Organohalogen Compounds, Volume 60, Pages 271-274 (2003)

COMPARISON BETWEEN A RAPID BIOLOGICAL SCREENING METHOD (EPA 4425) FOR TCDDs/TCDFs AND CHEMICAL ANALYTICAL METHODS

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Introduction

Seven polychlorinated dibenzo-p-dioxins (PCDDs), ten polychlorinated dibenzofurans (PCDFs) as well as twelve polychlorinated biphenyls (PCBs) are collectively referred to as dioxin-like compounds. The World Health Organization toxic equivalency factors (TEFs)[1] for these persistent chlorinated organic compounds and their measured concentrations are used to produce the toxic equivalency quotient (TEQ) of a sample. TEF values are partially based on a common mechanism involving binding of the chemical to the aryl hydrocarbon receptor (AhR). Biological methods for the determination of TEQs are based on the assumption that all dioxin-related compounds act through the AhR signal transduction pathway. Based on the biochemical response of CYP1A activation via the AhR, *in vitro* systems that utilize a reporter gene under transcriptional control of CYP1A have been developed. Several investigations have reported on the success of utilizing biological test systems to detect PCDDs, PCDFs, PCBs in environmental samples [2,3,4,5]. The P450 Human Reporter Gene System assay (EPA Method 4425) utilizes a human hepatoma cell line (HepG2) in which a plasmid containing the human CYP1A1 promoter and 5′-flanking sequences with three xenobiotic responsive elements (XREs) fused to the luciferase reporter gene[6]. The enzyme luciferase is produced in the presence of compounds that bind the XREs, and can be detected by a simple assay that measures relative light units with a luminometer. Method 4425, used by Columbia Analytical Services (CAS), has gained acceptance as a rapid and inexpensive approach for screening solvent extracts of environmental samples of soil, sediment, tissue, and water to detect compounds that activate the AhR [7,8,9]. Investigations in the U.S. and Japan comparing the results of Method 4425 and standard high-resolution GC/MS (HRGC/HRMS) will be reported here. The purpose of making these comparisons is to determine whether risk assessments for large dioxin sites both before and after remediation can be safely evaluated by the combination of analyses over a wide area by Method 4425 and selected samples confirmed by HRGC/HRMS. Such an approach would allow a more comprehensive evaluation of a site, while saving considerable time and costs.

Material and Method

The 101L cells were grown as monolayers in an atmosphere of 5% CO_2 and 100% humidity at 37° C in Eagle's Minimum Essential Media (Mediatech, Herndon, VA), supplemented with 10% fetal

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bovine serum, 2% *L*-Glutamine, and 1% sodium pyruvate. Geneticin is added to the media at 0.4 mg/mL to select for cells that contain the plasmid (which also confers neomycin resistance). All cell culture reagents were obtained from Sigma (St. Louis, MO). The dioxin standard (2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) were obtained from Ultra Scientific (North Kingstown, RI) at 97-99% purity. A standard mixture of the 17 dioxins and furans with TEF values was obtained from Cambridge Isotope Labs Inc. (Andover, MA). The detailed test methodology used by Nihon Environmental Services in Japan and Columbia Analytical Services has been described elsewhere [10,11]. Test solutions (10 µL) were applied to two or more replicate wells (six-well plates) with approximately one million cells in 2 mL of medium. After a 16-hour incubation, the cells were washed with Hank's Balanced Salt Solution (Mediatech, Herndon, VA), and lysed. Cell lysates were centrifuged to remove cell debris, and 50 µL of the supernatant was mixed with 100 µL of a buffer (pH 7.8) before reactions were initiated by injection of 100 µL of luciferin. Luminescence in relative light units (RLUs) was measured using a ML2250 Luminometer (Dynatech Laboratories, Chantilly, VA).

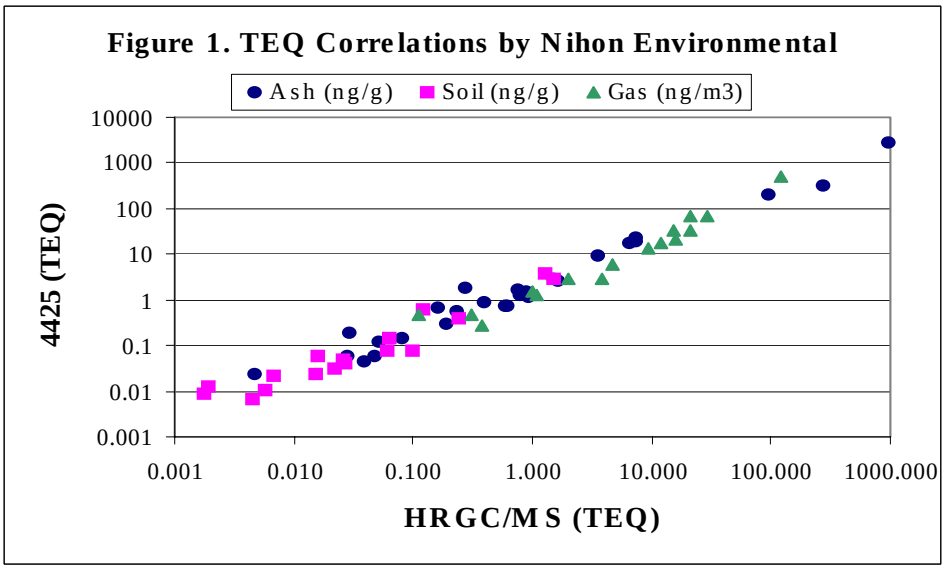
With each test run, replicate wells were tested with a solvent blank (using 10 µL of the solvent mixture), a TCDD concentration of 50 pg/mL, and four concentrations of a standard dioxin/furan mixture (at 5, 10, 25 and 40 pg/mL). Mean fold induction of the solvent blank was set equal to 1, and the fold induction of each solution was determined by dividing the mean RLUs for each solution by the mean RLUs produced by the solvent blank. The standard deviation and coefficient of variation were recorded for each test solution. Comparisons between the results of Method 4425 analyses and HRGC/HRMS results were made in the U.S. using EPA Method 8290 and the official method (JMS700D) in Japan. Nihon Environmental split the same sample extract for testing by both methods, but CAS later obtained a second sample from the jars for HRGC/HRMS confirmation, which could produce more variability.

