Mathematical Model Predictions of GC/MS Data for Dioxin Contaminated Environmental Samples Using CALUX® by XDS

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Introduction

The CALUX® by XDS bioassay is a mechanistically based recombinant cell bioassay designed to be a rapid screening tool to evaluate the level of polychlorinated dioxin-like contaminants (i.e., dibenzo-p-dioxins, dibenzofurans and biphenyls) present in sample extracts. While the methodological aspects of the bioassay system has been streamlined and optimized, the bioassay system is currently undergoing extensive validation using a wide variety of matrices. However, one aspect that is still evolving is the development and validation of mathematic approaches for the accurate estimation of these chemicals from bioassay analysis. Development of an appropriate mathematic model designed to predict GC/MS TEQ results from cell bioassay data would both improve and validate the comparability of GC/MS and CALUX® by XDS methods for determination of polychlorinated dioxin/furan and biphenyl concentrations from a Total equivalence perspective. Such a mathematical model was applied to this analysis.

Methods and Materials

Environmental samples for analysis. In March 2004, the U.S. EPA and the Battelle Corporation conducted a rigorous cross validation SITE study comparing CALUX® by XDS screening estimates to high resolution gas chromatography/mass spectrometry (GC/MS) analysis of chlorinated dioxins/furans and biphenyls in environmental samples. The SITE study consisted of 209 samples analyzed by both methods. For CALUX analysis, forty of these samples were analyzed in a mobile lab stationed in Saginaw, Michigan MI, while the remaining 169 were analyzed on-site at XDS in North Carolina.

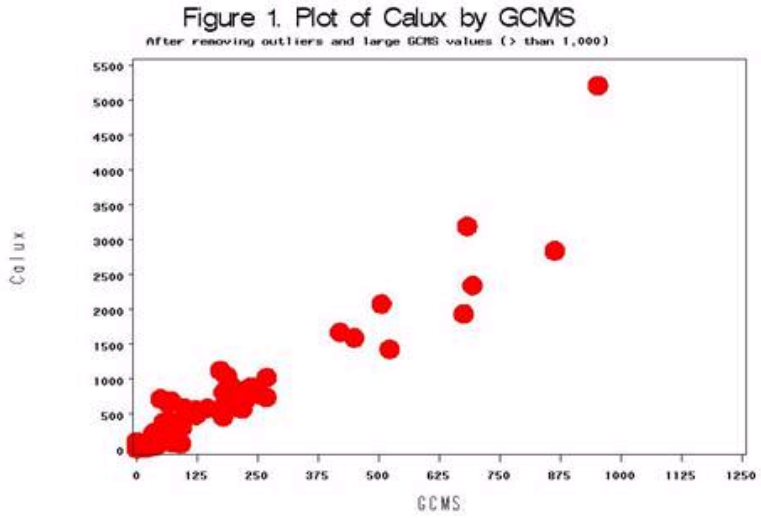
Sample preparation and CALUX® by XDS *bioassay.* The CALUX® by XDS bioassay is a reporter gene assay in which a mouse hepatoma (Hepa1c1c7) cell has been stably transfected with a vector that contains the luciferase gene under transactivational control of the Ah receptor[1-3]. Combined with a patented sample processing system to reduce contamination with non-dioxin-like agonists for the Ah receptor, the CALUX® by XDS bioassay can be used for estimation of TEQ contamination with dioxin-like chemicals of environmental, biological, and chemical samples[4-6]. In this study, a 0.5 g sub-sample of each soil sample or solution was extracted with toluene and processed through our patented cleanup procedure[4]. The isolated extract was exchanged into dimethylsulfoxide and applied to 96 well plate monolayers of our genetically engineered cells and incubated for maximal induction of the firefly luciferase within the cells. Light produced by the firefly luciferase enzyme was measured with an assay kit from Promega and quantified on a Berthold Orion MicroplateLuminometer. Dilution analysis was performed for range finding of the approximate concentration of the processed sample that induced maximal luciferase activity for quantification in the CALUX® by XDS bioassay. Dioxin-like TEQ activity was estimated from a standard curve of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin using a four-parameter Hill equation as previously described[6,7].

To model GC/MS as a function of CALUX® by XDS, we fitted a statistical model with generalized estimating equation (GEE)[7] that accounts for the correlation due to replicates (observations from the same sample) using a base 10 logarithm transformation of the two variables. Observations that contain no dioxins were removed from the model. Similarly, observations that contain CALUX® by XDS values that would have been removed under standard

test protocol were not included in the model.

