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, PCBs and BFRs in Feed Fat 2020

EURL-PT-DPB-2003-FF

FEED

Report

PCDD/Fs and PCBs

(Version 1.0)

19 March 2021

Office Building
Bissierstrasse 5
79114 Freiburg

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

E-Mail
EURL: eurl-pops@cvuafr.bwl.de

Internet
www.eurl-dioxin-freiburg.eu

Summary

Test samples (feed)	Palm fatty acid distillate – 2003-FFA Rapeseed oil – 2003-FFB
Analytes of interest	Mandatory for NRLs: - PCDD/Fs (17 2,3,7,8-substituted PCDD/Fs) - PCBs (12 DL-PCBs, 6 NDL-PCBs)
Methods	PCDD/Fs, DL-PCBs: GC-HRMS, GC-MS/MS and alternative methods; Bioanalytical screening methods Indicator PCBs: Any kind of method
Participants	NRLs, OFLs, other official laboratories, commercial laboratories performing the analysis of samples taken by feed business operators
Statistical evaluation	ISO 13528:2015, IUPAC Protocol, Positive scoring system
Report	19 March 2021 (Version 1.0)

1. Structure of the PT, test material and analytes

This proficiency test (PT) on the determination of PCDD/Fs, PCBs, PBDEs and HBCDDs in feed fat was organized by the EURL for halogenated Persistent Organic Pollutants (POPs) in Feed and Food to be performed between June and October 2020. The objective was to assess analytical performance of laboratories and the interlaboratory comparability of results from analyses of PCDD/Fs, PCBs, PBDEs and HBCDDs in one sample of palm fatty acid distillate and one sample of rapeseed oil.

National Reference Laboratories (NRLs) for Halogenated POPs in Feed and Food from EU member states were requested to participate as part of their work programme for 2020. NRLs were invited to encourage the participation of Official Laboratories (OFLs) from their member states as part of their duties following Article 101 of regulation (EU) 2017/625 of the European Parliament and of the Council of 15 March 2017. Furthermore, participation of OFLs will allow the extension of the data basis for calculation of assigned values and evaluation of results.

This PT was also open for other official laboratories and commercial laboratories per-forming the analysis of samples taken by feed business operators for official control or self-control in order to check the comparability of results not only within the EURL/NRL/OFL network, but also with official and private laboratories.

The evaluated results were discussed by representatives of EU Commission, NRLs and the EURL at the COM/EURL/NRL workshop in November 2020.

1.1 Samples and coding

The feed fat test samples were prepared of regular market feed and partly fortified with the analytes of interest using PCDD/F standards and technical mixtures of PCBs, PBDEs and HBCDDs.
The concentrations for PCDD/Fs, PCBs, PBDEs and HBCDDs in the test samples were partly in the lower concentration range reflecting also possible lowering of legal limits for PCDD/Fs and PCBs in the future.

Palm fatty acid distillate	**Sample no. 2003-FFA-xxx**
Rapeseed oil	**Sample no. 2003-FFB-xxx**

Each participant received about 20 g of each test sample.

1.2 Analytes of interest

NRLs for halogenated POPs in feed and food are requested to determine the following parameters:

PCDD/Fs and PCBs:

- 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, if applicable

1.3 Coding of laboratories and confidentiality

The laboratory codes 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.

1.4 Results of PCDD/Fs and PCBs determined by physico-chemical methods

Laboratories should:

- use their own reference standards for identification and quantification,
- report results for each analyte,
- report the limit of quantification (LOQ), at least for each non-quantified analyte,
- report upper, middle and lower bound results for WHO-PCDD/F-PCB-TEQ, WHO-PCDD/F-TEQ, WHO-PCB-TEQ and sum of six indicator PCBs,
- report if sample exceeds respective EU maximum or action levels for WHO-PCDD/F-PCB-TEQ, WHO-PCDD/F-TEQ and/or WHO-PCB-TEQ or the maximum

level for the sum of six indicator PCBs beyond reasonable doubt taking into account the measurement uncertainty,

- report the measurement uncertainty, applied for checking of compliance, for WHO-PCDD/F-PCB-TEQ, WHO-PCDD/F-TEQ, WHO-PCB-TEQ and the sum of six indicator PCBs,
- give method information and
- give information about the accreditation of the laboratory according to ISO/IEC 17025 *(for metrological traceability of consensus values of participants used as assigned values)*.

Results had to be reported in ng/kg, relative to a feed with a moisture content of 12 %, for PCDD/Fs and dioxin-like PCBs, and in µg/kg, relative to a feed with a moisture content of 12 %, for indicator PCBs, PBDEs and HBCDDs. TEQ-based results had to be calculated using the WHO-TEFs of 2005 [3].

1.5 Results of PCDD/Fs and PCBs determined by bioanalytical screening methods

Laboratories should:

- use their own reference standards;
- report if the samples are suspected to be non-compliant with EU legal limits and confirmation is required
- report PCDD/F and/or PCB results in BEQ, if applicable,
- report the reporting limit, maximum level / action level, which the evaluation is based on, and the bioassay cut-off, if applicable,
- give method information
- give information about the accreditation of the laboratory according to ISO/IEC 17025.

Results had to be reported in ng BEQ/kg, relative to a feed with a moisture content of 12 %, for PCDD/Fs and dioxin-like PCBs.

