European Union Reference Laboratory
for Dioxins and PCBs in Feed and Food

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

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EU-RL Proficiency Test on Determination of PCDD/Fs, PCBs, PBDEs and HBCDDs in Liver of Cattle

2017

EURL-PT-DP_1702-LC

FOOD

Announcement

12 June 2017

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Summary

Test samples (food)	Liver of Cattle - 1702-LC
Analytes of interest	PCDD/Fs, PCBs, PBDEs, HBCDDs
Methods	PCDD/Fs, DL-PCBs: GC-HRMS, GC-MS/MS and alternative methods; Bioanalytical screening methods Indicator PCBs: Any kind of method PBDEs: Any kind of method HBCDDs: Any kind of method
Participants	NRLs, OFLs, other official laboratories, commercial laboratories
Statistical evaluation	ISO 13528:2015, IUPAC Protocol, Positive scoring system
Participation fee	Participation fee for OFLs, other official and commercial laboratories
Registration	Return of registration form: 31 July 2017
Reporting of results	Deadline for reporting of results: 22 October 2017

1. Introduction

This proficiency test (PT) on the determination of **PCDD/Fs**, **dioxin-like PCBs**, **indicator PCBs**, **PBDEs** and **HBCDDs** in **liver of cattle** is organized by the EU-RL for Dioxins and PCBs in Feed and Food to be performed between August and October 2017. The objective is to assess analytical performance of laboratories and the interlaboratory comparability of results from analyses of all relevant PCDD/F and PCB parameters (17 PCDD/F, 12 dioxin-like PCBs, 6 indicator PCBs) and additionally PBDEs and HBCDDs in one sample of **liver of cattle**.

National Reference Laboratories (NRLs) for Dioxins and PCBs from EU member states are requested to participate as part of their work programme for 2017. Analysis of PBDEs and HBCDDs is purely voluntary.

NRLs are invited to encourage the participation of **Official Laboratories (OFLs)** from their member states as part of their duties following Article 33 of Council Regulation 882/2004. Furthermore, participation of OFLs will allow the extension of the data basis for calculation of assigned values and evaluation of results.

In addition, this PT is focusing on the reliability of analytical results for PCDD/Fs, PCBs, PBDEs and HBCDDs in a more complex food matrix. Therefore, this PT is 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 will be discussed by representatives of EU Commission, NRLs and the EU-RL at the COM/EU-RL/NRL workshop in November 2017 in Freiburg, Germany.

Participating laboratories will receive the evaluation of the PT results in preliminary and final reports.

2. Test sample (food)

The liver of cattle test sample is prepared of regular market food. The test sample is fortified with PCDD/F standards and PCB technical mixtures.

Liver of cattle	**Sample no. 1702-LC-xxx**

Each participant will receive about 100 g of the test sample.

3. Analytes of interest

Participants are requested to determine at least one of the following parameters:

- 17 2,3,7,8-substituted PCDD/Fs
- WHO-PCDD/F-TEQ (using WHO_{2005}-TEF)
- 12 dioxin-like PCBs
- WHO-PCB-TEQ (using WHO_{2005}-TEF)
- WHO-PCDD/F-PCB-TEQ (using WHO_{2005}-TEF)
- Six indicator PCBs: PCB 28, 52, 101, 138, 153, 180
- Sum of six indicator PCBs: Sum of PCB 28, 52, 101, 138, 153, 180
- PCDD/F-PCB-BEQ, PCDD/F-BEQ and/or PCB-BEQ (using bioanalytical screening methods)

Additionally results for the following parameters can be reported:

- PBDEs: BDE 28, BDE 47, BDE 49, BDE 99, BDE 100, BDE 138, BDE 153, BDE 154, BDE 183, BDE 209
- HBCDDs: α-HBCDD, β-HBCDD, γ-HBCDD

4. Methods

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

- GC-HRMS-, GC-MS/MS-methods for PCDD/Fs and dioxin-like PCBs
- Other alternative methods for GC-HRMS, GC-MS/MS for PCDD/Fs and dioxin-like PCBs
- Bioanalytical screening methods for PCDD/Fs and dioxin-like PCBs
- Any kind of method for indicator PCB
- Any kind of method for PBDEs and HBCDDs

5. Participants

The PT is mandatory for NRLs for PCDD/Fs and PCBs (free of charge) and open for official laboratories of EU member states (OFL), other official and commercial laboratories (with fee for participation). NRLs are encouraged to inform OFLs in their member states to participate.

