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Preliminary Evaluation of Hair as a Matrix for Monitoring Environmental Exposure

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The search for evidence of exposure to environmentally hazardous chemicals is one of the most important areas of environmental toxicology today. The most accessible markers of exposure are the actual environmental compounds. However, problems of metabolic conversion, excretion or storage make detection difficult. Some compounds (*i.e.* PCB's, DDT, DDE, dioxins, chlorophenols, and nonylphenol), are believed to disrupt endocrine systems at extremely low levels (Sonnenschein 1995). Therefore, it is very important to develop methods of analysis to ascertain chronic exposure to a variety of pollutant compounds at trace levels. There are a variety of testing methods available for determining individual exposure levels (Baselt 1988; Richter et al. 1986). However, these methods usually involve invasive sample collection of whole blood, plasma, or urine. Although these methods are successful in determining exposures to specified xenobiotic materials, they often are insufficient for analysis several days or even hours after the initial exposure (Baselt 1988). These methods may also fail to outline any pattern of exposure to a specific environmentally "unsafe" compound (Baselt 1988). In this short communication, we propose and evaluate a method of hair analysis that may prove to be quicker and less invasive than other methods used for analysis of environmental pollutants in biological systems.

It is well documented that hair is a reliable sample matrix for analysis of compounds such as, cocaine and benzoyl ecognine (cocaine metabolite) (Graham et al. 1989; Moller et al. 1993; Welch et al. 1993), β_2-antagonists (Polettini et al. 1996), phencyclidine (Sramck et al. 1985), nicotine and cotinine (nicotine metabolite) (Haley et al. 1985), methamphetamine and amphetamine (Suzuki et al. 1984), and morphine (Valente et al. 1981). The above reports demonstrate the ability of hair to incorporate and maintain levels of compounds of interest within the hair fiber. Therefore hair may also serve as a matrix for the analysis of exposure to environmentally important compounds. Hair has a β-pleated sheet structure (typical of structural proteins), which makes the hair very durable and also allows hair strands to solvate many analyte species within this hydrophobic environment. Since hair is an easily obtained, pain free

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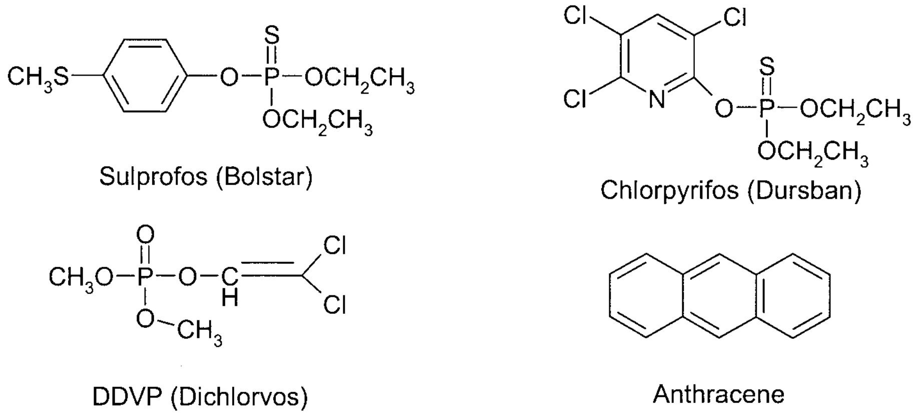
Sulprofos (Bolstar)

Chlorpyrifos (Dursban)

DDVP (Dichlorvos)

Anthracene

Figure 1. Structures of the compounds utilized for spiking experiments.

sample, hair analysis would be an ideal way to characterize exposure to environmentally hazardous compounds especially in pediatric situations. Since the levels of incorporation will be low, sensitivity of the analytical method becomes the primary concern.

Two model systems were selected to evaluate the analysis of xenobiotics incorporated in hair samples. Specifically, anthracene was chosen to represent the PAHs, and three organophosphate insecticides (OPs) were used to represent current use agricultural compounds (Figure 1).

MATERIALS AND METHODS

Hair used in this study was obtained from volunteers including personnel associated with this project. Aliquots of hair were weighed and cut into 2 cm sections to ensure complete emergence in spiking and digestion solutions. Hair samples used in the experiments varied in weight from 9 mg to 23 mg.

