IMMUNOASSAY ANALYSIS OF DIOXIN IN SOIL: VALIDATION OF TEQ SCREENING AT 500 PPT USING RAPID EXTRACTION AND CLEANUP

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Introduction

During the past decade immunoassays for industrial wastes have become widely used for site assessment and for monitoring the progress of remediation projects (1). Regulatory acceptance in the US has expanded to include many of the most common and troublesome analytes, such as PCB's and PAH's (2). But the development of a practical immunoassay method for dioxin has lagged behind because of 3 unsatisfied critical needs, including: A) increased sensitivity compared to other industrial waste tests, B) a particular specificity pattern to allow measurement of TEQ in real samples, and C) rapid sample preparation methods validated at environmentally significant levels to take full advantage of the speed and simplicity of the immunoassay itself. Recently we described an enzyme immunoassay (EIA) capable of satisfying needs A and B (3). Subsequently we showed the ability of this EIA to measure dioxin/furan TEQ at ppt to ppb levels in fully cleaned soil and fly ash extracts (4,5). At the same time we demonstrated the first progress ever toward need C by showing TEQ measurement at ppb levels in oxidized conventional fly ash extracts as well as an immunoassay specific extraction and oxidative cleanup at 10 ppb in soils.

The goal of this study is to provide preliminary validation of an immunoassay based method for rapid screening of dioxin/furan TEQ at sub ppb levels in soil samples. Two rapid cleanup methods were compared using the same dimethylformamide (DMF) extracts. Both methods used a simple oxidation of the DMF extract by SO_3 in conc. H_2SO_4. In the first method the oxidized extract was analyzed by EIA directly after solvent exchange. In the second method the oxidized extract was cleaned further by carbon mini-column before EIA analysis. Preliminary results from both methods indicate success, but with a possibility for improvement of the oxidative cleanup. Work toward this goal is in progress and data based on the final protocol will be presented in September.

Materials and Methods

Soil samples were analyzed by HRGC-HRMS using US EPA Method 1613 after full cleanup. Solvents for EIA sample preparation were HPLC grade or better.

Rapid soil extraction: A 5 g subsample of each soil was extracted with DMF as described previously (4). Extracts were stored in glass vials with Teflon lined caps in the dark at 18-24°C.

EIA protocol modifications: The EIA was performed as described previously (3) with modifications as described elsewhere (6). Sample incubations were typically either 2 or 12 to 20 hours. For Method B, the sample incubation step also included approximately 1.5% tetraethylene

glycol (TEG) from the sample keeper, as described below. Standards for Method B were adjusted to the same final concentration of TEG during the sample incubation.
Method A- oxidation only: DMF extracts were oxidized (4) using 7% SO_3 in conc. H_2SO_4 using 180 µL of DMF extract (equivalent to 60 mg soil) per 1.6 mL of oxidant. Further processing and EIA analysis was as described previously (6,7) except that only a single extraction step was used.
Method B- carbon column cleanup after oxidation: Disposable carbon columns were prepared by packing sections of PTFE tubing with 100 mg of 8% PX-21 activated carbon on Celite 545. Columns were attached to the Luer tip of a 6 mL glass syringe barrel reservoir, the top of which was fitted with a PTFE adapter which contained a female Luer port. Reservoirs were manually pressurized with a polypropylene syringe to maintain a flow rate of 0.5 to 1.5 mL/min. Columns were washed with hexane then loaded with oxidized sample (hexane supernatant from Method A). Columns were reversed, washed with 1:1 dichloromethane:hexane, eluted with toluene into glass tubes containing 25 µL of TEG, then evaporated under a gentle nitrogen stream at 70-75°C. The TEG keeper was collected at the bottom of the tube by brief centrifugation, mixed with 125 µL of 100 ppm Triton X-100 in methanol, and 50 µL aliquots were removed for EIA analysis.
Calculation and semiquantitative interpretation: A standard curve consisting of 0, 3.2, 10, 32, and 100 pg of 2378-TCDD per EIA tube was included with each EIA batch. Duplicate EIA tubes were run for all samples and standards and duplicate optical density values were averaged. Standard curve fitting was performed for each EIA run based on the sigmoid four parameter equation used by commercial immunoassay software such as SoftMax (Molecular Devices Corp.), but using the Solver optimization procedure within Microsoft Excel (8). Based on the calculated curve, the raw TEQ value was determined for each sample. An empirically determined adjustment factor was then applied uniformly to raw TEQ values across all runs and the adjusted results were scored as greater than or less than 500 ppt. Different adjustment factors were used for each sample cleanup method.

