LEVELS OF TOTAL TEQ IN VARIOUS FOOD, FEED AND SEDIMENTS MATRICES AS DETERMINED BY DR CALUX® BY BDS

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Introduction

New stringent EU limit values for dioxins and for the first time also for dioxin-like PCBs in food and feedingstuffs are in force end of the year 2006 for public health protection[1, 2]. In the last few years faster procedures have been established and evaluated to speed up the Total TEQ analysis including therefore dioxin and dioxin-like PCB analysis[3]. These screenings methods are cheaper and offer higher measurement capacities which is important in case of dioxin crisis situations[3]. Especially, the CALUX technologies have shown in the past few years in several dioxin crisis situations[3] their usefulness. To permit bioassays to be used for screening foodstuffs for the presence of dioxins and related compounds, the EU has laid down general requirements for the determination of dioxins and dioxin-like PCBs in foodstuffs and specific requirements for cell-based bioassays[4]. To ensure reliability the DR CALUX® bioassay has several validated methods for extraction, clean-up and measurement all kinds of sample matrices[5, 6]. Several round robin studies have been performed to evaluate the DR CALUX technology for sediments, feed and food[7, 8]. Also guidelines for Total TEQ in sediments are at the moment discussed in the Netherlands (50 ng TEQ/kg d.w.) and in Norway (25 ng TEQ/kg d.w.)[9, 10].
Within the last decade the service laboratory of BioDetection Systems has analyzed all kinds of feed, food and sediments by DR CALUX® method for Total TEQ. In this paper we present the results of around 490 food samples, 100 feed samples and 634 sediment samples. In the cases of food samples only 6.5% of the samples were found to exceed the EU guideline for only dioxins, while in case of feed samples 17% exceeded these EU limits. For sediment samples our evaluation study shows that 11% of the here tested samples would exceed the Dutch guidelines.

Methods and materials

Food, feed and sediment samples All samples analyzed were received in the period from the end 2004 to early 2006. In total we report here median results of 490 food samples, 100 feed samples and 634 sediment samples. The results shown in Figure 1, 3 and 5 describes in times weather or not the samples match to the corresponding EU limit for the specific food or feed sample matrix. Figures 2, 4 and 6 describes the distribution of levels for food, feed and sediment samples.

DR CALUX® bioanalysis The procedure for the DR CALUX® by BDS bioassay is described in detail previously[3]. Briefly, H4IIE cell, 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. sediment, foodstuffs, feedingstuffs). Following a 24 hour incubation period, cells were lysed. A luciferine containing solution was added and the luciferase activity was measured using a luminometer equipped with 2 dispensers. Overall, 1224 sample were evaluated.

Results and Discussion

End of the year 2006 the limit values will be revised by adding for the first time dioxin-like PCBs. Therefore the Total TEQ levels for food and feed are expected to increase significantly as

compared to the PCDD/F results only. In case of the DR CALUX technology in the past mostly the Total TEQ was requested by our clients, not only the dioxin TEQ, which was mostly requested for the HRGC/HRMS laboratories. By using this approach the Total TEQ measured by DR CALUX® did already implement both the dioxin- and dioxin-like PCB TEQ indicating a better risk assessment.

Figure 1 and 3 are presenting the distribution of Total TEQ data from food and feed based on the times they match to the corresponding EU feed/food guidelines or in case of Figure 5 for the Dutch guideline for sediments (50 ng TEQ/kg d.w.). In the figures 2, 4 and 6 also the median levels, the % below 0.5, % below 1.0, % above 1.0 the corresponding EU PCDD/F limit values and the total number of samples are listed.

Figure 2 and 4 are presenting the histograms of Total TEQ data from food and feed samples based on weather or not they match to the corresponding EU feed/food guidelines. This figure shows the different percentile of the sample levels.