Two methods of spiking hair were evaluated. The first method involved submerging the hair into spiking solutions prepared by dissolving selected analytes at various concentrations in dimethyl sulfoxide (DMSO). The hair was allowed to equilibrate in the DMSO solution for up to 6 days. The second method consisted of placing ≈ 25µl of a spiking solution directly onto the hair sample (surface spike). The solvent was allowed to evaporate at ambient temperature. The hair recovered from either spiking method was thoroughly washed with water, followed by acetone to remove any residual analytes adhering to the exterior of the hair.

For enzyme digestion, a solution was prepared by dissolving 3 mg Proteinase K (Sigma Chemical Corp.) and 20mg dithiothreitol (Sigma Chemical Corp.) in 10mL of tris buffer at a pH of 6.2. The hair samples were incubated in this enzyme solution at 35°C for at least 72 hours (Baselt 1988). After digestion, the sample was extracted three times with 1 mL aliquots of methylene chloride (Burdick and Jackson). The hair extracts were concentrated and analyzed on a Varian Saturn 4D GC/MS system equipped with MS/MS and SIS capabilities (Varian, Walnut Creek, CA.). The Varian 3400Cx GC equipped with a 1078 temperature programmable injector and a Varian 8200Cx autosampler. All separations were performed using a 60m x 0.25mm x 0.25μm(film thickness) DB-5MS capillary column manufactured by J&W Scientific (Folsom, CA.). The GC temperature program for the hair analysis was: 50°C for 1 min.; temperature programmed from 50°C to 160°C at 20°C/min; followed by an increase from 160°C to 300°C at 10°C/min.; and finally 300°C isothermal for 2 min. The following mass spectrometer conditions were used: manifold temperature 250°C; filament current 35μA; multiplier voltage 1950V; and AGC target set to 15000. A SIS waveform was used to isolate m/z 165 to m/z 185 for anthracene.

RESULTS AND DISCUSSION

In this preliminary study, we utilized two ion trap mass spectrometry techniques, selected-ion storage (SIS) and tandem mass spectrometry (MS/MS), to increase the sensitivity of the analysis portion of the method. These ion manipulation techniques greatly improve signal to noise ratios observed from ion trap mass spectrometers by eliminating matrix ions (chemical noise) during analysis. In the selected-ion storage (SIS) process, a window of masses is selected for isolation and detection. This process is similar to the selected ion monitoring (SIM) methods available with quadrupole instruments. Improvements in sensitivity observed with the SIS method results from elimination of background ions during the ionization process that allows more ions of interest to be stored in the ion trap. The MS/MS technique increases sensitivity by elimination of matrix ion interferences during the analysis. While the absolute ion abundance observed from a MS/MS experiment is typically less than that of a full scan experiment, the signal-to-noise ratio becomes quite large through elimination of matrix interference. In this study, a nonresonant form of the MS/MS experiment was used (Wang et al. 1996). Nonresonant excitation involves application of DC square waveform (50 to 500 Hz) in a dipolar fashion to the endcaps of the ion trap assembly. This DC waveform causes a prompt shift in the locus of the ion cloud in the trap, thus instantaneously increasing the kinetic energy of all ions before collisional damping. Removal of the field reverses the location of the ion cloud again increasing the kinetic energy of the ions. Repeating this cycle for 20 ms imparts sufficient excitation for dissociation to occur. With DC amplitudes as high as 100V, even relatively stable ions can be induced to dissociate. The benefit of this approach over the resonant forms of excitation is the ease of implementation since knowledge of the precursor ion's secular frequency is not required. All ions in the trap experience the restoring force of the DC waveform and thus this approach is mass independent. Applications of SIS and MS/MS methods are critical for the trace analysis of compounds in complex matrices. The optimized MS/MS parameters for the organophosphates are given in Table 1.

Table 1. Retention times, optimized MS/MS parameters and product ions for the organophosphates used in this study.

