AN ENZYME-LINKED IMMUNOSORBENT ASSAY (ELISA) FOR DETERMINING DIOXINS IN SEDIMENT AND SOIL SAMPLES

Jeanette M. Van Emon[1], Jane C Chuang[2], Robert A. Lordo[2], Mikaela Nichkova[3], Shirley J. Gee[3], and Bruce D. Hammock[3]

[1]U.S. EPA, ORD, NERL, MDAB, Las Vegas, Nevada, [2]Battelle Memorial Institute, Columbus, Ohio, [3]University of California, Davis

Background

Dioxins are highly toxic environmental contaminants that have been linked to cancer, liver damage, and various reproductive and developmental diseases (1). Environmental contamination by the dioxins (polychlorinated dibenzo-p-dioxins [PCDDs]) and the related polychlorinated dibenzofurans (PCDFs) is of great concern due to their persistence in the environment and adverse effects on wildlife and humans.

There are 75 different PCDD and 135 different PCDF congeners each having its own chemical and toxic characteristics. The most widely known congener is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) which is also the most toxic. The dioxins usually exist as mixtures complicating the issues of waste management, toxicity assessment, human exposure and environmental impact. Total releases are not the best measure of the actual toxicity of these compounds because each compound has its own level of toxicity. Toxic equivalency factors (TEFs) are used to represent the fraction of TCDD toxicity exhibited by a congener or congener group. A Toxicity Equivalence (TEQ) is then derived from the concentration of each of the toxic congeners in the mixture.

An early symptom of dioxin exposure is a persistent but non-fatal skin rash called chloracne.

The analysis of PCDDs and PCDFs is of great importance for environmental monitoring and human exposure assessment. Unfortunately, the analysis of these compounds is complex and expensive, limiting the number of samples that can be analyzed in a timely and cost-effective manner. Gas chromatography with high resolution mass spectrometry (GC-HRMS) is the reference analytical method for the dioxins. However, GC-HRMS requires intensive sample cleanup procedures and expensive instrumentation. Analysis costs range from $1,500-$5,000/sample (2). Immunochemical methods such as the enzyme-linked immunosorbent assay (ELISA) are based on specific antibodies combining with a target compound or group of compounds. ELISA methods have been developed for many compounds of environmental and human health concern providing improvements in cost, sensitivity and sample throughput relative to instrumental methods (3).

References

(1) National Primary Drinking Water Regulations, Technical Fact Sheet on Dioxin (2,3,7,8-TCDD), EPA 821-B-94-005.

(2) G. Shan, W.R. Leeman, S.J. Gee, J.R. Sanborn, A.D. Jones, D.P.Y. Chang and B. D. Hammock, Anal. Chim. Acta 444 (2001) 169-178.

(3) J.M. Van Emon, JOAC, vol. 84, no. 1 (2001) 125-133.

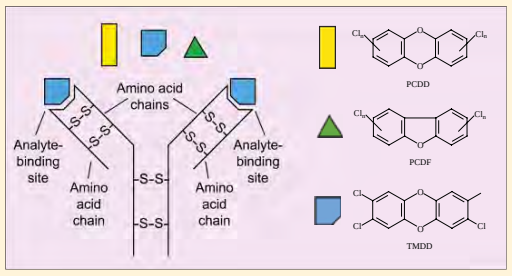
ELISA

An ELISA was developed to detect dioxins in environmental samples (2). The method uses the less toxic 2,3,7-trichloro-8-methyl-dibenzo-p-dioxin (TMDD) as an alternative standard for TCDD. The ELISA is based on a competitive reaction between dioxin in the sample and a protein-dioxin conjugate adsorbed to the sides of a microwell ELISA plate. An enzyme provides a color reaction to measure the amount of dioxin in the sample. The ELISA will detect many of the PCDDs and PCDFs that have a high TEF value. Study results indicate that the ELISA method offers improvement in speed, sample throughput and cost when compared to GC-HRMS.

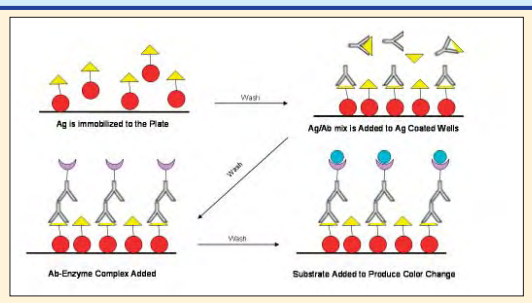
Objectives

- Develop an immunoassay method to substantially reduce the complexity and cost of dioxin toxic congener analysis
- Determine TCDD equivalent concentrations in real-world sediment and soil samples by the ELISA
- Determine whether the ELISA-derived TCDD equivalent results are statistically equivalent to the TEQ results derived from the 17 PCDDs/PCDFs GC/HRMS generated concentrations using the World Health Organization TEF values

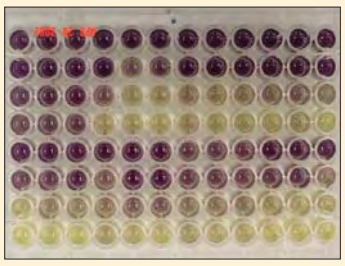
A Dioxin Antibody



Indirect Competitive ELISA



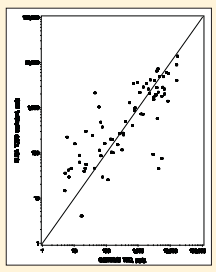
96-Microwell ELISA Plate



ELISA and GC/HRMS Performance Comparison

Summary Statistics	ELISA, TCDD equivalent pg/g	GC/HRMS, TEQ pg/g
Sample Size[a]	75	75
Arithmetic Mean	1690	2400
Standard Deviation	2430	3690
Geometric Mean	458	408
Minimum	<8[b]	6.82
25th Percentile	93	60
50th Percentile	493	661
75th Percentile	2590	3780
Maximum	13900	17000

ELISA and GC/HRMS Data Comparison



Ranking of Samples Based on Dioxin TEQ Levels Near the 1000 pg/g Cleanup Level

		GC/HRMS Analysis	
		# samples $ 1000 pg/g	# samples < 1000 pg/g
ELISA Analysis	# samples $ 1000 pg/g	27	5
	# samples < 1000 pg/g	6	38

Conclusions

- The dioxin TEQ levels by GC/HRMS and the TCDD equivalents derived by ELISA were highly linearly correlated suggesting the ELISA data could be used to indicate dioxin TEQ levels
- Sample-specific differences between methods were not significant at the 0.05 level indicating that the two methods yield statistically similar outcomes (<8 to 14000 pg/g by ELISA and 6.8 to 17000 pg/g by GC/HRMS)
- The ELISA method can be used as an alternative quantitative monitoring tool for determining dioxin TEQs in contaminated sediment/soil samples providing a more cost-effective and timely analysis for site monitoring and exposure related research

Acknowledgement

This work was funded by the U.S. Environmental Protection Agency (EPA) under contract number GS-10F-00275K. It has been subjected to Agency review and approved for presentation. Mention of trade names and commercial products does not constitute endorsement or recommendation for use. This research was also funded by grants from the National Institute of Environmental Health Sciences 5 P42 ES04699 and NIEHS Center for Environmental Health Sciences P30 ES05707.