

Behnisch Peter¹, Gras Nuri², Guerrero Pedro³, Besselink Harrie¹ and Brouwer Bram¹

¹BioDetection Systems BV (BDS), Science Park 406, 1098 XH Amsterdam, The Netherlands

²Labser, Camino Vecinal 950, Ruta H30, Casilla 251, Rancagua, Chile

³Pork Producers Trade Association of Chile, Isidora Goyenechea 2939 Ofic. 701, Las Condes, Santiago, Chile

Introduction

In the last few years animal feed and feed additives were the major sources of dioxin/dl-PCB contamination in the food chain and have caused severe crisis situation in the food chain¹. In such situations cell-based screening technologies have been often used for analyzing the total-PCDD/PCDF/dl-PCB-TEQ¹⁻⁴. In this paper we present the first results of a surveillance monitoring program of several food (milk, bovine, ovine) and feed items (minerals, fish oil, animal fat oil, vegetable oil fat) from Chile analysed by DR CALUX® technology.

Methods and materials

Food and feed samples: All samples were collected under supervision of Labser and shipped under frozen conditions from Chile to Amsterdam, The Netherlands.

DR CALUX® bioanalysis: The procedure for the DR CALUX® bioassay has been described in detail previously³. Briefly, H4IIE cells stably transfected with an AhR-controlled luciferase reporter gene construct, were cultured in MEM culture medium supplemented with 10% (v/v) FCS under standard conditions (37°C, 5% CO₂, 100% humidity). Cells were exposed in triplicate on 96-well micro titerplates 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). Following a 24 hour incubation period, cells were lysed. A luciferine containing solution was added and the luminescence was measured using a luminometer (Berthold Centro XS3).

Results and discussion

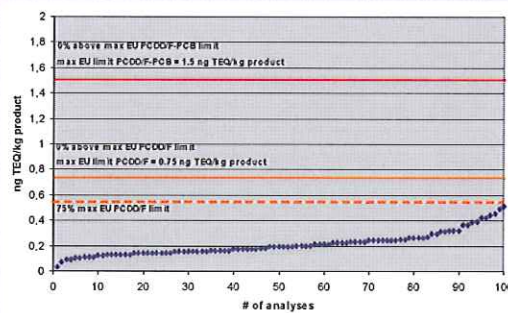
The last dioxins crisis case reported in Chile occurred in the year 2008 due to minerals used as animal feed⁵. This has led to an intensive monitoring program in 2009 for all kinds of food/feed items in Chile (Graph 1 and 2). Most of the food samples (such as milk, bovine, ovine) have been below the EC regulated levels for PCDD/PCDF-TEQ analysed by DR CALUX® bioassay (data not shown). In case of monitored fish oil, animal oil fat, and vegetable oil fat no significant dioxin/dl-PCB levels occurred (data not shown). The data shown in the Graph 1 presents the results of more than 100 different feed items. All samples have been below the applied screening level of 0.56 ng TEQ/kg product (EC/13/2006 guideline⁶ of 0.75 ng PCDD/F-TEQ/kg product minus 25% for the applied screening test from the EC/152/2009 guideline⁷).

It was reported in several countries that mineral premixes such as zinc oxide could be a source of dioxin contamination⁵. Contaminated zinc oxide from a smelting process was the primary source of dioxin⁵. Zinc oxide is used as a mineral supplement in feed. Therefore, the Chilean government carried out a surveillance program for dioxins and dioxin like PCBs by DR CALUX® in order to guarantee food safety. Our results in Graph 2 confirm the findings that zinc oxide and mineral clay are the main mineral ingredients which can cause higher Total-TEQ levels. From 92 tested minerals 5 (5.4%) have been higher than the EU limit for PCDD/PCDF-DL-PCB-TEQ of 1.5 ng/kg and 7 (7.6%) samples have been higher than the EU limit for PCDD/PCDF-TEQ of 0.75 ng/kg (Graph 2).

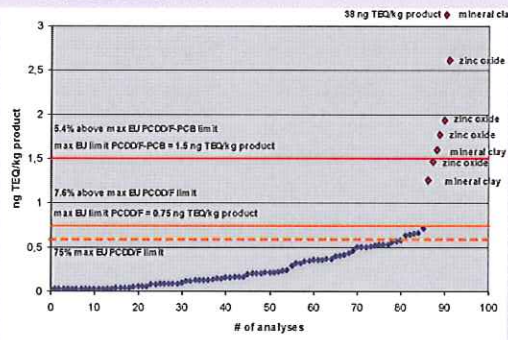
References

- Behnisch, PA. (2005) In Rapid Methods for biological and chemical contaminants in food and feed. Wageningen Academic Publishers. pp. 303-318.
- Behnisch, PA. (2005). Food. Issue 1, Spring 2005 pp. 13-17.
- Besselink H, Jonas A, Pijnappels M, Swinkels A, Brouwer B. (2004). Organohalogen Compounds; 66:677-681.
- Behnisch PA, Besselink H, Brouwer B (2009); Organohalogen Compounds; 71: 949-952
- Kim MK, Choi SW, Park JY, Kim DG, Bong YH, Jang JH, Song SO, Chung GS, Guerrero P (2009); Organohalogen Compounds; 71: 179-182
- Official Journal of the European Union. Commission Directive 2006/13 EC from 3 February 2006
- Official Journal of the European Union. Commission Directive 2009/152 EC from 27 January 2009

Graph 1 Distribution of Total TEQ data analysed by DR CALUX® from several feed items from Chile (n=100)



Graph 2 Distribution of Total PCDD/PCDF/dl-PCB-TEQ data analysed by DR CALUX® from several mineral items from Chile



In the present study we report the Total-TEQ analyzed by DR CALUX® technology of several food/feed items of a national surveillance program in Chile. It cannot be excluded, that some of these food and feed samples never were used as feeding stuff or food, due to the fact that they exceeded the limit values or the internal levels set by the client. Also in case of the feed samples, most of the here selected samples have been sorted out by the clients as critical samples. Thus they are not necessarily representative for the feeding stuff market. This explains the rather high percentage of exceeded EU limits for several kinds of minerals used as animal feed. Higher levels can arise due to local dioxin sources of mineral origin. Apart from these exceptional samples shown, the results presented here, are representative for the majority of background samples analyzed here in 2009 in Chile by DR CALUX® technology.

Conclusions

The results of this study show that the DR CALUX® bioassay for screening of dioxin and dioxin-like PCBs in feed and food is an efficient tool to separate the bulk of unpolluted samples from the few percentage of the regulated limit exceeding samples. The here reported case of a national surveillance monitoring of feed/food shows that screening of more samples also increases the chance to find the dioxin/PCB source and to prevent further damage to the food chain and the export of food and feed items.