Evaluation of the EURL proficiency test results on the determination of dioxin-like compounds by bioanalytical screening methods

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Introduction

The European Union Reference Laboratory (EURL) for halogenated persistent organic pollutants (POPs) in Feed and Food - until 2017 known as "EURL for Dioxins and PCBs in Feed and Food" - regularly organizes proficiency tests (PTs) for the determination of polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs), dioxin-like PCBs (DL-PCBs) and indicator PCBs (PCB 28, 52, 101, 138, 153 and 180) and partly also brominated contaminants (PBDEs, HBCDDs) in food and feed for laboratories using screening and confirmatory methods. The general objective of these PTs is the assessment of analytical performance of laboratories and the interlaboratory comparability of results.

This specific EURL proficiency test on the determination of dioxin-like compounds by bioanalytical screening methods focused on the evaluation of the results of bioanalytical screening methods in ten spiked samples. The main objective for the evaluation was the capability of these screening methods to reliably identify compliant samples and samples suspected to be non-compliant with the established maximum levels. Besides this the evaluation of the comparison of the reported BEQ-levels of bioanalytical screening methods with the TEQ-levels known from fortification and GC-MS analysis was of interest. This study design allowed a comprehensive overview of the discrepancies between TEQ- and BEQ-levels and gave an indication whether elevated BEQ levels are evidence for the presence of dioxin-like compounds other than PCDD/Fs and DL-PCBs.

Materials and methods

Test material

Ten oil test samples were prepared of regular market sunflower oil. For comparison of the contamination of the samples with maximum and action levels the samples are considered as egg oil and are therefore coded as Egg oil A (1803-EOA) to Egg oil K (1803-EOK).

The test samples were partly fortified with PCDD/F standards, technical mixtures of PCBs, brominated dibenzo-p-dioxin and dibenzofuran (PBDD/F) standards, mixed brominated-chlorinated dibenzo-p-dioxin and dibenzofuran (PXDD/F) standards, different technical mixtures of polybrominated diphenyl ethers (PBDEs) and different standards of chlorinated paraffins (CPs) according to the scheme shown in table 1.

Table1: Spiking scheme of ten test samples 1803-EOA to 1803-EOK. Spiked analyts are marked with "x". Sample 1803-EOA was used as not-spiked blank sample.

Analyte	1803-EOA	1803-EOB	1803-EOC	1803-EOD	1803-EOE	1803-EOF	1803-EOG	1803-EOH	1803-EOI	1803-EOK
PCDD/Fs		x	x	x	x	x	x	x	x	x
PCBs		x	x	x	x	x	x	x	x	x
PBDD/Fs					x		x	x	x	x
PXDD/Fs						x	x	x	x	x
PBDEs					x				x	x
CPs									x	x

The following individual standards and technical mixtures were used for spiking in different concentrations and/or patterns:

- PCDD/Fs: all 17 2,3,7,8-chlorinated congeners
- PCBs: Aroclor 1254 (Analabs inc, North Heaven, USA)

- PBDD/Fs: 2,3,7,8-TBDD, 1,2,3,7,8-PeBDD, 1,2,3,4,7,8-HxBDD, 1,2,3,6,7,8-HxBDD, 1,2,3,7,8,9-HxBDD, OBDD, 2,3,7,8-TBDF, 1,2,3,7,8-PeBDF, 2,3,4,7,8-PeBDF, 1,2,3,4,7,8-HxBDF, 1,2,3,4,6,7,8-HpBDF, OBDF
- PXDD/Fs: 2-B-378-TriCDD, 3-B-278-TriCDF, 23-DiB-78-DiCDD, 1-B-2378-TCDD, 2-B-1378-TCDD, 1-B-2378-TCDF, 2-B-36789-PeCDD, 1-B-236789-HxCDD, 1-B-2346789-HpCDD
- PBDEs: Great Lakes DE-71, Pentabromodiphenyl Oxide, Great Lakes DE-79, Octabromodiphenyl Oxide, Great-Lakes DE-83R, Decabromodiphenyl Oxide (Wellington Laboratories Inc., 345 Southgate Dr. Guelph, Canada)
- CPs: Chloroparaffin C14-C17 57% Cl (Dr. Ehrenstorfer standards, LGC Standards GmbH, Germany), Chloroparaffin C11 59% Cl, Chloroparaffin C17 61% Cl (University of Hohenheim, Germany)

The test for sufficient homogeneity was performed according to ISO 13528:2015[1] and the International Harmonized Protocol for the Proficiency Testing of Analytical Chemistry Laboratories[2]. Therefore, 10 portions of the test sample 1803-EOG were analysed in duplicate for PCDD/Fs and PCBs. This test material showed sufficient homogeneity for this proficiency test. The used PCDD/Fs / PCBs spiking solution contained all other contaminants, hence sufficient homogeneity can be concluded for all compounds. Additionally, two portions of the other test samples, prepared and spiked in exactly the same way as 1803-EOG, were analysed in triplicate and showed also no indication for inhomogeneity.

