# VALIDATION STUDY FOR THE USE OF THE DIOXIN RESPONSIVE CALUX™ ASSAY FOR ANALYSIS OF JAPANESE ASH AND SOIL SAMPLES

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## Introduction

In Japan incineration is a common method for disposing of municipal waste and it is estimated that more than 10,000 incinerators of various capacities are currently in operation. In the past couple of years there has been an increased concern regarding the emission from these incinerators and other the emissions of other industries. In particular the concern has focused on the inadvertent production and release of chlorinate aromatic compounds such as polychlorinated biphenyls (PCBs), polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs). In August 1997 the Japanese government addressed these concerns by amending the cabinet orders of the Air Pollution Control Law and Waste Management and Public Cleansing Law. These amendments implemented stricter regulations on incinerators and other industries that emit PCBs, PCDDs and PCDFs[1].

These amendments placed an immediate limit on emissions from new facilities that will emit these compounds and provided for gradually more strict limits for existing facilities over a five-year period. In order to comply with these new limits it was expected that monitoring by chemical analysis would have to increase. This raised concerns that the chemical analysis by HRGCMS for compliance might be an economic hardship for some of the regulated industries and that the increased demand might outstrip the capacity of the existing analytical laboratories. Based on these concerns the Japanese government and private corporations began to examine the possibility of using alternative testing methods to monitor for the presence of these compounds. In this article we report the results from a preliminary validation study conducted by Hiyoshi Corporation and Xenobiotic Detection Systems, Inc (XDS). This study used a blinded format to compare the results from the dioxin responsive CALUX™ assay with HRGCMS data.

## Materials and Methods

***Study Design.*** Twenty-five ash and soil samples were split and given a code number. The split samples were analyzed by HRGCMS and by the CALUX™ assay. The results from these two methods were sent to an independent statistician for analysis.

***HRGCMS.*** Sediment and ash samples were spiked with $^{13}C_{12}$-labeled PCDD/PCDF standards and analyzed for congener-specific PCDD/PCDFs at Hiyoshi Corporation. I-TEQs for PCDDs/PCDFs were calculated using TEF values[2].

*CALUX™ Assay*. XDS has a patented genetically engineered cell line (mouse hepatoma H1L1) that contains the gene for firefly luciferase under transactivational control of the aryl hydrocarbon receptor[3]. This cell line can be used for the detection and relative quantificatation of a sample's total dioxin I-TEQ. Using a patent pending sample processing procedure it is also possible to use the CALUX™ assay to estimate the I-TEQ contributions of PCDDs/PCDFs or the I-TEQ contributions of the coplanar PCBs[4]. The assay that uses this cell line is called the Chemically-Activated Luciferase Expression or CALUX™ assay.

The samples were extracted using a modification of the EPA 8290 extraction method[5]. Briefly, the dried samples were ground and one gram aliquots were placed in solvent cleaned glass vials with PTFE lined caps. The sample was extracted with a 20% solution of methanol in toluene then twice with toluene. During each extraction step the samples were incubated in an ultrasonic water bath. The three extracts from each sample were filtered, pooled and concentrated by vacuum centrifugation. The sample extract was suspended in hexane and prepared for the bioassay by a proprietary clean up method. The eluate from the clean up method was concentrated under vacuum into dimethyl sulfoxide (DMSO). The DMSO solution was used to dose the genetically engineered cells in the CALUX™ assay.

Prior to dosing the cells, the sample extracts in DMSO were suspended in cell culture medium. This medium was then used to expose monolayers of the H1L1 cell line grown in 96 well culture plates. In addition to the samples, a standard curve of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) was assayed (161, 80.5, 40.2, 20.1, 10.1, 5.0, 2.5, 1.2 and 0.6 parts per trillion (ppt) TCDD). The plates were incubated for a time to produce optimal expression of the luciferase activity in a humidified $CO_2$ incubator. Following incubation, the medium was removed and the cells were examined microscopically for viability. The induction of luciferase activity was quantified using the luciferase assay kit from Promega.

