

# Measurement of PCDD/F TEQ by Immunoassay: Concept Development and Validation

**Robert E. Carlson**\* and **Robert O. Harrison**†

\* ECOCHEM Research, Inc., MD 23, 1107 Hazeltine Blvd., Chaska MN 55318

† CAPE Technologies, L.L.C., 3 Adams St., South Portland ME 04106

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ECOCHEM Research, Inc., MD23, 1107 Hazeltine Blvd., Chaska, MN 55318 and  
CAPE Technologies LLC, 3 Adams Street, South Portland, ME 04106

## INTRODUCTION

Polychlorinated dibenzo-p-dioxins and dibenzofurans (PCDD/Fs) are significant environmental pollutants (1). Comprehensive assessment of their environmental impact is difficult because the PCDD/Fs are a mixture of 210 possible chlorinated isomers (2). Moreover, PCDD/F analysis focuses on the determination of Toxicity Equivalence (TEQ) which is derived from the concentration of each of the 17 “toxic congener” 2,3,7,8-substituted PCDD/Fs and their respective Toxicity Equivalence Factors (TEFs) (3). TEQ determination currently requires the specificity, sensitivity and expense of High Resolution Gas Chromatography / High Resolution Mass Spectrometry (HRGC/HRMS) (4). Antibody based analytical methods have demonstrated utility for both high throughput screening and quantitative analysis of environmental samples (5). However, previous PCDD/F immunoassay development programs have not been particularly successful (5). There is a clear need for the development of a sensitive, PCDD/F toxic congener specific immunoassay (6). The goal of this project is to develop an immunoanalytical method which can be used to substantially reduce the overall complexity and cost of PCDD/F toxic congener analysis. This presentation will discuss the requirements which must be satisfied for the successful development of a PCDD/F Enzyme ImmunoAssay (EIA) and present data which demonstrates the determination of PCDD/F TEQ by EIA.

## MATERIALS AND METHODS

The EIA which is described in this report was based on a novel anti-PCDD/F polyclonal antiserum and enzyme competitor conjugate combination. This EIA has demonstrated picogram sensitivity to 2,3,7,8-TCDD and specificity for the 2,3,7,8-substituted PCDD/F toxic congeners (6). The EIA has been formatted for use in a coated tube method (7). Details of the development of this EIA will be reported elsewhere. Soil samples were analyzed by HRGC/HRMS using US EPA Methods 1613 or 8290 following full cleanup. Fly ash samples were analyzed by HRGC/HRMS following full cleanup (8).

## RESULTS AND DISCUSSION

A PCDD/F EIA which is TEQ responsive must satisfy three critical criteria: 1) the assay must be responsive to picogram quantities of the most toxic PCDD/F congeners (i.e., those with the highest TEF: 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD and 2,3,4,7,8-PeCDF) to support part-per-trillion analysis, 2) the assay must selectively respond to the 17 PCDD/F congeners which are used in the determination of TEQ and 3) the assay must respond to the 17 TEQ determinant congeners in proportion to their TEF values for EIA determination of TEQ. Demonstration that this PCDD/F EIA satisfies these critical criteria requires four key steps:



STEP 1. Establish that the EIA has low picogram sensitivity for 2,3,7,8-TCDD. A study of the 2,3,7,8-TCDD standard curve performed both at CAPE Technologies and three collaborator laboratories over a period of 10 months (data not shown) has defined the minimum detection limit ( $I_{85}$ ) for the assay as 3.9 (sd 1.4) picograms/assay and the assay midpoint ( $I_{50}$ ) as 21.9 (sd 7.4) picograms/assay. This result clearly demonstrates that this PCDD/F EIA has sufficient sensitivity to support part-per-trillion analyses.

STEP 2. Determine that the assays PCDD/F cross-reaction profile is consistent with TEF. Figure 1 shows that the cross-reactivity of this assay, with 2,3,7,8-TCDD defined as a cross-reaction of 1.00, is generally consistent with PCDD/F TEF values. In particular, the figure shows that the five congeners which contribute most to TEQ (2,3,4,7,8-PeCDF, 2,3,7,8-TCDD, 1,2,3,7,8-PeCDD, 2,3,7,8-TDCF, 1,2,3,6,7,8-HxCDD, mean total contribution to TEQ equals 56% over 13 different matrices, data not shown), have cross-reactivities which are comparable to their TEF values.

STEP 3. Demonstrate that the predicted EIA TEQ correlates with HRGC /HRMS derived TEQ. The correlation of HRGC/HRMS derived TEQ with predicted EIA TEQ would provide a clear demonstration of EIA performance. Nineteen (19) fly ash samples were analyzed by HRGC/HRMS. This data was used to derive each samples TEQ. Predicted EIA TEQ was calculated using a simple additive response model based on congener concentration times cross-reaction equals 2,3,7,8-TCDD equivalents and TEQ. Regression analysis of this data set [ $n = 19$ ; slope = 0.97;  $R^2 = 0.999$ ] demonstrated that this EIA effectively predicts TEQ. A similar study using thirteen soil samples [regression analysis:  $n = 13$ ; slope = 0.99;  $R^2 = 0.997$ ] gave a similar result. These results clearly establish that the EIA responds to the seventeen TEQ determinant PCDD/F congeners in proportion to their TEF values.

