

# EURL Interlaboratory Study on the Determination of Brominated Contaminants and PCNs

in Cod Liver Oil

2021

EURL-ILS-BC\_2104-CLO

## FOOD

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Final results

(Report Version 1.0)

26 August 2022



## Summary

Test sample (food)	Fortified cod liver oil [2104-CLO]
Analytes of interest	<p><b>PBDEs</b> (BDE-28, -47, -49, -99, -100, -153, -154, -183, -209)</p> <p><b>eBFRs</b> (PBBz, HBB, PBT, PBEB, BTBPE, TBX, DBDPE)</p> <p><b>HBCDDs</b> (<math>\alpha</math>-HBCDD, <math>\beta</math>-HBCDD, <math>\gamma</math>-HBCDD or total HBCDD)</p> <p><b>TBBPA</b></p> <p><b>PCNs</b> (PCN 27, 28, 31, 36, 42, 46, 48, 49, 50, 52, 53, 59, 60, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75)</p>
Methods	Any kind of method (on request information on sample preparation and measurement methods could be provided)
Participants	NRLs, OFLs, other official laboratories, commercial laboratories performing the analysis of samples taken by food business operators
Statistical evaluation	ISO 13528:2020, IUPAC Protocol
Report of final results	26 August 2022
Publication	EURL POPs reserves all rights to publish and present the anonymised results of the interlaboratory study in scientific journals and/or during conferences.



## 1. Structure of the ILS, test material and analytes

This interlaboratory study (ILS) on the determination of **brominated contaminants (BCons)** and **polychlorinated naphthalenes (PCNs)** in **cod liver oil** was organized by the European Union Reference Laboratory (EURL) for halogenated persistent organic pollutants (POPs) in Feed and Food to be performed between July and September 2021. The objective was to assess interlaboratory comparability of results from BCons and PCNs in a **fortified food sample**.

**National Reference Laboratories (NRLs), Official laboratories (OFLs), research laboratories** and **commercial laboratories** performing the analysis of samples taken by food business operators were invited to participate in this interlaboratory study. First results were discussed by members of the of the core working group on “Brominated Contaminants and PCNs” (CWG BCons and PCNs) and the EURL at a working group meeting on 13 and 14 October 2021. The evaluated preliminary results were discussed by representatives of European Commission, NRLs and the EURL at the COM/EURL/NRL workshops on 23 and 24 November 2021 and on 18 and 19 May 2022.

### 1.1. Samples and coding

The cod liver oil test sample was prepared from commercially available food. It was naturally contaminated with PBDEs, HBCDDs and PCNs and fortified with other analytes of interest using eBFR and TBBPA standards.

<b>Cod Liver Oil</b>	<b>Sample no. 2104-CLO-xxx</b>
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Each participant received about **20 g** of the test sample in a HDPE bottle.

Participants registered for analysing the listed **eBFR group** were asked to quantify the two test standard solution using their own quantification standards.

<b>eBFR test standard solution</b>	<b>eBFR 200 pg/μl</b>
<b>DBDPE test standard solution</b>	<b>DBDPE 500 pg/μl</b>

Participants received **300 μl** eBFR test standard solution containing PBBz, HBB, PBT, PBEB, BTBPE and TBX and **200 μl** DBDPE test standard solution containing solely DBDPE in glass vials. The gross weight (solution and vial, **without** the sealing film) was provided with the standard solution and should be checked for losses during sample shipment by the participants.



## 1.2. Analytes of interest

Participants were asked to determine at least one of the following parameters (as many analytes as possible from each group):

- **Polybrominated diphenyl ether (PBDE) group**
  - Individual congeners: BDE-28, -47, -49, -99, -100, -153, -154, -183, -209
  - Sum of 8 PBDEs (without BDE-209)
  - Sum of 9 PBDEs (with BDE-209)
- **Emerging brominated flame retardant (eBFR) group**
  - PBBz (1,2,3,4,5-Pentabromobenzene; CAS 608-90-2)
  - HBB (1,2,3,4,5,6-Hexabromobenzene; CAS 87-82-1)
  - PBT (1,2,3,4,5-Pentabromo-6-methylbenzene; CAS 87-83-2)
  - PBEb (1,2,3,4,5-Pentabromo-6-ethylbenzene; CAS 85-22-3)
  - BTBPE (1,1'-[1,2-Ethanediy]bis(oxy)] bis[2,4,6-tribromo]benzene; CAS 37853-59-1)
  - TBX (1,2,4,5-Tetrabromo-3,6-dimethylbenzene; CAS 23488-38-2)
  - DBDPE (1,1'-(1,2-Ethanediy]bis[2,3,4,5,6-pentabromo-benzene]; CAS 84852-53-9)
- **Hexabromocyclododecane (HBCDD) and Tetrabromobisphenyl A (TBBPA) group**
  - $\alpha$ -HBCDD,  $\beta$ -HBCDD,  $\gamma$ -HBCDD stereoisomers
  - Sum of  $\alpha$ -,  $\beta$ -,  $\gamma$ -HBCDD (using HPLC methods)
  - Total HBCDD (using GC methods)
  - TBBPA
- **Polychlorinated naphthalene (PCN) group**
  - PCNs 27, 31, 42, 46, 48, 49, 50, **53**, 59, 63, 65, **69**, 70, **73**, **74**, **75**
  - PCNs 28/36, **52/60**, **66/67**, 64/**68**, **71/72** (difficult to separate chromatographically)  
[It was recommended to focus at least on the 12 main congeners (in **bold**) when starting method development]

## 1.3. Methods

All kinds of detection and quantification methods could be applied.