In case of the 490 food samples (Figure 1 and 2) the median level was 0.4 times the EU PCDD/F limit. More than 60% of the sample were below half of the EU limit value and only 6.5% exceeded the EU limit for only dioxins. Regarding the Total TEQ level including therefore the dioxin-like PCBs only 1.6% exceeded the in most cases corresponding double EU limit (PCDD/Fs and dioxin-like PCBs).

Figure 1. Distribution of food samples (n=490)

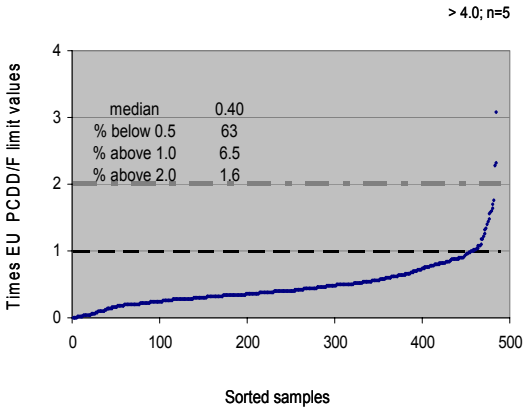
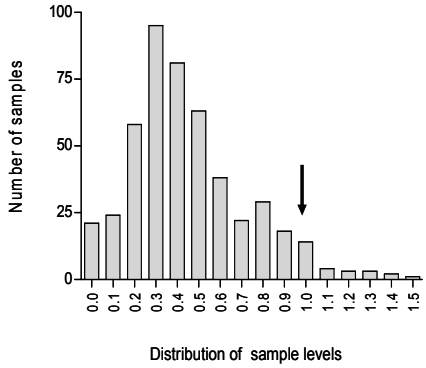


Figure 2. Histogram of food samples (n=490)



The same approach for around 100 feed samples (Figure 3 and 4) shows that the median level is around 0.4-times the EU PCDD/F limit. Around 60% of the sample were below half of this EU limit value and 17% exceeded the EU limit for only dioxins. Regarding the Total TEQ level including the dioxin-like PCBs only 6% exceeded in most cases the corresponding double EU limit.

Figure 3. Distribution of feed samples (n=100)

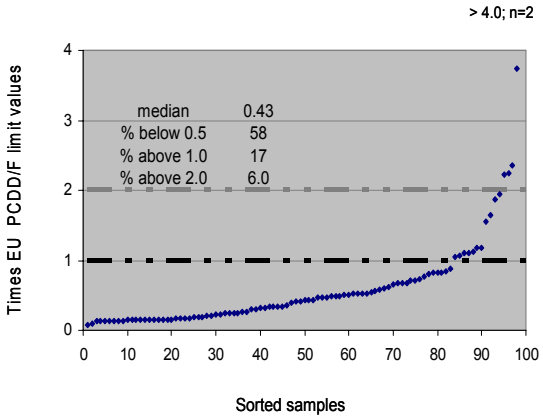
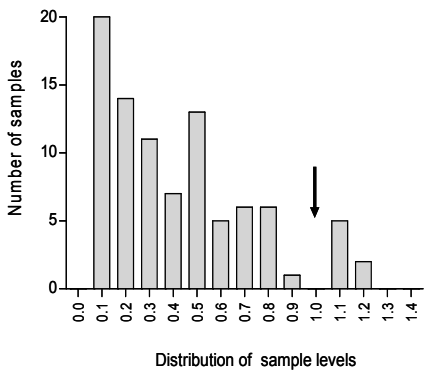


Figure 4. Histogram of feed samples (n=100)



For the 634 sediments samples evaluated here, 25% exceeded the Dutch guideline for sediments of 50 ng TEQ/kg dry weight (Figure 5 and 6).

