RESULTS OF THE FIRST INTERNATIONAL INTER LABORATORY DR CALUX® by BDS COMPARISON STUDY FOR FOOD AND FEED (BICS 2005).

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Introduction

Food and feed safety is a high priority issue for the food and feed sector as it directly impacts on human and animal health. Stringent EU limit values are in force for dioxins in food- and feedingstuffs [1,2] for animal and public health protection. The use of the DR CALUX® by BDS bioassay for monitoring dioxins in food and feed allows the (pre)-selection of samples suspected of being contaminated above limit values with dioxins. To permit bioassays to be used for screening of food- and feedingstuffs, the EU has laid down general requirements for the determination of dioxins and dioxin-like PCBs in food- and feedingstuffs and specific requirements for cell-based bioassays[3,4]. To ensure the reliability and performance of the DR CALUX® by BDS bioassay for monitoring food and feedingstuffs, an interlaboratory comparison study (ringtest) is mandatory.

In the present paper, the results of the first international DR CALUX® interlaboratory comparison study (BICS 2005) organized by BioDetection Systems BV (BDS) are described. A total of 21 laboratories world wide using the DR CALUX® bioassay in house participated in the BICS-2005 study. The protocol of the BICS-2005 study was divided into three phases. The complexity of analyses increased with the phases.

Methods and materials

A total of 21 laboratories were invited and participated in the BICS-2005 study (AgriQuality Ltd., Lower Hutt, New Zealand; BioDetection Systems, Amsterdam, The Netherlands; Bureau of Food and Drug, NangangTaipei, Taiwan; C.A.R.T.-University of Liege, Liege, Belgium, CCL B.V., Veghel, The Netherlands; CEFAS, Burnham-on-Crouch, United Kingdom; DWR, Amsterdam, The Netherlands; EMPA, Dübendorf, Switzerland; Environmental Analysis Laboratory of EPA, Chung Li City, Taiwan; Instituto Superiore di Sanita, Rome, Italy; Kaneka Techno Research Co., Ltd., Takasago-city, Japan; Keuringsdienst van Waren, Zutphen, The Netherlands; LABTRASA, Murcia, Spain; Masterlab BV, Boxmeer, The Netherlands; NIES, Tsukuba-city, Japan; Public Analyst's Laboratory, Galway, Republic of Ireland; RIKILT, Wageningen, The Netherlands; SGIT-INIA, Madrid, Spain; State Veterinary and Food Institute, Kosice, Slovakia; Veterinary Research Institute, Brno, Czech Republic; VITO, Mol, Belgium). The participating laboratories received all samples to be analysed, a full 2,3,7,8-TCDD calibration range and all appropriate protocols for extraction, clean-up and DR CALUX® bioanalysis.

Phase I. Phase I consisted of the DR CALUX® analysis of chemical standards (PCDDs, PCDFs, PCBs) dissolved in DMSO. One sample consisted of a PCDD/PCDF mixture (Campro, scientific (cat no. DF-ST-A)) dissolved in DMSO (Across). The mixture was diluted until a DR CALUX® TEQ of 0.996 nM. A second sample consisted of a PCDD/PCDF/PCB mixture (Campro, scientific (cat no. DF-ST-A); (cat no. C-WHO-01) dissolved in DMSO (Across). Again, the mixture was diluted until a DR CALUX® TEQ of 1.50 nM. The participants were asked to dilute both stock samples and determine the DR CALUX® TEQ according to supplied protocols. In addition, the participants received 2 vials containing a 3-times and 10-times dilution of the stock PCDD/PCDF mix and 1 vial containing a 10-times dilution of the stock PCDD/PCDF/PCB mix, prepared by the organizer. The participants were asked to determine the DR CALUX® response in these vials undiluted.

Phase II. Phase II consisted of the DR CALUX® analysis of cleaned sediment extracts dissolved in DMSO and a cleaned food/feed extract dissolved in DMSO. Aliquots of both extracts in DMSO were send to the participants of the study. The participants were asked to dilute both cleaned extracts and

determine the DR CALUX® TEQ according to supplied protocols. In addition, the participants received 1 vial containing a 10-times dilution of the sediment extract and 2 vials containing a 3-times and 10-times dilution of the food/feed extract, prepared by the organizer. The participants were asked to determine the DR CALUX® response in these vials undiluted.

