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APPLICABILITY OF MODIFIED EPA METHOD 4025 (IMMUNOASSAY) FOR DIOXIN SITE ASSESSMENT

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INTRODUCTION

Conventional testing for polychlorinated dibenzodioxins and polychlorinated dibenzofurans (hereafter referred to as dioxins) uses EPA Method 8290[1] for low pg/g levels. This method is expensive and has a relatively slow analytical cycle time. This constrains the number of samples that can be economically tested in projects, leading to uncertainties in the application and interpretation of results for management of site remediation. Kit based analysis, such as embodied by the US EPA's 4000 series of SW-846 methods[2,3], is one way of overcoming these problems.

In 2002 the US EPA Office of Solid Waste and Emergency Response (OSWER) accepted Method 4025 (Dioxin Screening in Soil by Immunoassay) for inclusion in the SW-846 Compendium of Solid Waste Methods[4]. Because the one-step cleanup of Method 4025 was designed for semiquantitative soil analysis at 500 pg/g and is not appropriate for low pg/g levels, highly oily soils, or quantitative analysis, a simple kit based adaptation of the Method 8290 cleanup has been developed to allow immunoassay analysis in these situations. The resulting integrated approach (here referred to as modified Method 4025 or Method 4025m) deals effectively with high levels of various interferences while maintaining both low cost and high sample throughput. The extraction and clean-up require no expensive equipment, use simple hardware and protocols, and can be done on a batch of 20 samples per analyst per day.

This paper presents information arising from the use of Method 4025m at a former sewage treatment plant near Melbourne, Australia, where the samples contained a mixture of contaminants (including oily residues) and a variable organic carbon content. Method 4025m data were combined with selected Method 8290 data to develop immunoassay calibration adjustment factors specific to three distinct congener profiles found on the site. The combination of careful immunoassay calibration and classification by congener profile enable quantification of dioxin concentrations in the range of approximately 30 pg/g to 500 pg/g, which spans the clean-up criteria for the site.

MATERIALS AND METHODS

All materials in contact with samples at any stage of the sample preparation were either fluoropolymer (PTFE or FEP), borosilicate glass, or stainless steel, with the exception of medical grade wooden tongue blades for initial sample weighing. All solvents were HPLC grade or better. Toluene was ultrapure grade for residue analysis (Burdick & Jackson). Immunoassay kits and

sample preparation materials, including acid silica columns, activated carbon mini-columns, and hardware for rapid manual processing of sample extracts, were provided by CAPE Technologies (Figure 1).

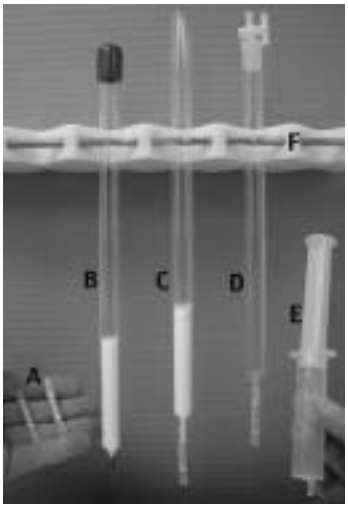


Figure 1. Hardware used for soil extract cleanup

A) disposable activated carbon mini-columns (Teflon housing)
B) acid silica column with shipping seals (glass housing)
C) acid silica column with carbon mini-column attached
D) glass reservoir for washing and eluting carbon mini-columns, with stopper-stopcock assembly in top and carbon mini-column attached to bottom
E) polypropylene syringe for manual pressurization
F) 12 position spring rack for holding acid silica columns and reservoirs

Samples were extracted by mixing 20 g with sodium sulfate and shaking with 50 mL of 1:1 hexane:acetone for 4 hours. After brief centrifugation, the supernatant extracts were removed and exchanged to approx. 100 µL paraffin oil, for acetone removal and for shipping or holding before analysis. Tetradecane (1 to 2 mL) was later added and the extract sonicated for 15 min. Aliquots of extract representing 1.0 to 1.3 g of original sample were removed for cleanup and immunoassay analysis. Extract cleanup was performed using the acid silica-activated carbon coupled column system described in CAPE Technologies Application Note AN-008[5].