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	25
	Other Countries	2
Official Laboratories	European Union	41
	Other European Countries	0
	Africa	0
	Americas	2
	Asia	0
	Oceania	1
Commercial Laboratories	European Union	10
	Other European Countries	2
	Africa	0
	Americas	3
	Asia	2
	Oceania	0
	Total	**88**

2.1 Number of reported results

Table 2: Reported results for PCDD/F and PCB sum parameters

Reported results	WHO-PCDD/F-PCB-TEQ	WHO-PCDD/F-TEQ	WHO-PCB-TEQ	Sum of six indicator PCBs	PCDD/F-PCB-BEQ [Bioanalytical screening methods]
Palm fatty acid distillate (2003-FFA)	61	62	61	67	10
Rapeseed oil (2003-FFB)	63	63	63	77	9

2.2 Accreditation

Table 3: Reported accreditation according to ISO/IEC 17025 by participants for PCDD/Fs and PCBs

Palm fatty acid distillate (2003-FFA)	**PCDD/Fs, PCBs** [Physico-chemical methods]	**PCDD/Fs, PCBs** [Bioanalytical screening methods]
yes	60	9
no	8	0

Rapeseed oil (2003-FFB)	**PCDD/Fs, PCBs** [Physico-chemical methods]	**PCDD/Fs, PCBs** [Bioanalytical screening methods]
yes	68	10
no	7	0

3. Detection methods

The following detection methods were applied:

- GC-HRMS-, GC-MS/MS-, GC-LRMS-methods for PCDD/Fs and non- ortho PCBs
- GC-HRMS-, GC-MS/MS-, GC-LRMS-, GC-ECD-methods for mono-ortho-PCBs and indicator PCBs
- Bioanalytical screening methods for PCDD/Fs and dioxin-like PCBs

Table 4: Overview of physico-chemical detection methods for PCDD/Fs and PCBs in palm fatty acid distillate (2003-FFA) and rapeseed oil (2003-FFB)

Detection methods	**PCDD/Fs**	**non-ortho-PCBs**	**mono-ortho-PCBs**	**Indicator PCBs**
HRMS	46 / 45	46 / 47	42 / 44	34 / 35
MS/MS	7 / 8	5 / 8	6 / 8	13 / 18
LRMS	2 / 2	2 / -	2 / 2	7 / 9
ECD	-	-	-	2 / 4

4. Homogeneity and stability of the test material

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

Therefore, 10 portions of the test samples 2003-FFA and 2003-FFB 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, WHO-PCB-TEQ, the sum of six indicator PCBs and individual congeners. The test materials showed sufficient homogeneity for this proficiency test.

The stability check of the analytes of interest applying room temperature storage was performed according to ISO 13528:2015 [2]. The test materials showed sufficient stability for this proficiency test.

5. Determination of the assigned values

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

The determination of the assigned value was performed according [1] by estimating of the assigned value as the consensus of participants' results (using only results of physico-chemical methods). 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, if necessary.

The assigned value was 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 limits of quantification(LOQs)), if possible. Additionally the median of all values was calculated.

For individual congeners (including LOQs) assigned values were 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 were only taken for evaluation and calculation, if these levels are equal to or above the LOQ; otherwise the LOQ was taken.

Assigned values could not be calculated for 2 PCDF congeners in sample 2003-FFA and the lower bound WHO-PCDD/F-TEQ, 2,3,7,8-TCDD, 2 PCDD/F congeners and PCB 169 in sample 2003-FFB due to the high variation of participants' results.

Since there are no traceable reference values available, the assigned values in this PT were calculated based on the Huber robust mean of the participants' results. Therefore,

the assigned values are only traceable to these submitted results. Additionally the results of all participants reporting results and the results of participants having accreditation according ISO/IEC 17025 were compared for PCDD/F and PCB sum parameters. No significant differences between the assigned values calculated for both data sets were observed.

Table 5: Comparison of assigned values for all participants and participants with reported accreditation according to ISO/IEC 17025 for PCDD/F and PCB sum parameters

Palm fatty acid distillate (2003-FFA)	**Assigned value** **All participants**	**Assigned value** **ISO/IEC 17025 accreditation**	**Deviation**
	ng/kg, µg/kg product*	ng/kg, µg/kg product*	%
WHO-PCDD/F-PCB-TEQ ub rep	0.984	0.977	1
WHO-PCDD/F-TEQ ub rep	0.717	0.711	1
WHO-PCB-TEQ ub rep	0.276	0.279	1
Sum Indicator PCBs ub rep	1.81	1.82	1

* relative to a feed with a moisture content of 12 %

Rapeseed oil (2003-FFB)	**Assigned value** **All participants**	**Assigned value** **ISO/IEC 17025 accreditation**	**Deviation**
	ng/kg, µg/kg product*	ng/kg, µg/kg product*	%
WHO-PCDD/F-PCB-TEQ ub rep	0.597	0.583	2
WHO-PCDD/F-TEQ ub rep	0.303	0.297	2
WHO-PCB-TEQ ub rep	0.289	0.285	1
Sum Indicator PCBs ub rep	8.44	8.43	0

* relative to a feed with a moisture content of 12 %

5.1 PCDD/Fs and PCBs – Sum parameters

The assigned values for the test samples 2003-FFA and 2003-FFB were calculated as consensus of participants' results for the PCDD/F and PCB sum parameters.

Table 6: Assigned values for physico-chemical methods for PCDD/Fs and PCBs (rounded to three significant figures)

Test sample	WHO-PCDD/F-PCB-TEQ upper bound	WHO-PCDD/F-TEQ upper bound	WHO-PCB-TEQ upper bound	Sum Indicator PCBs upper bound
	ng/kg product*	ng/kg product*	ng/kg product*	µg/kg product*
Palm fatty acid distillate (2003-FFA)	0.984	0.717	0.276	1.81
Rapeseed oil (2003-FFB)	0.597	0.303	0.289	8.44

* relative to a feed with a moisture content of 12 %

Table 7: Assigned values for PCDD/Fs and DL-PCBs for comparison with BEQ results of bioanalytical screening methods (rounded to two significant figures)

Test sample	WHO-PCDD/F-PCB-TEQ upper bound	WHO-PCDD/F-TEQ upper bound	WHO-PCB-TEQ upper bound
	ng/kg product*	ng/kg product*	ng/kg product*
Palm fatty acid distillate (2003-FFA)	0.98	0.72	0.28
Rapeseed oil (2003-FFB)	0.60	0.30	0.29

* relative to a feed with a moisture content of 12 %

5.2 PCDD/Fs and PCBs – Individual congeners

The assigned values for the test samples 2003-FFA and 2003-FFB for individual congeners were calculated as consensus of participants' results taken into account criteria for calculation as described above.