## 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.

The confidentiality between NRLs and their OFLs will be kept unless a Member State initiated a cooperation between the NRL, OFLs and the EURL.





## 1.5. Results of BCons and PCNs

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,
- 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 **µg/kg fat** for PBDEs, eBFRs, HBCDDs and TBBPA and in **pg/g fat** for PCNs.

## 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

29 laboratories registered for this proficiency test.

**Table 1:** Participating laboratories

Participating laboratories	Region	No. of participants
<b>National Reference Laboratories</b>	European Union	14
	Other Countries	2
<b>Official Laboratories</b>	European Union	8
	Other European Countries	2
	Africa	0
	Americas	1
	Asia	0
	Oceania	1
<b>Commercial Laboratories</b>	European Union	1
	Other European Countries	0
	Africa	0
	Americas	0
	Asia	0
	Oceania	0
	<b>Total</b>	<b>29</b>

## 2.1. Number of reported results

**Table 2:** Reported results for PBDEs for cod liver oil (2104-CLO)

Reported results	All laboratories	NRLs
BDE-28	23	11
BDE-47	23	11
BDE-49	20	11
BDE-99	23	11
BDE-100	23	11
BDE-153	23	11
BDE-154	23	11
BDE-183	22	11
BDE-209	19	8
Sum of 8 PBDEs (without BDE-209) (ub)	22	11
Sum of 8 PBDEs (without BDE-209) (lb)	22	11
Sum of 9 PBDEs (with BDE-209) (ub)	19	8
Sum of 9 PBDEs (with BDE-209) (lb)	19	8

**Table 3:** Reported results for HBCDDs and TBBPA for cod liver oil (2104-CLO)

Reported results	All laboratories	NRLs
$\alpha$ -HBCDD	14	8
$\beta$ -HBCDD	13	7
$\gamma$ -HBCDD	14	8
Sum of $\alpha$ -, $\beta$ -, $\gamma$ -HBCDD (ub)	13	8
Sum of $\alpha$ -, $\beta$ -, $\gamma$ -HBCDD (lb)	13	8
Total HBCDD (using GC methods)	3	2
TBBPA	8	5

**Table 4:** Reported results for eBFRs for cod liver oil (2104-CLO)

Reported results	All laboratories	NRLs
<b>PBBz</b>	6	1
<b>HBB</b>	10	3
<b>PBT</b>	9	3
<b>PBEB</b>	9	3
<b>BTBPE</b>	7	1
<b>TBX</b>	8	3
<b>DBDPE</b>	8	3

**Table 5:** Reported results for eBFRs for the standard solutions (eBFR 200 pg/μl and DBDPE 500 pg/μl)

Reported results	All laboratories	NRLs
<b>PBBz</b>	3	1
<b>HBB</b>	6	3
<b>PBT</b>	6	3
<b>PBEB</b>	6	3
<b>BTBPE</b>	3	1
<b>TBX</b>	5	3
<b>DBDPE</b>	5	3

**Table 6:** Reported results for PCNs for cod liver oil (2104-CLO)

Reported results (2104-CLO)	All laboratories	NRLs
<b>PCN 27</b>	9	5
<b>PCN 28</b>	7	4
<b>PCN 31</b>	3	2
<b>PCN 36</b>	4	4

Reported results (2104-CLO)	All laboratories	NRLs
PCN 42	9	5
PCN 46	6	3
PCN 48	6	3
PCN 49	6	3
PCN 50	6	3
PCN 52*	10	6
PCN 53*	10	6
PCN 59	3	2
PCN 60*	2	1
PCN 63	4	2
PCN 64	3	2
PCN 65	7	3
PCN 66*	9	6
PCN 67*	2	1
PCN 68*	8	5
PCN 69*	9	5
PCN 70	7	3
PCN 71*	9	6
PCN 72*	3	2
PCN 73*	10	6
PCN 74*	8	5
PCN 75*	10	6



## 2.2. Accreditation

**Table 7:** Reported accreditation according to ISO/IEC 17025 by participants for PBDEs, HBCDDs and PCNs

Cod Liver Oil	PBDEs	HBCDDs	PCNs
Accreditation	14	4	1
No accreditation	6	10	8

## 2.3. Detection methods

The following detection methods were applied:

- GC-HRMS-, GC-MS/MS-methods for PBDEs, PCNs and eBFRs
- GC-HRMS-, GC-MS/MS-, LC-MS/MS-, LC-HRMS-methods for HBCDDs and TBBPA

## 2.4. Chromatographic separation of PCNs

Because some PCN congeners are difficult to separate chromatographically under routine GC conditions (e.g., PCN 28/36, 52/60, 66/67, 64/68, 71/72), participants were asked to report the concentration of co-eluting congeners for only one of these congeners and to specify which congeners co-eluted in the "Co-eluting PCN congeners" column. Some laboratories reported results differently, resulting in large variation between participants' results for some congeners. Participants were asked to recheck their results and correct them, if necessary. A new evaluation of these results is part of this final report.

Co-eluting pairs:

28/36: Five labs did not report co-elution for PCN 28 and PCN 36, whereas only one lab reported values for both congeners (above the LOQ).

52/60: Only three laboratories reported no chromatographically co-eluting for PCN 52 and PCN 60 (Lab 6, 16 and 19), whereas only Lab 6 and 19 gave values for both congeners.