Acid silica columns contained neutral silica below the acid silica, with anhydrous sodium sulfate and an inert retaining filter at each end. The carbon mini-column was placed on the tip of the acid silica column so that solvent passed directly from the acid silica into the carbon mini-column. Samples and solvents were added to the top of the acid silica column and a silicone rubber stopper with a stopcock was placed on the top. Solvents were then driven through the coupled column system by injecting air through the stopcock to maintain a flow rate of 1-2 mL per minute.

Coupled column systems were prewashed with hexane, loaded with sample in less than 1 mL tetradecane and paraffin oil, then washed with hexane. The acid silica column was then discarded and the carbon mini-column was transferred to a clean glass reservoir. The carbon mini-column was washed in the forward direction with 6 mL of 1:1 toluene:hexane, then reversed on the same reservoir and eluted with 12 mL toluene. The extract cleanup procedure was typically performed in batches of 14 field samples and 4 quality assurance (QA) samples. Toluene eluates were evaporated under a gentle nitrogen stream after the addition of a keeper solution containing Triton X-100 detergent and tetraethylene glycol (TEG). After complete removal of the toluene, sample tubes were centrifuged briefly to collect the keeper in the bottom of the tube. Methanol was added to the sample tubes and after brief mixing, the sample was introduced to the immunoassay according to the kit insert[6]. The immunoassay was completed according to kit insert instructions. Data from standards and samples were entered into a specially built Microsoft Excel spreadsheet which performs sigmoid curve fitting and calculates sample concentrations based on the standard curve[7].

RESULTS AND DISCUSSION

Over 200 field samples were analyzed by this method in 8 weeks by one analyst in a laboratory no more sophisticated than a typical field lab. Numerous quality assurance samples were run within Method 4025m, including method blanks, spiked method blanks, duplicates, spiked samples, and controls on different parts of the sample preparation procedure. The results for these QA samples (summarized in Table 1) indicate good quantitative precision and accuracy.

Table 1. Quality assurance data within Method 4025m.

QA sample	n	mean±SD	units (comment)
Solvent exchange negative controls	24	1.9±1.0	pg
Unspiked method blanks	22	2.9±1.7	pg
Solvent exchange positive controls	22	102±20	% of nominal pg (generally 50 pg)
Spikes into method blank extracts	14	79±27	% of nominal pg/g (30 to 195 pg/g)
Spikes into sample extracts	32	67±28	% of nominal pg/g (30 to 390 pg/g)
Duplicate precision (from one extract)	23	13±14	% cv (range 5 to 750 pg/g)

Selected field samples (23 in total) were analyzed by both Method 4025m and Method 8290 to calibrate the immunoassay response against Method 8290. While the overall correlation for this set of samples was high (r = 0.95), uniform application of a single calibration adjustment factor (Figure 2) did not provide adequate accuracy at the project action levels of 500 pg/g and below. The calibration adjustment factor applied to Method 4025m raw data must correct for a variety of influences on the raw result. One of the most important factors is congener profile. Because the immunoassay cross-reactivity data[4] show disproportionately low sensitivity to some congeners, Method 8290 data were examined to determine if congener profile could be an issue. Assessment of 108 samples revealed three distinct congener profiles (Figure 3). Dioxin contamination in type A soils is dominated by 2378-TCDD and 12378-PeCDD. Type B soils are dominated by these two congeners plus 1234678-HpCDD and OCDD. Type C soils are completely dominated by 1234678-HpCDD and OCDD and have very little contribution from 2378-TCDD and 12378-PeCDD. None of the soils showed significant furan contamination.

