GERMAN DIOXIN CRISIS 2011 – EXPRESS ANALYSIS OF PCB- AND PCDD/F-TEQ IN FOOD AND FEED MATRICES BY THE HTPS DR CALUX® METHOD

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Introduction

Cell-based screening technologies such as the DR CALUX® technology are recommended e.g. by the European guideline EC/1883/2006 ("should be used") for analyzing total-PCDD/PCDF/dl-PCB-TEQ as well PCDD/PCDF-TEQ in feed and food. This type of rapid analysis methods have shown their usefulness and reliability in the past two decades in several dioxin crisis situations with short turn-around time, high weekly capacity and economic advantages compared to the chemical analysis[1-5]. Within the last two decades the DR CALUX® method has been involved almost yearly in several kinds of global feed and food crisis situations, as well as in routine National Monitoring Programs. In the present paper we present data from the recent German dioxin/PCB crisis at the start of 2011, where we analysed more than five hundred (500) pig meat, two hundred (200) chicken meat and other food/feed samples from Germany, using our high-through-put-out (HTPS) high capacity PCDD/F- and dl-PCB-TEQ cell-based screening method called DR CALUX®.

More than 97% of the here reported samples have been compliant according to the cut-off level (25% minus the EC EC/1881/2006 regulated level for the relevant matrices) for cell based screening technologies. In case of the analysed meat samples, more than 99,8% of the samples have been lower than the here relevant cut-of level of 25% of the EC regulation for the here relevant PCDD/F-TEQ for cell based screening technologies regarding the by EC/1881/2006 regulated levels. All feed samples have been below 50% of the regulated EC levels.

Cost savings between our internal DR CALUX analysis and our external offered HRGC/HRMS testing (via our ISO 17025 accredited partner institutes) have been around 70% (even when the 2-3 % of samples needed for confirmation analysis are included) and time savings have been up to ten (10) days compared to typical market turn-around-times due to our new automated cell culture/dosing robot, solvent evaporation and automated injection systems for the HTPS DR CALUX® method.

Methods and materials

Food and feed samples: All samples were analyzed according to the standard procedures of the DR CALUX® method from BDS[3,4].

DR CALUX® bioanalysis: The procedure for the BDS DR CALUX® bioassay is described in detail previously[3,4]. Briefly, H4IIE cells stably transfected with an AhR-controlled luciferase reporter gene construct, were cultured in α-MEM culture medium supplemented with 10% ($^{v}/_{v}$) FCS under standard conditions (37°C, 5% CO_2, 100% humidity). Cells were exposed in triplicate on 96-well microtiterplates containing the standard 2,3,7,8-TCDD calibration range, a DMSO blank, an internal reference material and various samples extracts at multiple dilutions (e.g. foodstuffs, feeding stuffs). The procedure of automated cell pipetting and dosing on 96 well plates by Mikrolab Star^{Let} robot (Hamilton) have been evaluated. Following a 24 hour incubation period, cells were lysed. A luciferine containing solution (Glow Mix) was added and the luminescence was measured using a luminometer (Berthold Centro XS3 or Mithras).

Results and Discussion

From Germany more than twenty meat exporters, ten chicken exporters and several feed producing companies send several weeks frequently samples to BDS for our express ISO 17025 accredited service analysis with usual turn around times (TAT) between 48 hrs and 120 hrs. At that time, the typical TAT from laboratories involved in this crisis reported from clients has been between 10 and 20 days. Sample reports have been sent direct to customers or in some cases direct to the local German governmental veterinary inspection services according to their local and Russian authority requirements.

In this time period more than five hundred (500) samples of pig meat, more than two hundred (200) chicken meat, more than forty (40) pig liver and several egg samples have been analysed. For more than 90% of the samples analysed by BDS, the PCB-TEQ and PCDD/F-TEQ have been reported separately by using a modified protocol of the DIN 2464 method based on the prior work of Behnisch (1997)[7].

In case of feeding materials (15 samples) and premixes (13 samples) tested by DR CALUX, all samples have been complaint with levels below 50% of the in the EC/152/2009 regulated levels (not presented here in table 1).

Table 1 PCDD/F-, dl-PCB- and total-TEQ results from pig meat, pig liver, eggs and chicken meat samples from Germany tested by DR CALUX technology (concentrations are in pg TEQ/g fat).