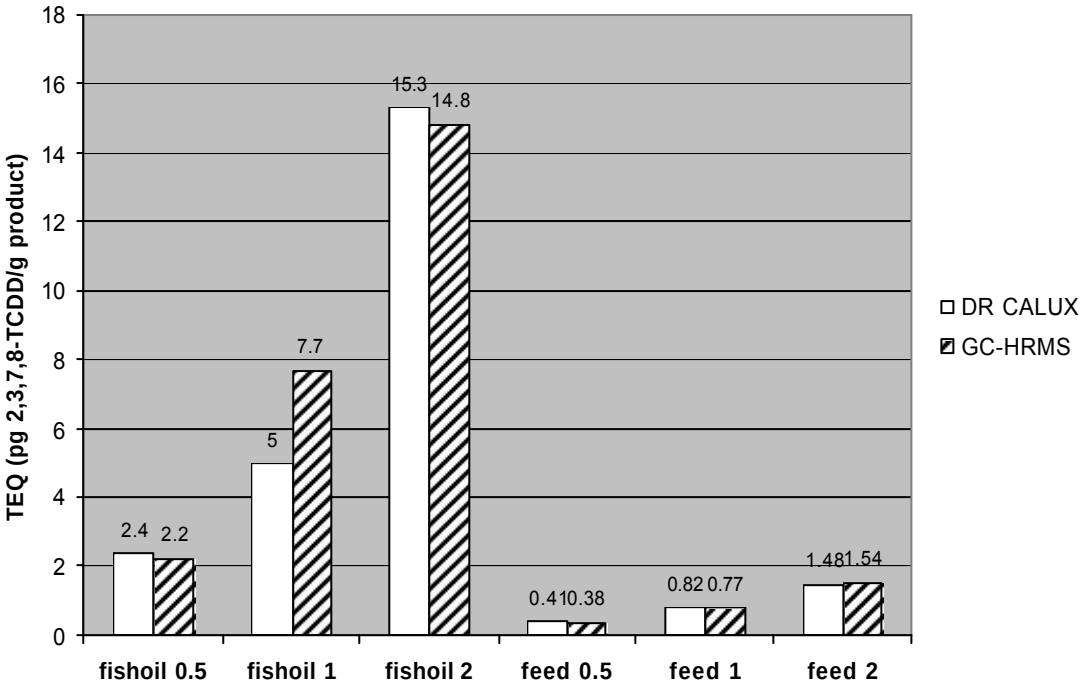

Figure 1 Comparison between DR CALUX® analysis results and GC-HRMS analysis results of fishoil reference samples and feed reference samples prepared for the DR CALUX® interlaboratory comparison study.

Table 1 Summarised DR CALUX® analysis results for phase I and phase II

	PCDD/F mix			PCDD/F/PCB mix		Sediment extract		Feed/food extract		
	Participants result (undiluted)	3*dilution	10*dilution	Participants result (undiluted)	10*dilution	Participants result (undiluted)	10*dilution	Participants result (undiluted)	3*dilution	10*dilution
Avg (nM)	1.05	0.31	0.087	1.72	0.15	0.21	0.076	0.50	0.16	0.06
SD (nM)	0.28	0.06	0.027	0.42	0.05	0.05	0.030	0.16	0.05	0.03
RSD(%)	26.9	18.9	30.6	24.2	35.3	23.5	39.5	31.2	30.6	49.6
Median (nM)	1.03	0.31	0.094	1.72	0.16	0.22	0.068	0.50	0.14	0.04
x^* (nM)	1.02	0.31	0.088	1.69	0.15	0.21	0.072	0.50	0.15	0.05
s^* (nM)	0.20	0.07	0.028	0.33	0.05	0.06	0.027	0.18	0.03	0.03

NOTE: Results marked in grey had an average result below 1 pM/well (LOQ of the DR CALUX®)