A coordination of the participation of OFLs through NRLs is required. The EU-RL will send the samples only to the NRLs, including the samples for the OFLs in the respective member state, if applicable.

6. Statistical evaluation of results

Statistical evaluation of the PT results is performed by the EU-RL for Dioxins and PCBs in Feed and Food according to ISO 13528:2015, 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 (IUPAC Technical Report, Pure Appl. Chem, Vol. 78, No. 1, pp-145-196, 2006).

The determination of the assigned value is performed according to “The international harmonized protocol for the proficiency testing of analytical chemistry laboratories" (IUPAC Technical Report, Pure Appl. Chem, Vol. 78, No. 1, pp-145-196, 2006) 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.

The assigned value is calculated for WHO-PCDD/F-PCB-TEQ, WHO-PCDD/F-TEQ, WHO-PCB-TEQ, the sum of six indicator PCBs and individual PCDD/F, PCB, PBDE congeners and HBCDD diastereomers (including limits of quantification(LOQs)), if possible. Additionally the median of all values is calculated.

For individual congeners (including LOQs) assigned values are 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 of z-scores, if these levels are equal to or above the LOQ; otherwise the LOQ will be taken.

6.1 Participants' results for physico-chemical methods

6.1.1 Z-scores

Criteria for successful participation of laboratories using physico-chemical methods are based on the evaluation of the results of the sum parameters WHO-PCDD/F-TEQ, WHO-PCB-TEQ, WHO-PCDD/F-PCB-TEQ and the sum of six indicator PCBs and evaluated individual congeners. The criteria will be applicable for sum parameter concentrations in the range (about 0.5 to 4 times) of the level of interest (maximum level or action levels).

For evaluation of results of physico-chemical methods the **z-scores** are 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 WHO-PCDD/F-TEQ, WHO-PCB-TEQ and WHO-PCDD/F-PCB-TEQ the standard deviation for proficiency assessment σ_p is defined as 10 %, for the sum of six indicator PCBs (PCB 28, 52, 101, 138, 153, 180) as 15 % and for evaluated individual PCDD/F, PCB, PBDE congeners and HBCDD diastereomers as 20 %.

Interpretation of z-scores:

$\lvert$z-score$\rvert \leq 2$	satisfactory performance
$2 < \lvert$z-score$\rvert < 3$	questionable performance (warning signal)
$\lvert$z-score$\rvert \geq 3$	unsatisfactory performance (action signal)

6.1.2 Z-scores for individual congeners

Participants' z-scores for individual congeners are calculated according to the procedure as described in chapter 6.1.1. In case of reporting of LOQs for individual congeners different approaches of evaluation apply:

- Participant's LOQ below assigned value:
  Calculation and reporting of z-score as for reported concentrations
- Participant's LOQ equal to or above assigned value:
  Calculated z-score < 3: Reporting of the calculated z-score
  Calculated z-score ≥ 3: Reporting of an allocated z-score of + 2.5

6.1.3 Positive scoring system

Additionally a scoring system covering results for sum parameters and individual congeners has been developed within the EU-RL/NRL network. This "**positive scoring system**" gives one assessment for each PT sample covering all relevant sum parameters and congeners.

The total score for the **positive scoring system** is calculated according to the following general principles:

- Calculation of z-scores for sum parameters and evaluated individual congeners
- Calculation of the positive scores according to the following table:

Positive scoring system	**I z-score I ≤ 2**	**2 < I z-score I < 3**	**I z-score I ≥ 3**
Individual congeners	**Positive score**	**Positive score**	**Positive score**
Contribution to sum parameter* > 10 %	12	6	0
Contribution to sum parameter* 3 – 10 %	8	4	0
Contribution to sum parameter* < 3 %	6	3	0
Not evaluated congeners	0	0	0

*separately for the respective sum parameters WHO-PCDD/F-TEQ, WHO-PCB-TEQ and the sum of six indicator PCBs

- Calculation of maximum achievable scores (I z-score I ≤ 2) for PCDD/F and DL-PCB and indicator PCB congeners separately:

$$Maximum\ Score = \sum_{i=1}^{n} Max.Score_{(>10\%)i} + \sum_{i=1}^{m} Max.Score_{(3-10\%)i} + \sum_{i=1}^{p} Max.Score_{(<3\ \%)i}$$

- Calculation of the participant's scores for PCDD/F and DL-PCB and indicator PCB congeners separately:

$$Participant's\ Score = \sum_{i=1}^{n} Score_{(>10\%)i} + \sum_{i=1}^{m} Score_{(3-10\%)i} + \sum_{i=1}^{p} Score_{(<3\ \%)i}$$