64/68: Two laboratories did not report co-elution for PCN 64 and PCN 68, whereas both labs did only give values for one congener.

66/67: All labs reported co-eluting for PCN 66 and PCN 67

71/72: All labs reported co-eluting for PCN 71 and PCN 72

For the evaluation of the data the sum of co-eluting PCN pairs was calculated by the EURL, when separation of co-elution was reported, if two identical values were reported and no chromatographic separation was stated only one value was taken as sum for the co-eluting congeners.



### 3. Homogeneity and stability of the test material

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

Therefore, 10 portions of the test samples 2104-CLO were analysed in duplicate for PBDEs, HBCDDs, PCNs and eBFRs. The test for sufficient homogeneity was performed for the individual congeners/stereoisomers. The test materials showed sufficient homogeneity for PBDEs, HBCDDs, PCNs and eBFRs in this proficiency test.

The stability check of the analytes of interest applying room temperature storage was performed according to ISO 13528:2020 [2] for PBDEs, PCNs, HBCDDs and eBFRs. The test material showed sufficient stability for PBDEs, PCNs, HBCDDs and eBFRs. Homogeneity and stability can be concluded also for TBBPA due to similar physico-chemical properties for this proficiency test.

### 4. Determination of the assigned value

Statistical evaluation of the ILS results was performed by the EURL for halogenated POPs in feed and food according to ISO 13528:2020 [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 is taken as assigned value after excluding extreme outliers (outside the range of  $\pm 50\%$  of the median of all reported results) and examination of the distribution of the remaining results using histogram and Kernel density estimation, if necessary.

Assigned values were calculated for individual congeners / stereoisomers / substances and sum parameters (upper and lower bound calculation), if possible. Additionally, the median of all values was calculated.

For individual congeners / stereoisomers / substances (including LOQs), assigned values were only calculated according to the abovementioned procedure if more than 2/3 of all results were above the LOQ and less than 1/3 of all results (including LOQs) were outside the range of  $\pm 50\%$  of the median of all reported results. Levels for individual congeners / stereoisomers / substances are only used for evaluation and calculation if these levels are equal to or above the LOQ; otherwise the LOQ will be used instead.

Assigned values could be calculated for individual PBDE congeners, sum of 8 (without BDE-209) and sum of 9 (with BDE-209) PBDEs, for individual HBCDD diastereomers, sum of  $\alpha$ -,  $\beta$ - and  $\gamma$ -HBCDD (including limits of quantification (LOQs)), TBBPA, HBB and PCN 42, PCN 52, PCN 69, PCN 73, PCN 75 and calculated PCN sum parameters.



Due to high variation of participants' results in the range of the respective LOQ or too few results, no assigned values could be calculated for:

- BDE-183, BDE-209
- $\beta$ - and  $\gamma$ -HBCDD, Total HBCDD (using GC-methods)
- PBBz, PBT, PBEB, BTBPE, TBX, and DBDPE
- All other PCN congeners except the above mentioned

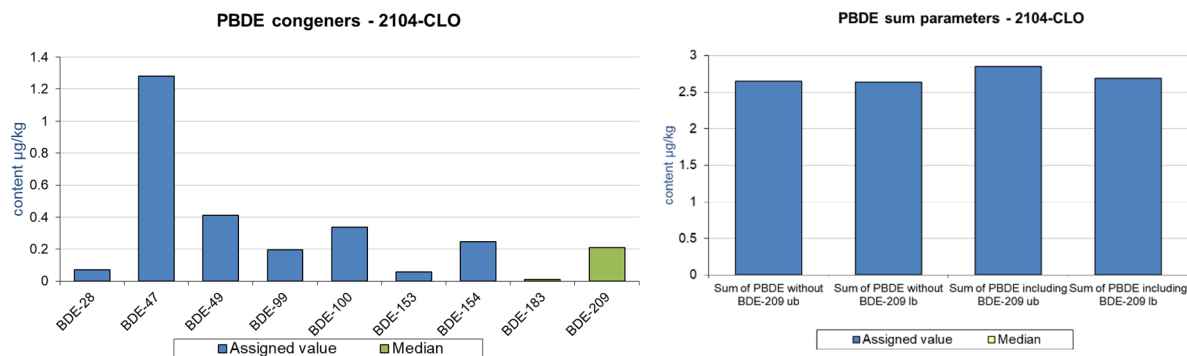
Six laboratories quantified the two test standard solutions using their own quantification standards. Assigned values for comparison and deviations from the prepared values were calculated, with the exception of BTBPE, because the reported values for BTBPE were only one-fourth of the prepared standard solution concentration.

#### 4.1. PBDEs – individual congeners and sum parameter

The assigned values for the test sample 2104-CLO were calculated as consensus of participants' results for individual PBDEs and sum parameters, taking into account the calculation criteria described above (Table 8 ; tabular summary see annex 1; Figure 1).

