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INTER-LABORATORY CALUX® COMPARISON DATA AT LOW LEVELS WITH TRADITIONAL GC-HRMS CONFIRMATIONS

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Introduction

Reports over the past few years[1,2,3] have shown comparison data between CALUX® and traditional GC-HRMS methodologies for PCDD/PCDF analyses. These studies have demonstrated good correlation between the two methodologies at elevated levels, with the CALUX® bioassay typically exhibiting higher TEQ values than traditional GC-HRMS methods at these elevated levels. One reason for this trend is that the CALUX® bioassay is sensitive to other analogous halogenated species as well as the chlorinated dioxins and furans.

PCDDs and PCDFs in animal feed and feed components need to be detected at the lowest concentrations possible. Currently the European Council[4] has a tolerance level of 0.5 pg/g for feed materials of plant origin and 0.75 pg/g for certain other feed materials[5]. Such thresholds may approach the limit of quantitation that the CALUX® bioassay can offer.

The purpose of this study was to determine if the CALUX® bioassay is useful in screening low level samples. This study involves the analysis of 35 samples using three separate instrumental systems: by CALUX® bioassay at the Food and Drug Administration's Arkansas Regional Laboratory (ARL), by CALUX® bioassay at Xenobiotic Detection Systems, Inc. (XDS), and by GC-HRMS at ARL. Two aspects of interest in comparing the data include the percentage of false negatives produced by CALUX® and the percentage of false positives found using the CALUX® technique.

Method

Thirty-five samples, including animal feeds and animal feed components, were collected by FDA field investigators in 2001 and analyzed at ARL for the seventeen 2,3,7,8-Cl-containing dioxins and furans using traditional GC-HRMS. These samples were extracted by Soxhlet and cleaned using multi-layered silica gel and alumina columns. Congener separation was achieved using an Agilent 6890 GC system equipped with a BPX-5 column (40m, 0.18µm film thickness, 0.18 mm i.d.) from SGE and a guard column (5m, 0.25 mm i.d., deactivated silica) from Agilent. Mass spectrometric analysis was completed with an Autospec Ultima HRMS from Micromass using a minimum mass resolution of 10000 at 10% signal height.

The same samples also served as CALUX® proficiency samples for an ARL analyst. This proficiency training using these samples took place at XDS in Durham, NC, USA. The recovery

method at XDS used a surrogate sample spiked with a $^{14}C_{12}$ 2,3,7,8-TCDD estimation of recovery was read via scintillation counting.

Once the CALUX® system became operational at ARL, these same samples were subsequently re-extracted and analyzed by CALUX® at ARL for inter-laboratory comparison. To measure recoveries, the samples required a surrogate extraction that was read using a luminometer, and the surrogate extraction concentration was used to determine recoveries of dioxin-like chemicals from the sample for each matrix.

Results & Discussion

Figures 1 and 2 show the comparison of 35 feed and feed components between GC-HRMS and CALUX® results obtained at XDS. The symbols shown in Figure 1 represent the comparison of TEQ values from CALUX® to that found by GC-HRMS. The TEQ determined using GC-HRMS was calculated from only those congeners confirmed to be present. However, one may also consider the TEQ contribution from the remaining congeners by multiplying the LOD of each congener by its respective TEF value and adding those values to the original TEQ. In this manner, congeners unconfirmed by GC-HRMS yet detected by CALUX® can be included in the comparison; these adjustments for the same 35 samples are represented in Figure 2.

