

RESULTS OF THE INTERNATIONAL INTERLABORATORY COMPARISON STUDY FOR MEAT SAMPLES USING DR CALUX by BDS (BICS 2011).

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Introduction

The protection of safety of human consumers is a high priority issue for the food and feed sector. For dioxins (PCDDs), furans (PCDFs) and dioxin-like PCBs (dl-PCBs), the European Commission has introduced new stringent limit values in food- and feedingstuffs^{1,2} for animal and public health protection. To permit bioassays such as the Dioxine Responsive (DR) CALUX, to be used for screening, the EU has laid down general requirements for the determination of PCDDs, PCDFs and dl-PCBs in food- and feedingstuffs and specific requirements for cell-based bioassays^{3,4} in which frequent participations in international intercalibrations testing is mandatory. To ensure the reliability and performance of the DR CALUX bioassay by BDS for monitoring food and feedingstuffs, interlaboratory comparison studies (ringtest) are mandatory.

In the present paper, the results of the international interlaboratory comparison study, using DR CALUX by BDS (BICS 2011) are described. A total of 12 laboratories worldwide using the DR CALUX bioassay in house participated in the BICS-2011 study. The inter-laboratory calibration study consisted of 3 meat samples supplied by BDS.

Methods and materials

The EC maximal level for pork meat is 1.5 pg PCDD/PCDF/dl-PCB TEQ per gram fat (according to COMMISSION REGULATION (EC) No 1881/2006). For confirmatory analytical methods, a sample is considered positive in case the PCDD/PCDF/dl-PCB levels are higher than 1.73 pg PCDD/PCDF/dl-PCB TEQ per gram fat (EC maximal level plus 15%). Three pork meat samples were prepared at 3 different PCDD/PCDF/dl-PCB levels which were sent for HRGC/HRMS analysis (Eurofins | GfA, Münster, Germany). The total TEQ content in the 3 pork meat samples showed to be for one sample (A) well below the level of interest (maximum EU level for PCDDs/PCDFs/dl-PCBs in pork meat), for another sample (B) just below the level of interest and for the third sample (C) well above the level of interest (see table 1) For calculating the TEQ value of the chemical HRGC/HRMS analysis the TEF (1998) have been used. Therefore, meat sample A and B can be considered as negative whereas meat sample C can be considered as positive.

A total of 12 laboratories located world-wide participated in the BICS-2011 study. Ten of the laboratories invited are using the DR CALUX and two are using CALUX bioassay in house for screening purposes of food and feed materials. Some laboratories are using the DR CALUX already for several years whereas others just implemented the DR CALUX bioassay. The participating laboratories received the 3 pork meat samples to be analyzed using the appropriate protocols for extraction, clean-up and analysis offered to them during training. The participants were asked to deliver the results in the calculation files provided by the organizer for evaluation of results. Only results that met the performance criteria of the CALUX bioassay were taken into account (maximum induction ≥ 6 ; RSD triplicate analysis $\leq 15\%$; R^2 of the fit ≥ 0.98 ; reported analysis results > 1 pM TEQ/well).

For evaluation of results, the international standard 13528:2005 (Statistical methods for use in proficiency testing by inter-laboratory comparison)⁵⁾ was used. For all samples the robust average (indicated as \bar{x}^*) and robust standard deviation (indicated as s^*) were calculated by the algorithm A as mentioned in 13528:2005. These were used to calculate the z-scores of the laboratories. Furthermore the average, the standard deviation, median and the relative standard deviation were calculated and presented for general purposes. Finally, the analysis results received from participants were classified as negative or suspected. A sample was considered negative in case the DR CALUX analysis result was below the cut-off level for bioassays. The cut-off level for DR CALUX analysis is 1.13 PCDD/PCDF/dl-PCB TEQ per gram fat (EC maximal level minus 25%). The statistical study evaluation was done by a third independent party, Institute for Interlaboratory studies, SGS Netherlands (ISS).