Figure 5. Distribution of sediment samples (n=634)

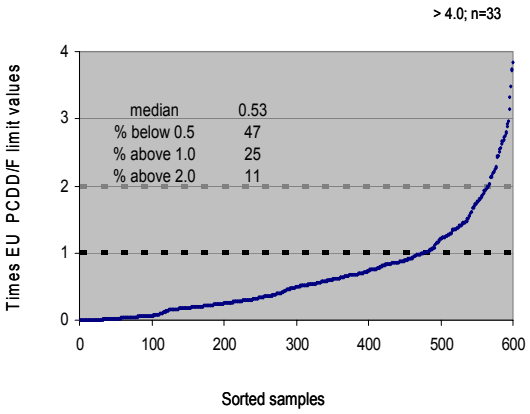
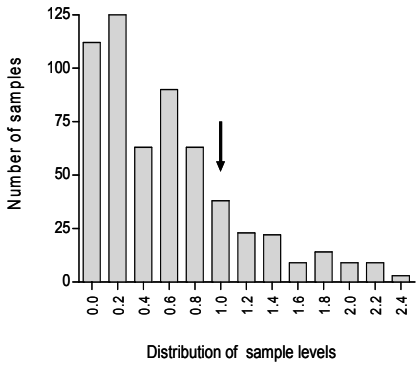


Figure 6. Histogram of sediment samples (n=634)



In the present study we report for the first time Total TEQ levels analyzed by DR CALUX® technology of various feed, food and sediment samples sent to a laboratory acting as international analytical service institution. Thus 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 feed samples. In case of the feed samples the animal fat and the fish oil is over represented. Unusual higher levels can arise due to local dioxin crisis situations. Apart from these exceptional samples shown, the results presented here, are surely representative for the majority of normal background samples we analyzed here by DR CALUX® technology.

Several other studies of laboratories using HRGC/HRMS showed also low numbers of samples exceeding the EU limit values for these kinds of feed and food samples[11, 12], while this is the first study confirming this findings for the testing by DR CALUX® technology as a screening method.

The demand for feed/food inspections is expect to rise not at least due to the EU legislation with the ultimate goal to reduce these dioxins and dioxin-like PCBs significantly. More efforts will be promoted by the EU Commission to receive more data about the dioxins and dioxin-like PCBs of all member states. The results of this study shows again that the DR CALUX® bioassay for screening of dioxin and dioxin-like PCBs in feed, food and sediments is an important tool to separate the bulk of unpolluted samples from the few percentage of the EU limit exceeding samples.

References

[1] Commission Directive 2006/13/EC of 3 February 2006. on undesirable substances in animal feed as regards dioxins and dioxin-like PCBs.
[2] Commission Regulation (EC) 199/2006 of 3 February 2006. amending regulation setting maximum levels for certain contaminants in foodstuffs as regards dioxins and dioxin-like PCBs.
[3] Behnisch, P.A. (2005) in Rapid Methods for biological and chemical contaminants in food and feed. Wageningen Academic Publishers. pp. 303-318.
[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] Besselink, H., Jonas, A., Pijnappels, M., Swinkels, A. and Brouwer, B. (2004). *Organohalogen Compounds*, 66:677-681.
[6] Besselink, H., Jonas, A., Pijnappels, M., Swinkels, A. and Brouwer, B. (2004). *Organohalogen Compounds*, 66:682-686.
[7] Besselink, H.T., Schipper, C., Klamer, H., Leonards, P., Verhaar, H., Felzel, E., Murk, A.J.,Thain, J., Hosoe, K., Schoeters, G., Legler, J. and Brouwer, B. (2004). *Environm. Toxicol. Chem*., 23:2781-2789.
[8] Besselink, H., Fezel E, Jonas, A.,. and Brouwer, B. (2006). Results of the first international interlaboratory DR CALUX® comparison study for food and feed. Organohalogen Compounds 2006.
[9] Rectificatie Chemie-Toxiciteit-Toets verspreiding zoute baggerspecie. Staatscourant 5 juli 2004, nr. 125.
[10] Veileder for risikovurdering av forurenset sediment (TA-2085/2005). Rev 1, juni 2005.
[11] Herrmann, T., Collingro C., and Paepke O., (2004) *Organohalogen Compounds*, 66:2122 – 2126.
[12] Behnisch, P. A. (2005). Food. Issue 1, Spring 2005 pp. 13-17.