- Calculation of achieved scoring percentage for each participant:

$$Participant's\ Scoring\ Percentage = \frac{Participant's\ score}{Maximum\ score} \cdot 100$$

- Criteria for successful participation:

Sum parameters:	≤ 1 parameter with | z-score | > 2, no parameter with | z-score | ≥ 3
PCDD/F congeners:	≥ 75 % of maximum score
DL-PCB congeners:	≥ 75 % of maximum score
Indicator PCB congeners:	≥ 75 % of maximum score

The assessment based on the positive scoring system is performed for each PT test sample. A laboratory participates successfully in a PT, if all above mentioned criteria for the reported analytes are met for each PT test sample.

6.2 Participants' results for bioanalytical screening methods

According to Commission Regulation (EU) 2017/644 of 5 April 2017, "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. Due to the focus of bioanalytical screening methods on the decision over compliance or potential non-compliance of a sample, 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.

Bioassay-scores are calculated according to the following formula:

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

x_a: assigned value (physical-chemical methods)

x: participants 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 %.

7. Quality control

The Deutsche Akkreditierungsstelle GmbH attests that the provider of proficiency testing Chemisches und Veterinäruntersuchungsamt Freiburg, EU-Reference Laboratory (EU-RL) for Dioxins and PCBs in Feed and Food is competent under the terms of DIN EN ISO/IEC 17043:2010 to carry out proficiency testing/interlaboratory comparisons in the testing field of chemical analysis and bioanalytical methods for determination of PCDD/Fs and PCBs in food and feed (Accreditation number: D-EP-18625-01-00).

8. Confidentiality

The identity of participating laboratories will be kept confidential.

For NRLs of EU member states, 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.

For OFLs of EU member states cooperating with NRL, the respective NRLs will inform the EU-RL for Dioxins and PCBs about the participating OFLs and will receive the respective laboratory codes, invoices for participation fee and certificates of participation of the OFLs.

9. Participation fee

The participation of **NRLs** of EU member states is free of charge.

For **OFLs of EU member states (in cooperation with NRLs)** the following participation fees have to be paid:

- 250 € for determination of PCDD/Fs and/or DL-PCBs, NDL-PCBs
- 150 € for determination of PCDD/Fs, DL-PCBs using bioanalytical screening methods only
- 150 € for determination of NDL-PCBs only
  - 50 € for additional analysis of PBDEs and/or HBCDDs in addition to PCDD/Fs or PCBs
- 150 € for determination of PBDEs and/or HBCDDs only

The participation fees for **other official laboratories and commercial laboratories** are:

- 350 € for determination of PCDD/Fs and/or DL-PCBs, NDL-PCBs
- 250 € for determination of PCDD/Fs, DL-PCBs using bioanalytical screening methods only
- 250 € for determination of NDL-PCBs only
  - 50 € for additional analysis of PBDEs and/or HBCDDs in addition to PCDD/Fs or PCBs
- 250 € for determination of PBDEs and/or HBCDDs only

Invoices for participation of OFLs and other official and commercial laboratories will be sent before sending of the final report and the certificate of participation.

10. Registration

For registration for this proficiency test, participants are asked to fill out the respective registration form and return it to the EU-RL. Registration forms are included as attached documents.

NRLs of EU-member states, including additional information on **participating OFLs** cooperating with the NRL, if applicable:

- EU-RL-PT_DP_1702-LC_Registration_NRL.xls 

Other official and commercial laboratories:

- EU-RL-PT_DP_1702-LC_Registration_Other.xls 

Please return the filled out registration form until July 31st, 2017 to eurl-dioxin@cvuafr.bwl.de.

Registration for this PT and reporting of results/method information is only possible by e-mail using the above mentioned e-mail address.

11. Time schedule

EU-RL	Announcement	12 June 2017
Participant	**Return of registration form**	**Until 31 July 2017**
EU-RL	Shipment of test material, instructions and spreadsheets	22 August 2017
Participant	Confirmation of receipt of test material	Within 7 days
Participant	**Reporting of results** **(There will be no extension of the deadline.)**	**By 22 October 2017**
EU-RL	Evaluation and preparation of a preliminary report	November 2017
EU-RL/ NRLs	Discussion at COM/EU-RL/NRL workshop with NRLs	November 2017
EU-RL	Sending of final report to all participants	March 2018

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