NEW MASS SPECTROMETRIC APPROACHES FOR THE ANALYSIS OF PCBs AND PERSISTENT PESTICIDES IN HUMAN SERUM

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INTRODUCTION

Public health efforts to effectively prevent disease associated with exposure to organic toxicants depend on identification of the toxicant, risk assessment, and risk management. Since there is little information on the risk to human health from environmental exposure to organic toxicants (both exposure assessment and dose-response assessment for humans), risk assessment is currently the most difficult part in the prevention of environmental disease. Comparisons of environmental exposure data from people in the general population can greatly decrease the uncertainties of human risk assessment, especially if exposure assessment in human studies is improved.

Polychlorinated biphenyls (PCBs) and persistent pesticides are examples of two persistent organic pollutants (POP) that are found in the environment. These compounds can be found all over the world and due to their stable lipophilic nature tend to bioaccumulate in humans and animals. Generally, human exposure to these compounds has been monitored with blood serum measurements. Serum is the main matrix for measuring human body burden of PCBs and persistent pesticides because it is readily accessible unlike adipose tissue. The disadvantage of analyzing serum is the relatively low concentrations of PCBs and persistent pesticides compared to adipose tissue.

To determine health effects from exposure to POPs it is useful to have measurements on many different compounds and classes of compounds from a single individual. It is also cost-effective to obtain the greatest amount of information on exposure to POPs from a single sample especially in reference range studies. We have recently developed serum cleanup methods for sample preparation of dioxins, furans, coplanar PCBs, persistent pesticides and PCB congeners in a single sample. These POPs are eluted in two different fractions. One fraction contains the dioxins, furans and coplanar PCBs while a separate fraction includes the PCB congeners (38 compounds) and the persistent pesticides (13 compounds). Efficient analysis of the PCB-persistent pesticide fraction offers a great analytical challenge.

Many groups have analyzed these POPs using GC with electron capture detection (ECD). However, many advantage are realized by using high resolution mass spectrometry (HRMS) for detection instead. HRMS offers an excellent degree of sensitivity and specificity for trace organic analysis, and unlike ECD detection, is ideally suited for isotope-dilution which automatically corrects for variable recoveries. Therefore, we have developed several isotope dilution GC/HRMS methods for the quantification of PCBs and persistent pesticides from extracts of human serum. These methods include: 1) Analysis of all 38 PCBs and 13 persistent pesticides in a single GC/HRMS run using one column; 2) Analysis of PCB congeners and persistent pesticides in two separate GC/HRMS runs; 3) Analysis of PCB congeners and

persistent pesticides in two separate fast GC/HRMS runs; and 4) Analysis of the PCB-persistent pesticide mixture using parallel capillary columns in the same GC/HRMS run.

EXPERIMENTAL

Mass Spectrometry

In each case, the two most intense chlorine isotope peaks in the PCB molecular ion clusters were monitored. Molecular ions for the chlorine isotopes of all the PCBs were monitored (from trichloro to decachloro PCB). Molecular ions for aldrin and hexachlorobenzene were also monitored. Fragment ions were monitored for op'-DDT, pp'-DDT, DDE, β-HCCH, γ-HCCH heptachlor epoxide, oxychlordane, *trans*-nonachlor, dieldrin and mirex. All spectra were recorded with low energy (30 eV) electron impact ionization with a resolution of 10,000 (10 % valley definition).

Analysis of 38 PCBs and 13 persistent pesticides in a single GC/HRMS run using one column

Spectra were obtained on a Micromass 70-SE equipped with HP 5890 GC. A 30 m x 0.25 mm DB-5MS column with a 0.25 μm film thickness was employed. The initial linear velocity of helium was 40 cm/sec. The injector and transfer lines were both set to 260°C. The initial column temperature was 100°C and was held for 1 minute. The oven was then heated to 150°C at 25°C/min , and then to 270°C at 3°C/min.