**Table 8:** Assigned values for PBDEs (rounded to three significant figures)

Cod Liver Oil (2104-CLO)	Assigned value $\mu\text{g}/\text{kg}$ fat
BDE-28	0.0722
BDE-47	1.28
BDE-49	0.412
BDE-99	0.196
BDE-100	0.338
BDE-153	0.0569
BDE-154	0.247
Sum of 8 PBDEs (without BDE-209) (ub)	2.65
Sum of 8 PBDEs (without BDE-209) (lb)	2.64
Sum of 9 PBDEs (with BDE-209) (ub)	2.85
Sum of 9 PBDEs (with BDE-209) (lb)	2.69



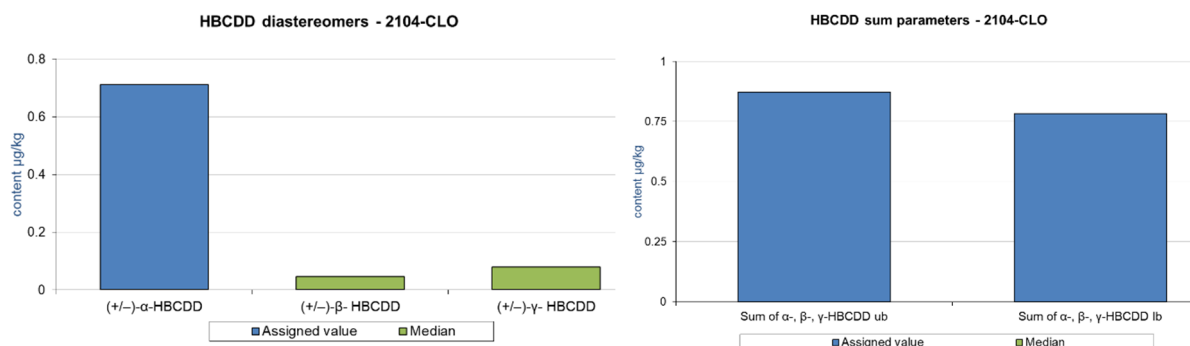
**Figure 1:** Assigned values (blue) and median values (green) for PBDE individual congeners and sum parameters for cod liver oil (2104-CLO) [µg/kg fat]

#### 4.2. HBCDDs – individual stereoisomers and sum parameter

The assigned values for the test sample 2104-CLO were calculated as consensus of participants' results for individual HBCDDs and sum parameters, taking into account the calculation criteria described above (Table 9; tabular summary see annex 1; Figure 2).

**Table 9:** Assigned values for HBCDDs (rounded to three significant figures)

Cod Liver Oil (2104-CLO)	Assigned value µg/kg fat
(+/-)-γ- HBCDD	0.711
Sum of α-, β-, γ-HBCDD (ub)	0.873
Sum of α-, β-, γ-HBCDD (lb)	0.783



**Figure 2:** Assigned values (blue) and median values (green) for HBCDD individual congeners and sum parameters for cod liver oil (2104-CLO) [µg/kg fat]

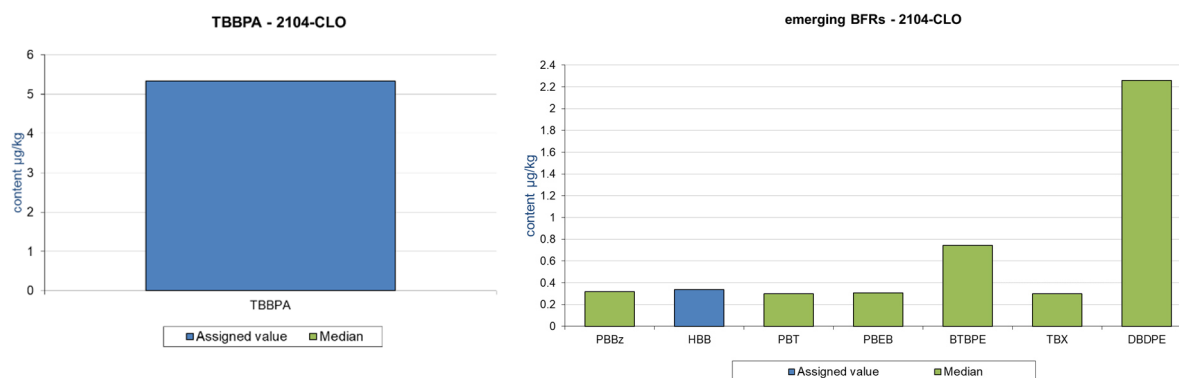


### 4.3. TBBPA and eBFRs – individual substances

The assigned values for the test sample 2104-CLO were calculated as consensus of participants' results for TBBPA and individual eBFR substances, taking into account the calculation criteria described above (Table 10 ; tabular summary see annex 1; Figure 3).

**Table 10:** Assigned values for HBCDDs (rounded to three significant figures)

Cod Liver Oil (2104-CLO)	Assigned value $\mu\text{g/kg fat}$
TBBPA	5.33
HBB	0.336



**Figure 3:** Assigned values (blue) and median values (green) for TBBPA and individual emerging BFRs for cod liver oil (2104-CLO) [ $\mu\text{g/kg fat}$ ]

