COST-EFFECTIVE DIOXIN SITE CHARACTERIZATION USING A P450 HUMAN REPORTER GENE SYSTEM (HRGS; EPA 4425)

Jack W. Anderson[1], Jennifer M. Jones[2], and Daniel L. McCoy[1]

[1] Columbia Analytical Services, 1185 Park Center Dr., Suite A, Vista, CA 92083
[2] Environmental Science and Engineering Program, School of Public Health, University of California, Los Angeles, CA 90095-1772.

Introduction

Dioxins, furans, coplanar PCBs, and some high molecular weight PAHs are known to bind to the Ah receptor (AhR) and subsequently mediate induction of the CYP1A gene, resulting in the production of cytochrome P4501A. This biochemical event is widely used as an indicator of exposure to potentially harmful contaminants[1,2]. Using a transgenic human cell line (101L), stably transfected with a plasmid containing firefly luciferase linked to the human CYP1A1 promoter, the P450 Human Reporter Gene System (HRGS) can detect the presence of CYP1A1-inducing compounds in solvent extracts of water, sediment, soil, and tissue. Solvent extracts are applied directly to the cells and responses, measured as relative light units in a luminometer, are expressed as HRGS Toxic Equivalents (TEQ in ng/g). Approximately one-thousand marine sediment samples from the three coastal areas of the U. S. have been screened for inducing compounds for NOAA[3]. HRGS responses to tissue extracts of deployed mussels correlated highly with measured PAHs[4]. Testing procedures of this assay have been described by ASTM, APHA, and recently by EPA as Method 4425[5,6,7]. This paper will define the QA/QC steps used in the assay, the method used to screen numerous field samples, and illustrate the correlations between the results of method 4425, and the high resolution Mass Spectrometer method (EPA 8290), using EPA TEF values.

Material and Methods

Culturing of the transgenic human cell line and the conduct of testing are described in the methods cited above. Approximately 250,000 cells are added to each well of a 6-well plate in 2 mL of medium on Mondays and Fridays. The cells are allowed to grow for three days in number to approximately one million per well attaching to the bottom, before sample extracts are applied in 20 µL of a solvent mixture (2 DMSO: 1 toluene: 1 isopropyl alcohol). Exposure time is 16 hours, and then the lysate of the cells is measured for light production by adding luciferin. Extraction of samples follow EPA methods for water (3510) and solids (3540C), but for projects only concerned with dioxins and coplanar PCBs, the extracts are now passed through a silica gel column to remove PAHs. The results shown in this paper are on extracts not cleaned of PAHs, so values are generally higher than those from EPA 8290. Quality assurance and quality control procedures used with each set of samples now include testing of: 1) an extraction method blank; 2) five concentrations (1-50 pg/mL) of a standard mixture of dioxins and furans; 3) 2,3,7,8-TCDD at 50 pg/mL; and 4) a Standard Reference Material (1944) extracted with the samples.

Results and Discussion

Figure 1 shows the response of 4425 to TCDD over a period of about two months, with a mean response of 89 fold induction (times background). Over approximately the same time period, the responses of 4425 to the five concentrations of the standard mixture of dioxins and furans are shown in Figure 2. The large standard deviations at the 50 pg/mL concentration demonstrate one reason for not

utilizing data for extracts producing fold induction over 100. These extracts are diluted and re-tested. Figure 3 shows the type of data provided to a project manager charged with cleaning up a contaminated site. Low, high and intermediate level samples are then selected for confirmation by the more time- consuming and expensive EPA method 8290.

Figures 4 and 5 illustrate the results of two projects where only a portion of the samples screened by 4425 were selected for confirmation. At the wood-treating site the 4425 values were about twice as high as those produced by chemical methods and the use of EPA TEF values (Fig. 4). For soil samples collected from a site on the Island of Guam, the 4425 values were about 3 times higher than those by 8290 (Fig. 5). The correlations for both of these sets of samples were sufficiently high to provide confidence in the remaining screening data produced by method 4425, thus providing a significant cost saving for the project.