STEP 4. Demonstrate that actual EIA TEQ data correlates with predicted EIA TEQ. The results given in Table I establish that the EIA is responsive to the seventeen PCDD/F congeners. However, the 193 PCDD/F congeners which are not included in the calculation of TEQ may have a significant effect on the EIA. The same group of 19 fly ash samples were subjected to a HRGC/HRMS sample extraction and clean-up protocol. The results of EIA analysis of these samples are presented in Figure 2. Figure 2 illustrates that the actual EIA TEQ determined for these fully cleaned samples correlates with the predicted EIA TEQ [ $n = 19$ ; slope = 1.04;  $R^2 = 0.97$ ]. A similar result was obtained with a set of soil samples [ $n = 13$ ; slope = 0.64;  $R^2 = 0.96$ ]. These results clearly establish that this EIA specifically responds to the seventeen TEF/TEQ congeners in the presence of the other PCDD/F congeners which do not contribute to TEQ.

## CONCLUSION

The results presented here establish the validity of the concept of TEQ measurement by immunoassay. Moreover, these results demonstrate the use of this EIA for the measurement of PCDD/F TEQ in real samples.

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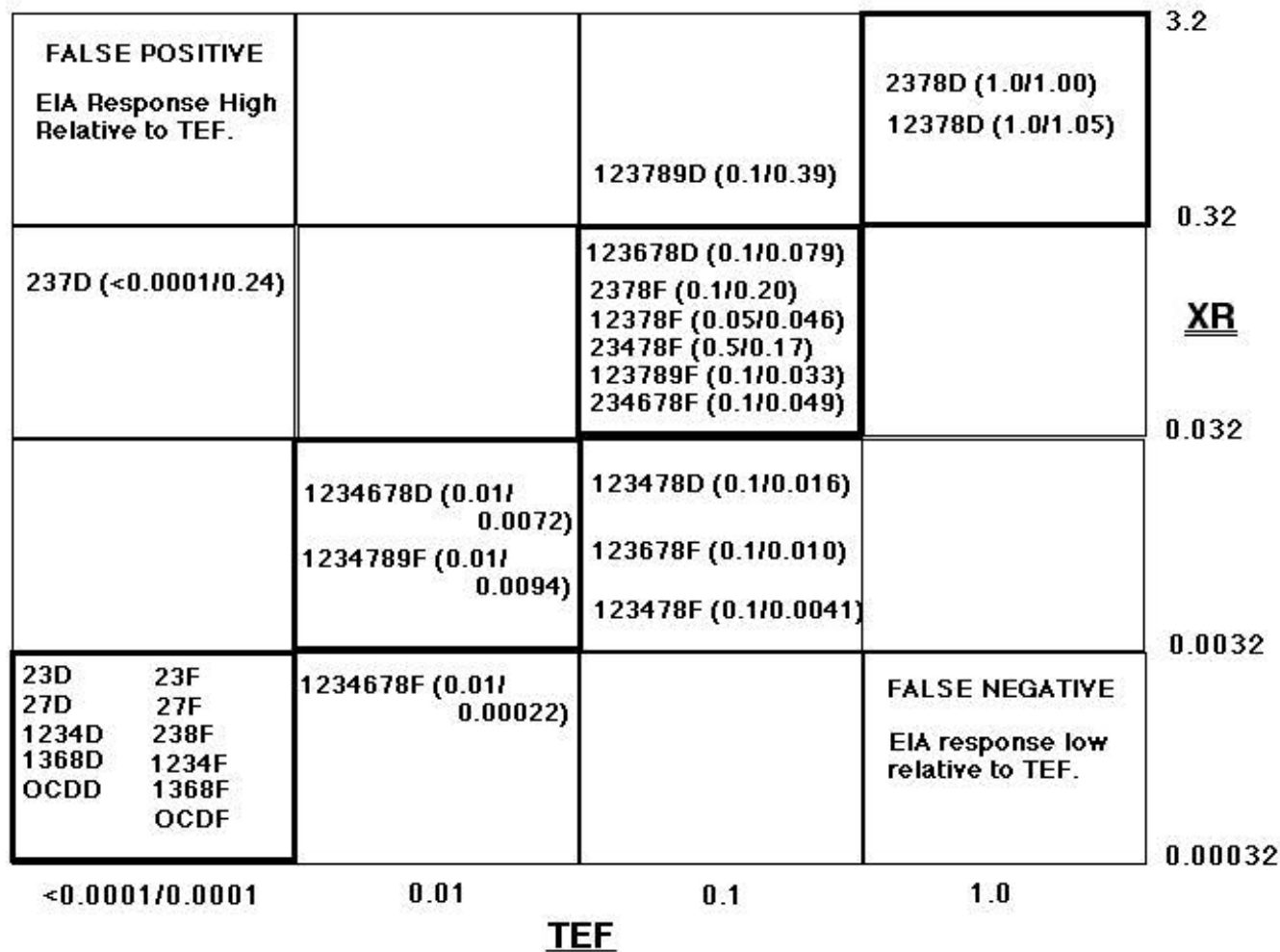