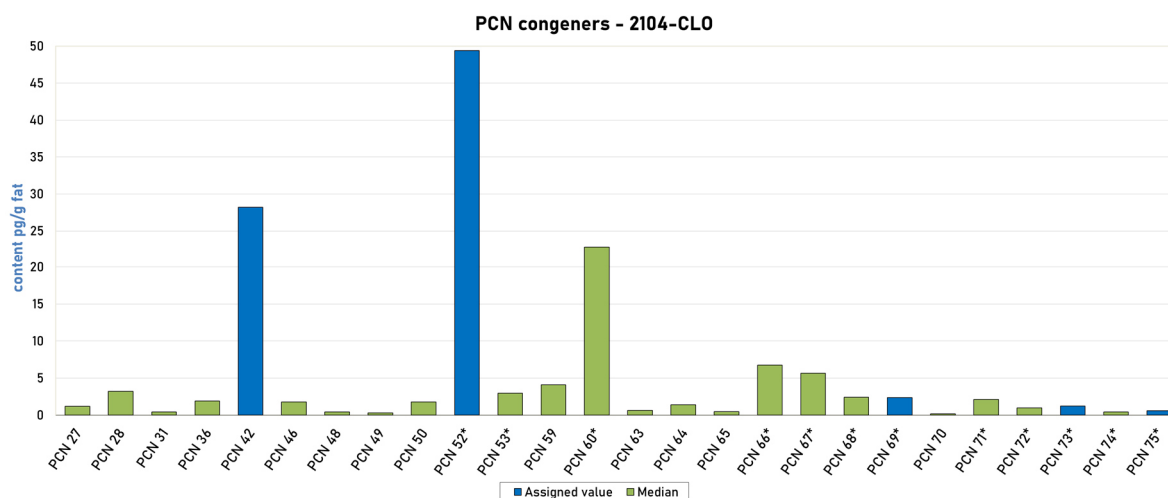
#### 4.4. PCNs – individual substances and sum parameters

The assigned values for the test sample 2104-CLO were calculated as consensus of participants' results for individual PCN congeners and calculated sum parameters, taking into account the calculation criteria described above (Table 11 ; tabular summary see annex 1; Figure 4 and 5).

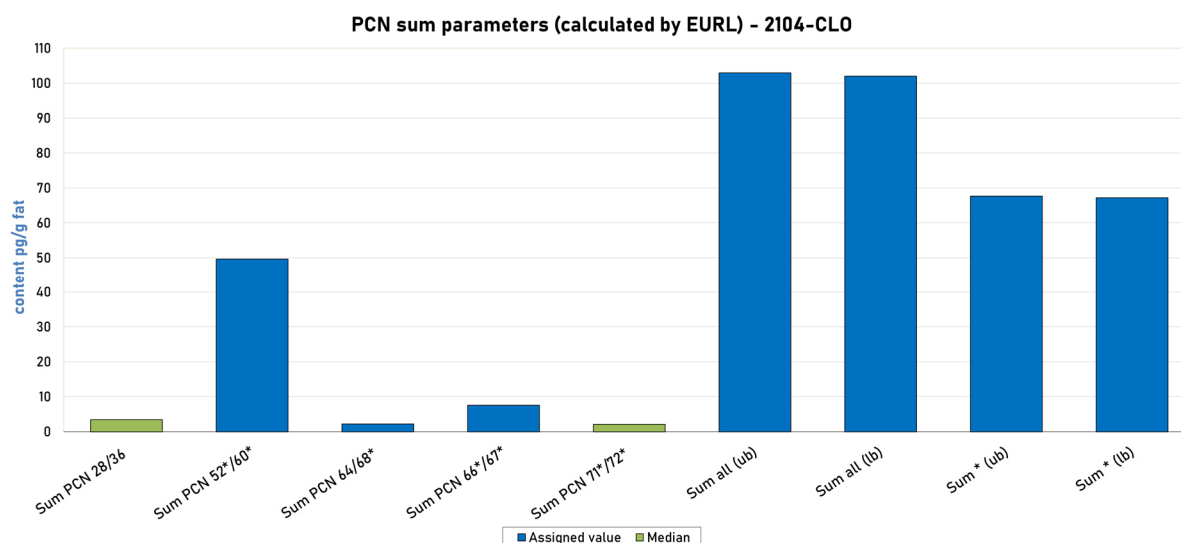
**Table 11:** Assigned values for PCNs (rounded to three significant figures)

Cod Liver Oil (2104-CLO)	Assigned value pg/g fat
PCN 42	28.2
PCN 52*	49.4
PCN 69*	2.38
PCN 73*	1.24
PCN 75*	0.581
Sum PCN 52*/60*	49.7
Sum PCN 64/68*	2.22
Sum PCN 66*/67*	7.54
Sum all 26 PCN congeners (ub)	103
Sum all 26 PCN congeners (lb)	102
Sum main 12 congeners* and PCN 64 (ub)	67.7
Sum main 12 congeners* and PCN 64 (lb)	67.2

\*recommended 12 main congeners



**Figure 4:** Assigned values (blue) and median values (green) for individual PCN congeners for cod liver oil (2104-CLO) [pg/g fat]



**Figure 5:** Assigned values (blue) and median values (green) for PCN sum parameters for cod liver oil (2104-CLO) [pg/g fat]

#### 4.5. Comparison of assigned values with recommended LOQs

The limits of quantification are currently based on the values specified in Commission Recommendation of 3 March 2014, on the monitoring of trace levels of brominated flame retardants in food (2014/118/EU). For PBDEs the recommended LOQ value is 0.01 µg/kg w.w. for individual congeners (Table 12). However, it was discussed in the meetings of the core working group “Brominated Contaminants and PCNs” of the EURL/NRL network that a lower LOQ value of 0.001 µg/kg w.w. is preferable for all congeners except BDE-209, given that some foods show concentrations below this level (Table 13). Valid data on the background contamination of foodstuffs with BFRs is particularly important for a reliable risk assessment. For HBCDDs the recommended LOQ value is 0.01 µg/kg w.w. for α-, β- and γ-stereoisomers (Table 12). For total HBCDD measured by GC-MS, the corresponding LOQ value is 0.003 µg/kg (as cumulative response of all possible HBCDD diastereomers, Table 13).