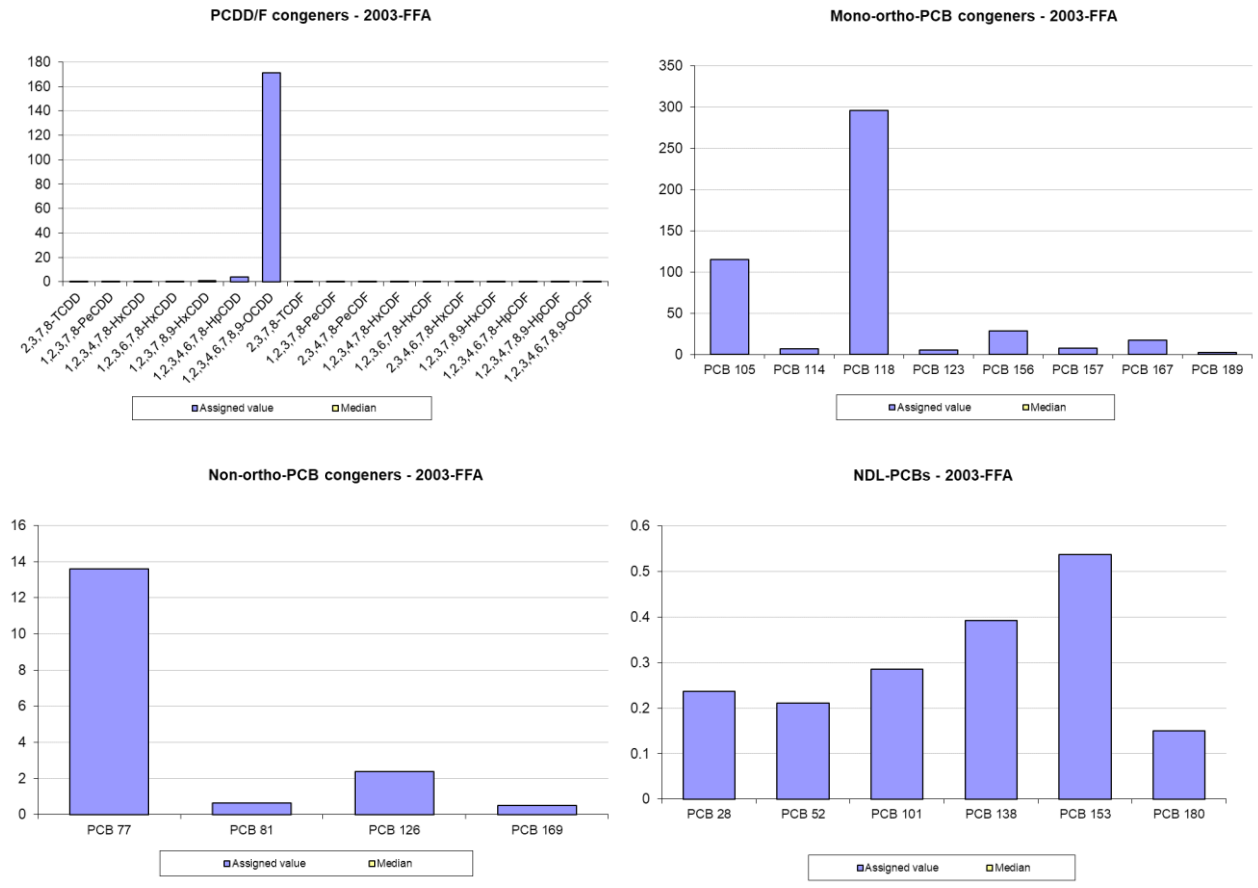

Figure 1: Assigned values (blue) for PCDD/F and PCB congeners for palm fatty acid distillate (2003-FFA) [ng/kg and µg/kg product, relative to a feed with a moisture content of 12 %]

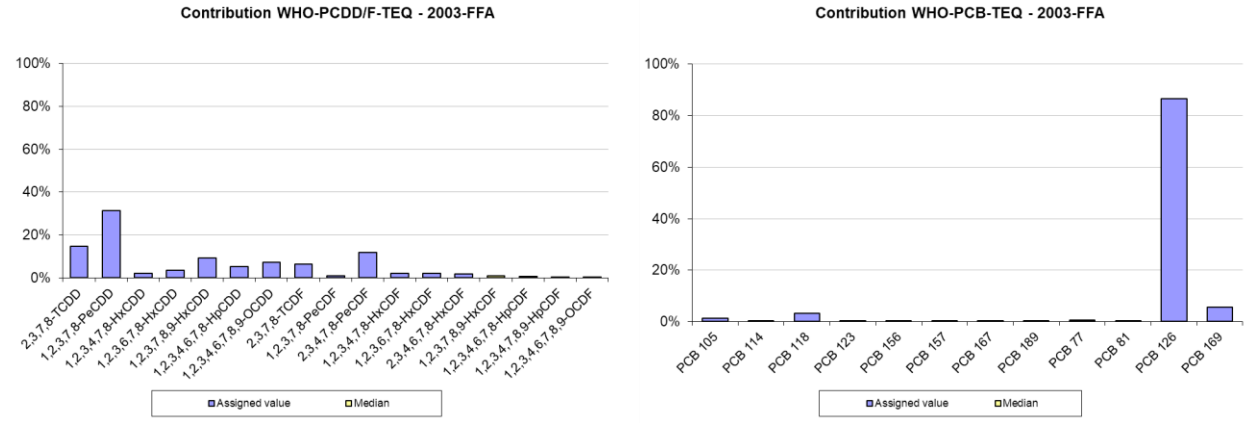

Figure 2: Contributions in % to WHO-PCDD/F-TEQ and WHO-PCB-TEQ for PCDD/F and PCB assigned and median values for palm fatty acid distillate (2003-FFA)

