DIOXIN SCREENING RESULTS IN SOIL AND SEDIMENT USING THE PROCEPT ARYL HYDROCARBON BASED POLYMERASE CHAIN REACTION ASSAY

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Introduction

The United States Agency for Toxic Substances and Disease Registry guideline screening concentration for polychlorinated dibenzo-*p*-dioxins and furans (PCDD/F) in residential soils near or on hazardous waste sites is 50 pg/g (50 parts per trillion, ppt) toxicity equivalent quotient (TEQ) of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (2,3,7,8-TCDD).[1] The standard method for measuring PCDD/F is gas chromatography-high resolution mass spectrometry (GC-HRMS). However, a more rapid technique capable of screening at 50 ppt TEQ, could allow more detailed and efficient assessment of contaminated sites and more efficient use of GC-HRMS resources. The Procept Rapid Dioxin Assay (Eichrom Technologies, Inc.) is an Aryl hydrocarbon-Receptor (AhR) based assay which utilizes Polymerase Chain Reaction (PCR) to quantify levels of PCDD/F in samples[2] in either a quantitative or screening mode.

Materials and Methods

The Procept Rapid Dioxin Assay was obtained from Eichrom Technologies, Inc. and Hybrizyme Corporation. Analytical standards were obtained from Cambridge Isotope Laboratories. HPLC grade solvents and silica gels were obtained from Sigma Aldrich. Diatomaceous earth and accelerated solvent extraction supplies were obtained from Dionex. Deionized water was obtained from a Milli-Q2 water purification system. PCR reagents were obtained from Stratagene, Inc.. Spiked soils and sands were prepared by drying and homogenizing playground sand and potting soil, suspending the sand or soil in hexane in a glass round bottom flask, adding a mixture of tetra- to octa- chlorinated dibenzo-*p*-dioxins and furans, mixing the suspension by rotation on a rotary evaporator, and carefully removing the hexane under vacuum. The samples were then placed into sealed glass containers and further homogenized by shaking thoroughly. Real world samples were obtained from Battelle, through the US EPA Superfund Innovative Technologies Evaluation (SITE) program.

Soil samples were extracted and prepared as described previously,[3,4] using accelerated solvent extraction (ASE) with a 7:3 mixture of toluene:acetone. Multilayer silica and Florisil columns were used to isolate the PCDD/F fraction from interfering compounds, such as polycyclic aromatic hydrocarbons (PAH) and polychlorinated biphenyls (PCB). Purified soil extracts were then measured using the Procept assay and TEQ values obtained using the following formula:

$$TEQ_{sample} = \frac{(TEQ_{extract})(DF)(V)(RF)}{M} \qquad \text{(eqn. 1)}$$

$$RF = \frac{\text{TEQ of Recovery Standard by GC-HRMS}}{\text{TEQ of Recovery Standard by Procept}} \qquad \text{(eqn. 2)}$$

Where $TEQ_{extract}$ = TEQ of the purified extract output by the PCR, DF = dilution factor of purified extract, V = volume of purified extract (in mL), RF = recovery factor, M = dry weight of sample (in grams). The recovery factor is calculated using a recovery standard processed through the same sample preparation as the unknown samples. This recovery standard can be a soil of known TEQ or a mixture of standards of known TEQ. The recovery standard, depending on its composition, can serve several purposes, including a chemical yield monitor (in place of carbon-13 labeled standards, which cannot be used with this method because they respond identically to native PCDD/F compounds).

Results and Discussion

Figure 1 depicts the comparison of TEQ measured by the Procept assay vs TEQ measured by GC-HRMS for nearly 200 determinations of spiked soil and sand and real world samples from US EPA superfund sites using a recovery standard consisting of a sample from the same site which had been analyzed by GC-HRMS (site-specific recovery standard). The Procept assay results directly correlate (R^2 = 0.94) to the GC-HRMS results in a nearly 1:1 ratio (slope = 0.97) and yielded 5 false positive results and no false negative results.

Figure 2 depicts the comparison of the TEQ measured by the Procept assay vs the TEQ measured by GC-HRMS using a single recovery standard sample for all of the samples consisting of a mixture of tetra- to octa-chlorinated dibenzo-*p*-dioxins and furans (no site-specific recovery standard). Using a single recovery standard for all samples, the correlation is still high (R^2 = 0.94), but the slope of the best fit line through the data decreases to 0.78 and the incidence of false positive results increases to 4.2%.

Figure 3 depicts the comparison of the TEQ measured by the Procept assay vs the TEQ measured by GC-HRMS without using a recovery standard. The TEQ measured by the Procept Assay is still highly correlated to the GC-HMRS TEQ (R^2 = 0.94, slope 0.78). However, the Procept assay tends to overestimate the TEQ of samples by a factor of ~3. This overestimation is due to a combination of factors including differences in the cross-reactivity on the Procept assay and the WHO TEF values of individual PCDD/F congeners and the presence of PCDD/F-like compounds which have a response on the Procept assay, but have not been assigned a TEF value.[2,5]