**Table 12:** Recommended LOQs for PBDEs and HBCDDs from COMMISSION RECOMMENDATION of 3 March 2014 on the monitoring of traces of brominated flame retardants in food (2014/118/EU)

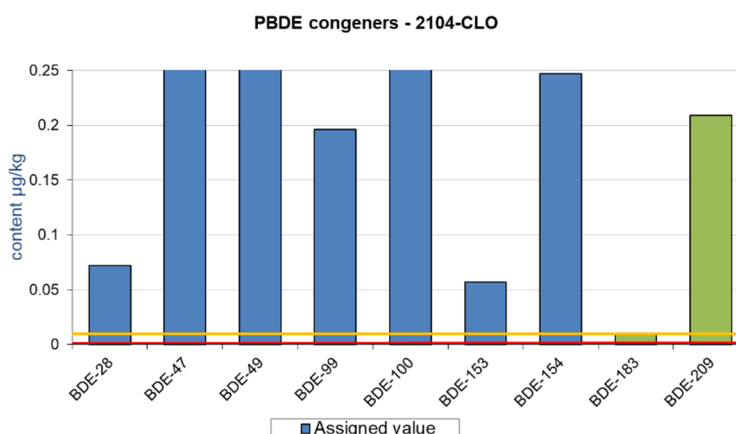
Food	Limit of quantification per congener/stereoisomer ng/g wet weight
PBDEs	≤ 0.01
HBCDDs	≤ 0.01

**Table 13:** Analytical recommendations from “Guidance document on analytical parameters for the determination of organobromine contaminants in food and feed” (CWG “BCons and PCNs”)

Food	Limit of quantification per congener/stereoisomer µg/kg wet weight
PBDEs	0.01 0.001 (all congeners except BDE-209)
HBCDDs	0.01 0.01 (sum of HBCDDs) 0.003 (total HBCDD)

**PBDEs:**

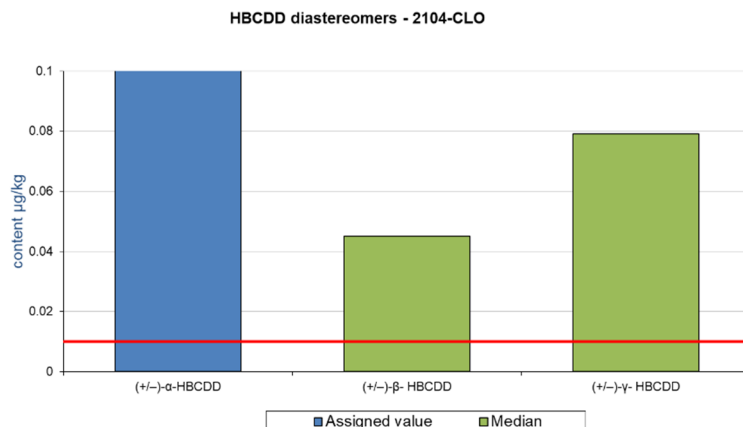
All calculated assigned values for individual PBDE congeners were above the recommended LOQ. For BDE-183 no assigned values could be calculated. The median value of all results for BDE-183 was at the recommended LOQ of 0.01 µg/kg. For BDE-209, with a median value of 0.21 µg/kg fat, no assigned value could be calculated as well, indicating, that some laboratories are not able to determine BDE-209 above the recommended LOQ.



**Figure 6:** Comparison of assigned values for PBDE congeners with recommended LOQs (yellow line at 0.01 µg/kg and red line at 0.001 µg/kg) in cod liver oil (2104-CLO)

**HBCDDs:**

For β- and γ-HBCDD no assigned values could be calculated, because less than 2/3 of all reported results were above the LOQs (see calculation criteria section 4). Therefore, the median values were taken for comparison with the recommended LOQs. For β-HBCDD (0.045µg/kg) and γ-HBCDD (0.079 µg/kg) both median values were 5 to 7-times above the recommended LOQs of 0.01 µg/kg w.w., showing that the majority of the laboratories in this PT could not reliably report results for HBCDDs in cod liver oil in this low concentration range.



**Figure 7:** Comparison of assigned values for HBCDD stereoisomers with recommended LOQs (red line at 0.01 µg/kg product) in cod liver oil (2104-CLO)

## 5. Evaluation of results

### 5.1. Z-scores calculation

Criteria for successful participation of laboratories were based on the evaluation of the results of individual congeners and sum parameters. For evaluation of results of physico-chemical methods the z-scores were calculated according to the following formula:

$$z = \frac{(x - x_a)}{\sigma_p}$$

$x$ : participant's result

$x_a$ : assigned value

$\sigma_p$ : fitness-for-purpose-based standard deviation for proficiency assessment

For PBDE and PCN congeners, eBFR and TBBPA individual substances, HBCDD diastereomers and PBDE, HBCDD and PCN sum parameters, the standard deviation for proficiency assessment  $\sigma_p$  is defined as 20 %.