				Sample compliant	Sample compliant	Sample compliant	Sample compliant
	PCB-TEQ	PCDD/F-TEQ	Total-TEQ	PCDD/F-25% below	Total-25% below	PCDD/F-50% below	Total-50% below
1) Pig Meat: N	496	496	502	99,8%	97,6%	94%	89%
1) Pig Meat: Mean	0,23	0,21	0,43				
1) Pig Meat: Range	0,1-1,1	0,1-0,77	0,1-1,47				
2) Pig Liver: N	32	37	34	99%	97,3%	93,2%	90%
2) Pig Liver: Mean	0,80	0,78	1,57				
2) Pig Liver: Range	0,1-2,1	0,1-3,4	0,1-5,2				
3) Eggs: N	9	9	36	100%	100%	44%	100%
3) Eggs: Mean	0,67	1,21	1,48				
3) Eggs: Range	0,1-1,0	0,1-2,3	0,1-2,6				
4) Chicken: N	201	201	207	99,5%	98%	93%	88%
4) Chicken: Mean	0,74	0,56	1,29				
4) Chicken: Range	0,3-3,3	0,3-1,7	0,6-3,8				

As presented in table 1, the pig meat samples have a mean value of 0,23 pg PCB-, 0,21 pg PCDD/F and 0,43 PCDD/F/dl-PCB-TEQ/g fat and therefore have been far below the regulated EC levels (1.0 pg for PCDD/F-TEQ and 1.5 pg for PCDD/F/dl-PCB-TEQ/g fat). The range of the PCB-, PCDD/F-TEQ- and mostly calculated Total-TEQ results are also as expected. In case of the pig liver samples, the analysed results have been as expected higher with

mean values of 0,80 pg PCB-, 0,78 pg PCDD/F- and 1,57 pg PCDD/F/dl-PCB-TEQ/g fat, but still far below the regulated EC levels (4 pg for PCDD/F-TEQ/g fat and 12 pg for PCDD/F/dl-PCB-TEQ/g fat). The range of the results for the PCB-, PCDD/F-TEQ- and mostly calculated Total-TEQ are also as expected.
The eggs samples have been also as expected higher due to the accidental here sometimes occurred contamination by German food fat and used feed (table 1). In case of the chicken meat samples the analysed results have been as expected with mean values of 0,74 pg PCB-, 0,56 pg PCDD/F and 1,29 PCDD/F/dl-PCB-TEQ/g fat, but still below the regulated EC levels (3 for PCDD/F-TEQ and 6 for PCDD/F/dl-PCB-TEQ).
More than 97% of the here reported samples have been compliant according to the cut-off level (25% minus the EC EC/1881/2006 regulated level for the relevant matrices) for cell based screening technologies. In case of the analysed meat samples, more than 99,8% of the samples have been lower than the here relevant cut-of level of 25% of the EC regulation for the here relevant PCDD/F-TEQ for cell based screening technologies regarding the by EC/1881/2006 regulated levels. In case of the analysed meat samples more than 88% of the samples have been even lower than 50% of the EC regulation for cell based screening technologies regarding the by EC/1881/2006 regulated levels.
Please remember that basis of the EC guided level is the 95% percentile and therefore the number of non-compliant samples are in accordance to the expectations.

Figure 1 Distribution of the PCDD/F-TEQ by DR CALUX from more than five hundred (500) German pig meat samples analysed by BDS DR CALUX technology during the first weeks of 2011. (concentrations are in pg PCDD/F-TEQ/g fat).