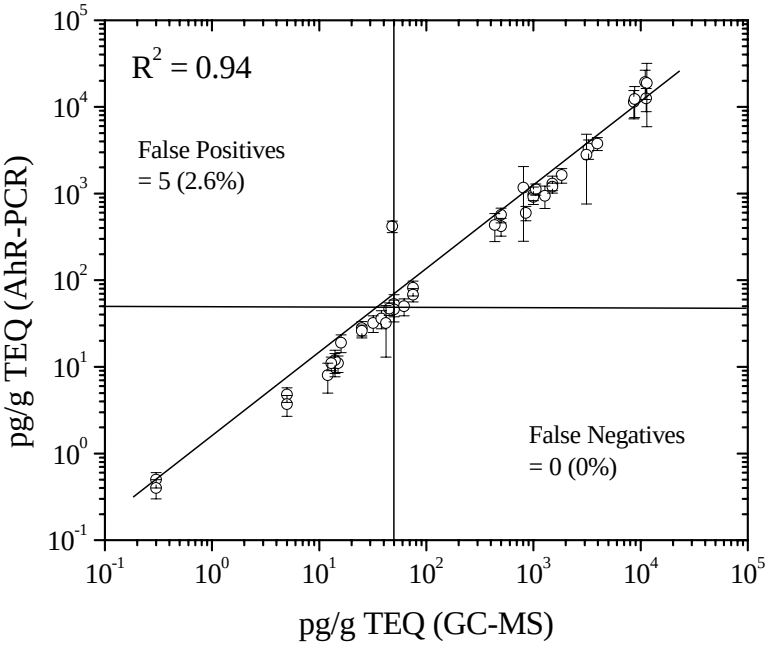

Figure 1. Soil Screening Results using Procept Assay (Site Specific Recovery Standard)

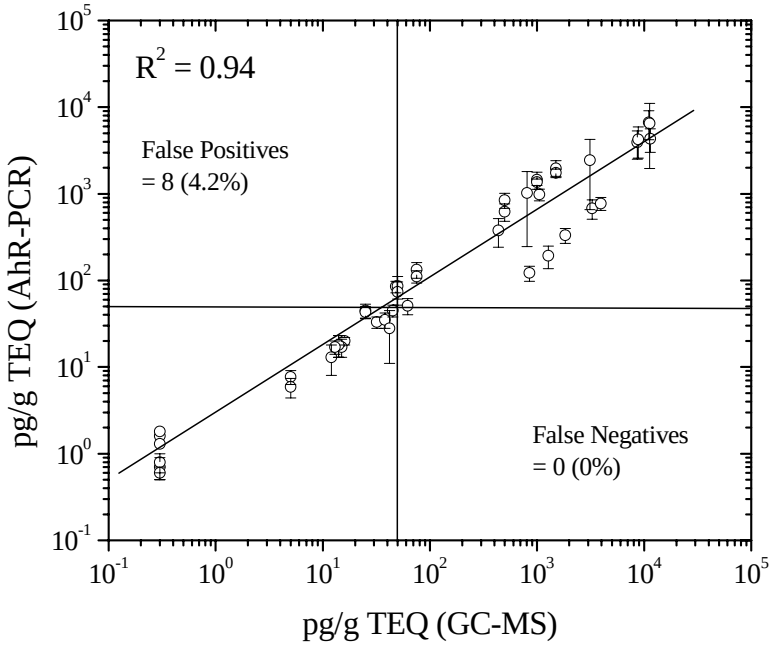

Figure 2. Soil Screening Results using Procept Assay (Single Recovery Standard for all samples)

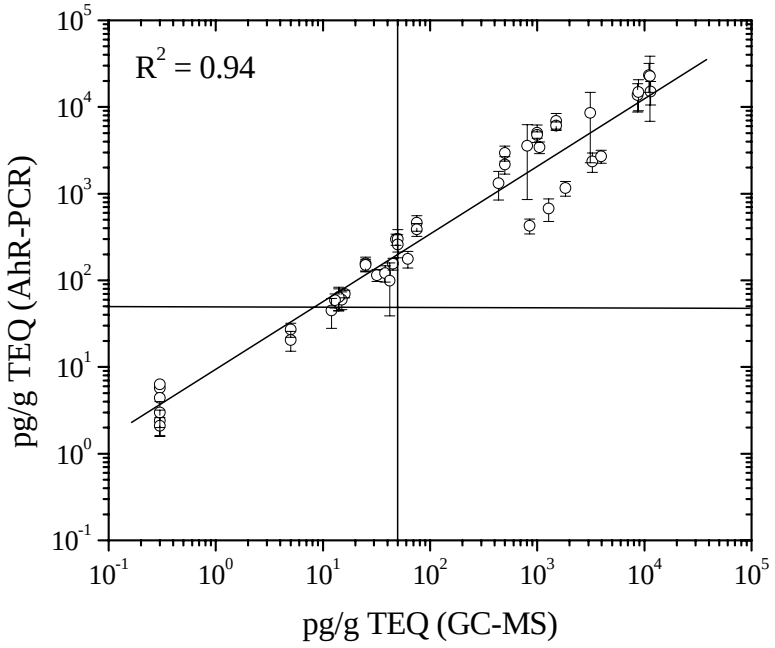

Figure 3. Screening Results using Procept Assay (No recovery standard)

In summary, using either a site-specific recovery standard or a single recovery standard to normalize the data, effective screening results at 50 pg/g 2,3,7,8-TCDD TEQ can be achieved using the Procept assay. The best correlation with GC-HRMS data is obtained when a site specific recovery standard is used. Running the Procept Assay without a recovery standard yields results which are still highly correlated to GC-HRMS, but which can exhibit a large positive bias.

References

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