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

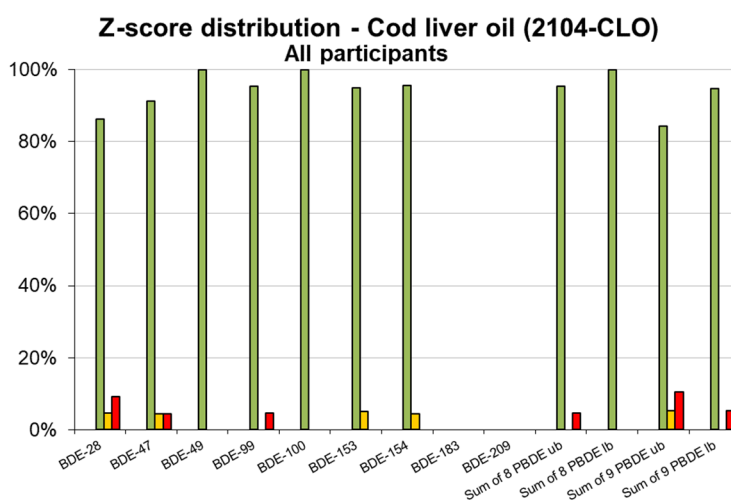
Interpretation of z-scores:

$ z\text{-score}  \leq 2$	<i>satisfactory performance</i>
$2 <  z\text{-score}  < 3$	<i>questionable performance (warning signal)</i>
$ z\text{-score}  \geq 3$	<i>unsatisfactory performance (action signal)</i>

## 5.2. PBDEs - Participants' z-scores

**Table 14:** Distribution of participants' z-scores for PBDEs for cod liver oil (2104-CLO)

Percentage of participants' results	$ z\text{-score}  \leq 2$	$2 <  z\text{-score}  < 3$	$ z\text{-score}  \geq 3$
BDE-28	86%	5%	9%
BDE-47	92%	4%	4%
BDE-49	100%	-	-
BDE-99	96%	-	4%
BDE-100	100%	-	-
BDE-153	95%	5%	-
BDE-154	96%	4%	-
Sum of 8 PBDEs without BDE-209 (ub)	96%	-	4%
Sum of 8 PBDE including BDE-209 (lb)	100%	-	-
Sum of 9 PBDE including BDE-209 (ub)	84%	5%	11%
Sum of 9 PBDE including BDE-209 (lb)	95%	-	5%



**Figure 7:** Distribution of participants' z-scores for PBDE congeners / sum parameters for cod liver oil [Green bars:  $-2 \leq z\text{-score} \leq 2$ , orange bars:  $-3 < z\text{-score} < -2$ , red bars:  $2 < z\text{-score} < 3$ , red bars:  $z\text{-score} \leq -3$ ,  $z\text{-score} \geq 3$ ]

### 5.3. HBCDDs - Participants' z-scores

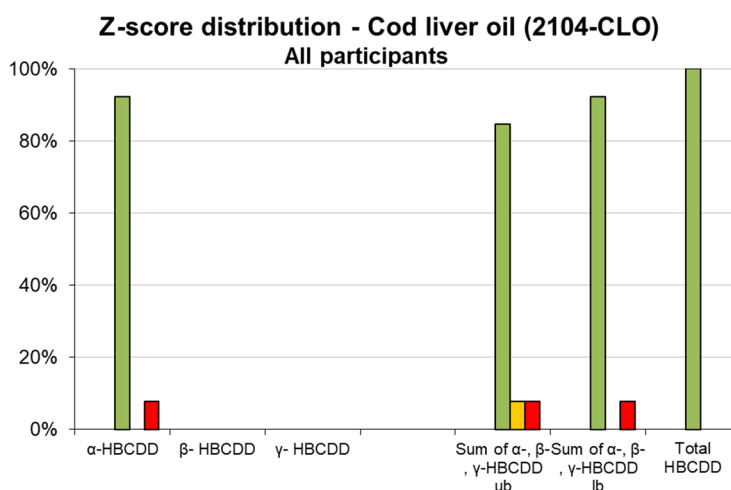
HBCDD stereoisomers undergo thermal isomerization at temperatures above 160 °C. With GC elution temperature of these compounds of normally above 160 °C a separation of HBCDD stereoisomers using GC analysis is not possible. Only one unresolved peak is obtained. Additional thermal decomposition of HBCDDs is reported for temperatures above 240 °C. Therefore, in case of applying GC-MS methods for HBCDD analysis determination of total HBCDD (as sum of all originally present HBCDD diastereomers) is possible only.

Due to the low numbers of results for total HBCDD the sum of  $\alpha$ -,  $\beta$ -,  $\gamma$ -HBCDD (using LC separation) was taken for comparison.

**Table 15:** Distribution of participants' z-scores for HBCDD for cod liver oil (2104-CLO)

Percentage of participants' results	$ z\text{-score}  \leq 2$	$2 <  z\text{-score}  < 3$	$ z\text{-score}  \geq 3$
$\alpha$ - HBCDD	92%	-	8%
Sum of $\alpha$ -, $\beta$ -, $\gamma$ -HBCDD (lb)	84%	8%	8%
Sum of $\alpha$ -, $\beta$ -, $\gamma$ -HBCDD (ub)	92%	-	8%
Total HBCDD*	100%	-	-

\*Comparison of participants' results for total HBCDD with assigned value for sum of  $\alpha$ -,  $\beta$ -,  $\gamma$ -HBCDD (ub)



**Figure 8:** Distribution of participants' z-scores for HBCDD stereoisomers / sum parameters for cod liver oil (2104-CLO) [Green bars:  $-2 \leq z\text{-score} \leq 2$ , orange bars:  $-3 < z\text{-score} < -2$ ,  $2 < z\text{-score} < 3$ , red bars:  $z\text{-score} \leq -3$ ,  $z\text{-score} \geq 3$ ]



## 5.4. TBBPA

**Table 16:** Distribution of participants' z-scores for TBBPA for cod liver oil (2104-CLO)

Percentage of participants' results	$ z\text{-score}  \leq 2$	$2 <  z\text{-score}  < 3$	$ z\text{-score}  \geq 3$
<b>TBBPA</b>	88%	12%	-