Results and Discussion

These screening data points do not demonstrate a strict one to one correspondence as shown in Figure 1. This is expected since the TEQ estimates for CALUX® by XDS receptor based technology provides an estimate of receptor-dependent activation of the Ah receptor by chemicals present in the extract and many biological responses are logarithmically related to concentration. The plotting of this relationship generates one model that describes the relationship.



The deviation from a direct relationship occurs for a number of reasons including such factors as the presence of other halogenated dioxins and furans and other Ah receptor agonists in the fractionated sample, differences in the REP values of XDS cells versus the TEF values used to scale the GC/MS estimates of TEQ, and the kinetics of binding and activation of the receptor. Modeling the data we can derive a formula to transform the CALUX® by XDS data to provide a better estimate of the GC/MS data. The relationship between CALUX® by XDS and GCMS is given by the following equation:

$$LOG(GC/MS)=0.6093*LOG(CALUX)+0.0584*[LOG(CALUX)]^2$$

Figure 2 (dots representing observed and the line representing the predicted) shows the relationship between predicted and observed values from the model. Overall, the values for the predicted and observed are close to the identity line indicating a good fit. For larger dioxin levels (greater than 375), the model tends to underestimate the true GC/MS value.

Figure 2. Observed versus predicted GCMS

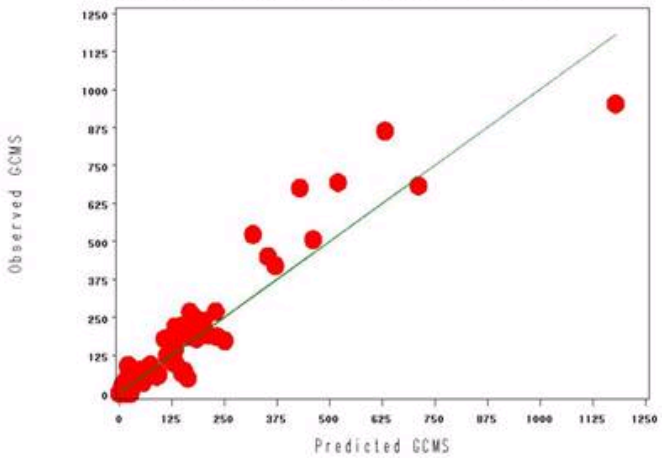
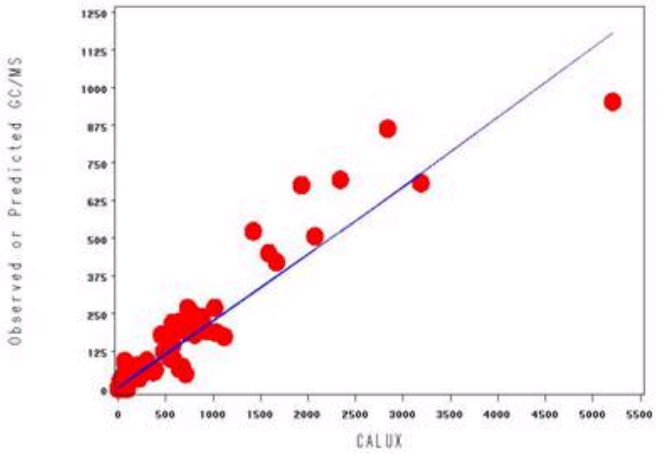


Figure 3 (dots representing observed and the line representing the predicted) shows fitted values from the model against CALUX® by XDS values. The graph shows that the model overall is a good fit for values of CALUX® by XDS less than 1,500. For values beyond 1,500, the model fits the data reasonably well although the value of GC/MS tends to be estimated.

Figure 3. Observed or Predicted GC/MS and CALUX



This modeling exercise shows how values of GC/MS can be predicted from CALUX® by XDS for dioxins. Several limitations need to be noted. First, the model appears to underestimate the true GCMS values over some range of the data. Accordingly, there may be a need to refine the model using splines (that is by assuming for example that the slope of the line is not constant). Second, values beyond 1000 for GC/MS were excluded. Hence, care should be exercised when using the model for samples that contain high concentration of dioxin/furans. When there is a high concentration of dioxins in the sample (beyond 1,000), the CALUX® by XDS assay yields higher than usual values, which would result in overestimation of the true GC/MS value. Thus, with the CALUX® by XDS bioassay, there is unlikely to be a false negative result with a concentration of dioxin/furans beyond the regulatory threshold. Finally, the model has not yet been determined as to whether it also applies to other dioxin-like chemicals such as the polychlorinated biphenyls.

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