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

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EU-RL Proficiency test on Determination of PCDD/Fs and PCBs in Cod Liver and Fish Liver Oil

2014

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Announcement

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1. Introduction

This proficiency test (PT) on the determination of PCDD/Fs, dioxin-like PCBs and indicator PCBs in cod liver and fish liver oil is organized by the EU-RL for Dioxins and PCBs in Feed and Food to be performed between August and October 2014. The objective is to assess analytical performance of laboratories and the interlaboratory comparability of results from analyses of all relevant parameters (17 PCDD/F, 12 dioxin-like PCBs, 6 indicator PCBs) in one sample of cod liver and one sample of fish liver oil.

National Reference Laboratories (NRLs) for Dioxins and PCBs from EU member states are requested to participate as part of their work programme for 2014. 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.

The evaluated results will be discussed by representatives of EU Commission, National Reference Laboratories and the EU-RL at the COM/EU-RL/NRL workshop in November 2014 in Freiburg, Germany.

2. Participants

The PT is mandatory for NRLs (free of charge) and open for official laboratories of EU member states (OFL) and other official 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.

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

- PCDD/F-PCB-BEQ, PCDD/F-BEQ and/or PCB-BEQ (using bioanalytical screening methods)
- Measurement uncertainty for WHO-PCDD/F-TEQ, WHO-PCB-TEQ, WHO-PCDD/F-PCB-TEQ and sum of six indicator PCBs

4. Test samples

The cod liver and the fish liver oil sample is prepared of regular market food and is not fortified with analytes of interest.

Cod liver	**Sample no. 1402-CLA-xxx**
Fish liver oil	**Sample no. 1402-CLB-xxx**

Each participant will receive about 75 g of cod liver and 20 g of fish liver oil.

5. 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

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 13528:2005, 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 exclud-

ing 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 and PCB congeners (including LOQs), if possible.

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 or action level).

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 and PCB congeners as 20 %.

Acceptable z-scores are between - 2 and + 2. Not acceptable are z-scores not inside the range of - 3 to + 3.

6.1.2 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:

$$\text{Maximum score} = \Sigma\text{max. score}_{(> 10\ \%)} + \Sigma\text{max. score}_{(3\text{-}10\ \%)} + \Sigma\text{max. score}_{(< 3\ \%)}$$

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

$$\text{Participant's score} = \Sigma\text{score}_{(> 10\ \%)} + \Sigma\text{score}_{(3\text{-}10\ \%)} + \Sigma\text{score}_{(< 3\ \%)}$$

- Calculation of achieved scoring percentage for each participant:

$$\text{Participant's scoring percentage} = \text{Participant's score} / \text{Maximum score} \bullet 100$$

- 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 (EC) No 589/2014, "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:

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

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 %

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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:

- 400 € 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

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

- 500 € for determination of PCDD/Fs and/or DL-PCBs, NDL-PCBs
- 350 € for determination of PCDD/Fs, DL-PCBs using bioanalytical screening methods only
- 350 € for determination of NDL-PCBs only

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

10. Registration

Please fill out the registration form. NRLs of EU member states are asked to give also additional information on participating OFLs from their member state, if applicable.

A registration form is included as separate attached document:

- EU-RL_PT_Cod_Liver_2014_Registration.xls 

Please return the filled out registration form until August 8th, 2014 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	03 July 2014
Participant	**Return of registration form**	**Until 8 August 2014**
EU-RL	Shipment of test material, instructions and spreadsheets	26 August 2014
Participant	Confirmation of receipt of test material	Within 7 days
Participant	**Reporting of results and method information** **(There will be no extension of the deadline.)**	**By 19 October 2014**
EU-RL	Evaluation and preparation of a preliminary report	November 2014
EU-RL/ NRLs	Discussion at COM/EU-RL/NRL workshop with NRLs	November 2014
EU-RL	Sending of final report to all participants	February 2015

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