Analytical Advances in Biological Monitoring: a New Era of High Speed in both Sample Preparation and Analysis

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Introduction

Over the past several years at the Centers for Disease Control and Prevention the demand for high quality laboratory measurements for highly toxic environmental contaminants in human populations has increased dramatically. The increased demand is in part due to recent reports that certain kinds of synthetic chemicals that are in the environment (many of which are found in human tissues) are capable of disrupting animal hormone systems. The increased demand is also due to results from a number of recent epidemiologic studies showing a very poor correlation between epidemiologic exposure indices and actual human body-burden laboratory measurements. A body-burden measurement reduces misclassification and greatly increases the probability of finding an association (if one really exits) between exposure to an environmental toxicant and any potential human health effects. In response to the increased demand on the laboratory, we have developed a number of new high-speed single and multidimensional techniques as well as methods compatible with direct measurements in human samples. These new techniques have dramatically increased the laboratory throughput while maintaining high specificity and increased sensitivity (e.g. a signal-to- noise ratio of 15 for 350 attograms of 2,3,7,8 - tetrachlorodibenzo-p-dioxins).

Results and Discussion

Figure 1 shows the fast single-dimensional GC/high resolution mass spectrometry analysis for the chlorinated dioxins, furans, and coplanar biphenyls normally found in humans. This analysis previously took 60 minutes. The separation of the two hexachlorinated dioxins and furans has been lost using the fast chromatography. These congeners have the same toxic equivalency factors, however, and the loss of resolution for these congener pairs is not a serious compromise in gaining the additional separation speed. Figures 2 and 3 show the newer faster analysis for nonpersistent and persistent