Table 1 PCDD, PCDF and PCB congener pattern in meat samples A, B and C as determined by HRGCMS

Congener	Meat A (pg/g fat)	Meat B (pg/g fat)	Meat C (pg/g fat)
2,3,7,8-TCDD	<0.1	<0.1	<0.1
1,2,3,7,8-PeCDD	<0.1	<0.1	<0.1
1,2,3,4,7,8-HxCDD	<0.2	<0.2	<0.2
1,2,3,6,7,8-HxCDD	<0.2	<0.2	<0.2
1,2,3,7,8,9-HxCDD	<0.2	<0.2	<0.2
1,2,3,4,6,7,8-HpCDD	<0.3	<0.3	<0.3
OCDD	1.3	1.0	1.1
2,3,7,8-TCDF	<0.1	<0.1	0.79
1,2,3,7,8-PeCDF	<0.1	<0.1	0.44
2,3,4,7,8-PeCDF	<0.1	1.3	21
1,2,3,4,7,8-HxCDF	<0.2	0.5	9.7
1,2,3,6,7,8-HxCDF	<0.2	<0.2	3.7
1,2,3,7,8,9-HxCDF	<0.2	<0.2	<0.2
2,3,4,6,7,8-HxCDF	<0.2	<0.2	2.1
1,2,3,4,6,7,8-HpCDF	<0.3	<0.3	1.4
1,2,3,4,7,8,9-HpCDF	<0.3	<0.3	<0.3
OCDF	<0.5	<0.5	<0.5
PCB 77	<10	<10	<10
PCB 81	<1	<1	<1
PCB 126	<1	<1	1.2
PCB 169	<1	<1	1.2
PCB 123	<10	<10	11.4
PCB 105	<100	<100	<100
PCB 118	<200	<200	258
PCB 114	<10	<10	<10
PCB 167	<10	<10	68.2
PCB 156	<20	39.1	1030
PCB 157	<10	<10	127
PCB 189	<10	10	10

Table 2 Summarised DR CALUX[®] analysis results in pg TEQ/g fat of the BICS 2011 study.

	Meat A	Meat B	Meat c
Study assigned parameters for calculation			
x^*	0.53	1.2	13
s^*	0.31	0.62	6.4
Additional parameters			
Average***	0.59	1.2	13
Stdev	0.42	0.56	6.3
RSD(%)	71	48	48
Median***	0.46	1.2	14
HRGCMS (WHO-1998) pgTEQ/g fat	0.67	1.3	13

Table 3 Classification of analysis result. A sample is classified negative in case the analysis result is more than 25% below the maximum EU limit for PCDDs/PCDFs/dl-PCBs in pork meat.

	laboratory	Meat A	Meat B	Meat C
LAB	A	negative	negative	suspect
	B	negative	negative	suspect
	C	negative	negative	suspect
	D	negative	suspect	suspect
	E	negative	negative	suspect
	F	negative	negative	suspect
	G	negative	suspect	suspect
	H	suspect	suspect	suspect
	i	negative	suspect	suspect
	J	negative	suspect	suspect
	K	negative	n.a.	suspect
	L	negative	n.a.	suspect
	HRGCMS (WHO-1998)	negative	negative	positive