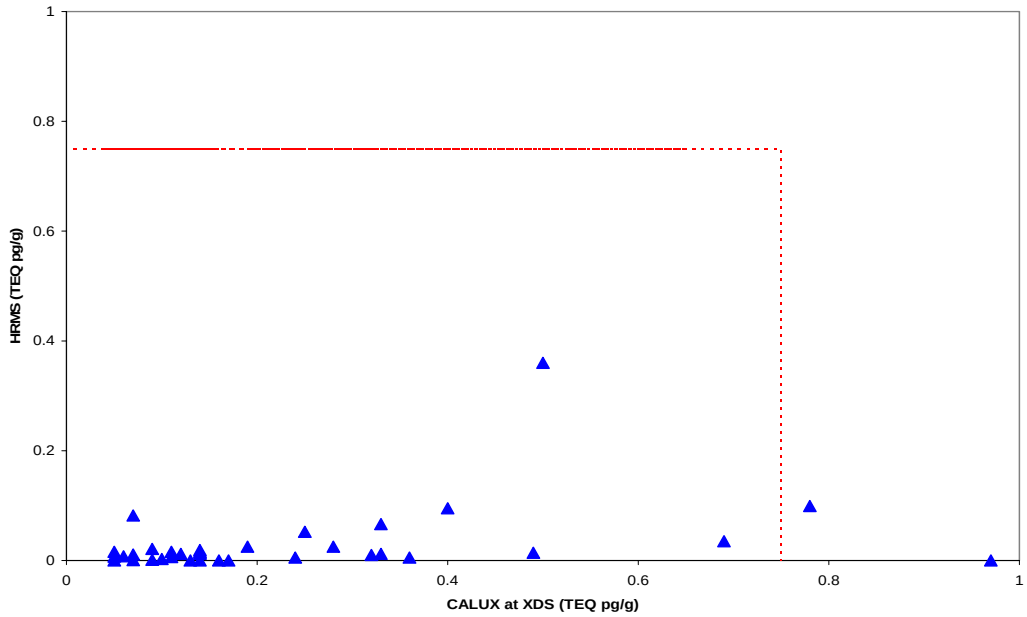

Figure 1. GC-HRMS data versus CALUX® data obtained at XDS for 35 feed and feed component samples. The GC-HRMS TEQ for all congeners confirmed and for all confirmed congeners. The dotted line at 0.75 pg TEQ/g represents the maximum level set by the European Council in most complete feedingstuffs.

Useful information is gained by plotting these data. First, there appears be a rather poor linear correlation between GC-HRMS and CALUX® in animal feed samples with low dioxin levels. Second, while all of the values using GC-HRMS were less than 0.75 pg TEQ/g, only 2 of the 35 samples (about 6%) exceeded 0.75 pg TEQ/g by the CALUX® bioassay. This suggest that roughly 6% of the samples may give false positive results using CALUX®, indicating its usefulness as a screening technique in the feed industry.