Figure 1

FAST SINGLE-DIMENSIONAL GC/HRMS OF PCDDs, PCDFs, cPCBs FOUND IN HUMAN SAMPLES

20m DB-5; 0.1 mmID; 0.1u FILM; HP 6890 CONSTANT FLOW 23 cm/sec

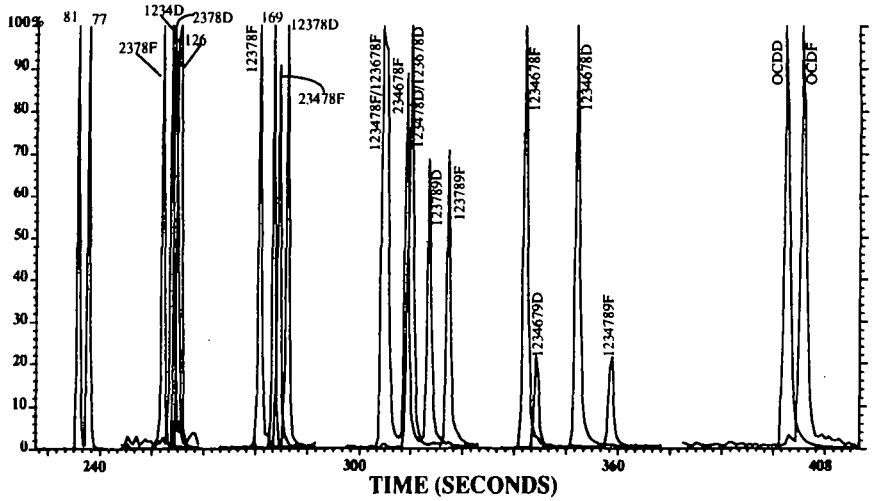


Figure 2

FAST GC/HRMS OF COMMONLY USED NON-PERSISTENT PESTICIDES

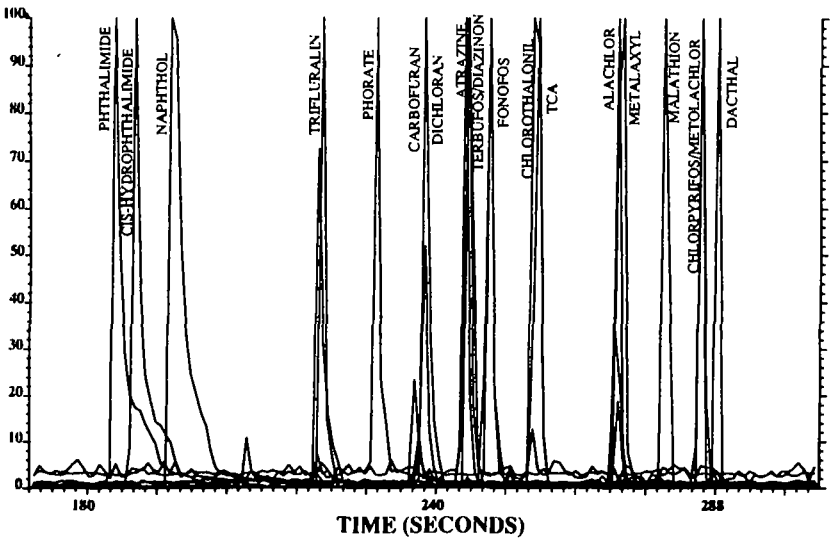
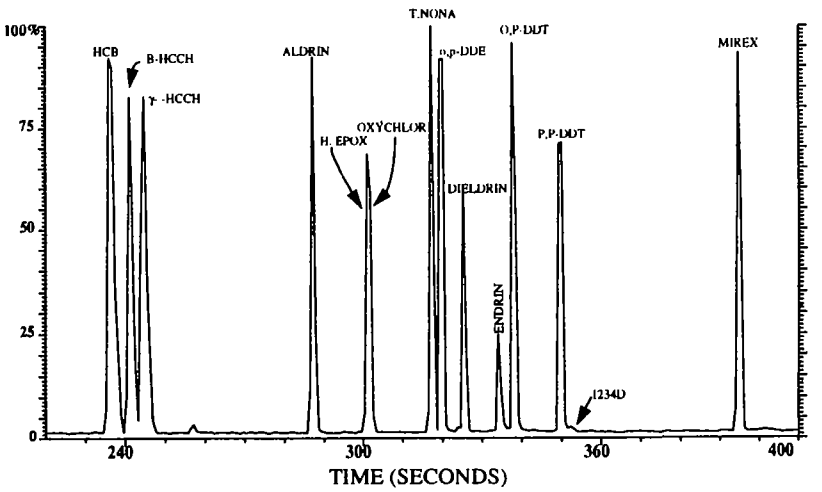


Figure 3 FAST GAS CHROMATOGRAPHY HRMS ANALYSIS FOR PERSISTENT PESTICIDES

GC - start 100; 50/min-->185; 30/min-->320



pesticides found in human samples from the general population. The separation of these pesticides using the 60m, 0.25 mm I.D. columns required 40 minutes. All of the pesticides are separated either by accurate mass or by GC retention time. We have also developed fast GC separations for chlorinated toxaphenes and 38 PCB's found in human serum.

Over the years, we have attempted to add more and more compounds to our sample cleanup procedure. The number of compounds and compound classes has grown steadily since 1983 (Figure 4). In epidemiologic studies where only one small sample is available from each individual, it is desirable to analyze for as many compounds as possible. We are continuing to add analytes to our automated sample preparation system, with more than 250 analytes in various stages of validation (Figure 4). While the number of analytes has steadily increased, we have also attempted to decrease the amount of serum required for the analysis as shown in Figure 5. Reducing the amount of serum allows a wide participation in epidemiologic studies by potentially adding the elderly, children, or individuals too sick to normally give blood. Reducing the sample size also potentially allows us to increase the throughput in sample preparation. As the sample size decreases, however, the detection limits also become higher. In addition, levels of a number of analytes of interest appear to be decreasing in human populations. This decrease puts an added burden on analytical sensitivity requirements.