Table 2 Summarised DR CALUX® analysis results for phase III

	Fishoil 0.5	Fishoil 1	Fishoil 2	Feed 0.5	Feed 1	Feed 2
Avg (ng TEQ/kg product)	3.78	8.81	15.98	0.802	0.939	1.407
SD (ng TEQ/kg product)	2.19	5.69	4.78	0.64	0.55	0.689
RSD(%)	58.1	64.6	29.9	79.8	58.6	49.0
Median (ng TEQ/kg product)	4.26	8.21	15.66	0.566	0.852	1.147
x^* (ng TEQ/kg product)	3.65	8.81	16.2	0.679	0.902	1.333
s^* (ng TEQ/kg product)	2.14	3.93	4.73	0.497	0.532	0.633

Table 3 The participant z-scores for all samples analysed

Participant code	PCDD/F			PCDD/F/PCB		Sediment extract		Feed/food extract			Fishoil			Feed		
	Participants result (undiluted)	3*dilution	10*dilution	Participants result (undiluted)	10*dilution	Participants result (undiluted)	10*dilution	Participants result (undiluted)	3*dilution	10*dilution	Fishoil 0.5	Fishoil 1	Fishoil 2	Feed 0.5	Feed 1	Feed 2
A	-0.9	0.3	-0.2	0.1	0.5	-1.4	-1.0	-1.0	-0.8	-1.1	-0.5	-1.0	0.2	-0.7	-0.5	-0.3
B	---	---	---	---	---	---	---	---	---	---	---	---	---	-0.9	-0.9	---
C	1.9	1.0	1.9	-0.3	0.3	1.2	2.3	1.6	3.5	1.9	-1.0	-1.0	1.0	-0.4	0.5	0.8
D	-0.4	-1.5	-1.1	-0.5	-0.8	-0.8	-0.1	-0.9	-0.4	-0.6	-1.2	-1.8	-0.5	-1.1	-1.2	-1.3
E	0.1	-1.1	-1.4	-0.7	-1.0	0.0	1.0	0.8	-0.7	1.5	0.2	0.4	0.4	1.8	-0.5	-0.6
G	0.3	0.0	0.4	0.3	0.3	-0.7	-0.6	-0.5	-0.5	-0.8	2.5	2.2	1.3	0.4	1.3	2.5
i	-0.6	0.4	-0.6	-0.9	-1.5	---	---	-1.2	-2.9	---	---	---	---	---	-1.0	-0.7
J	0.0	-0.1	0.3	-1.1	-0.7	0.9	0.8	1.2	2.2	1.3	-0.4	-0.8	-0.1	1.3	1.3	1.0
K	3.3	1.2	0.9	1.2	0.9	1.5	-0.7	0.9	1.2	0.2	0.5	3.4	-0.5	1.3	0.7	1.3
L	2.4	0.8	0.7	0.6	0.5	0.6	0.4	0.9	2.2	1.0	---	---	---	---	---	---
M	-0.5	0.2	0.2	0.7	0.2	0.1	-0.1	-0.5	-0.7	-0.4	0.4	1.0	0.7	3.0	2.6	0.9
O	-0.5	-0.2	-0.5	0.2	0.8	0.6	-0.4	-0.2	-0.4	-0.8	0.5	0.2	-0.1	-0.3	0.0	-0.3
Q	1.2	1.3	0.9	2.7	1.7	0.4	1.2	0.3	1.6	0.9	0.9	0.7	1.4	-0.2	0.3	1.6
R	0.3	-0.1	0.3	0.1	0.3	0.1	-0.3	0.0	0.2	-0.4	-0.7	-1.1	-2.2	-0.3	-0.2	-0.7
S	-1.9	-0.9	-1.2	-1.5	-1.0	-0.8	-1.0	0.4	1.3	-0.4	---	-0.9	-1.3	2.3	0.3	-0.7
T	-0.9	0.5	0.7	2.7	1.2	0.6	2.2	0.2	1.2	1.3	-1.1	-1.1	-0.9	-0.8	-0.9	-0.3
U	-2.2	-1.7	-1.8	-2.0	-2.0	-1.5	-1.5	-0.9	-0.8	-0.7	---	---	---	-1.1	-1.1	-1.1
W	0.2	0.0	0.2	-0.2	0.3	-0.6	-0.2	-1.2	-1.0	-1.2	0.7	-0.2	-0.1	-0.2	0.5	-0.2