Results and Discussion

Figure 1 shows semiquantitative EIA results for the 56 soil samples prepared by both Methods A and B. Figure 1A shows 75% correct results, 0% false negatives, and 25% false positives. Figure 1B shows 70% correct results, 2% false negatives, and 28% false positives. Repeat analyses were performed for 11 of the samples of Figure 1A with no change in the semiquantitative interpretation. Repeat analyses were performed for 8 of the samples of Figure 1B with no change in the semiquantitative interpretation for 7 samples. One sample (39 ppt TEQ) gave different results, both of which are plotted in Figure 1B. These data clearly demonstrate the ability of the EIA to identify soil samples above 500 ppt TEQ by both cleanup methods.

Because of the high (35%) initial false positive rate for Method A, two samples above 500 ppt TEQ and ten samples below 500 ppt were chosen for repeat analysis using a modified oxidation procedure. These samples received a second treatment with fresh oxidant before solvent exchange and EIA. Both positive samples were correctly scored the second time and 7 of 10 negative samples were changed from false positive to correct negative. These results are included in Figure 1A. The improved procedure was not applied to any of the Method B samples (Figure 1B).

This decrease in the false positive rate illustrates that very simple improvements in sample cleanup can have large effects on test performance. Further experiments to optimize oxidation conditions

are in progress. The final oxidation procedure determined through these investigations will be applied to the same set of 56 samples and the resulting data presented at Dioxin '99 in Venice.
Sample preparation by Method A is simpler and faster than by Method B, but it may not be suited to all samples. About 10% of the samples in this study appeared milky during the sample incubation step of the EIA. Some of these same samples also left a clear oily residue after oxidation and hexane evaporation. The samples which left the most oil residue did not go completely into solution in methanol-Triton during the redissolving step, probably giving only partial recovery of analyte for introduction into the EIA.

Milkiness during the EIA sample incubation indicates that the amount of oil extracted by the DMF and carried through the oxidation step exceeds the capacity of the EIA's solvent-detergent system, leading to phase separation. When this occurs, the TEQ as measured by the EIA can be reduced by partial sequestering of the analyte in the oil phase. In the present study however, all the oily samples were scored correctly by Method A at the 500 ppt decision level. In contrast to Method A, none of the samples prepared by Method B retained enough oil after the carbon mini-column cleanup to cause milkiness during the EIA, indicating effective oil removal. Therefore Method B can be used to address high oil content in the EIA due to highly oily samples or large matrix loads.

Conclusion

This preliminary study is the first description of rapid immunoassay screening of dioxin in real samples at sub ppb levels. The false negative rates are acceptably low by both sample preparation methods. However, because of the striking improvement in false positive rate with a minor protocol change, these results must be viewed as a work in progress. Preliminary data from optimization of the oxidative cleanup procedure indicate that a significantly lower false positive rate should be obtainable with relatively minor changes.
This method can be performed in a simple field lab and should allow most of the benefits of industrial waste immunoassays to be extended to dioxin analysts. The novel extraction and simple cleanup methods described allow same day batchwise analysis of many samples. The low cost, rapid turnaround time, and portability of this system offer a completely different approach to dioxin analysis which should provide an excellent complement to conventional methods.

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Figure 1.
Preliminary EIA screening results for 56 soils with two sample preparation methods. Samples are grouped according to their semiquantitative EIA result (above or below 500 ppt), then by TEQ within each group. The GC-MS TEQ values for samples over 1000 ppt ranged from 1088 ppt to 1.5 ppm. A) DMF extracts of soils were oxidized, exchanged from hexane to methanol, then analyzed by semiquantitative EIA. An empirically determined adjustment factor was applied to each raw concentration value before scoring against the 500 ppt target level. B) DMF extracts of soils were oxidized, cleaned by carbon mini-column, exchanged from toluene to methanol, then analyzed by semiquantitative EIA. An empirically determined adjustment factor was applied to each raw concentration value before scoring against the 500 ppt target level. One sample (39 ppt TEQ) gave different results in two runs, both of which are plotted. Results for 12 samples based on an improved oxidation protocol (see text) are incorporated into Figure 1A, but not Figure 1B.