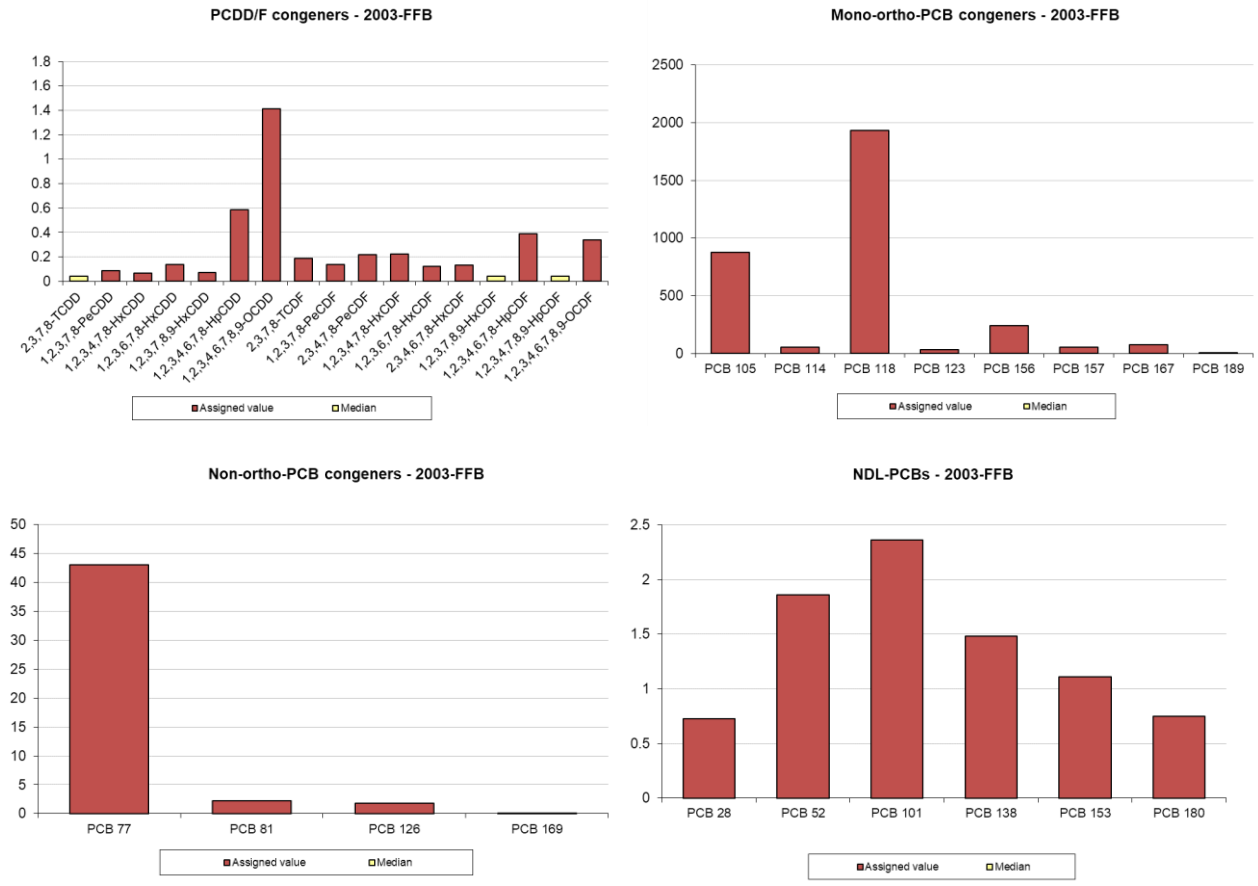

Figure 3: Assigned values (red) for PCDD/F and PCB congeners for rapeseed oil (2003-FFB) [ng/kg and µg/kg product, relative to a feed with a moisture content of 12 %]

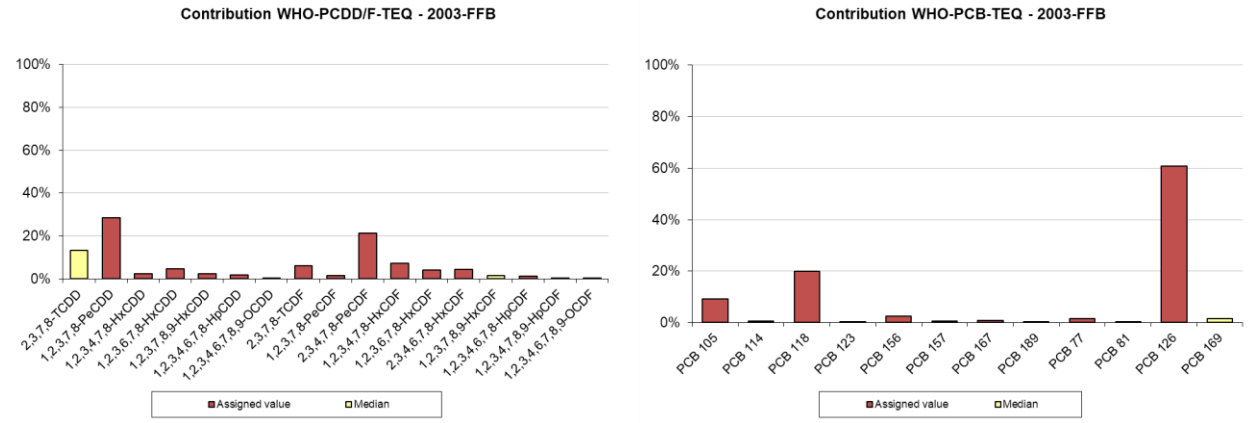

Figure 4: Contributions in % to WHO-PCDD/F-TEQ and WHO-PCB-TEQ for PCDD/F and PCB assigned and median values for rapeseed oil (2003-FFB)

5.3 Comparison of assigned values with legal limits

Maximum levels and action thresholds for feed are defined in Directive 2002/32/EC of the European Parliament and of the Council of 7 May 2002 on undesirable substances in animal feed.