Analysis of PCB congeners and persistent pesticides in two separate GC/HRMS runs

PCBs. The same analytical system and conditions described above were used for PCB analysis with the exception of the oven temperature program. The initial column temperature was 100°C and was held for 1 minute. The oven was then heated to 200°C at 25°C/min and held for 10 min. The temperature was then increased to 200°C at 7°C/min, held for 3 min. and finally ramped to 320°C at 25°C.

Persistent Pesticides. Chromatograms were recorded on a Micromass Autospec equipped with an HP 5890 GC. The same column as above was used and the linear velocity was 30 cm/sec. The initial column temperature was 100°C and was held for 0.75 min. The oven was then heated to 200°C at 18°C/min and held for 1 min. The temperature was then increased to 206°C at 1°C/min and finally ramped to 300°C at 25°C/min.

Analysis of PCB congeners and persistent pesticides in two separate Fast GC/HRMS runs

PCBs and Persistent Pesticides were analyzed in separate mass spectral runs where different ions were monitored, but each run employed the same GC conditions. This was done on a Micromass 70-4SE equipped with a HP 6890 GC. The injector and transfer line were set to 280°C. The linear velocity of helium was held constant at 23 cm/sec throughout the entire run. The initial column temperature was 100°C and was then increased to 185°C at 50°C/min, and then to 320°C at 30°C/min.

Analysis of the PCB-persistent pesticide mixture by parallel columns in one GC/HRMS run

Analysis was performed on a Micromass Autospec equipped with an HP 5890 GC. Two columns were placed in the same injector and oven and were installed in parallel into the mass spectrometer. The first column was a 2 m x 0.1 mm DB-1 column with a 0.1 μm film. The second column was a 30 m x 0.25 mm DB-5MS column with a 0.25 μm film. The linear velocity was 16 cm/sec on the DB-1 column and 30 cm/sec on the DB-5MS column. The initial column temperature was 100°C and was held for 1 min. The oven was then heated to 150°C at 12°C/min. The temperature was then increased to 320°C at 7°C/min.

RESULTS and DISCUSSION

The analysis of PCBs and persistent pesticides from human serum is an analytical challenge. GC/HRMS provides a high degree of specificity and selected ion monitoring (SIM) yields high sensitivity. We were able to successfully separate and analyze all 38 PCBs and 13 persistent pesticides (see Figure 1). However, the mass difference between closely eluting peaks was sometimes too great to be successfully scanned in the same group by the mass spectrometer. One example of this was DDT and the heptachloro PCB-178 which were only separated by ten seconds. This creates a problem since virtually no molecular ion is seen for DDT and the highest weight major fragment ion is m/z 235.0081. The M+2 and M+4 ions of the molecular ion cluster for PCB-178 are the major ions recorded and the M+2 ion has a molecular weight of 393.8025. This gives a ratio between these two ions of almost 1.7 which is higher than the practical ratio limit of 1.6. In SIM, the magnet is held constant for every ion in a single group and the accelerating voltage is varied to record different masses. The accelerating voltage for the lowest mass ion in a group is generally 8 KV. Decreasing the accelerating voltage below 5.5 or 6 KV leads to a huge loss in sensitivity and considerable loss of resolution. The magnetic field must therefore be changed between these peaks. This requires a gap between eluting peaks since it is a relatively slow process (taking up to 1 second) and changes in retention times (often seen with serum extracts) can cause peaks to be missed.

The analysis time for the PCBs and persistent pesticides was greater than 40 minutes in a single GC/HRMS run due to constraints on the mass spectrometer. We discovered that by performing two separate GC/HRMS analyses on each serum extract (each taking about 20 minutes) that the total analysis time was about the same and was more reliable. Thus, one GC/HRMS run was used to record ions for the 38 PCB congeners and a second GC/HRMS run was employed to record ions for the 13 persistent pesticides. Chromatograms for a 30 meter 0.25 mm GC/HRMS run for both these can be found in figures 2A and 2B respectively. This method has now been validated and is currently in use.