Results and Discussion

The results of dioxin and furan analyses of bottom/fly ash, exhaust gas and soil or sediment to produce TEQs by both Method 4425 and the HRGC/HRMS method used in Japan are shown in Figure 1 on log scales. Excellent correlations were obtained, independent of the sample matrix. There was a tendency for the Method 4425 data to be somewhat higher (1.51-2.97 times) than HRGC/HRMS results, particularly for soil, where the presence of other compounds with an affinity for the AhR would be likely. A recent study using two *in vitro* biological measurements of PCDDs/PCDFs TEQs in fly ash after various thermal treatment conditions, produced TEQs 1.4 to 5.1 times higher than those obtained by HRGC/HRMS[12].

Testing conducted in the U.S. by CAS since 1994 first followed procedures described in ASTM[13]. In more recent years (since 2000) additional quality assurance and quality control measures have been taken to increase confidence in the consistency of the biological response. Five concentrations (including the solvent blank) of a standard dioxin/furan mixture are added to replicate test wells to produce the curve of the day (Figure 2). The equation from this curve is used to adjust the responses of the test system to produce the final TEQ values for that sample set. Table 1 lists the results of the studies conducted in Japan and the seven different investigations in the U.S. in which the results of Method 4425 analyses were compared with those of HRGC/HRMS analyses on a subset of samples. Some of the earlier testing by Method 4425 did not include silica gel cleanup of the sample extracts before application to the cells. There was

apparent co-contamination by PAHs or other CYP1A-inducing compounds at one of the sites (Pacific Island B), where the ratio of 4425 to 8290 TEQs was high (20). Even with the cleanup step, samples from a municipal waste dump site (ash) likely containing unidentified inducing compounds, produced a higher ratio (21). Since the correlation coefficients for these projects have been reasonably high, the equations from the curves have been used to adjust the Method 4425 data to predicted Method 8290 TEQ values. Substantial project savings can result from the approach of first conducting a comprehensive screening of a large site by Method 4425, before conducting confirmation analyses on 10-20% of the samples by the more expensive HRGC/HRMS methods and then using the correlation curve to predict all other TEQs.



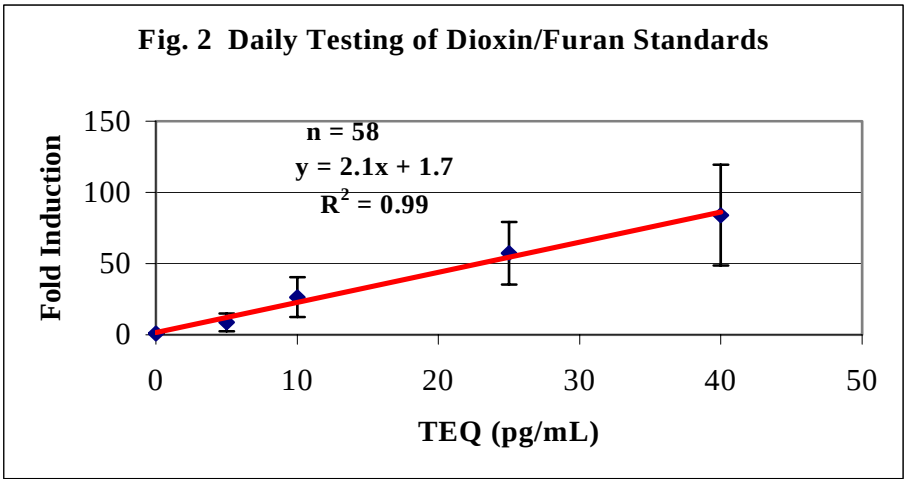


Table 1. Summary of Comparisons Between the TEQ Results of Analyses by Methods 4425 and HRGC/HRMS.

Project Type	Numbers of Samples by Method		Ratio of Results 4425/GC-MS	Correlation Coefficient (r^2)
	4425	8290/JIS		
Bottom Fly Ash	27	27	2.2	0.97
Exhaust Gas	16	16	1.5	0.94
Soil and Sediment	17	17	3.0	0.92
NW Woodtreating Site*	64	13	1.8	0.79
Pacific Island A*	20	5	3.3	0.99
Pacific Island B*	49	6	21	0.97
SE Woodtreating Site	163	14	1.2	0.89
Pilot Study Penta Site	4	4	1.6	0.99
Municipal Dump A -ash	44	14	20	0.79
Municipal Dump B-ash	3	3	1.6	0.99

*Previous extraction methods did not include removal of PAHs by silica gel.

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