APPLICATION OF THE CALUX BIOASSAY FOR THE DETERMINATION OF LOW TEQ VALUES IN MILK SAMPLES

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Introduction

The CALUX method has been evaluated earlier on its applicability as screening assay for food matrices.[1] Several samples of largely different matrices and variable contamination levels (ranging from 0.5 to > 1000 pg TEQ/g lipid) were analyzed by GC-HRMS and by the CALUX assay in order to compare and correlate obtained results.
This preliminary comparison has now been extended by new analyses of milk samples with low-level TEQ values. The objective was to qualitatively and quantitatively screen those samples, taking into account the compulsory norm of 5 pg TEQ/g lipid. Table 1 presents existing regulation for food items in Belgium.

Table 1

Food matrix	Limit
Milk, bovine- and chicken meat, eggs, animal fats/oils + derived products (> 2 % fat)	5 pg WHO-(PCDD/F)-TEQ/g lipid
Pork meat + derived products (> 2 % fat)	3 pg WHO-(PCDD/F)-TEQ/g lipid

Concentrations in milk that were previously determined by GC-HRMS rarely exceed 5 pg TEQ/g lipid. These values were obtained within the framework of a monitoring program launched in 1990.
The aim of this study was to investigate whether comparable results were obtained for poorly contaminated milk samples (background levels). Therefore a number of samples, already analyzed by GC-HRMS, were reanalyzed by CALUX.

Materials and Methods

Samples

Samples were taken at the point of transport from farm to factory (mixed milk, MM) or at individual farms (farm milk, FM). TEQ values, determined by GC-HRMS, were calculated according to WHO-TEF values and are based on lower bound detection.

Sample preparation

The milk samples (60 g) were transferred to hexane-rinsed glass Erlenmeyers with PTFE-lined caps and were shaken and extracted 4 times with an acetone/hexane mixture. The pooled extracts were evaporated under nitrogen, and the dried residues were weighed to determine organic extractables ("milk lipid"). The dried extracts were further cleaned up in combination with PCDD/F isolation using a patent pending clean-up procedure.[2]

CALUX assay

In the CALUX assay a genetically engineered cell line is used, which contains the firefly luciferase gene under trans-activational control of the aryl hydrocarbon receptor.[3]
The purified sample extracts in DMSO were suspended in cell culture medium prior to dosing monolayers of H1.L1.6 mouse hepatoma cells that were grown in 96-well culture plates. In addition to the samples, a 2,3,7,8-TCDD standard curve was generated on each plate. The plates were incubated for optimal induction of luciferase activity in a humidified CO_2 incubator. After incubation, the medium was removed and the cells were examined microscopically for viability. The induced luciferase activity was quantified using the luciferase assay kit from Promega.

Data and statistical analysis

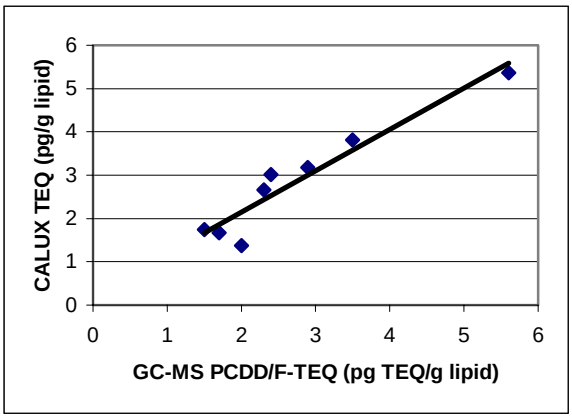
The data for the 2,3,7,8-TCDD standard curve were fit to a sigmoid curve described by the Hill equation using least squares best fit modeling and responses of unknown samples were calculated as TCDD-TEQs by interpolation.
The Spearman rank correlation coefficient was calculated in STATISTICA.

Results and discussion

Correlation between CALUX and GC-MS results

8 Milk samples with dioxin levels between 1 and 6 pg TEQ/g lipid, as determined by GC-HRMS, were analyzed in the CALUX assay. The correlation between the results obtained by CALUX and by GC-HRMS is shown in Figure 1.

Figure 1



The Spearman rank correlation coefficient was found to be 0.94 ($p = 0.0048$) indicating a good correlation between the results obtained by both techniques.
This correlation is in good agreement with results from a previous study using CALUX for the determination of TEQ levels in breast milk.[4] The TEQ values ranged from 25-60 pg TEQ/g lipid, what is significantly higher than the results of our study.

Differences between chemo- and bio-analytical techniques

A few aspects must be considered when GC-MS and CALUX results are compared:

- It has been demonstrated that CALUX-REPs differ from the WHO-TEFs.[5,6]

- Chemo-analysis focuses on a limited number of dioxins (17 congeners with assigned TEFs). The CALUX assay, however, detects all Ah agonists, such as other chlorinated and brominated congeners.

Implications for using the bioassay as a screening method

The CALUX assay is a promising tool for sample screening. A first objective of reference labs is to provide statistical evidence for levels exceeding the compulsory norm concentration. Secondly, the technology could be used for quantitative determination, requiring extensive validation effort.

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