Compounds	RT (min)	Precursor ion (m/z)	rf storage level m/z)	CID (volts)	Product ions (m/z)
dichlorvos	10.30	185	66	57	93
chlorpyrifos	18.41	314	172	83	258; 286
sulprofos	21.84	322	109	69	156; 198

The principle goal of this preliminary study was to determine if environmentally important molecules once incorporated into hair could be subsequently extracted and detected by mass spectrometry. In an initial experiment, a mixture of OPs was spiked into the enzyme solution to determine the stability of the OPs under digestion conditions. The OP/enzyme solution was incubated for 72 hours and then extracted with methylene chloride. Comparison of the data from the OP/enzyme extract with that of the original spiking solution indicated excellent recoveries (typically > 70%). Therefore, the enzymatic digestion was concluded to have little or no effect on the OPs. In the next experiment, 18 mg of hair was soaked for 3 days in 1mL of DMSO containing OP insecticides at 20 ng/component. The DMSO solvent is known for its unique ability to transport materials across biological membranes and has been used for spiking hair with illicit drugs (Richter et al. 1986). After the spiking equilibration period, the hair was recovered by filtration and washed with water and then acetone to remove any OPs adhering to the exterior of the hair. The hair sample was dried and subjected to enzymatic digestion for 72 hours in a sealed vial at 37°C. After 72 hours, the hair was extracted with methylene chloride, concentrated and analyzed by GC/MS/MS. Dichlorvos (DDVP), chlorpyrifos (Dursban), and sulprofos (Bolstar), were all observed in the MS/MS chromatograms obtained from the extract as shown in Figure 2. An intense signal was observed in a full scan spectra between 11.5 and 14.1 minutes resulting from matrix interferences. However, these interferences were not observed in the MS/MS reconstructed ion chromatogram. The reconstructed ion chromatogram (RIC) area counts associated with these three compounds indicated 50 pg for DDVP, 10 pg for chlorpyrifos, and 30 pg for sulprofos, injected on-column. These results demonstrate two important points: the ability of hair

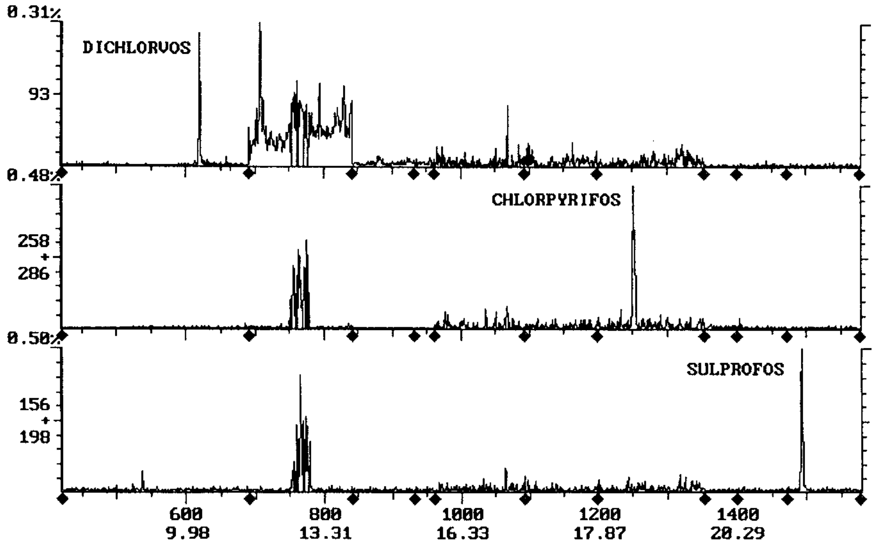

Figure 2. Reconstructed ion chromatograms for dichlorvos, chlorpyrifos, and sulprofos resulting form MS/MS analysis of an extract obtained from enzymatic digestion of an organophosphate spiked hair sample.

to incorporate pesticides and the viability of GC/MS/MS analysis for the analysis of these agriculturally and environmentally important compounds in hair.

The PAHs were next evaluated. Since the fragmentation of anthracene under EI conditions (70eV electrons) is limited, only very intense m/z 178 (molecular ion) was monitored. Once again, the submerging technique was employed to spike a hair sample with anthracene. When 20 mg of hair was submerged into a 0.5 mg/mL solution of anthracene in DMSO, analysis of the extract produced a RIC with a S/N ration of 246 indicating favorable incorporation into the hair.

