New Methods and New Challenges for Measuring Human Exposure to Environmental Toxicants

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A number of recent epidemiologic studies have shown that when an exposure index is constructed using the best available information (environmental sampling data; questionnaire data; routes of exposure, etc.), it may still be poorly correlated with laboratory body burden measurements of the toxicant. Most recent epidemiologic studies involving exposure to Polychlorinated Dibenzo-p-dioxins (PCDDs) and Dibenzofurans (PCDFs) have used the laboratory measurement as the measure of exposure. Some studies have measured PCDD and PCDF levels in all participants in the study, while others have validated the exposure index by measuring levels in a statistical subsample of the study participants. In general, epidemiologic studies have been limited in the extent of their exposure assessment and in their statistical power. These limitations are due in part to the high cost of the laboratory measurement and the length of time necessary to make the measurement. In order to increase the sample size for larger epidemiologic studies and thereby increase the statistical power of the study, it will be necessary to greatly decrease the cost of the laboratory analysis and increase the laboratory sample throughput. The major challenge facing analytical chemists is to achieve these two objectives while maintaining trace level sensitivity, specificity, and very high quality assurance of the exposure assessment data.

Over the past 7 years significant progress has been made in increasing sample throughput (thereby reducing the cost of the analysis). In 1985, it required one week for a chemist to cleanup five human samples. The amount of time was reduced to two days by semiautomating the cleanup procedure and just recently cut to one day by a fully automated procedure. Even though a five-fold increase in sample throughput has been achieve, it is still not fast enough to provide cost effective analyses for large epidemiologic studies. The samples processed by one chemist requires a full day to analyze by high-resolution mass spectrometry (HRMS). At the current mass spectrometry sample throughput rate it would require one HRMS for each sample cleanup chemist in order to increase a laboratories throughput. This is clearly not cost effective since high resolution mass spectrometers cost in excess of $500,000 each and require thousands of dollars per year to maintain. The manufacturers of high resolution mass spectrometers have dramatically increased the

sensitivity of their instruments (3 orders of magnitude, see Table 1) over the last 7 years. This increase in instrumental sensitivity has allowed the laboratory measurements to keep pace with the declining PCDD and PCDF levels in normal population samples (see Table 1). The increase in sensitivity has also allowed a decrease in the amount of sample matrix required for the analysis as shown in Table 1. The decreased sample size has therefore contributed to faster sample cleanup and some reduction in cost. It is unlikely, however, that any more dramatic sensitivity increases will be produced in the foreseeable future. In order to achieve any further reduction in cost per sample and higher laboratory throughput, new approaches will be necessary. Some of these new approaches are outlined below and will be discussed in more detail during the presentation.

Table 1. PCDD and PCDF Analytical Capabilities Over Time

	1984-1986 Adipose Tissue 20g	**1987 Serum 200g**	**1988-1989 Serum 50g**	**1990-1991 Serum 10g**	**1992-1993 Serum 2g**
DL[a]($3S_o$)	10 pg	2 pg	300 fg	60 fg	10 fg
Concentration DL($3S_o$)	0.5 ppt	19 ppq	6 ppq	6 ppq	5 ppq
Concentration QL[b]($7S_o$)	1.2 ppt	23 ppq	14 ppq	14 ppq	12 ppq
Concentration DL for 200g of Serum	--	10 ppq	1.5 ppq	300 ppqt	50 ppqt
Normal Levels in pooled Serum	1985 1986 25.8 22.1 ppq	1987 20.8 ppq	1988 1989 18.8 16.5 ppq	1990 15.0 ppq	1992 13.0 ppq
S/N (Amount On-Column)	8 (1 pg)	--	6 (100 fg)	34 (100 fg)	125 (100 fg)
Time to Cleanup 5 Serum Samples	5 Days	--	2 Days	--	1 Day

[a]DL = Detection Level. [b] QL=Quantification level.

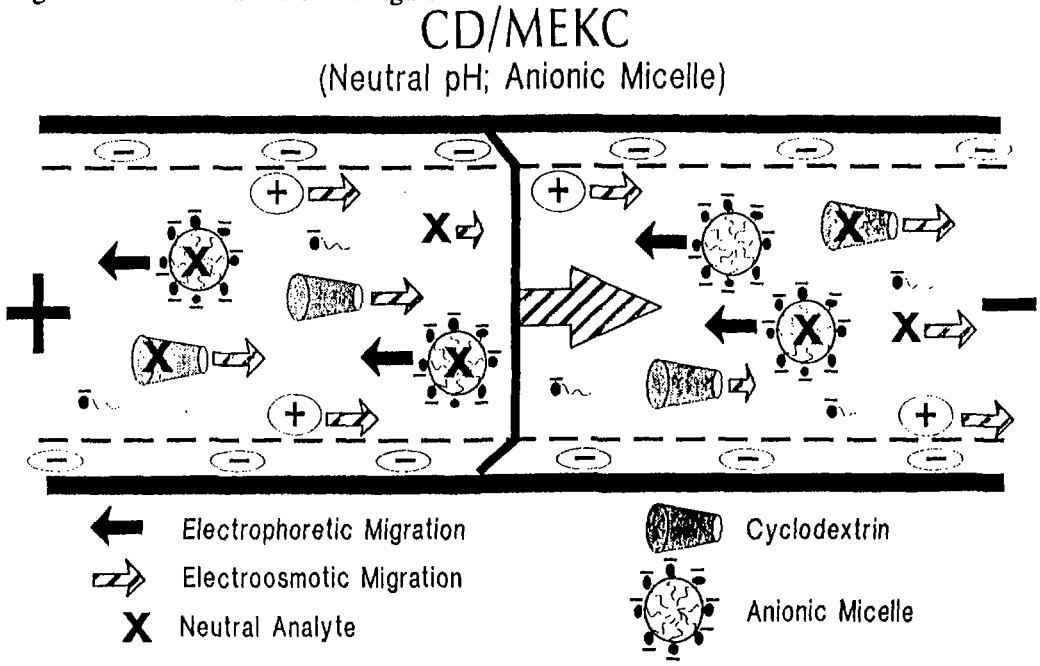
Comprehensive Two-Dimensional Gas Chromatography. A two-dimensional gas chromatogram can be generated by passing all of the effluent from a conventional primary column directly onto a short, high-speed secondary column. A thermal desorption modulator chops the primary column effluent into a series of concentration pulses each of which generates a secondary chromatogram. Secondary chromatograms with total retention times as

