SCREENING OF FOOD SAMPLES FOR DIOXIN LEVELS – COMPARISON OF GC/MS DETERMINATION WITH THE CALUX BIOASSAY

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Introduction

Dioxin-like compounds are recognized as widespread, persistent and hazardous contaminants that accumulate in the food chains and biological matrices, including human adipose tissue, blood and milk. More than 90% of the human exposure is estimated to come from dietary intake, with food of animal origin usually being the predominant sources[1]. With the acceptance of the new maximum residue limits for PCDDs and PCDFs in food and feedstuff within the EU, there is an increasing demand for rapid and less expensive screening methods for these compounds. The CALUX (Chemical Activated Luciferase Expression) bioassay is a reporter gene assay that determines the total Ah receptor activity of dioxin-like compounds present in a sample. However, in order to maximize the utility of this bioassay for use as a first screening step for food and feedstuff control purposes, the whole assay procedure needs to be fully optimised and additional analyses of various types of food samples have to be validated.

The aim of this study was to examine the correlation between TEQ values obtained in the CALUX bioassay and determined by GC/MS for different types of food samples. Furthermore the clean-up procedure and bioassay was validated by extracting varying amount of fat from two food samples and a cod liver oil used as reference sample.

Methods and Materials

Sample preparation

Seven different food samples of animal origin collected in Denmark including bovine milk, eggs, oil from fish farms, and fat from pork, beef, chicken and turkey were selected for screening in the CALUX bioassay and compared to GC/MS determinations. Samples of 1.0 gram lipid were cleaned up on a column packed with silica gel coated with 30 % H_2SO_4, 2 g of activated silica gel and 2 g of Na_2SO_4. The column was eluted with 40 ml n-pentane and after solvent removal by evaporation the residue was dissolved in 30 ml DMSO. A cod liver oil was used as reference sample and was prepared as the food samples. Three of the food samples and the cod liver oil were prepared with varying amount of lipid (0.3, 0.6 and 1.0 g). In addition, the n-pentane extract of the cod liver oil (1.0 g lipid) was divided into fractions of 1, 2, 4, 8, 16 and 32 ml (63 ml total) before evaporation and dilution in DMSO. Aliquots of 25 ml from each DMSO extract were added to 1.25 ml MEMa-medium and tested in the CALUX bioassay.

CALUX bioassay

Rat H4IIE hepatoma cells stably transfected with the AhR-controlled luciferase reporter gene plasmid pGudLuc1.1 (Biodetection Systems) were grown in 96-well white culture plates (Packard) containing 100 ml MEMa culture medium supplemented with 5% fetal bovine serum (BioWhittaker) and 1 % penicillin/streptomycin. The density of the cells was $2.2 * 10^4$ cells/well. After an incubation period of 24 hours (37 °C with humidified atmosphere of 5 % CO_2/air) the cells reached 90-100 %

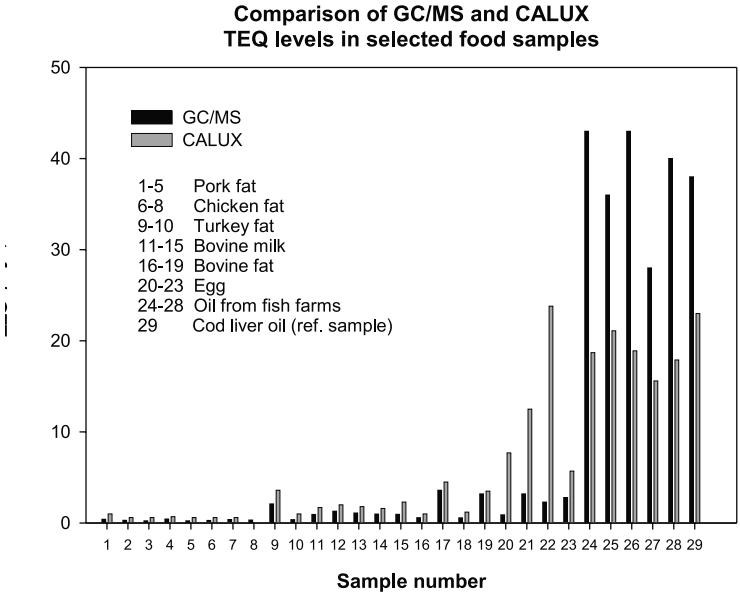

Figure 1. TEQ values in 28 food samples tested in the CALUX bioassay and determined by GC/MS (including PCDDs, PCDFs and dioxin-like PCBs