n.a. : result not reported by respective laboratory

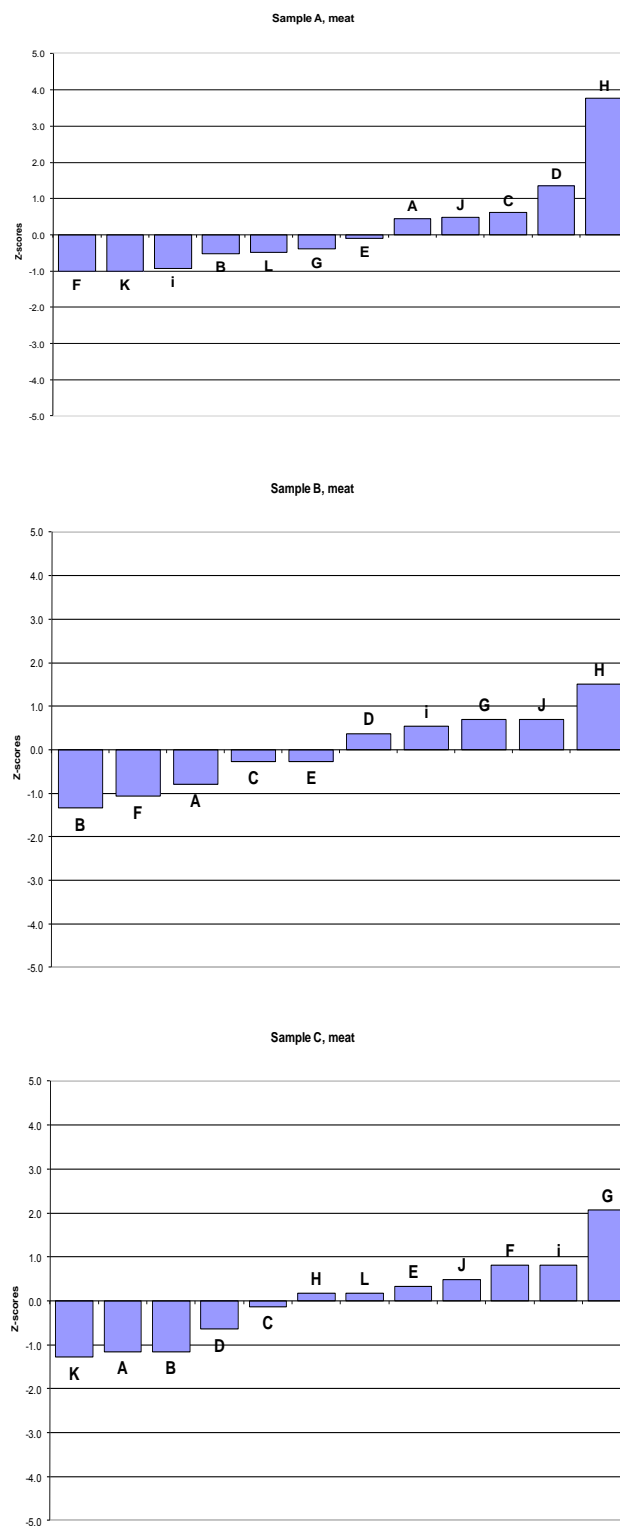


Figure 1. Graphical representation participants z-scores

Results and Discussion

In table 1, the congener patterns as determined by HRGC/HRMS of the 3 prepared meat samples are given. These analyses were performed prior to sending the samples to the participants. In table 2, the PCDD/F/dl-PCB TEQ content as determined by HRGC/HRMS, is given. The TEQ content was calculated to be 0.67, 1.3 and 13 pg TEQ/g fat for meat sample A, B and C respectively (using TEF 1998 values).

In table 2, a summary of the CALUX analysis results obtained for the meat samples are given. The participants were asked to extract, clean-up and determine the total TEQ content by CALUX analysis. Most of the laboratories were following the basic principles of the EC/1883/2006 guideline and the performance criteria's for cell based screening methods. From the participating laboratories, 2 participants did not send in their analysis results for meat sample B. Following evaluation of the analysis results, the total CALUX TEQ (pg TEQ/g fat) was calculated. The relative standard deviation for meat samples varied from 71% in the meat samples with low TEQ content to 48% in the medium with high TEQ meat samples. In figure 1, the z-scores for all participants and all samples tested are given. Thirty four z-scores were calculated of which 2 were above a score of [2Z] (representing 3% of the results).

In table 3, sample CALUX analysis results are classified as negative or suspect. Following evaluation of the analysis results, it can be concluded that no false negative results were reported. For sample A, one laboratory reported elevated levels of PCDDs/PCDFs/dl-PCBs thus reporting a false-positive analysis result. For meat sample B, which was just below the cut off level for confirmative methods, five out of ten laboratories reported a result above the cut off level for the CALUX screening method thereby classifying the sample as suspected. Although very close to the maximum EU limit value for pork meat, the sample could be classified negative as determined by one HRGC/HRMS analysis result. All participants classified sample C as suspected. The HRGC/HRMS analysis result shows that this sample indeed has high levels of PCDDs/PCDFs/dl-PCBs and therefore is a positive sample according to the EU directives.

Acknowledgements

The organisers of the DR CALUX interlaboratory comparison study like to thank all participants for their collaboration and participation.

References

- ¹ COMMISSION DIRECTIVE 2006/13/EC, of 3 February 2006 amending Annexes I and II to Directive 2002/32/EC of the European Parliament and of the Council on undesirable substances in animal feed as regards dioxins and dioxin-like PCBs
- ² COMMISSION REGULATION (EC) No 1881/2006 of 19 December 2006, setting maximum levels for certain contaminants in foodstuffs
- ³ COMMISSION REGULATION (EU) No 252/2012 of 21 March 2012 laying down methods of sampling and analysis for the official control of levels of dioxins, dioxin-like PCBs and non-dioxin-like PCBs in certain foodstuffs and repealing Regulation (EC) No 1883/2006.
- ⁴ COMMISSION REGULATION (EU) No 278/2012 of 28 March 2012 amending Regulation (EC) No 152/2009 as regards the determination of the levels of dioxins and polychlorinated biphenyls
- ⁵ Statistical methods for use in proficiency testing by interlaboratory comparison. ISO 13528:2005(E).