short as two seconds and peaks as sharp as 30 msec are continuously generated as the primary chromatogram is developed. The peaks scatter throughout the accessible two-dimensional retention plane. This 2D technique can separate very complex mixtures in very short times and has the potential (when coupled with mass spectrometry) of increasing mass spectrometry throughput by more than a factor of 10.

Superficially Hydrophilic Reversed Phase Liquid Chromatography (SHRP-LC). These new LC columns allow the direct injection of a large amount of a biological matrix (or other aqueous samples) onto the LC column. The analytes are retained by the hydrophobic inner surfaces of the LC support while the hydrophilic proteins elute from the column. The analytes are then eluted with an organic solvent and collected or passed onto a second LC column for further separation. This procedure provides a rapid, automated cleanup of biological or aqueous samples for off-line GC/MS analysis.

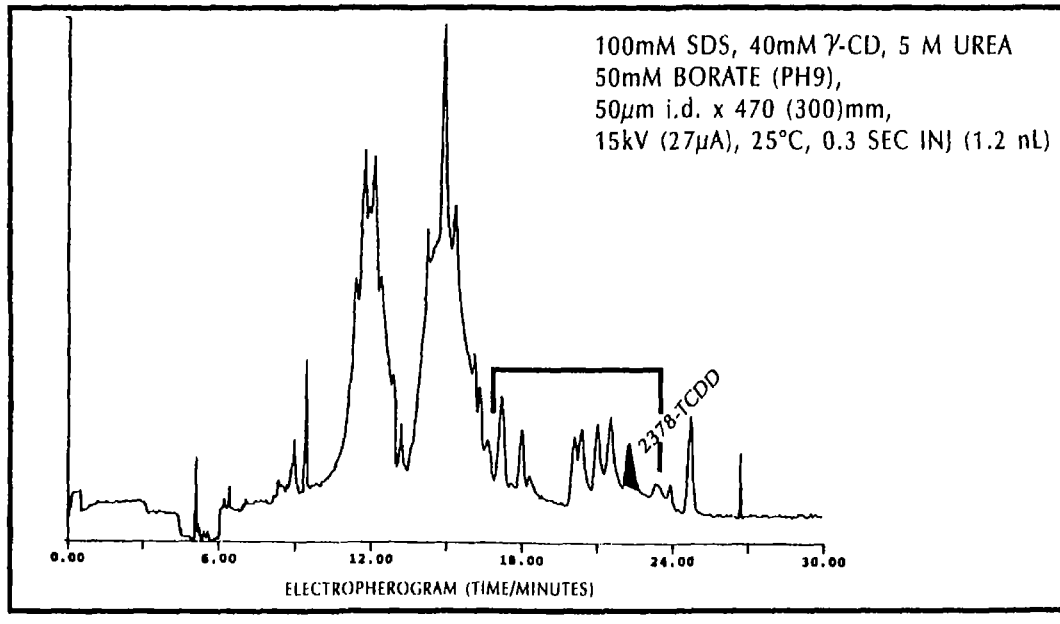
High-Speed, Thermally Modulated Supercritical Fluid Extraction/Gas Chromatography. A coupled supercritical fluid extraction (SFE) and high-speed gas chromatography system permits simultaneous rapid sample extraction and rapid analysis. SFE can be more efficient and faster than conventional liquid extraction because of more rapid mass transfer and better penetration into the sample matrix. This technique can be used with solid samples (e.g., soil) as well as biological samples which have been adsorbed onto a solid material.

Cyclodextrin Modified Micellar Electrokinetic Chromatography. This technique which relies on the partitioning of hydrophobic analytes between neutral cyclodextrins and negatively charged micelles is illustrated in Figure 1.



This technique is compatible with aqueous based matrices (like HPLC) but also retains the high column efficiency of capillary GC. An electropherogram produced by directly analyzing serum is shown in Figure 2. This technique is isomer-specific for 2378-TCDD.

γ-CD/MEKC SEPARATION OF 0.2μm FILTERED HUMAN SERUM (SPIKED WITH 15 TCDDs)



A number of other potential techniques for rapid sample extraction and analysis will also be discussed: Comprehensive Two-Dimensional Supercritical Fluid Chromatography/Gas Chromatography; Immunoaffinity Column Chromatography; Magnetic Reagent Technology; and others.