confluency. Samples and 2,3,7,8-TCDD standard series from 0.3 to 1000 pM were added in a final concentration of 2.0 % DMSO and tested in triplicates. After 20-24 hours of exposure the cells were washed with PBS and lysed with 25 ml cell lysis reagent. The luciferase activity was measured using a Galaxy Orbit luminometer.

Standard curves for TCDD concentrations ranging from 0.6-10 pM were fitted using a linear regression. Samples were interpolated on this curve calculating the total amount of TCDD-equivalents (CALUX-TEQ) for each sample. TEQ values obtained in the CALUX bioassay were compared to TEQ values determined by GC/MS.

GC/MS-determination

Before fat extraction and clean up, samples were spiked with a mixture of carbon-13 labelled PCB, PCDD and PCDF internal standards. The final extracts were dissolved in 20 ml toluene and 2.5 µl was injected by a Fisons AS800 autosampler into a Fisons 8065 gas chromatograph interfaced to a Micromass AutoSpec Ultima high-resolution mass spectrometer.

Results and Discussion

The limit of detection for 2,3,7,8-TCDD was 0.6 pM, which resulted in a limit of detection for the food samples on 0.3 pg TCDD eq./g fat. When determined by GC/MS the samples ranged from 0.23 to 43 pg WHO-TEQ/g fat and ranged from 0.6 to 23.8 pg TEQ/g fat when tested in the bioassay (Fig 1). Of the 28 food samples analysed, only one sample (#8) was not detectable in the bioassay but contained only 0.24 pg TEQ/g fat when determined by GC/MS. The cod liver oil reference sample together with the five fish oil samples all showed lower TEQ levels when determined by CALUX than by GC/MS. In fish oil the contribution of PCBs are generally very high and the fact that the relative potencies of

certain mono-ortho PCBs such as PCB118 are much lower in the CALUX bioassay than their TEF-values assigned by the WHO might explain these underestimated TEQ values in the bioassay[2,3]. Furthermore, a high content of di-ortho PCBs might antagonise the total Ah receptor activity of the dioxin-like compounds present in the sample extract when tested in the bioassay[4]. In contrast the four egg samples contained up to ten times higher TEQ values when tested in the bioassay, which may indicate the presence of a persistent Ah receptor agonist in this sample type that is not included in the GC/MS analysis.

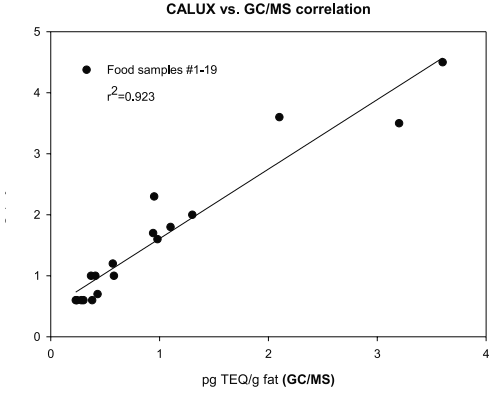

Figure 2. Correlation between TEQ values (pg TEQ/g fat) obtained in the CALUX bioassay and determined by GC/MS for food sample 1-19.Apart from the egg and fish oil samples, the correlation between GC/MS- and CALUX-TEQs in the food samples showed a clear linear trend, with all bioassay values being highest (Fig 2).

Extraction of varying amount of fat from the cod liver oil and two food samples (#9 and #17) was performed as part of a validation of the whole screening procedure. Results showed that TEQ values increased, as the amount of fat was reduced but only for the cod liver oil reference sample (Table 1). Running the samples through two serial silica columns instead of one resulted in identical TEQ values (data not shown) indicating that the capacity of the column was sufficient. Instead the higher amount of extracted fat and thereby higher amount of di-ortho PCBs might result in an increased antagonistic effect on the AhR and thus a reduced total TEQ value.