Table 8: Maximum levels and action thresholds for feed materials of plant origin – vegetable oils and their by-products:

Feed materials of plant origin – vegetable oils and their by-products		**Maximum level**	**Action threshold**
WHO-PCDD/F-PCB-TEQ	ng/kg product*	1.5	
WHO-PCDD/F-TEQ	ng/kg product*	0.75	0.5
WHO-PCB-TEQ	ng/kg product*		0.5
Sum of 6 Indicator PCBs	µg/kg product*	10	

* relative to a feed with a moisture content of 12 %

For both test samples 2003-FFA and 2003-FFB the assigned values for the sum parameters WHO-PCDD/F-PCB-TEQ, WHO-PCDD/F-TEQ, WHO-PCB-TEQ and the sum indicator PCBs were below the respective maximum levels and/or action thresholds, except for WHO-PCDD/F-TEQ in sample 2003-FFA (above the action threshold).

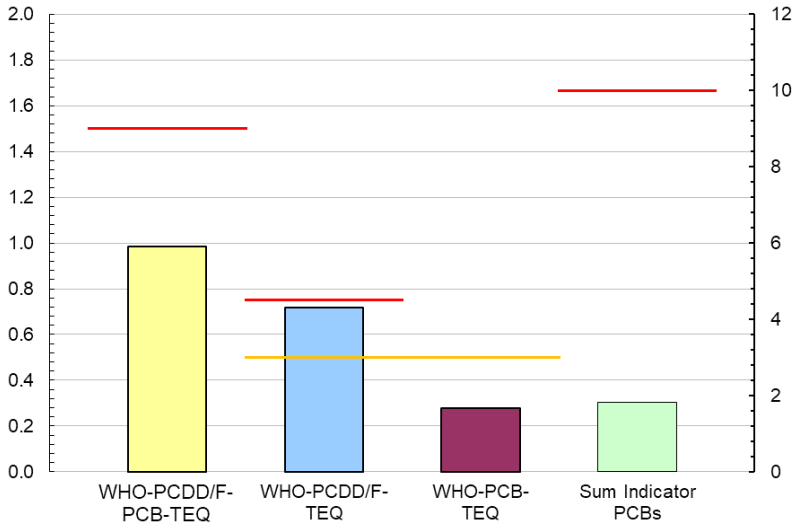

Figure 5: Comparison of assigned values for sum parameters for palm fatty acid distillate (2003-FFA) with maximum levels (red line) and action thresholds (yellow line) [ng/kg and µg/kg product, relative to a feed with a moisture content of 12 %]

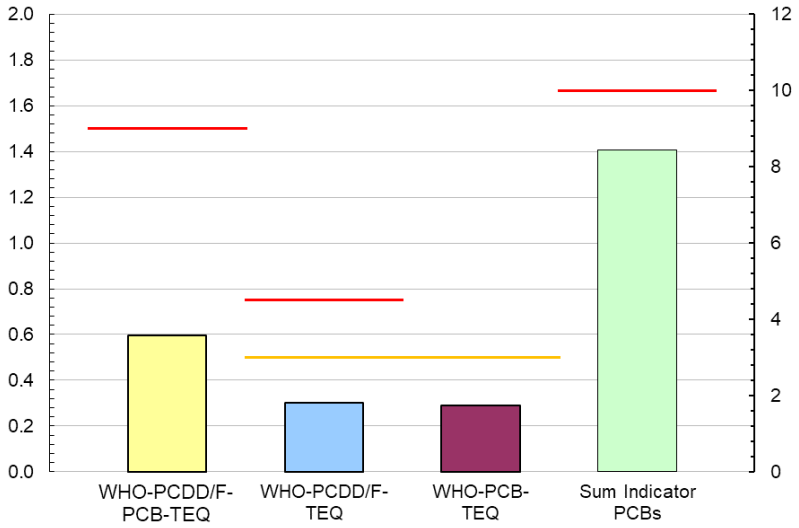

Figure 6: Comparison of assigned values for sum parameters for rapeseed oil (2003-FFB) with maximum levels (red line) and action thresholds (yellow line) [ng/kg and µg/kg product, relative to a feed with a moisture content of 12 %]

6. Evaluation of results

6.1 Physico-chemical methods

6.1.1 Z-score calculation

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 threshold).

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: participant's 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 as 20 %.

Z-scores for individual congeners are only calculated and reported if levels for these congeners are equal to or above the LOQ. Otherwise, no z-scores will be given.

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 PCDD/Fs and PCBs - Participants' z-scores

The concentrations of the sum parameters for both test samples 2003-FFA and 2003-FFB are in the range (about 0.5 to 4 times) of the respective maximum levels or action thresholds, except for the sum of indicator PCBs in sample 2003-FFA and WHO-PCDD/F-PCB-TEQ in sample 2003-FFB.

Table 9: Distribution of participants' z-scores for sum parameters

Palm fatty acid distillate (2003-FFA)	**WHO-PCDD/F-PCB-TEQ**	**WHO-PCDD/F-TEQ**	**WHO-PCB-TEQ**	**Sum of six indicator PCBs**
$\lvert$z-score$\rvert \leq 2$	79 %	73 %	74 %	76 %
$2 < \lvert$z-score$\rvert < 3$	6 %	8 %	8 %	8 %
$\lvert$z-score$\rvert \geq 3$	15 %	19 %	18 %	16 %

Rapeseed oil (2003-FFB)	**WHO-PCDD/F-PCB-TEQ**	**WHO-PCDD/F-TEQ**	**WHO-PCB-TEQ**	**Sum of six indicator PCBs**
$\lvert$z-score$\rvert \leq 2$	81 %	69 %	78 %	94 %
$2 < \lvert$z-score$\rvert < 3$	5 %	9 %	13 %	2 %
$\lvert$z-score$\rvert \geq 3$	14 %	22 %	9 %	4 %

