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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION I
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Memorandum

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Subj: Dioxin/Furan Immuno Assay Test Kit Correlation Study

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Introduction:

On May 15 - May 23, 2000, a study to compare high resolution gas chromatography - high resolution mass spectrometry dioxin/furan analytical results to enzyme immunoassay test kit dioxin/furan screening results was conducted in the NERL Mobile Laboratory on a set of dioxin laden soils/sediments from the Centredale Manor site in Providence, RI.

The objective of this correlation study was to determine if the CAPE Technology Enzyme Immunoassay Test Kits are a viable option for generating quick turn-around, quality screening data for future investigations at the Centredale Manor site, and possibly other dioxin contaminated sites. A secondary purpose of the study was to use the exercise to train NERL in the use of the CAPE test kit.

A total of forty-six soil and sediment samples were screened using CAPE Technologies High Performance Dioxin/Furan Immunoassay Kit, which is an Enzyme Immunoassay (EIA) for analysis of Polychlorinated Dibenzo Dioxins and Polychlorinated Dibenzofurans (PCDD/Fs) in prepared sample extracts. EIA screening results were compared to EPA Method 8290A (Polychlorinated Dibenzodioxins and Polychlorinated Dibenzofurans by High-Resolution Gas Chromatography/High-Resolution Mass Spectrometry) results generated by one of two commercial laboratories (Quanterra or Triangle Laboratories).

PCDD/DFs are a family of compounds with the same general structure. There are 75 dibenzodioxin congeners and 135 dibenzofuran congeners, containing from 1 to 8 chlorine atoms on the dibenzodioxin or dibenzofuran nucleus. Only 7 of the 75 PCDD congeners and 10 of the 135 PCDF congeners contain the 2,3,7,8 chlorination pattern thought to be required for dioxin-like toxicity. Only these 17 of the 210 total PCDD/F congeners contribute to the Toxic Equivalency (TEQ) of a sample, which is generally the critical analytical target. Based on a variety of toxicity tests, these 17 congeners have been assigned Toxic Equivalency Factors (TEFs) of 1.0 to 0.0001 relative to 2,3,7,8-tetrachlorodibenzo-p-dioxin.

Methodology:

For this study, the CAPE Technologies EIA Test Kits (see method reference below) were used to determine if a sample contained PCDD/Fs above or below a 500 part per trillion (ppt) Toxic Equivalency (TEQ). The EIA results were compared to EPA reference Method 8290A (8290A). Soil and sediment samples for the comparison study were selected from the large number available from prior work at Centerdale. An attempt was made to insure that the majority of samples selected had PCDD/DF concentrations of approximately 1 part per billion (ppb). All samples were taken from archived material that had previously been analyzed using 8290A by one of the two contract laboratories used in the Centerdale investigation of 1998-99.

EIA assay samples were weighed out, dried with sodium sulfate and extracted using dimethyl formamide (DMF). A portion of sample extract was then added to hexane and oxidized with fuming H_2SO_4 . A final solvent exchange from hexane to methanol was followed by an immunoassay incubation procedure. Samples were analyzed in four groups, generating four data sets (one per each immunoassay procedure) of EIA data.

Results:

Four separate groups of soil/sediment samples were extracted, oxidized and analyzed using EIA. Tables 1-4 show the correlation between the immunoassay screening analysis and TEQ as determined by 8290A, in of each of the fours sample sets.

In the Tables EIA results are reported as: CP = confirmed positive (>500ppt), CN = confirmed negative (<500 ppt), FP = false positive (>500ppt when 8290A results were reported as <500ppt), and FN = false negative (<500 ppt when 8290A results were reported as >500ppt).

Table 1**Sample Set 1 Correlation**

<u>Sample #</u>	<u>ppt TEQ by EPA Method 8290A</u>	<u>EIA Results</u>
D00867	956	CP
D00984	729	CP
D01033	2280	CP
D01425	325	FP
D00992	530	CP
D00982	3340	CP
D00852	323	CN
D00852 Assay Dup	323	FP
CMS237B	1200	CP
CMS237B Assay Dup	1200	CP

Table 2**Sample Set 2 Correlation**

<u>Sample #</u>	<u>ppt TEQ by EPA Method 8290A</u>	<u>EIA Results</u>
D01065	508	CP
D01398	169	FP
D01055	1380	CP
D01069	3260	CP
CMS423D	120	CN
CMS237A	7100	CP
D00993	792	CP
D01448	534	CP
D01448 Assay Dup	534	CP
CMS417A	2700	CP
D00863	2.9	CN
D00863 Lab Dup	2.9	CN
D00983 Lab Dup	3160	CP

Table 3**Sample Set 3 Correlation**

<u>Sample #</u>	<u>ppt TEQ by EPA Method 8290A</u>	<u>EIA Results</u>
D00983	3160	CP
CMS025A	33000	CP
D01093	1500	FN
D01429	418	FP
D01468	148	CN
D00995	531	FN
D00870	118	CN
D00862	176	CN
D01070	3090	CP
D00861	120	CN
D01057	2380	CP
D01159	7.6	CN
D01422	388	CN
CMS417C	1900	CP
CMS417C Assay Dup	1900	CP
D01062	591	FN
D01062 Assay Dup	591	FN