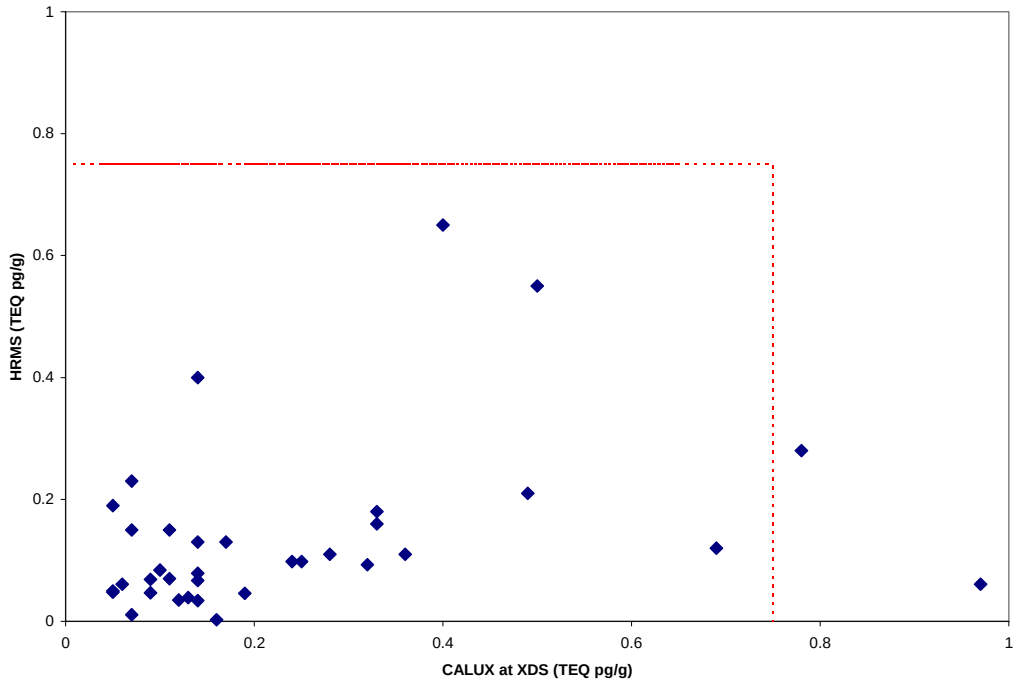

Figure 2. GC- HRMS data versus CALUX® data obtained at XDS for 35 feed and feed component samples. The GC-HRMS TEQ for all congeners confirmed plus the LOD contribution from unconfirmed congeners. The dotted line at 0.75 pg TEQ/g represents the maximum level set by the European Council in most complete feedingstuffs.

Figure 3 depicts the inter-laboratory CALUX® comparison data consisting of 35 feed and feed component samples that were collected in 2001. Although a rather poor linear correlation exists between these data, nearly identical conclusions can be made:

- Fewer than 6% of false positives with a TEQ greater than 0.75 pg/g were observed
- CALUX can be successfully used as a screening tool for animal feeds at 0.75 pg TEQ/g
- This study shows inter-laboratory agreement, using cut-off values, in the low level range

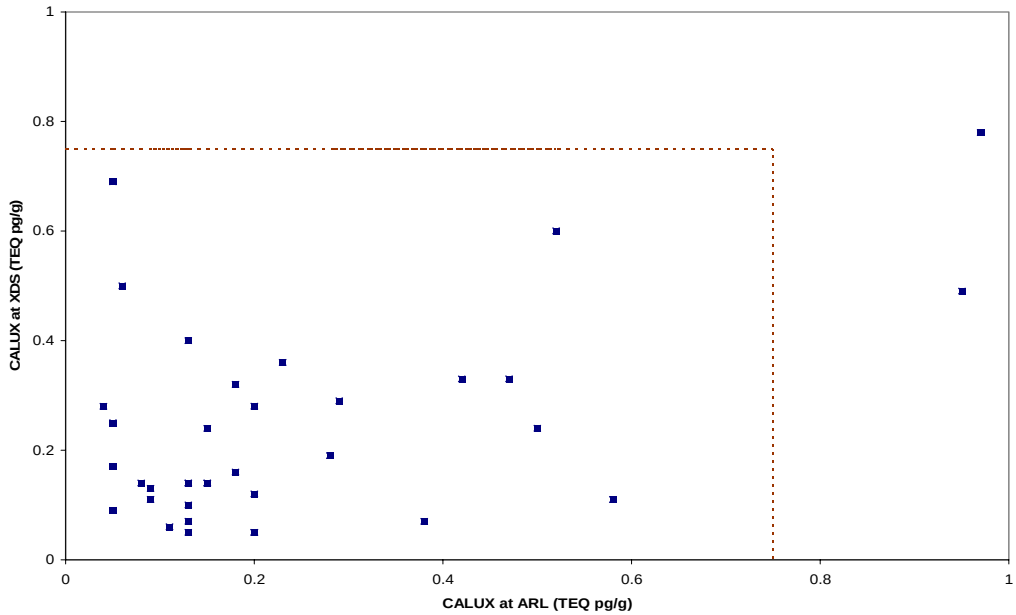

Figure 3. Plot showing TEQ values for the same samples analyzed by CALUX® at ARL and XDS. The dotted line at 0.75 pg TEQ/g represents the maximum level set by the European Council in most complete feedingstuffs.

Acknowledgments
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