Phase III. Phase III consisted of the DR CALUX® analysis of whole fishoil and feed samples to be extracted and cleaned-up by the participants according to the supplied protocols. Three fishoil samples were prepared at 0.5, 1 and 2 times the maximum EU limit value for fishoil (6 ng TEQ/kg product) by selecting naturally contaminated fishoil at the indicated TEQ concentrations. Three feed samples were prepared at 0.5, 1 and 2 times the maximum EU limit value for feed materials (0.75 ng TEQ/kg product) by enrichment with contaminated fishoil and further dilution with low contaminated feed. The fishoil and feed materials are intended to be available as DR CALUX® reference materials. Prior to sending the samples to the participants, the TEQ content of the prepared fishoil and feed samples was determined by

DR CALUX® bioassay (BDS) and GC-HRMS analysis (Eurofins¦ GfA, Münster, Germany). The participants were asked to extract, clean-up and determine the DR CALUX® response in the fishoil and feed samples according to the supplied protocols.

Data handling The participants were asked to deliver the results in the calculation files provided by the organizer. Only results that met the performance criteria of the DR CALUX® bioassay were taken into account (maximum induction = 6; RSD triplicate analysis = 15%; R^2 of the fit = 0.98; reported analysis results > 1 pM TEQ/well). In case only 1 measurement of the requested three-fold was reported or taken into account, this analysis result was not included in the calculations (phase I and II only). For all samples, the sample average, the standard deviation of the sample standard, the relative standard deviation of the sample standard, the mean, the robust average (x^*) and the robust standard deviation (s^*) were calculated. All data calculation and evaluation was performed according to ISO 13528:2005(E)[5].

Results and Discussion

In figure 1, the DR CALUX® analysis results and GC-HRMS analysis results of the prepared fishoil and feed samples are given. Both analyses were performed prior to sending the samples to the participants. From the participating 21 laboratories, 3 participants did not send in their analysis results or their analysis results were incorrect.
In table 1, a summary of the DR CALUX® analysis results obtained for phase I and phase II are given. The participants were asked to prepare and analyse dilution series for the stock solutions of chemical standards (phase I) and samples extracts (phase II). Following evaluation of their analysis results, the total DR CALUX® TEQ (nM 2,3,7,8-TCDD) in the stock solution was calculated. The participants also received chemical standards and extracts already diluted. In table 1, the analysis results for the diluted samples are given. The relative standard deviation for the phase I and phase II samples varied from 18.9% until 35.3% (analysis results > LOQ only). Samples on average below the LOQ of 1 pM/well showed slightly elevated %RSD, ranging from 30.6% until 49.6%.
In table 2, a summary of the DR CALUX® analysis results obtained for phase III are given. The participants were asked to extract, clean-up and determine the total TEQ content by DR CALUX® analysis. Following evaluation of the analysis results, the total DR CALUX® TEQ (ng 2,3,7,8-TCDD/kg product) was calculated. The relative standard deviation for phase III samples varied from 29.9% until 79.8%.
In table 3, the z-scores for all participants and all samples tested are given. A small percentage of the participating laboratories had z-scores above 2Z (7%). Two participating laboratories encountered structural problems (induction < 6; EC50 > 18 pM).

Acknowledgements

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References

[1] Commission Directive 2003/57/EC, amending Commission Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed
[2] Council Regulation (EC) No 2375/2001 of 29 November 2001, amending Commission Regulation (EC) No 466/2001 setting maximum levels for certain contaminats in foodstuffs
[3] Commission Directive 2002/69/EC of 26 July 2002. Laying down the sampling methods and methods of analysis for the official control of dioxins and the determination of dioxin-like PCBs in foodstuffs
[4] Commission Directive 2002/70/EC of 26 July 2002. Establishing requirements for the determination of levels of dioxins and dioxin-like PCBs in feedingstuffs
[5] Statistical methods for use in proficiency testing by interlaboratory comparison. ISO 13528:2005(E).