A COMPARATIVE STUDY OF GC-HRMS AND CALUX™ TEQ DETERMINATIONS IN FOOD SAMPLES BY THE BELGIAN FEDERAL MINISTRIES OF PUBLIC HEALTH AND AGRICULTURE

Ilse Van Overmeire [1], Leo Goeyens [1], Hedwig Beernaert [1], Sarah Srebrnik [1], Geert De Poorter [2], Willy Baeyens [3], George Clark [4], Michael Chu [4], Andrew Chu [4], David Chu [4], Richard Morris [5], and David Brown [4]

[1] Scientific Institute of Public Health, Juliette Wytsmanstraat 14, 1050 Brussels, Belgium; [2] Belgian Federal Ministry of Agriculture, WTC3 Boulevard S. Bolivar 30, 1000 Brussels, Belgium; [3] Vrije Universiteit Brussel, Laboratory for Analytical and Environmental Chemistry, Pleinlaan 2, 1050 Brussels, Belgium; [4] Xenobiotic Detection Systems Inc., 1601 East Geer St., Durham, NC 27704; [5] Analytical Sciences Inc., 2605 Meridian Parkway, Durham, NC 27113

Introduction

In January of 1999 the Belgian food supply was contaminated with transformer fluids containing polychlorinated biphenyls (PCBs). The Belgian government public health response to this contamination incident was detailed by G. De Poorter at the 19 th International Symposium of Halogenated Environmental Organic Pollutants (Dioxins 99) in Venice, Italy on September 17, 1999. The source of contamination was a storage tank at the Verkest fat-rendering works. PCB transformer oil was included in fat that was being recycled for a feed additive in composite feed. The transformer oil contained predominantly Aroclor 1254 and 1260 that was contaminated with traces of chlorinated dibenzofurans (PCDFs) and chlorinated dibenzo-*p*-dioxins (PCDDs). The contamination incident was highlighted by a sudden drop in egg production, reduced egg hatchability and increased mortality of chicks.

The Belgian government mobilized a major public health effort aimed at: a) identifying the contaminated feed materials; b) placing quarantine on those farms that were identified as being contaminated with PCBs; c) undertaking a major analytical effort to identify the extent of the contamination; d) isolating and destroying animals determined to be contaminated. The extent of this public health disaster resulted in a commitment from the Belgian government that a system would be identified to prevent a reoccurrence of contamination of the food supply with these toxic environmental contaminants.

The Belgian Ministries of Public Health and Agriculture made a public tender for proposals for a screening methodology that would allow for the detection of contamination of fat, feed stuffs, animal fat, milk products, egg products and processed feed stuffs at the level of 5 pg TEQ/g fat. The competition involved the analysis of 23 samples for contamination on a TEQ basis of PCBs, and PCDDs/PCDFs. If possible, an analysis of the contribution of TEQ by the PCBs versus PCDDs/PCDFs was requested.

Described in this report are the results of the CALUX™ analysis, as developed by Xenobiotic Detection Systems Inc., versus detection using gas chromatography/ high resolution mass spectrometry (GC-HRMS) provided by various laboratories contracted by the Belgian government.

Methods and Materials

The samples were prepared by the Belgian Ministries of Public Health and Agriculture. They included 2 animal fat samples, a chicken fat sample, 5 porcine fat samples, 3 samples of feed stuff, 4 samples of eggs, 4 milk samples and 4 serum samples. A subsample of all of these samples and one serum sample were sent to each participating laboratory for analysis.

CALUX™ Analysis: 1 gram aliquots of each sample were weighed out and extracted with a mixture of methylene chloride/hexane; "total organic extractable lipids" were quantified by gravimetric analysis. The extract was processed through a patent pending sample preparation procedure to concentrate contaminating PCBs or PCDDs/PCDFs in the sample.[1] The sample extract in DMSO was applied to the patented genetically engineered cell line which contains the firefly luciferase gene under trans-activational control of the aryl hydrocarbon receptor for the detection and relative quantification of PCDDs, PCDFs, and coplanar PCBs.[2]

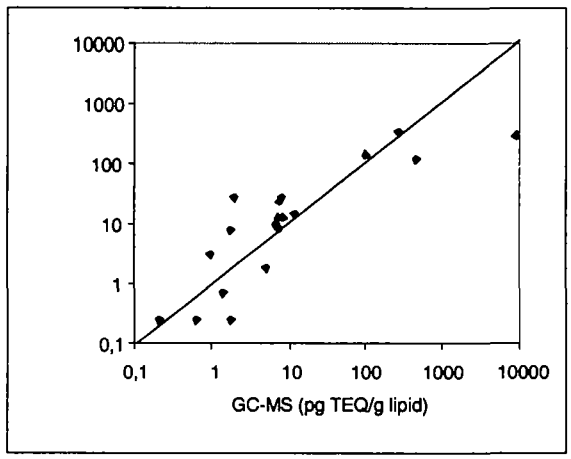
GC-HRMS Analysis: GC/HRMS determinations of the contamination of the samples were performed by standard methods for quantification of isomer specific concentrations in the sample such as EPA method 8290.[3] TEQ contamination of the sample was estimated by multiplying congener specific concentrations by the TEF values of the WHO.[4]

Results and Discussion

CALUX™ versus GC-HRMS data

In Figure 1 the results of the analyses of 19 samples (fat (8), feed stuff (3), eggs (4), and milk (4)) by the CALUX™ method and by GC-HRMS are represented on a lipid adjusted basis. A value of 0.25 pg TEQ/g lipid (1/2 DL) was assigned to 3 results that were not detected by CALUX™. The Spearman's Rank correlation coefficient between the CALUX™ and GC-HRMS values is 0.8875 ($p < 0.0001$). This demonstrates that the results of both analysis methods are highly correlated. It suggests that the CALUX™ analyses are predictive for GC-HRMS results and may serve as a rapid and sensitive screen for dioxin contamination in feed and food samples.