Figure 6 is a plot of the serum whole-weight detection limits for various serum sample sizes on our most sensitive HRMS, the Micromass Ultima. The normal background level of 2378-TCDD is about 10 parts-per-quadrillion and requires new technology in order to measure this level in one milliliter of serum (Figure 6). We have previously published a comprehensive two-dimensional GC/HRMS method for TCDD at this low level [1]. Figure 7 summarizes the increase in sample throughput for both sample preparation and mass spectrometry. Currently, we can analyze 40 samples per day for PCDD's, PCDF's and Coplanar PCB's by HRMS. The sample preparation throughput is 10 samples per day per cleanup person (Figure 7).

Figure 4

NUMBER OF ANALYTES FROM A SINGLE HUMAN SERUM SAMPLE USING UNIVERSAL AUTOMATED CLEANUP SYSTEM

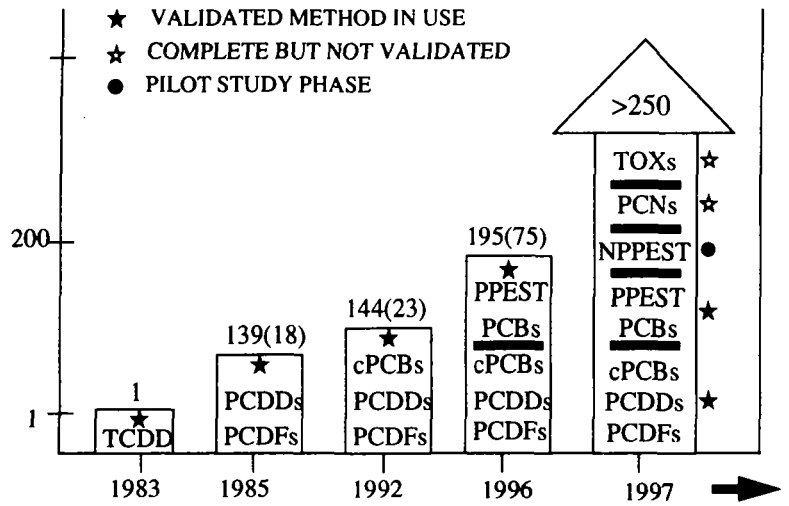


Figure 5 CHANGE IN NUMBER OF ANALYTES AND AMOUNT OF SERUM REQUIRED OVER TIME

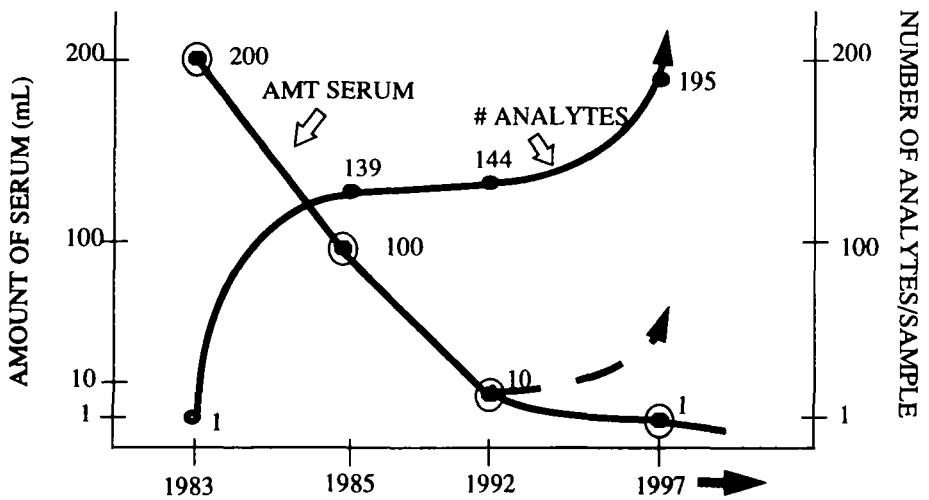


Figure 6 2378-TCDD DETECTION LIMITS VS AMOUNT OF SERUM

PARTS-PER-QUADRILLION (WHOLE WEIGHT)