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FIGURE 1. CORRELATION OF PCDD/F TEF VALUE TO IMMUNOASSAY CROSS-REACTIVITY. TEF - PCDD/F Congener Toxicity Equivalence Factors; XR - Immunoassay Cross-reactivity; Congeners shown as e.g., 2378D is 2,3,7,8-TCDD.

**[CONGENER (TEF/XR)]**



### FLY ASH DATA: Predicted versus Cleaned EIA

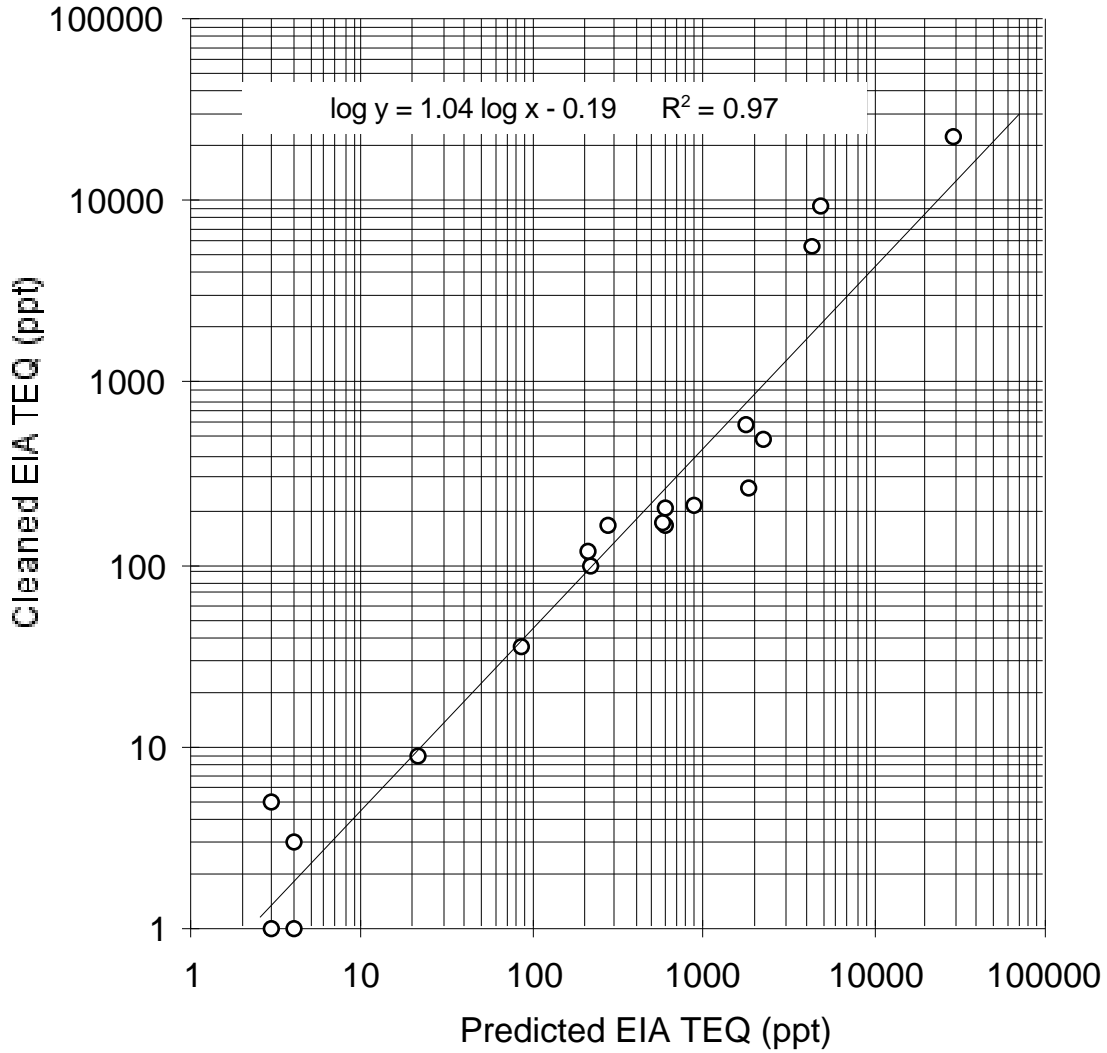


FIGURE 2. CORRELATION OF PREDICTED EIA TEQ TO CLEANED EXTRACT EIA TEQ FOR FLY ASH SAMPLES. Regression linefit as shown.