## 5.5. eBFRs z-scores

**Table 17:** Distribution of participants' z-scores for eBFR for cod liver oil (2104-CLO)

Percentage of participants' results	$ z\text{-score}  \leq 2$	$2 <  z\text{-score}  < 3$	$ z\text{-score}  \geq 3$
<b>HBB</b>	100%	-	-

**Table 18:** Deviation in percentage of participants' results from standard solutions prepared concentration (eBFR 200 pg/μl and DBDPE 500 pg/μl)

Deviation from prepared concentration in %	PBBz 200 pg/μl	HBB 200 pg/μl	PBT 200 pg/μl	PBEb 200 pg/μl	BTBPE 200 pg/μl	TBX 200 pg/μl	DBDPE 500 pg/μl
<b>LC3</b>		5	2	1	72	0	-19
<b>LC13</b>	12	12	11	12	64	19	17
<b>LC14</b>		10	11	10			
<b>LC19</b>		10	5	-5		-5	18
<b>LC20</b>	14	18	18	10		17	21
<b>LC25</b>	13	4	8	12	74	2	-9

Participants registered for analysis of the listed eBFR group were asked to quantify the seven eBFR analytes in prepared standard solutions of 200 and 500 pg/μl using their own quantification standards. Reported concentrations had a deviation of up to 21% (DPDPE) for all analytes except BTBPE. For BTBPE, deviations of up to 74% from the prepared concentration were calculated. This will be followed up at a later stage.



## 5.6. PCN z-scores

**Table 19:** Distribution of participants' z-scores for PCNs for cod liver oil (2104-CLO)

Percentage of participants' results	$ z\text{-score}  \leq 2$	$2 <  z\text{-score}  < 3$	$ z\text{-score}  \geq 3$
<b>PCN 4</b>	100%	-	-
<b>PCN 52</b>	70%	10%	20%
<b>PCN 69</b>	89%	-	11%
<b>PCN 73</b>	78%	-	22%
<b>PCN 75</b>	88%	-	12%
<b>PCN 52*/60*</b>	90%	-	10%
<b>PCN 64/68*</b>	80%	-	20%
<b>PCN 66*/67*</b>	70%	20%	10%
<b>Sum all 26 congeners (ub)</b>	80%	-	20%
<b>Sum all 26 congeners (lb)</b>	80%	-	20%
<b>Sum 12 main congeners &amp; PCN 64 (ub)</b>	80%	10%	10%
<b>Sum 12 main congeners &amp; PCN 64 (lb)</b>	80%	10%	10%

## 6. Participants' feedback

A questionnaire for feedback from participants of this EURL proficiency test was available as online survey between 12 November 2021 and 21 December 2021. The survey was anonymous, but participants could also give their laboratory name. The identity of the laboratories is kept confidential. The survey included several 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, 5 laboratories (17 % of all PT participants) replied to this survey.

**Table 20:** Participating laboratories in the feedback survey

Type of laboratory	Answers
National Reference Laboratory (NRL)	4
Official Laboratory (OFL)	1
Commercial laboratory / Other (e.g. research and development) / no answer	0

**General aspect**

How satisfied are you with the organization of this proficiency test in general? Please rate the parts below according to your experience, with 0 stars meaning "no opinion" and 5 stars meaning "full satisfaction".



**Did the proficiency test meet expectations?**



**Specific aspects of this proficiency test**

We would like to know a bit more about specific aspects of this proficiency test. Please rate the aspects below according to your experience, with 0 stars meaning "no opinion" and 5 stars meaning "full satisfaction".

Was all necessary information for participation and performance of the PT provided in an understandable way?	
Was the time frame acceptable?	
Was the handling of EUSurvey as webtool for reporting and source of instructions manageable?	
Was the evaluation of participant's results and the information in the preliminary report clear and comprehensible?	

Was the selected sample adequate for the goal to assess analytical performance of laboratories in relevant matrices?

**Choice of matrix**



**Level of contamination (BCon)**



**Level of contamination (PCNs)**



No further comments or any suggestions for improvements were submitted.

## 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 PBDE, eBFR, HBCDD, TBBPA and PCN results for the ILS test sample cod liver oil (2104-CLO) are given in the following annexes. Laboratories are coded according to the laboratory codes sent after registration.

## 9. 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 13528:2020, Statistical methods for use in proficiency testing by interlaboratory comparisons, International Organization for Standardization



## 10. Annex

(Please download the report and open it with a common pdf reader. After that you can open the annexes by double clicking the pdf icons. )

Cod Liver Oil – 2104-CLO		
1	Assigned values – PBDEs, HBCDDs, TBBPA, eBFRs, PCNs	
2	Participants' results – Tables – PBDEs, HBCDDs, TBBPA, eBFRs, PCNs	
3	Participants' z-scores – Tables – PBDEs, HBCDDs, TBBPA, HBB, PCNs	
4	Participants' z-scores – Charts – PBDEs, HBCDDs, TBBPA, HBB	
5	Participants' z-scores – Charts – PCNs	
6	Test for sufficient homogeneity and stability	
eBFR and DBDPE test standard solutions		
7	Assigned values – eBFRs standard solutions	
8	Participants' results – eBFRs standard solutions	
9	Participants' z-scores – eBFRs standard solutions	
Analytical Methods		
10	Participants' methods 1 for PBDEs, HBCDDs, TBBPA, eBFRs	
11	Participants' methods 2 for PCNs	

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