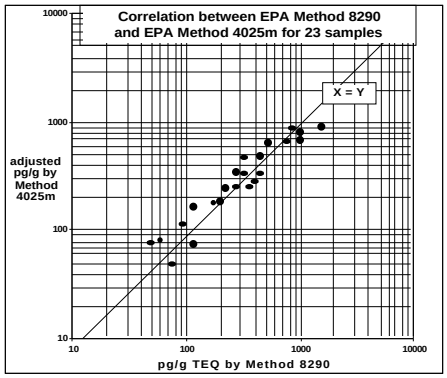
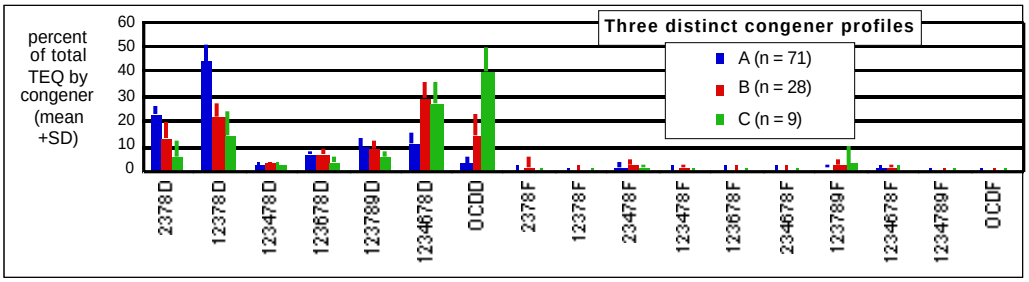


Figure 2. Correlation between Methods 4025m and 8290. All 4025m results were adjusted by a uniform calibration factor, chosen to minimize the total difference between 4025m and 8290.

The presence of multiple distinct congener profiles means that proper interpretation of Method 4025m data for this site requires consideration of the congener composition. Since the majority of site soils were type A, 10 of the 23 paired 4025m and 8290 results representing type A were used for calibration of Method 4025m below 500 pg/g. The correlation shown in Figure 4 (r=0.87) indicates a useful quantitative relationship. Linear regression analysis of the 10 unadjusted 4025m



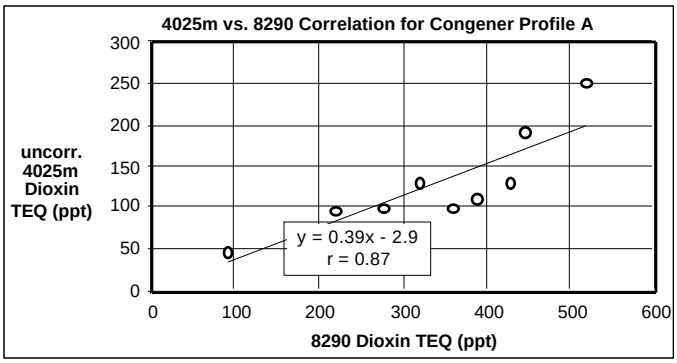


Figure 3 (above). Method 8290 data from 108 samples demonstrate three distinct congener profiles.

Figure 4 (left). Subset of Figure 2 data representing congener profile A samples containing approx. 500 pg/g TEQ and less, plotted on linear-linear scale with calculated regression line and no calibration adjustment.

results and their matched 8290 results gave an intercept near zero (2.9) and a slope of 0.39. The calibration adjustment factor used for any of the 3 soil types from this site will ultimately account for congener profile effects, as well as extraction efficiency and cleanup recovery. This calibration adjustment approach parallels that used by earlier US EPA 4000 series methods[2,3], but differs importantly in allowing site specific correction based on Method 8290 data from each site.

In conclusion, modified EPA Method 4025 has been demonstrated here to be an effective approach for assessing dioxins at sewage treatment plants. It remains simple, low cost, has improved turnaround times compared to 8290, and allows more data to be generated for a specified budget than possible using Method 8290 alone. Information about congener composition (from Method 8290) allow this method to be calibrated against Method 8290 TEQ concentrations so that 4025m may be used as a screening tool. The method is simple enough to be performed in a small field lab, potentially allowing next day or same day results for rapid on site decision-making.

REFERENCES

1. US EPA Method 8290 (http://www.epa.gov/epaoswer/hazwaste/test/pdfs/8290.pdf)
2. US EPA 4000 series Methods (http://www.epa.gov/epaoswer/hazwaste/test/4_series.htm)
3. Immunoassay Techniques in Environmental Analyses (2000) B. Lesnik in Wiley Encyclopedia of Analytical Chemistry, p. 2653, and www.cape-tech.com, Technical Reference TR-005
4. US EPA Method 4025 (http://www.epa.gov/epaoswer/hazwaste/test/new-meth.htm#4025)
5. CAPE Technologies Application Note AN-008 (http://www.cape-tech.com)
6. CAPE Technologies DF1 Kit Insert IN-DF1 (http://www.cape-tech.com)
7. CAPE Technologies Calculation Module C (http://www.cape-tech.com)