**Results and Discussion**

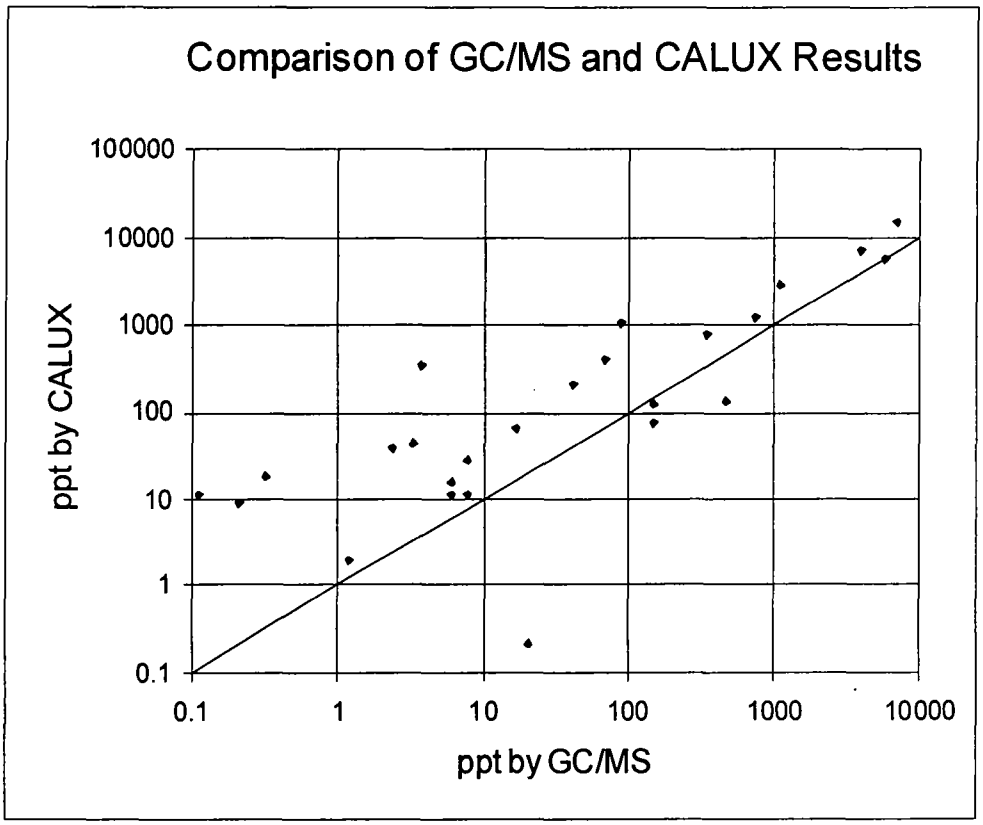
From the GC/MS analysis of the samples, the I-TEQs were calculated using the TEF values for the individual congeners. The sample I-TEQs were estimated by the CALUX™ assay by comparing the response of the sample extract to the standard curve for 2,3,7,8-TCDD.

The correlation coefficient between the results is acceptable, ($r = 0.94$), however, there were several samples where the CALUX™ assay over-estimated the sample I-TEQ relative to the GC/MS results. These over estimates of sample I-TEQ by the CALUX™ assay were probably due to the fact that the contribution of the coplanar PCB was included in the CALUX™ estimates while the GC/MS estimates only include the contributions from the PCDDs and PCDFs. We plan to test this hypothesis by reanalyzing these samples using our patent pending clean up process that allows us to differentiate between the contributions of the coplanar PCBs and PCDDs/PCDFs.

Figure 1 provides a graphical comparison of the results for the 25 samples from GC/MS and CALUX™ assay. A diagonal line representing a 1 to 1 relationship has been drawn on the graph to help with comparisons. By examining the graph it is evident that there is a strong correlation between the CALUX™ results and the GC/MS data. It is also evident that the CALUX™ I-TEQ estimates tend to be higher than the GC/MS data (21 of 25 samples), especially for lower level samples. Part of this could be related to the point made above: as currently reported the *CALUX™ I-TEQ* includes the contribution from the coplanar PCBs while the GC/MS results do

not include the coplanar PCB contribution to I-TEQ. Over estimation by the CALUX™ assay is not a significant concern. The CALUX™ assay is intended to be a screening assay that can be used to identify samples that need to be analyzed by more time consuming and expensive chemical analysis methods (GC/MS). In general it is better for a screening assay to provide a high estimate as false positives are more acceptable than false negatives for a screening assay. Of the four samples that were underestimated by the CALUX™ assay, the results for three were very similar to the GC/MS results. One of the samples that was underestimated by the CALUX™ assay would be considered a false negative (1 of 25 or 4%). This sample will be reanalyzed to determine whether the incidence of false negatives can be further reduced.

Figure 1.




**Conclusions**

The data presented in this report showed the usefulness of the biologically based CALUX™ as an alternative method to GC/MS for estimating TEQ levels in soil and ash samples. The CALUX™ assay is a rapid and cost effective method that showed good correlation with GC/MS results with a minimal number of false negatives. Based on these results the CALUX™ assay should be useful as a screening assay for estimation of TEQ in ash and soil samples.

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