Having determined that hair could be spiked with certain environmentally interesting compounds, it was of further interest to determine the extent of incorporation. This would allow us in the future to be able to develop standard reference hair samples. To accomplish this task, spike solutions containing chlorpyrifos and anthracene were prepared at concentrations ranging from 200 pg/mL to 1mg/mL and used to surface spike aliquots of hair. Figure 3 shows RICs obtained from an experiment where 25 ng anthracene and 50 ng chlorpyrifos were spiked on 15 mg of hair. Comparison of the peak areas obtained from these RICs to external standard calibration curves indicated 30 pg chlorpyrifos and 1 pg anthracene. Since the total spike was 50,000 pg, a recovery of 30 pg for

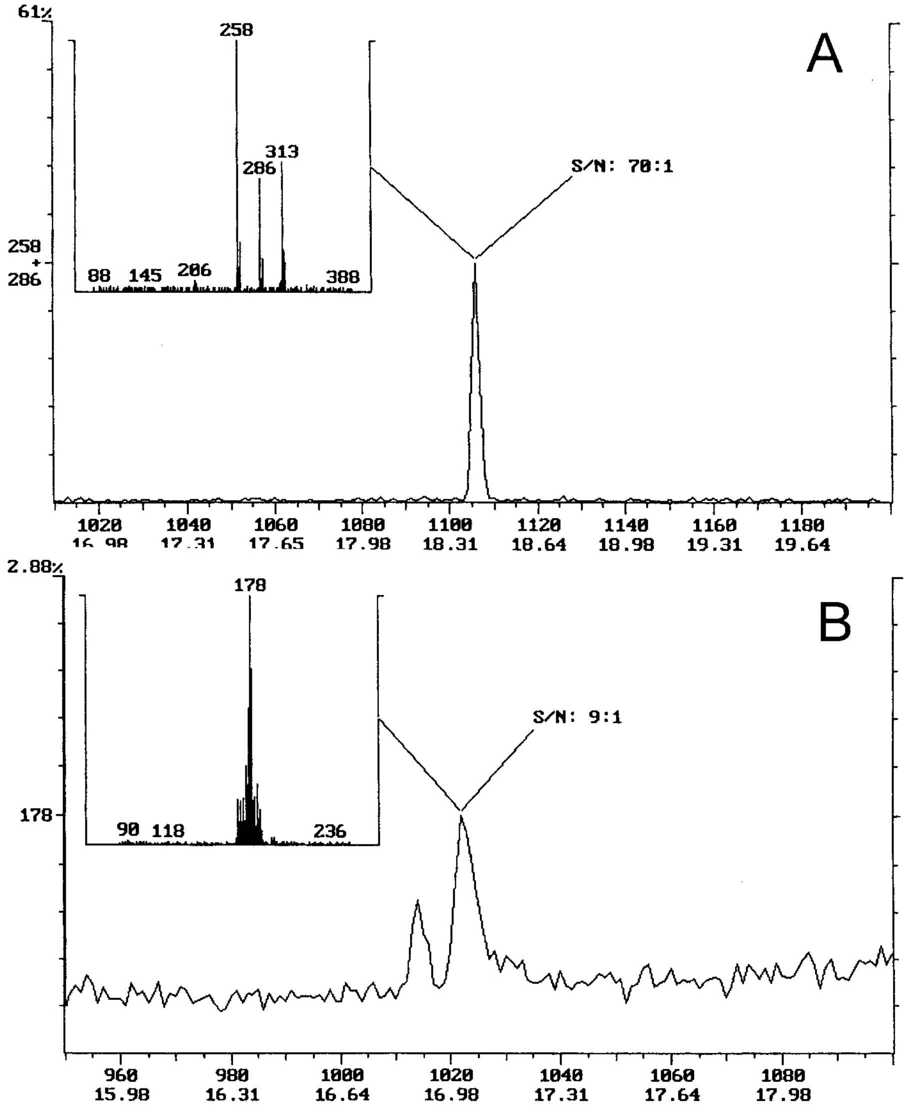

Figure 3. Comparison of spiking efficiencies of chlorpyrifos and anthracene: a) MS/MS chromatogram of chlorpyrifos at an estimated level of 30 pg on column. b) SIS chromatogram of anthracene at an estimated level of 1 pg on column. The spiking procedure for these compounds consisted of 50 ng for chlorpyrifos and 25 ng anthracene (surface spike protocol).

chlorpyrifos indicated an incorporation of approximately 0.06% of the total concentration into the hair fiber. Subsequent analysis of the acetone wash indicated a significant amount of pesticide was removed at that stage of the experiment. