Measurement of PCDD/F TEQ by Immunoassay: Demonstration Using Real World Samples

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INTRODUCTION

The use of enzyme immunoassay (EIA) kits to rapidly screen environmental samples has dramatically simplified field analysis of PCBs and other difficult analytes over the last 8 years (1). Several kit based methods have received regulatory approval and are now widely used in the assessment and remediation of hazardous waste sites (2). For more than 2 decades, researchers have sought to advance immunoassays for polychlorinated dibenzo-*p*-dioxins and polychlorinated dibenzofurans (PCDD/Fs) to a similar level of utility (1,3). One dioxin EIA kit demonstrated acceptable specificity for TEQ measurement (4,5,6), but its sensitivity was marginal and it is no longer available. Another dioxin immunoassay was successfully used for analysis of a limited number of real samples (7), but only with extraction and cleanup procedures nearly equal in rigor to those required for HRGC-HRMS analysis. None of these previous immunoassays provided the sensitivity necessary to justify an ongoing investment in immunoassay specific sample preparation protocols.

A more recent EIA (8) has demonstrated low picogram sensitivity, which is an improvement of at least ten-fold over the immunoassays cited above. The PCDD/F congener recognition profile of this test also correlates with congener toxicity (8), potentially allowing TEQ measurement. The use of this EIA for measurement of TEQ in real samples has been validated theoretically (8,9) and by analysis of fully cleaned sample extracts (9). The present study extends the validation of the performance of this EIA to the analysis of TEQ in real samples using an immunoassay specific extraction method with a minimal cleanup step.

MATERIALS AND METHODS

Soil samples were analyzed by HRGC-HRMS using US EPA Method 8290 or 1613 following full cleanup. Fly ash samples were analyzed by HRGC-HRMS following full cleanup (10). Crude toluene extracts of fly ash were oxidized using concentrated sulfuric acid/sulfur trioxide (10). Fully cleaned and oxidized extracts were evaporated onto a detergent keeper. The residue was redissolved in methanol and analyzed directly by EIA (8).

Immunoassay specific extraction of soil samples was performed by shaking with dimethylformamide (DMF). A five gram soil sample and 15-20 g of anhydrous sodium sulfate were blended with a wooden spatula in a 40 mL glass vial. Reagent grade DMF (15 mL) and 3 hexane washed steel mixing beads were added. Extraction vials were capped with Teflon lined caps, laid on their sides, and shaken for 2 hours at 350 rpm on an orbital platform shaker. Vials were centrifuged for 10 minutes at 2000 x g and a portion of the DMF supernatant removed. An aliquot of this extract was shaken for 5 minutes with concentrated sulfuric acid containing 7% (w/w) sulfur trioxide. The mixture was centrifuged, the hexane recovered, and the extraction was repeated twice more. For EIA analysis, a Triton X-100 keeper was added and the extract was exchanged to methanol. Oil content was determined for the soils extracted with DMF. A separate subsample of soil was mixed with sodium sulfate, then extracted by shaking with 1:1 hexane:acetone. An aliquot of this extract was evaporated and the residue weighed.

RESULTS AND DISCUSSION

A multi-laboratory collaboration is currently in progress for evaluation of EIA kit performance and sample preparation methods. This program has 4 goals which must be completed in the following sequence: 1) validate the concept of TEQ measurement by EIA through use of a simple additive model of EIA response to calculate predicted EIA response from HRGC-HRMS data, 2) extend validation to TEQ measurement in real samples through EIA analysis of HRGC-HRMS characterized samples which have the minimum level of interferences, i.e. samples which have been subjected to the full HRGC-HRMS cleanup protocol, 3) extend validation to the analysis of conventionally prepared extracts which have been subjected to reduced levels of sample cleanup, 4) development and validation of immunoassay specific sample preparation procedures which will maximize both the throughput and cost-effectiveness of EIA based PCDD/F analysis. Achievement of the fourth and ultimate goal may require extraction and cleanup procedures which are quite novel with respect to current HRGC-HRMS based PCDD/F analysis procedures. Each matrix for which an EIA application is validated must independently meet all of these four goals.

Results for soil are presented here and results for fly ash are presented both here and elsewhere (10). Other current projects include fish tissue analysis (11) and sediment analysis. The correlation between EIA results and TEQ for fully cleaned soils is shown in Figure 1.

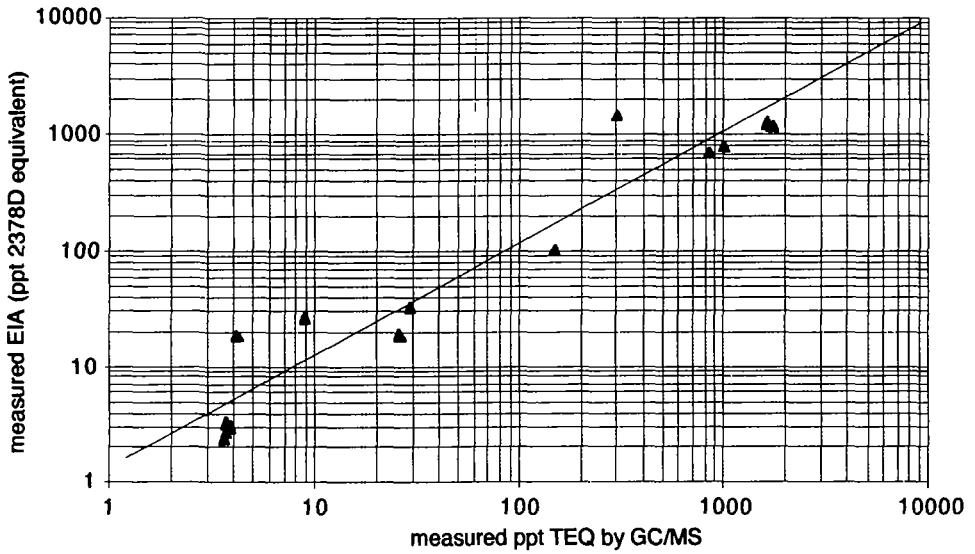

Figure 1. Correlation between EIA and TEQ for 15 fully cleaned soil sample extracts. Fully cleaned conventional soil extracts were analyzed by quantitative EIA and HRGC-HRMS. Individual congener TEF values (12) were used to calculate the TEQ for each congener and these values were summed to give the sample TEQ. These TEQ values were then plotted against ppt measured by EIA. Five soils were below the HRGC-HRMS detection limits for all toxic congeners. For TEQ calculation, the individual concentrations of toxic congeners in these samples were assumed to be one quarter of the individual congener detection limit. The sample TEQ detection limits calculated in this way ranged from 3.5 to 3.8 ppt for these 5 samples. The calculated regression line is shown (R^2 = 0.93). These results clearly establish the ability of the immunoassay to measure TEQ at ppt levels in fully cleaned soil extracts.

Results obtained for fully cleaned fly ash samples were similar to the results of Figure 1 (data not shown; TEQ range from 3 to 22,600 ppt, n = 19, R^2 = 0.94). These fly ash results and the results shown in Figure 1 confirm that this EIA can measure TEQ in real samples in the absence of significant matrix interferences. These results also validate the use of the kit for analysis of fully cleaned soil and fly ash samples at low ppt levels.

A novel rapid sample extraction and extract cleanup method was applied to EIA analysis of 9 soil samples from a site contaminated with burned electrical debris. The HRGC-HRMS data from these soils showed a PCDF dominated congener pattern consistent with PCB contamination of the electrical debris prior to burning. Correlation between EIA and HRGC-HRMS derived TEQ is shown in Figure 2.