Table 4**Sample Set 4 Correlation**

<u>Sample #</u>	<u>ppt TEQ by EPA Method 8290A</u>	<u>EIA Results</u>
CMS024B	75000	CP
D00849	1050	CP
D00981	2070	CP
D01141	237	CN
D01033	2280	CP
CMS025B	350	FP
CMS417A Lab Dup	2700	FN
D01403	1110	CP
D01139	6800	CP
CMS237C	350	CN
D00864	982	CP

Table 4 (cont'd)

<u>Sample #</u>	<u>ppt TEQ by EPA Method 8290A</u>	<u>EIA Results</u>
D00866	2960	CP
CMS417B	1400	CP
CMS417B Assay Dup	1400	CP
D01052	4250	CP
D01052 Assay Dup	4250	CP

Result Summary:

Fifty-six actual immunoassay assays were performed on the forty-six field samples. The 10 replicate analyses included lab duplicates (taking a duplicate sample completely through the analysis scheme) and assay duplicates (taking a duplicate of the final prepared extract through the final stage [immunoassay step] of the analysis). In summarizing the results of the fifty-six assays performed we had:

34 Confirmed Positives
12 Confirmed Negatives
5 False Positives
5 False Negatives

In evaluating a real scenario situation for these forty-six samples, where if a sample was positive and a duplicate was negative, the worst case result would be chosen, we had:

28 Confirmed Positives
10 Confirmed Negatives
5 False Positives
3 False Negatives

Table 5 summarizes the false positive and false negative results from the complete set of assays performed.

Table 5**False Positive and False Negative Correlations**

<u>Sample #</u>	<u>ppt TEQ by EPA Method 8290A</u>	<u>EIA Results</u>
D01398	169	FP*
D00852 Assay Dup	323	FP@
D01425	325	FP@
CMS025B	350	FP@
D01429	418	FP@
D00995	531	FN@
D01062	591	FN@
D01062 Assay Dup	591	FN@
D01093	1500	FN*
CMS417A Lab Dup	2700	FN*

*Lack of correlation between methods and outside of 8290A RPD range

@Lack of correlation between methods but within 8290A RPD range

Discussion:

There are several confounding factors that must be considered when interpreting this inter-method comparison data:

- ▶ The field precision of the 8290A PCDD/DF analyses in Centerdale soils averaged 41% and ranged from near 0 to well over 100 percent for field replicates
- ▶ The actual percent(%) moisture was not determined during the EIA analytical episode. Percent moisture of the sample was assumed based upon data provided by the contract laboratories that performed the 8290A analyses. Actual sample moisture content at the time of EIA screening might have been different if the samples lost or gained moisture in storage between analyses.

If the observed 41% average RPD between 8290A field duplicates is factored into the above correlation, then, for example, the 531 ppt result for D00995 could range from 313 ppt to 749 ppt. Therefore the EIA negative result (<500ppt) may in fact be correct and the observed difference between the EIA and 8290A assays may simply be the result the imprecision of the 8290A measurements.

The observed average 41% RPD for 8290A data could not however fully explain the false negative at 1500 and 2700 ppt nor false positive at 169 ppt. Several replicate pairs for the 8290A work exceeded 100% RPD suggesting that even these contradictions might be largely attributable to the imprecision of the 8290A measurement. The observed RPD between field duplicates represents a combined measure of the homogeneity of the sample as well as inherent acceptable error in two different methods

The possibility of error due to using the incorrect % solids may also enter into the interpretation for the relatively close comparisons but this effect cannot be quantitated.

All of the above confounding factors must enter into the interpretation of this data set. Using the RPD factor alone could account for 7 out of the 10 observed contradictory results. The actual comparability of the methods could be further clarified with additional field duplicates and more comparison analyses, however this study has demonstrated the suitability of EIA for field screening at the 500 ppt level.

In summary:

The CAPE Technologies EIA Test Kits can generate data that compares favorably with EPA Method 8290A. For future work the comparability of the data will be enhanced by 1.) having a fully proficient analyst (this study was, in part, a training exercise) , 2.) carefully homogenizing soil and sediment samples before analyses - to reduce sample homogeneity errors, 3.) performing percent moisture analysis at the time of sample preparation for each sample, 4.) developing a confirmatory analysis plan for a small percentage of field screened samples.

Using EIA it is estimated that 60-80 samples could be analyzed per week by a single experienced analyst. This would offer the investigator high quality, quick turn-around screening data for 15-20 samples per day with near real time information at roughly 10% of the cost of a full EPA Method 8290A analysis.

In this study, the correlation between the CAPE Technologies Immunoassay kit and the full protocol EPA Method 8290A looks good and quite acceptable for a field screening methodology. In the real life scenario, approximately 83% of the samples were assayed correctly, 11% of the results were false positives and 6% of the results were false negatives. When the mitigating factors listed above are considered the overall performance of EIA seems exceptionally good.

Reference

Cape Technologies High Performance Dioxin/Furan Immunoassay Kit, DF1 Kit Insert (IN-DF1), page 1, 2/2/00.