The final project shown (Fig. 6), conducted for the Atlanta EPA, included samples that were all confirmed by chemical methods (8290). We were concerned that 4425 data were 53 times higher than the 8290 values, even though the correlation coefficient was relatively high. Since we suspected the presence of PAHs in these samples, our laboratory selected one low and one high level sample for chemical analyses. The sum of 27 parent and alkylated PAHs in the two samples was approximately 50 ng/g, which would not be expected to significantly enhance the 4425 responses. However, the alklyated phenanthrene, retene, was found at 410 and 450 ng/g in these two samples. Other studies have demonstrated that retene induces CYP1A, and our testing showed it was a somewhat stronger inducer than benzo[a]pyrene. These findings aid in explaining the higher level responses of 4425 in the Florida study, as compared to 8290 results.

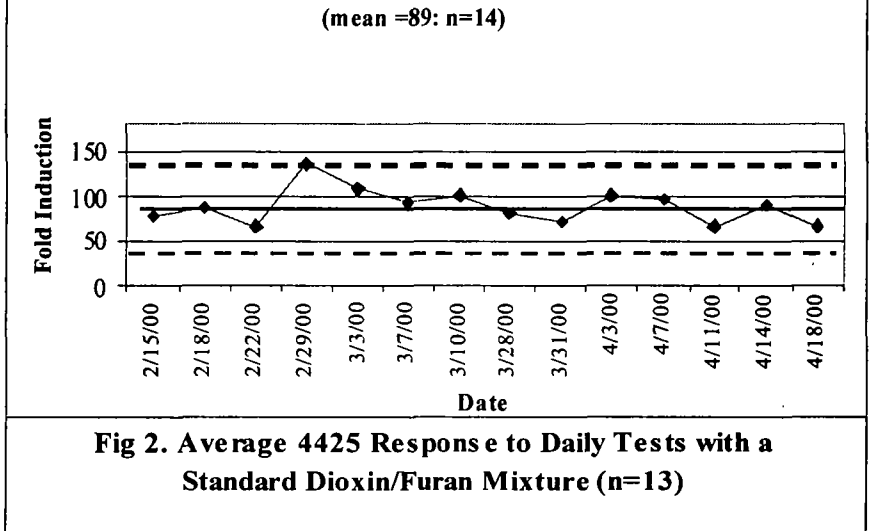
Use of a standard mixture of dioxins and furans has shown that fold induction of the HRGS assay matches closely the TEQ of the mixture (in pg/mL), thus making the estimation of sample TEQs straightforward. The data presented show that correlations with chemical analyses are always strong, while the level of 4425 response to one class of chemicals may be enhanced by the presence of another class. The objectives of some projects (NOAA-bioeffects) are to find the stations containing the highest levels of all inducing compounds, which may produce chronic toxicity for organisms living or feeding in the area. For these studies it is more appropriate to use non-cleaned extracts, but for projects only concerned with dioxin/furan contamination, silica gel columns are used to remove PAHs. We believe the use of 4425 to first screen a large number of samples, before selecting a portion for chemical characterization is a valuable cost-saving approach that can be applied to many different types of programs. When the purpose of an investigation is to monitor changes over time, as in remediation or discharge monitoring, 4425 can also be a valuable tool.

Acknowledgements
We thank Joe Wiegel of Columbia Analytical Services in Kelso, WA for his aid in extracting these samples, and also for measuring PAHs, including retene, in the selected samples.

References

1. Bucheli, T.D., Fent, K. (1995) Crit. Rev. Environ. Sci.Technol. 25, 201.
2. Collier, T.K., Anulacion, B.F., Stein, J.E., Goksoyr, A., Varanasi, U. (1995) Environ. Toxicol. Chem. 14, 143.
3. Anderson, JW, Jones, JM, Hameedi, J, Long, E, and Tukey, R. (1999) Marine Environ. Res. 48:407-425.