We are also exploring other GC/HRMS techniques to improve the efficiency of the PCB-persistent pesticide analysis. The first approach is fast GC analysis using shorter narrow bore capillary columns. Recently, we have successfully coupled this technique to HRMS. This has allowed us to greatly increase the speed of analysis and has provided some increase in sensitivity because of the narrower, higher peaks. As can be seen in Figure 3, the persistent pesticides (3A) can be separated and analyzed by HRMS in about 6.5 min., and the PCBs (3B) in about 7 min. This technique offers fast sensitive analysis in which both sets of compounds can be analyzed in less than 15 min. We have also analyzed serum extracts of the PCBs and persistent pesticides and found that there were no interferences or problems with the analysis.

An alternate approach is the use of two columns in parallel. In the analysis of PCBs and persistent pesticides, problems arose because of the large difference in masses of closely eluting peaks. We, therefore, have explored the use of two parallel columns for the analysis. The first

column was a short (2 m) 0.1 mm DB-1 column. The second column was a longer (30 m) 0.25 mm DB-5MS column. Using parallel columns, the short column completes a chromatogram before any analyte elutes from the second column. The shorter, less polar microbore column does not have the resolution of the longer column. We, therefore, monitored the masses of the persistent pesticides from 3 to 10 min., as they eluted from the first column, and the masses for the PCBs from 10 to 19 min (Figure 4). The first and second columns facilitated the time or mass separation of all of the persistent pesticides and most PCBs, respectively. Also, we found the last eluting PCBs (206 and 209) could easily be monitored as they eluted from the first column, therefore, further shortening the run time. Additionally, the elution of the peaks from the first column can be manipulated to elute at different times by changing the diameter and length of the column.

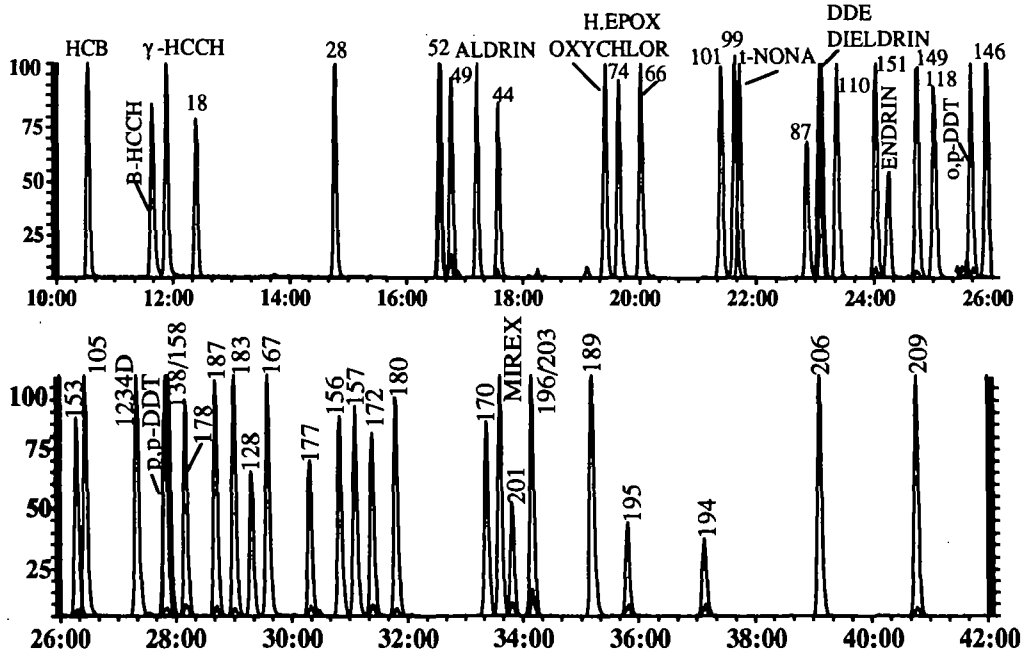

Figure 1: GC/ID/HRMS Chromatogram of PCBs and Persistent Pesticides on a 30 meter DB-5 column.