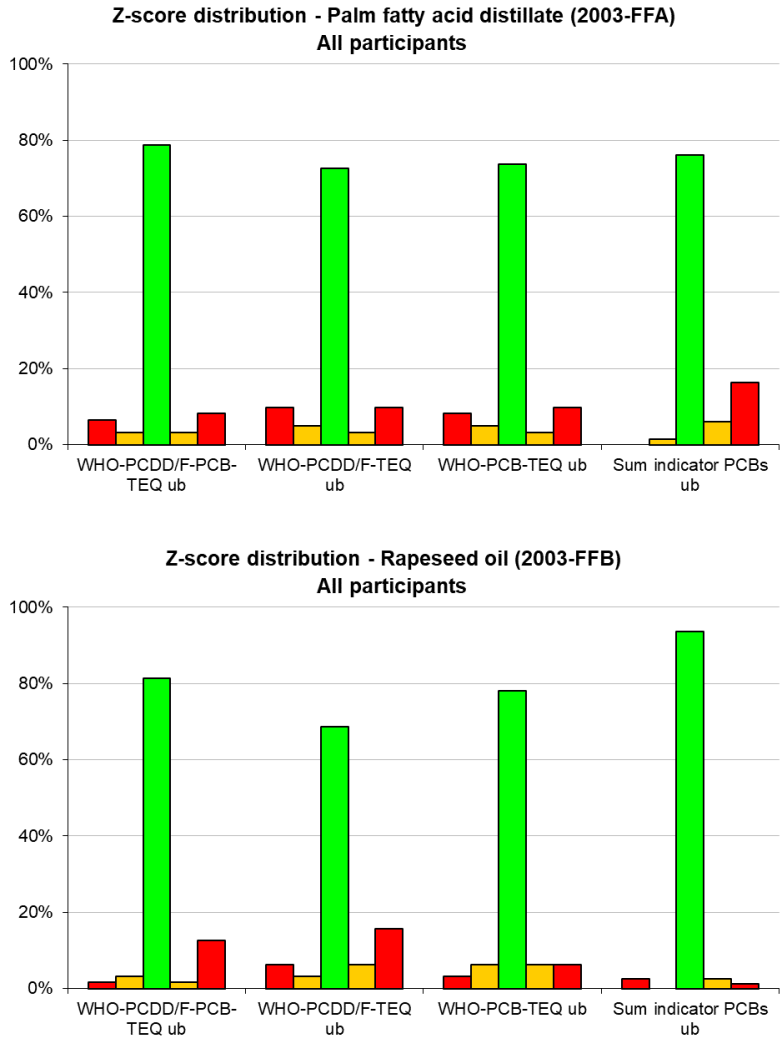

Figure 7: Distribution of participants' z-scores for sum parameters for palm fatty acid distillate (2003-FFA) and rapeseed oil (2003-FFB)
[Green bars: -2 ≤ z-score ≤ 2, yellow bars: -3 < z-score < -2, 2 < z-score < 3, red bars: z-score ≤ -3, z-score ≥ 3]

6.1.3 PCDD/Fs and PCBs - Positive scoring system

The “positive scoring system” gives one assessment for each PT sample covering all relevant PCDD/F and PCB 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:

Table 10: Positive scores allocated to z-scores achieved for all parameters

Positive scoring system	**| z-score | ≤ 2**	**2 < | z-score | < 3**	**| z-score | ≥ 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 (| z-score | ≤ 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 participants’ 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
Difference between reported and calculated results for sum parameters	≤ 10 %

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

Table 11: Successful participation rate according to positive scoring system for palm fatty acid distillate (2003-FFA) and rapeseed oil (2003-FFB)

Scoring system	**Successful participation**		**Reason for not successful participation**			
Percentage of participants' results	**yes**	**no**	**Only sum parameters**	**Sum parameters + individual congeners**	**Only individual congeners**	**Calculation of sum parameters**
2003-FFA	60 %	40 %	36 %	40 %	12 %	44 %
2003-FFB	68 %	32 %	30 %	41 %	15 %	30 %

6.1.4 Comparison of reported and calculated sum parameters

In addition to the calculation of the sum parameters for reported individual PCDD/F and PCB congener values, the calculated sum parameters for PCDD/Fs and PCBs by the EURL are compared with the ones reported by each participant. As the reported sum parameters are decisive to compare the results with the legal limits, an incorrect calculation might lead to a wrong assessment of a sample. Therefore, in case of a significant deviation of the reported sum parameter value from the (EURL) calculated one (deviation > 10 %), the respective results will be marked as incorrect and no z-score will be given in the certificate of participation.

Table 12: Difference between reported and calculated sum parameters for PCDD/Fs and PCBs for palm fatty acid distillate (2003-FFA) and rapeseed oil (2003-FFB): Percentage of participants' results

Palm fatty acid distillate (2003-FFA)	**WHO-PCDD/F-PCB-TEQ**	**WHO-PCDD/F-TEQ**	**WHO-PCB-TEQ**	**Sum of six indicator PCBs**
Deviation ≤ 10 %	92 %	92 %	93 %	96 %
Deviation > 10 %	8 %	8 %	7 %	4 %

Rapeseed oil (2003-FFB)	**WHO-PCDD/F-PCB-TEQ**	**WHO-PCDD/F-TEQ**	**WHO-PCB-TEQ**	**Sum of six indicator PCBs**
Deviation ≤ 10 %	95 %	95 %	92 %	99 %
Deviation > 10 %	5 %	5 %	8 %	1 %

6.1.5 Difference between upper and lower bound calculation

According to Commission Regulation (EC) No 152/2009 the difference between upper bound level and lower bound level shall not exceed 20 % for confirmation of exceedance of maximum level or in case of need of action thresholds for PCDD/Fs and DL-PCBs. For indicator PCBs the difference between upper bound and lower bound levels for the sum of six indicator PCBs shall be ≤ 20 % at the level of interest.

For both test samples 2003-FFA and 2003-FFB the assigned values for all sum parameters are below the respective maximum levels and/or action thresholds, except for WHO-PCDD/F-TEQ in sample 2003-FFA (above the action threshold).