Table 1. TEQ values determined for 4 samples after extraction of 0.3, 0.6 and 1.0 g of fat.

g fat extracted	Bovine fat #17 n=2	Turkey fat #9 n=1	Cod liver oil (ref.) n=2
0.3	5.2	4.6	71.9
0.6	5.1	-	36.9
1.0	5.4	4.5	23.0

This antagonistic effect was additionally tested by dividing the pentane sample extract of the cod liver oil (Fig 3). Fractions corresponding to 62.5-500 mg fat all showed similar TEQ values when

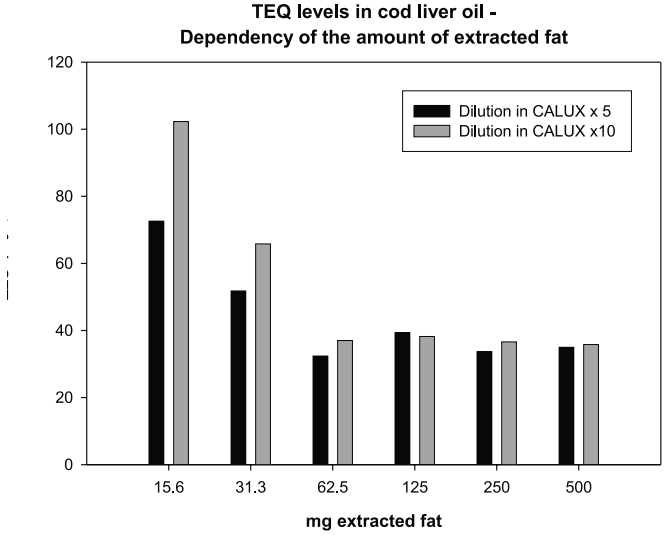

Figure 3. TEQ values in a cod liver oil sample with varying amount of fat (15.6-500 mg) in the sample extract, dividing the n-pentane extract (63 ml) into fractions of 1, 2, 4, 8, 16 and 32 ml. The sample was diluted 5 and 10 times before tested in the CALUX bioassay.

tested in CALUX (range 32.8-39.3) and dilution of the sample extracts in the bioassay had no influence on these values. However, fractions corresponding to 15.6 and 31.3 mg fat had markedly higher TEQ values and further dilution of the extracts in the assay also influenced the TEQ values. The presence of compounds able to antagonise the AhR activity of dioxin-like compounds in specific food samples such as fish oils might explain why the TEQ values observed depend on the amount of fat tested in the bioassay. However, for most food samples the CALUX bioassay seem to be a valuable screening tool prior to GC/MS determination.

Acknowledgements

Thanks Dr. Abraham Brouwer, Vrije University, Amsterdam, The Netherlands, for having provided the CALUX bioassay.

References

1. Liem, A. K. D., Fürst, P. and Rap. (2000). Exposure of populations to dioxins and related compounds. Food Addit. Contam. 17, 241-259.

2. Hoogenboom, L. A. P., Traag, W. A., Hoogerbrugge, R., Baumann, B. and De Vries, J. (2001). Control analysis of Eel using CALUX and GC/MS. Organohal Cmpds. 54, 23-27.

3. Laier, P., Cederberg, T., Larsen, J. C. and Vinggaard, A. M. Optimisation of a bioassay based on H4IIE-cells for testing of dioxin levels using human milk as example. Submitted

4. Aarts, J. M. M. J. G., Denison, M. S., Cox, M. A., Schalk, M. A. C., Garrison, P. M., Tullis, K., De Haan, L. H. J. and Brouwer, A. (1995). Species-specific antagonism of Ah-receptor action by 2,2',5,5'-tetrachloro- and 2,2',3,3',4,4'-hexachlorobiphenyl. Environ. Toxicol. Pharmacol. 293, 463-474.