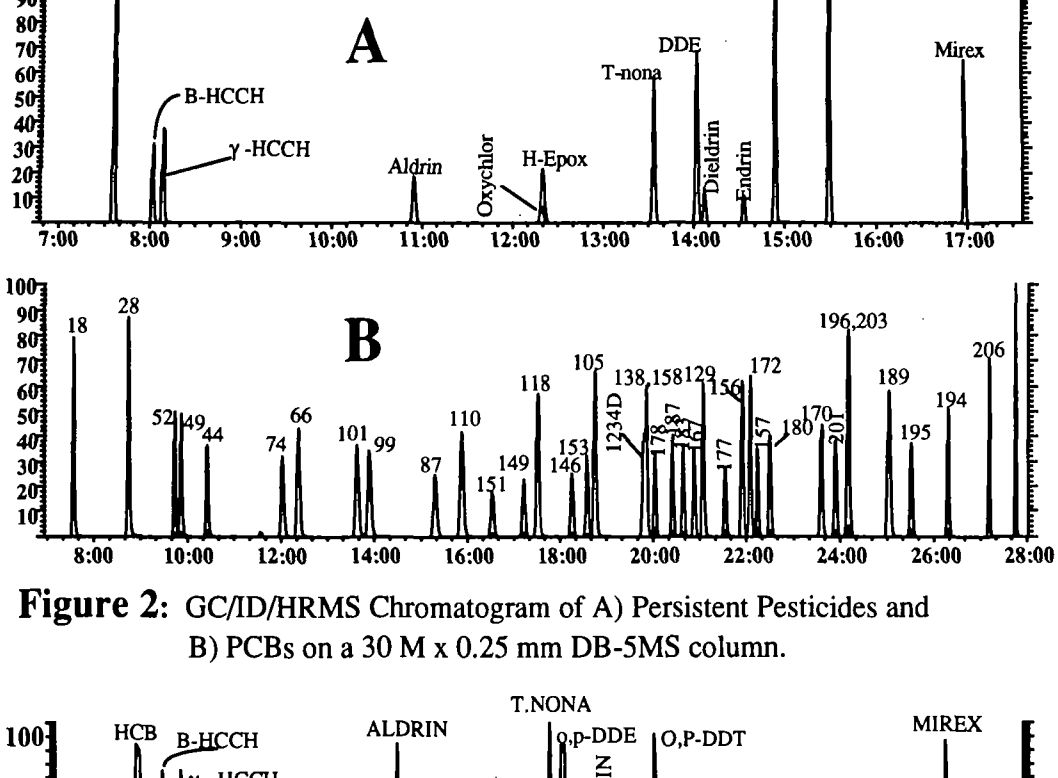

Figure 2: GC/ID/HRMS Chromatogram of A) Persistent Pesticides and B) PCBs on a 30 M x 0.25 mm DB-5MS column.

