables	
4	Participants' bioassay-scores (comparison with consensus values in BEQ of participants' results) – Tables	
5	Participants' bioassay-scores (comparison with GC-MS assigned values) – Charts	
6	Participants' bioassay-scores (comparison with consensus values in BEQ of participants' results) – Charts	
7	Homogeneity test	
8	Participants' methods	

EURL for halogenated Persistent Organic Pollutants (POPs) in Feed and Food
c/o State Institute for Chemical and Veterinary Analysis of Food Freiburg

Alexander Schächtele
(Head of EURL for halogenated POPs in Feed and Food)
Phone: +49 761 8855 500
E-Mail: eurl-pops@cvuafr.bwl.de