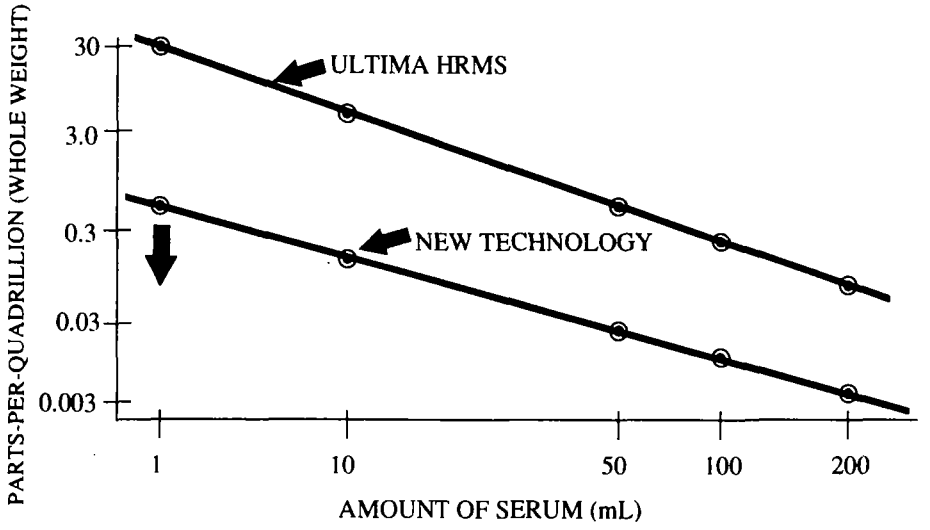
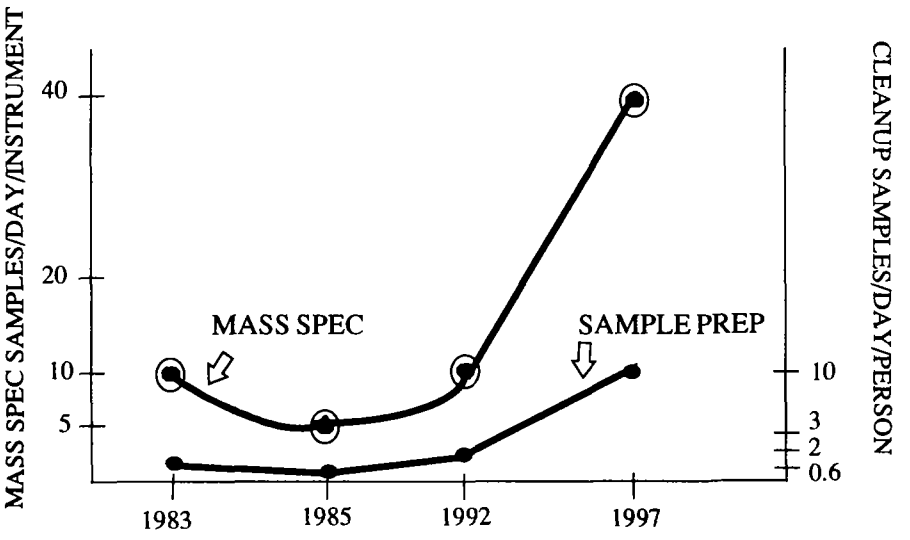


Figure 7 CHANGE IN SAMPLE CLEANUP AND MASS SPEC THROUGHPUT OVER TIME



These new methods have allowed a large number of new studies to be conducted particularly in the area of women's health. These studies include examining levels of dioxins, furans, PCBs, and pesticides in women who have developed breast cancer and in women with endometriosis. Other studies have centered around assessing exposure to families living around waste incinerators as well as farm families exposed to newer "non-persistent" pesticides.

Literature Cited

(1) Patterson, D.G., Jr., Barr, J.B., Dipietro, E. Et al. Organohalogen Compounds **1996**, 27, 309-314.