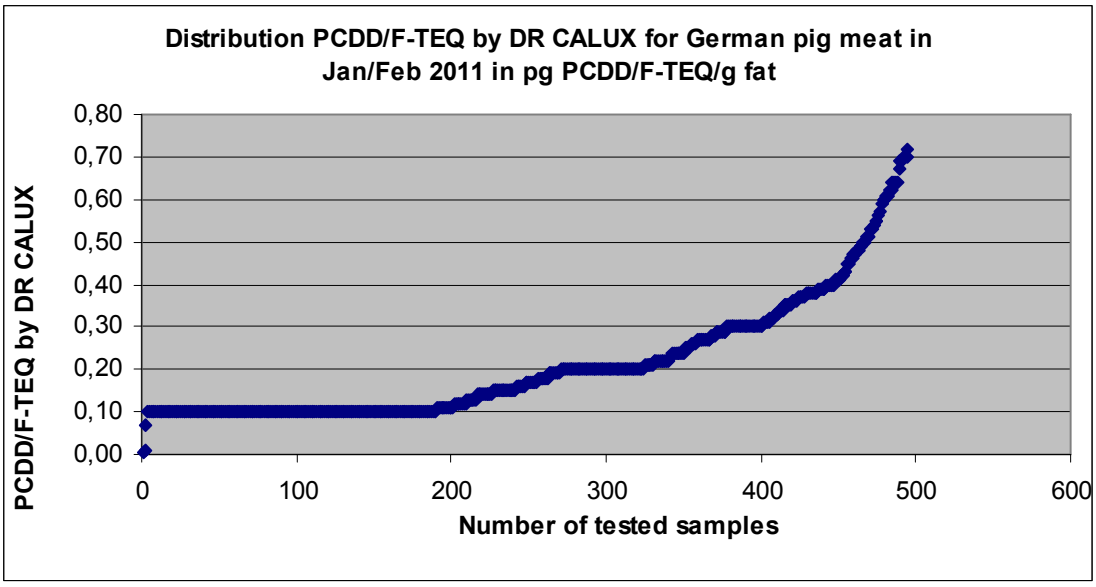
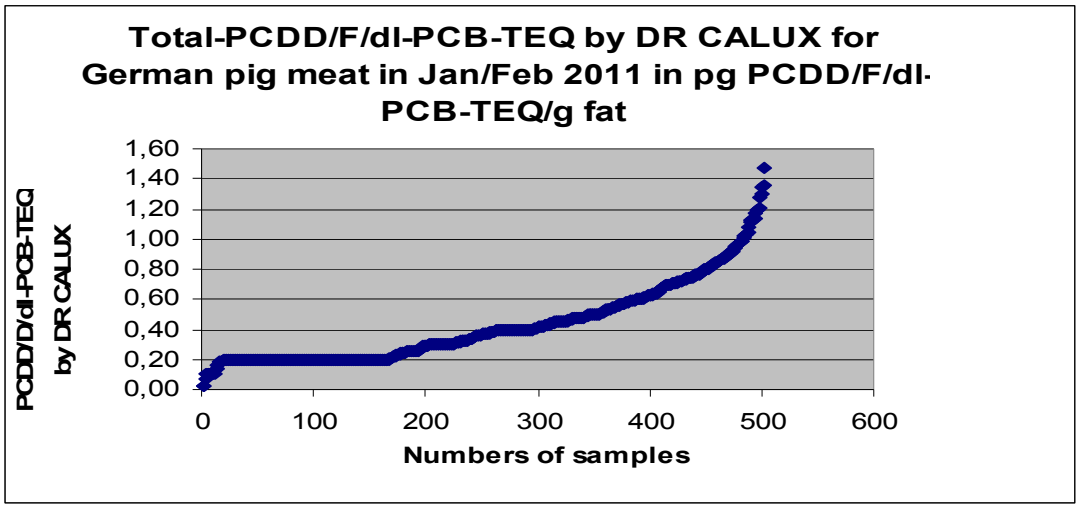


Figure 2: Distribution of the sum of the PCB-TEQ and PCDD/F-TEQ by DR CALUX from more than 500 pig meat samples in the first weeks of 2011. More than 90% of the samples have been reported as separate PCB-TEQ and separate PCDD/F-TEQ (concentrations are in pg PCDD/F/dl-PCB-TEQ TEQ/g fat).



This study shows similar distribution of results as reported earlier at previous DIOXIN conferences from the National Monitoring Program for food and feed from the Slovak Republic[3].
In the present study we report several food/feed sample analysis in a crisis situation in Germany. Separate PCB-TEQ, PCDD/F-TEQ and total TEQ levels analyzed by DR CALUX® technology of various feed and food samples showed that more than 97% of samples were below EC/1883 cut-of level. All feed samples have been below 50% of the regulated EC levels. The results of this study shows again that the DR CALUX® bioassay for screening of PCDD/Fs and dioxin-like PCBs in feed and food is an important device to identify the few percentage of the EU limit exceeding samples among the bulk of the compliant samples

References

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