Figure 1: CALUX™ versus GC-MS (pg TEQ/g lipid)



As seen in Figure 1, CALUX™ estimates of TEQ are often higher than GC-HRMS measurements. CALUX™ measurements exceed GC-HRMS measurements in 13 of the 19 paired observations. In 8 of 19 observations the values are very similar. A sign test does not reject the hypothesis that the difference between the two measurements made on the same biological sample is as likely to be positive as it is to be negative ($p = 0.167$).
Graphically represented in Figure 2 are the Log transformed data comparing CALUX™ determinations with GC-HRMS determinations of TEQ contamination in the samples on a lipid adjusted basis. The correlation coefficient amounts to 0.85; the Log transformed data follow a normal distribution (Shapiro-Wilk $W= 0.94296$, $p < 0.2979$).

Figure 2: Correlation between Log transformed CALUX™ and GC-HRMS TEQ data (CALUX™ and GC-HRMS in pg TEQ/g lipid)

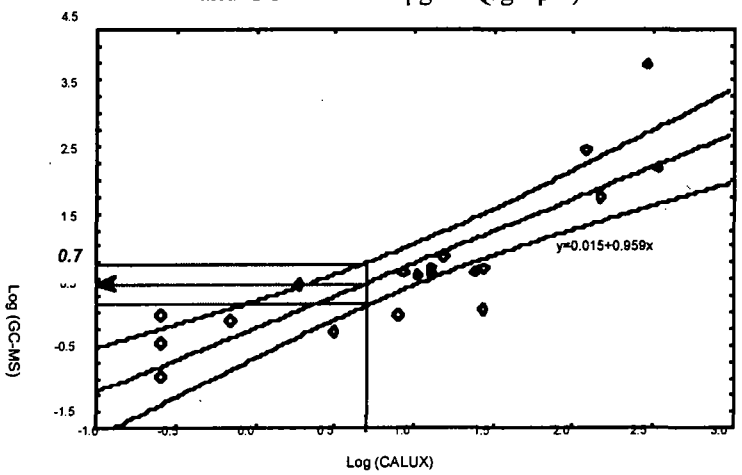


Figure 3: Correlation CALUX™ and GC-HRMS TEQ data around 5 pg TEQ/g lipid

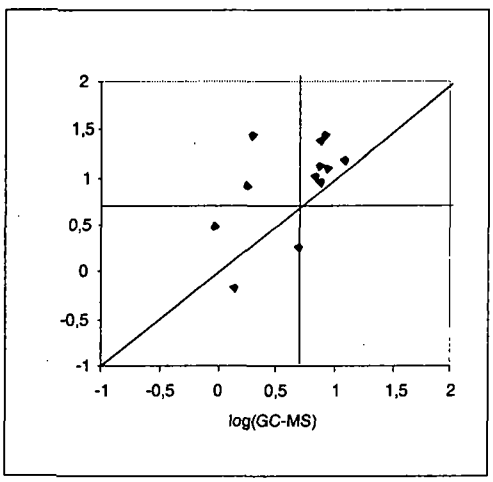


Figure 3 illustrates 12 measurements for observations in a region of interest near 5 pg TEQ/g lipid. Dotted reference lines at 5 pg TEQ/g lipid are drawn. Of 12 observations, 10 have CALUX™ measurements that exceed corresponding GC-HRMS measurements. Using the reference lines, decisions based on 5 pg TEQ/g lipid would be concordant between CALUX™ and GC-HRMS in 10 of 12 cases. The remaining 2 observations (in the upper left square) would be labeled as exceeding 5 pg TEQ/g lipid by CALUX™ but not by GC-HRMS.

Precision of the results

It is generally concluded from the evaluation of standard GC-HRMS procedures that an average uncertainty of 30% must be taken into consideration.[3,5] On the other hand standard deviations obtained from triplicate CALUX™ analyses, performed for this study, range from 2% to 48% (mean value 21%). These results indicate that CALUX™ and GC-MS results are comparable in terms of uncertainty on the results.

Prediction of GC-MS data from CALUX™ data

The regression line, obtained with the data of this study, can be used as a model to predict GC-HRMS data with their variability from CALUX™ data. In Figure 2 this is illustrated at a 5 pg TEQ/g lipid level. The log(CALUX) value of 0.71 is converted in a log(GC-MS) value of 0.7 (5 pg TEQ/g lipid). The corresponding variability of the GC-HRMS value ranges from 2.5 to 10.2 at 95% probability; there is a spread of ± 44% on the log value.

Conclusion

Biologically based systems of analysis for TEQ determination of contamination of food and feed have developed to the point that they are useful for risk assessment determinations of the hazards of exposure to this class of environmental contaminants. Other investigators have demonstrated the value of reporter gene technology in assessing dioxin and/or PCB contamination and the risk of exposure to this class of environmental contaminants with less stringent parametric correlations.[6,7]

The data in this report showed the usefulness of the CALUX™ method as an alternative method for GC-HRMS to determine TEQ levels in different matrices. It is a rapid and cost effective analytical system to determine planar PCB- and PCDD/PCDF- levels in various food products. We showed that a good correlation exists between GC-HRMS data and CALUX™ data and that a minimal amount of false positive results is obtained using the CALUX™ method. More data are required to obtain an improved model for predicting GC-HRMS data from CALUX™ results and to study matrix dependent influences.

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