Participants and methods

The PT was open for participation of National Reference Laboratories (NRLs) of EU member states and other countries, official and commercial laboratories. Twelve laboratories registered for this PT and ten reported results for the test samples applying CALUX bioassay. Participants were requested to determine the following parameters:

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

Assigned values and scoring of results

The estimation of the assigned values for PCDD/Fs and DL-PCBs was based on the GC-MS results, analysed at the EU-RL for halogenated POPs in Feed and Food. TEQ-based results were calculated using the WHO-TEFs of 2005[3]. For additional comparison of results, a consensus value of participants' BEQ results was calculated. The median of reported results without exclusion of outliers was taken as consensus value.

The main criterion for the 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 were applied. Therefore, the reported BEQ-values, derived from bioanalytical screening methods, were compared with the WHO-TEQ assigned values, which were calculated based on the results of physical-chemical and the BEQ consensus values of all participants.

Bioassay-scores were calculated according to the following formula:

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

x_a: assigned value (TEQ from GC-MS) or consensus value in BEQ of participants' results

x: participants result (BEQ from bioanalytical screening method)

$\sigma_{bioassay}$: bioassay target standard deviation (for BEQ-results $\sigma_{Bioassay}$ = 20 %)

Results and discussion:

Assigned / consensus values

A comparison of assigned values derived from GC-MS results and consensus values calculated from participants' BEQ results for the sum of PCDD/F and DL-PCB showed in most cases an overestimation of the results by bioanalytical screening methods, but for 70 % of the samples the deviations were below 30 %. Only in

one case (1803-EOK) an underestimation was observed and in two cases a significant overestimation of more than 50 % (1803-EOE, 1803-EOI) (see figure 1).

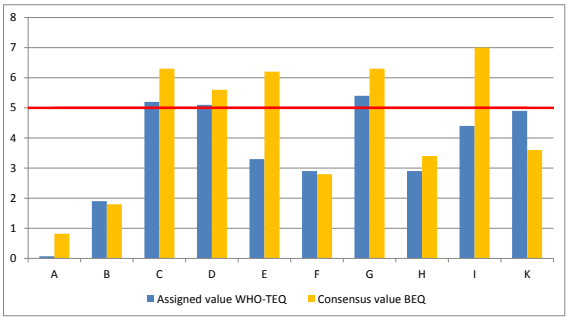

Figure 1: Comparison of WHO-TEQ assigned values (blue bar) with consensus values in BEQ from participants (yellow bar) for sum of PCDD/Fs and DL-PCBs in pg/g fat; comparison with maximum level of 5.0 pg/g fat for WHO-PCDD/F-PCB-TEQ in hen eggs and egg products (red line)

For PCDD/Fs deviations of the consensus values from the assigned values were in all cases above +40 %, except for one (1803-EOD). These deviations could partly be explained by the additional presence of PBDD/Fs and/or PXDD/Fs in the samples, but not in all cases. For sample 1803-EOC BEQ values were 70 % above the TEQ values although only PCDD/Fs and DL-PCBs were spiked in this sample.
Comparing the results for DL-PCBs showed a quite good correlation for half of the samples, but in three cases a significant underestimation of the TEQ results by the CALUX-BEQ by more than 40 % was found (C, F, K).
Possible reasons for these differences in PCDD/Fs and DL-PCBs results could be the presence of additional substances with dioxin-like activity in the PBDE technical mixtures and/or possible antagonistic effects of impurities in some CP standards.
The contribution of the PBDD/Fs and PXDD/Fs was estimated by calculating a TEQ also for these analytes based on the WHO-TEF values for PCDD/Fs[3,4] and/or published REP-values[5,6] for brominated and mixed brominated/chlorinated congeners. The application of the different TEF/REP-values showed no considerable differences between the results of the different calculation approaches. For the test samples 1803-EOE to 1803-EOK the contribution of PBDD/Fs and/or PXDD/Fs ranged between 9% and 36%. A comparison of the deviation of the PCDD/F-BEQ values reported by participants from the calculated TEQ (WHO-PCDD/F-TEQ and Dioxin-TEQ based on chlorinated, brominated and mixed chlorinated/brominated congeners) is given in figure 2. For most samples, the higher PCDD/F-BEQ values could be explained by the additional presence of PBDD/Fs and/or PXDD/Fs.