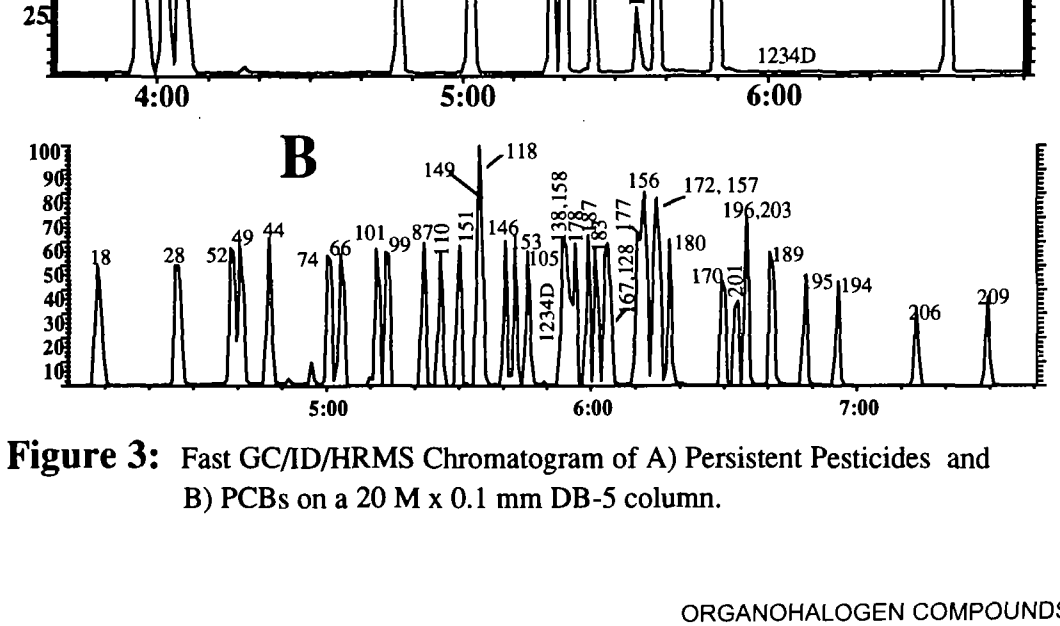

Figure 3: Fast GC/ID/HRMS Chromatogram of A) Persistent Pesticides and B) PCBs on a 20 M x 0.1 mm DB-5 column.

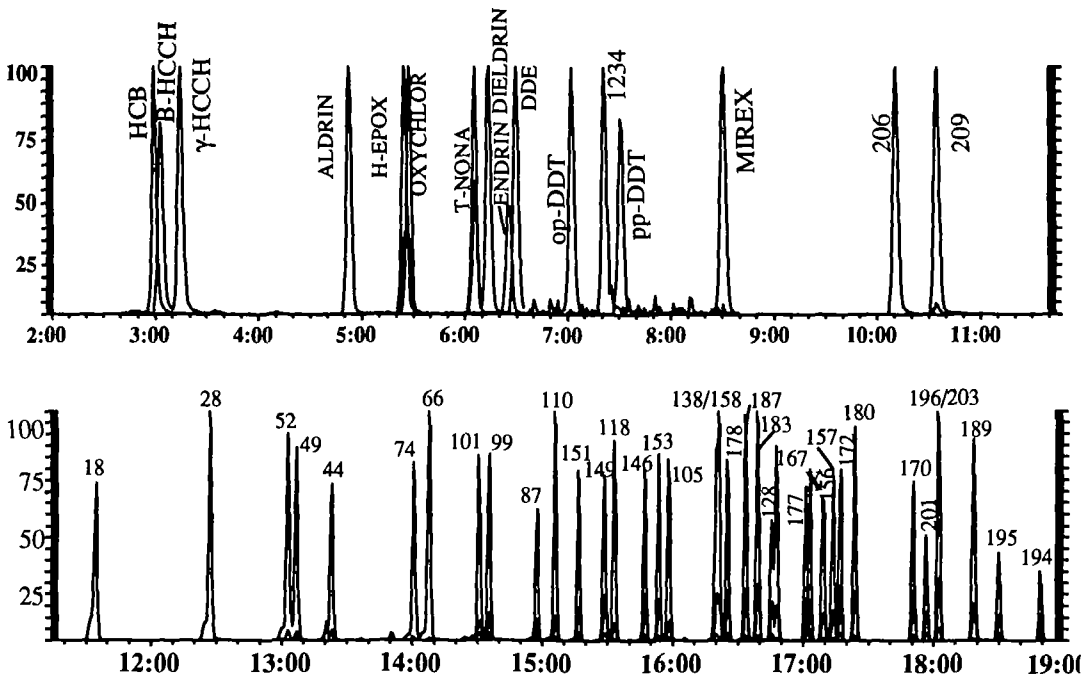

Figure 4: Parallel Column (Same Injector) GC/ID/HRMS of Persistent Pesticides and PCB Congeners. DB-1; 2m; 0.1mm ID; 0.1u FILM/DB-5; 30m; 0.25mm ID; 0.25u FILM