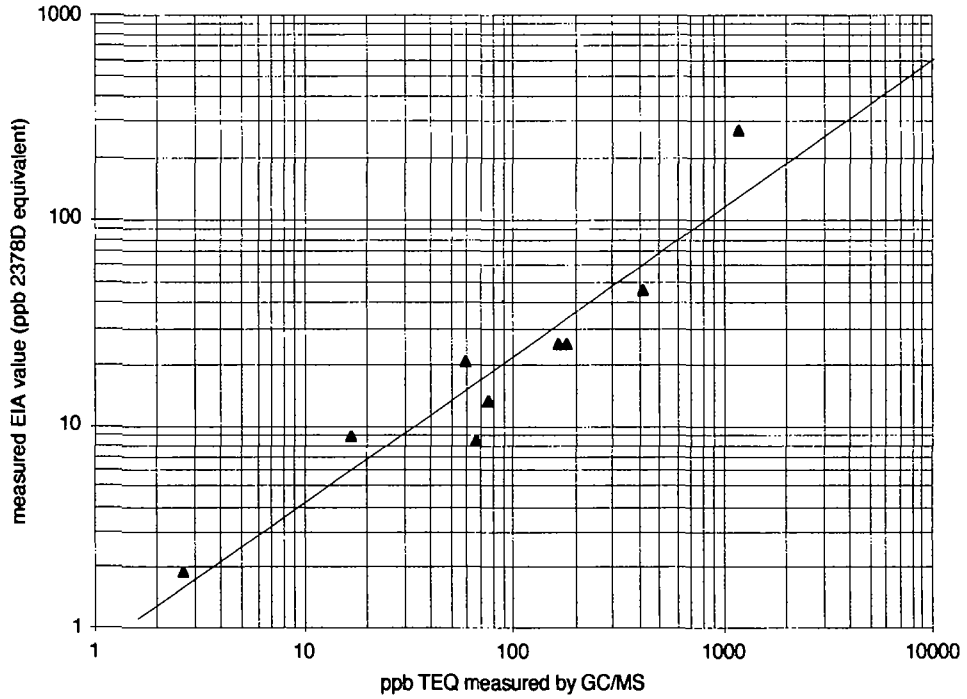

Figure 2. Correlation between EIA specific extracts and HRGC-HRMS derived TEQ for 9 soil samples. DMF extracts of soils were oxidized and analyzed by quantitative EIA. Conventional extracts of the same soils were analyzed by HRGC-HRMS. Sample TEQ values were calculated as for Figure 1. The calculated regression line is shown (R^2 = 0.90). These results clearly establish the ability of the immunoassay to measure TEQ at ppb levels in soil samples using an immunoassay specific sample extraction and cleanup.

Dimethylformamide (DMF) was selected for development of the EIA specific extraction because it is immiscible with aliphatic hydrocarbons and is an excellent solvent for PCDD/Fs. This approach was essential for these samples, which had a mean oil content of 3.5% (range 0.2 to 7.2). The oil content of these samples did not correlate with TEQ. Following the oxidation step, the residue after evaporation was not visibly different among the nine samples, confirming the value of the DMF extraction for removal of the residual oil. The results for these samples are shown in Figure 2. These results clearly establish the ability of the immunoassay to measure TEQ at ppb levels in soil samples using an immunoassay specific sample extraction and cleanup. The entire procedure of extraction, cleanup, and EIA analysis represented in Figure 2 can be completed in less than 8 hours.

CONCLUSION

Validation of this EIA for TEQ measurement has proceeded by the four steps outlined above. First, it has been demonstrated that PCDD/F congener profiles typically found in real samples are compatible with the prediction of TEQ using the EIA (8,9). Second, correlation between EIA response and TEQ has been demonstrated for both soil and fly ash samples here and elsewhere (10) using fully cleaned extracts to minimize matrix interferences. Third, correlation between EIA response and TEQ has been shown using an immunoassay specific cleanup for conventionally extracted fly ash samples (10). Finally, an immunoassay specific extraction/cleanup protocol has been demonstrated here for soils. These results clearly demonstrate the ability of this EIA to measure TEQ at meaningful levels in real samples and in the presence of significant amounts of sample matrix.

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