Table 13: Difference between upper and lower bound calculation for palm fatty acid distillate (2003-FFA) and rapeseed oil (2003-FFB): Percentage of participants' results

Palm fatty acid distillate (2003-FFA)	**WHO-PCDD/F-PCB-TEQ**	**WHO-PCDD/F-TEQ**	**WHO-PCB-TEQ**	**Sum of six indicator PCBs**
0 – 10 %*	87 %	89 %	86 %	80 %
10 – 20 %*	7 %	3 %	7 %	8 %
20 – 50 %*	3 %	3 %	3 %	2 %
> 50 %*	3 %	5 %	3 %	11 %

* Difference between upper and lower bound calculation

Rapeseed oil (2003-FFB)	**WHO-PCDD/F-PCB-TEQ**	**WHO-PCDD/F-TEQ**	**WHO-PCB-TEQ**	**Sum of six indicator PCBs**
0 – 10 %*	58 %	45 %	90 %	95 %
10 – 20 %*	21 %	23 %	8 %	1 %
20 – 50 %*	16%	21 %	0 %	3 %
> 50 %*	5 %	11 %	2 %	1 %

* Difference between upper and lower bound calculation

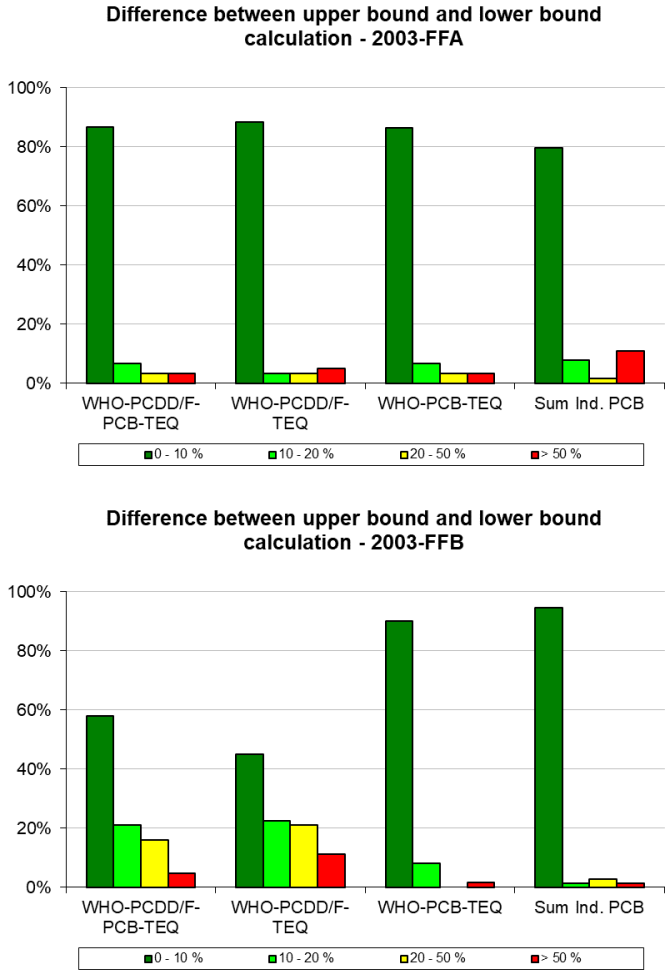

Figure 8: Difference between upper and lower bound calculation for palm fatty acid distillate (2003-FFA) and rapeseed oil (2003-FFB): Percentage of participants' results [Dark green bars: 0 – 10 %, light green bars: 10 – 20 %, yellow bars: 20 – 50 %, red bars: > 50 %]

6.1.6 Assessment of analytical results and measurement uncertainty

In addition, participants were asked to report the measurement uncertainty applied for checking of compliance for WHO-PCDD/F-PCB-TEQ, WHO-PCDD/F-TEQ, WHO-PCB-TEQ and the sum of six indicator PCBs and the assessment if the analytical results for the sample exceed the respective maximum levels and action thresholds beyond reasonable doubt taking into account the measurement uncertainty.

Table 14: Reported relative expanded measurement uncertainty for sum parameters for palm fatty acid distillate (2003-FFA) / rapeseed oil (2003-FFB); outlier removed (e.g. reporting of absolute values)

Reported measurement uncertainty	WHO-PCDD/F-PCB-TEQ	WHO-PCDD/F-TEQ	WHO-PCB-TEQ	Sum of six indicator PCBs
Minimum	10 % / 10 %	10 % / 10 %	10 % / 10 %	6 % / 6 %
Mean	24 % / 24 %	22 % / 23 %	23 % / 23 %	26 % / 26 %
Median	21 % / 22 %	20 % / 22 %	20 % / 22 %	23 % / 25 %
Maximum	50 % / 50 %	40 % / 41 %	48 % / 48 %	88 % / 55 %

Table 15: Number of participants reporting exceedance of legal limits for palm fatty acid distillate (2003-FFA) and rapeseed oil (2003-FFB)

Palm fatty acid distillate (2003-FFA)	WHO-PCDD/F-PCB-TEQ	WHO-PCDD/F-TEQ	WHO-PCB-TEQ	Sum of six indicator PCBs
Maximum level	1	12	-	0
Action threshold	-	39	0	-

Rapeseed oil (2003-FFB)	WHO-PCDD/F-PCB-TEQ	WHO-PCDD/F-TEQ	WHO-PCB-TEQ	Sum of six indicator PCBs
Maximum level	1	1	-	1
Action threshold	-	2	0	-

6.2 Bioanalytical screening methods

According to Commission Regulation (EC) No 152/2009, “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.

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 %.

6.2.1 Assessment of analytical results

As a consequence of the comparison of the assigned values with legal limits, the assessment of the analytical results using bioanalytical screening methods should read “suspected to be non-compliant with the action threshold for WHO-PCDD/F-TEQ, WHO-PCDD/F-TEQ” for the test sample 2003-FFA and “compliant” for the test sample 2003-FFB.