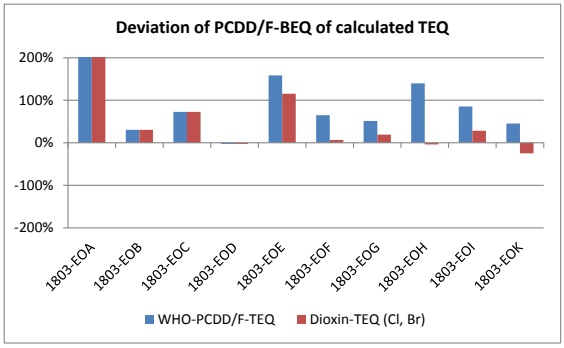

Figure 2: Deviation of the PCDD/F-BEQ values reported by participants from the GC-MS WHO-PCDD/F-TEQ (blue bar) and calculated Dioxin-TEQ (Cl, Br) (red bar) in %

Bioassay-scores

Bioassay-scores were calculated based on the assigned values from GC-MS analysis and the BEQ consensus values from participants. An overview of the bioassay-scores is given in figure 3.

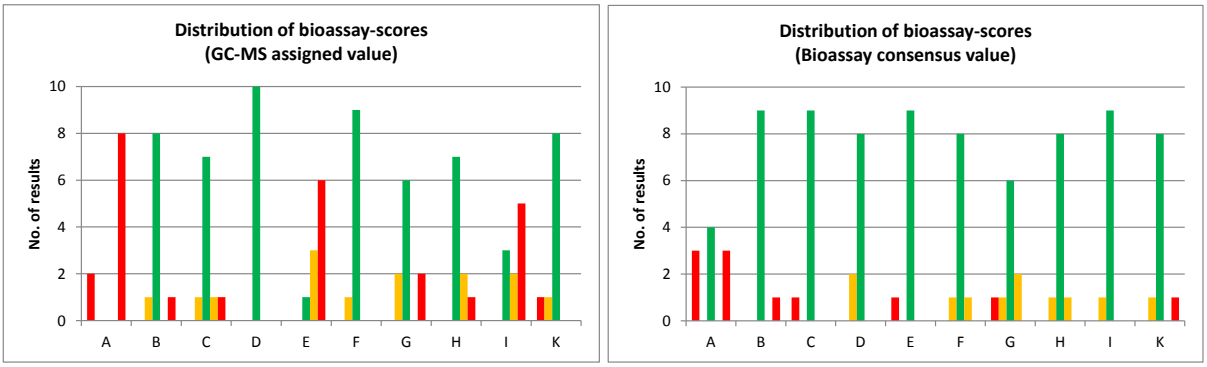

Figure 3: Distribution of the bioassay-scores of the sum of PCDD/Fs and DL-PCBs for comparison with the GC-MS assigned value (left) and the bioassay consensus value (right), $\sigma_{bioassay}$ = 20 %; |bioassay-score| ≥ 3 marked in red, |bioassay-score| between 2 and 3 marked in yellow, |bioassay-score| ≤ 2 marked in green

The distribution of the bioassay-scores for the sum of PCDD/Fs and DL-PCBs demonstrated a good comparability of the bioassay results with the derived consensus value, irrespective of the applied clean-up procedure and bioassay cell line. In most cases at least 80 % of results were within ± 2 bioassay-scores. The comparison with the WHO-TEQ value from GC-MS analysis showed considerably higher bioassay-scores of participants for samples 1803-EOE and 1803-EOI.

Assessment of analytical results

Commission Regulation (EC) No 1881/2006 of 19 December 2006 defines maximum levels for hen eggs and egg products as 5.0 pg/g fat for WHO-PCDD/F-PCB-TEQ and 2.5 pg/g fat for WHO-PCDD/F-TEQ. WHO-PCDD/F-PCB-TEQ levels for three samples (C, D, G) are only slightly above the respective maximum level, whereas three samples (D, G, I) exceed the maximum level for WHO-PCDD/F-TEQ by more than 30 %. Most laboratories identified these samples correctly as suspected to be non-compliant with the respective maximum levels, but one laboratory assessed the samples D, G and I as compliant with the maximum level for WHO-PCDD/F-TEQ resulting in a false compliant assessment of these samples.

Summary

The EURL proficiency test on the determination of dioxin-like compounds by bioanalytical screening methods showed a good comparability of the results of CALUX bioassay among each other. In comparison with the WHO-PCDD/F-PCB-TEQ based on GC-MS results differences could be partly linked to additional presence of PBDD/Fs and/or PXDD/Fs, but not in all cases. Other additionally spiked halogenated POPs (PBDEs, CPs) also could possibly contribute to an increasing or decreasing bioassay response due to agonistic or antagonistic effects. Overall participating laboratories were in most cases able to reliably identify respective samples correctly as suspected to be non-compliant.

Acknowledgements:

We would like to thank the European Commission for the financial support of the work of the European Union Reference Laboratory for halogenated POPs in Feed and Food, Freiburg, Germany, and the network of EURL and NRLs for halogenated POPs in feed and food for their scientific contribution.

References:

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