4. Anderson, JW, Jones, JM, Steinert, S, Sanders, B, Means, J, McMillin, D, Vu, T and Tukey, R. (1999) Marine Environ. Res. 48:389-405.
5. ASTM. (1999) Vol. 11.05:1999 Annual Book of ASTM Standards, American Society for Testing and Materials, West Conshohocken, PA.
6. APHA. (1998) 20th Edition of Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington, D.C.
7. EPA. (1999) Method 4425: EPA Office of Solid Waste, SW 846 Methods, Update IVB.



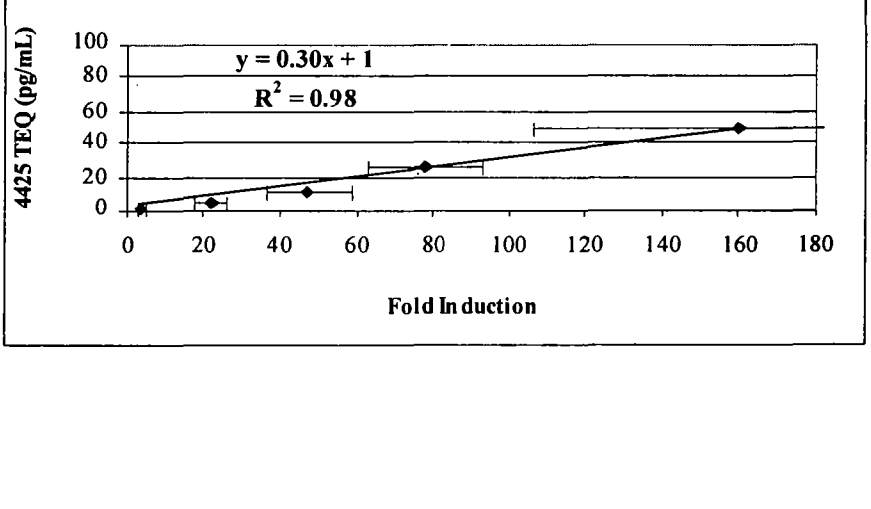


Fig. 3 4425 Responses to Soil Samples: Wood-Treating Site *(from 1:20 Dilutions)*

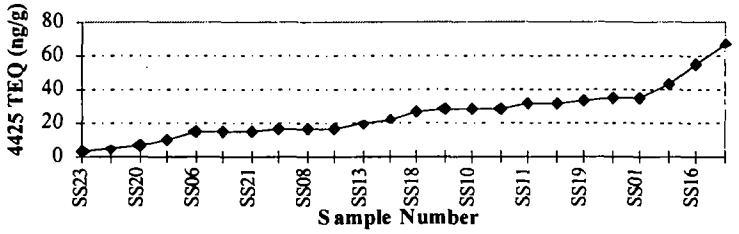


Fig. 4 4425 TEQs vs. Chemical TEQs: Wood-Treating Site

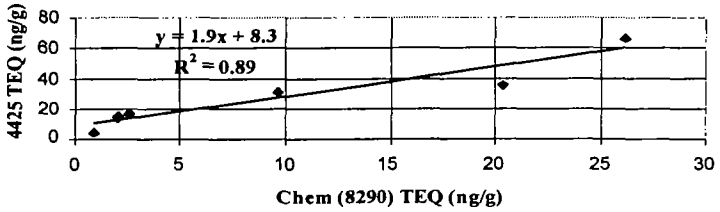


Fig. 5 4425 TEQs vs. Chemical TEQs for Guam Soils

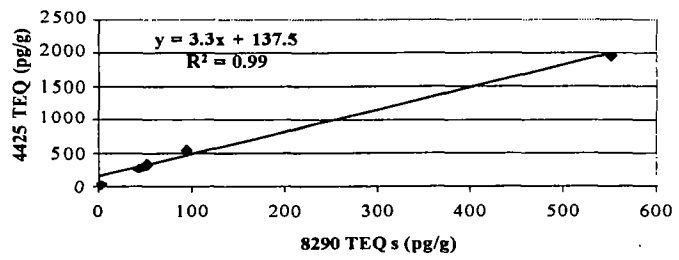


Fig. 6 4425 TEQs vs. Chemical TEQs for a Florida Bay (n=27)