Table 16: Participants' assessment of analytical results using bioanalytical screening methods for 2003-FFA

Laboratories' assessment of analytical results	**WHO-PCDD/F-PCB-TEQ** Maximum level	**WHO-PCDD/F-TEQ** Maximum level	**WHO-PCDD/F-TEQ** Action threshold	**WHO-PCB-TEQ** Action threshold
Suspected to be non-compliant	7	6	4	3
Compliant	2	0	1	1

Table 17: Participants' assessment of analytical results using bioanalytical screening methods for 2003-FFB

Laboratories' assessment of analytical results	**WHO-PCDD/F-PCB-TEQ** Maximum level	**WHO-PCDD/F-TEQ** Maximum level	**WHO-PCDD/F-TEQ** Action threshold	**WHO-PCB-TEQ** Action threshold
Suspected to be non-compliant	2	4	3	3
Compliant	8	3	2	1

6.2.2 Participants' bioassay-scores

Concentrations for WHO-PCDD/F-PCB-TEQ, WHO-PCDD/F-TEQ and WHO-PCB-TEQ in the test sample 2003-FFA are in the range (about 0.5 to 2 times) of the respective maximum levels and action thresholds. For the test sample 2003-FFB concentrations for WHO-PCDD/F-TEQ and WHO-PCB-TEQ are in this range.

Table 18: Distribution of participants' bioassay-scores for BEQ parameters for palm fatty acid distillate (2003-FFA)

Percentage of participants' results	**PCDD/F-PCB-BEQ**	**PCDD/F-BEQ**	**PCB-BEQ**
$\lvert$bioassay-score$\rvert \leq 2$	40 %	50 %	50 %
$2 < \lvert$bioassay-score$\rvert < 3$	10 %	-	17 %
$\lvert$bioassay-score$\rvert \geq 3$	50 %	50 %	33 %

Table 19: Distribution of participants' bioassay-scores for BEQ parameters for rapeseed oil (2003-FFB)

Percentage of participants' results	**PCDD/F-PCB-BEQ**	**PCDD/F-BEQ**	**PCB-BEQ**
$\lvert$bioassay-score$\rvert \leq 2$	67 %	50 %	33 %
$2 < \lvert$bioassay-score$\rvert < 3$	-	-	50 %
$\lvert$bioassay-score$\rvert \geq 3$	33 %	50 %	17 %

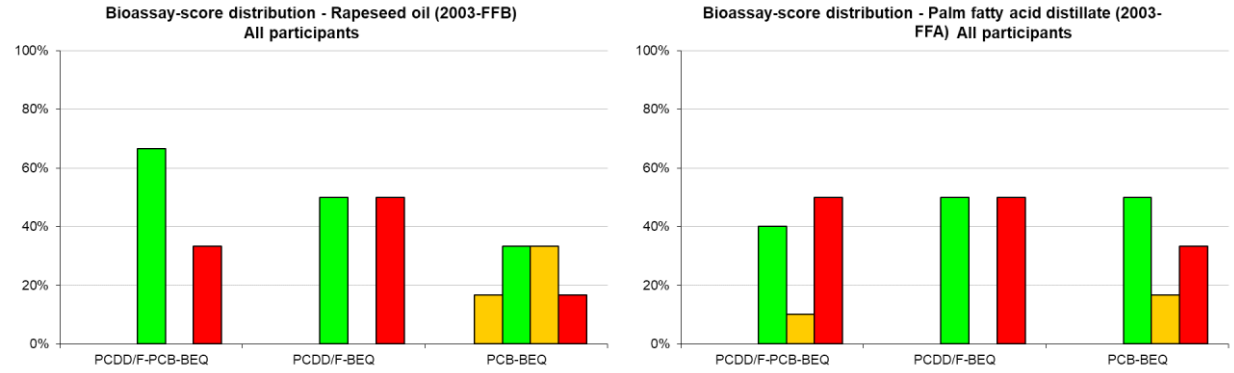

Figure 9: Distribution of participants' bioassay-scores for BEQ parameters for palm fatty acid distillate (2003-FFA) and rapeseed oil (2003-FFB) [Green bars: -2 ≤ bioassay-score ≤ 2, yellow bars: -3 < bioassay-score < -2, 2 < bioassay-score < 3, red bars: bioassay-score ≤ -3, bioassay-score ≥ 3]

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, 29 laboratories (33 % of all participants) participated in this survey.

7.1 Overview of questions and answers of participants

Participants' information (more than one answer possible):

National Reference Laboratory (NRL)	Official Laboratory (OFL)	Commercial laboratory	Other
61 %	32 %	11 %	0 %

Organization of proficiency test:

	Fully	Largely	Partly	Not at all	No opinion
Satisfied with organization of PT	71 %	29 %	-	-	-
Meeting of expectations	39 %	54 %	3.5 %	-	3.5 %
Information understandable	64 %	32 %	4 %	-	-
Time frame acceptable	57 %	36 %	3.5 %	-	3.5 %

PT test samples:

	Fully	Largely	Partly	Not at all	No opinion
Selection of matrix and level of contamination adequate	25 %	64 %	7 %	-	4 %

Evaluation of results and summary of data:

	Fully	Largely	Partly	Not at all	No opinion
Evaluation of results and report clear and comprehensible	75 %	18 %	3.5 %	-	3.5 %

7.2 Comments and suggestions

Comments referred to the presentation of the data in the reports. Options for filtering of results based on laboratory codes or parameters or presentation in Excel- or csv-format would be helpful for data evaluation.

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 PCDD/F and PCB results for the PT test samples palm fatty acid distillate (2003-FFA) and rapeseed oil (2003-FFB) and the evaluation of the results are given in the following annexes 1 - 14. 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.)

Palm fatty acid distillate – 2003-FFA		
1	Assigned values – PCDD/F, PCB	
2	Participants' results – Tables – PCDD/F, PCB	
3	Participants' z-scores and bioassay-scores – Tables - PCDD/F, PCB	
4	Participants' z-scores – Charts – PCDD/Fs, PCB	
5	Scoring system – PCDD/F, PCB	
6	Homogeneity and stability test – PCDD/F, PCB	
7	Participants' methods – PCDD/F, PCB	

Rapeseed oil – 2003-FFB		
8	Assigned values – PCDD/F, PCB	
9	Participants' results – Tables – PCDD/F, PCB	
10	Participants' z-scores and bioassay-scores – Tables - PCDD/F, PCB	
11	Participants' z-scores – Charts – PCDD/Fs, PCB	
12	Scoring system – PCDD/F, PCB	
13	Homogeneity and stability test – PCDD/F, PCB	
14	Participants' methods – PCDD/F, PCB	

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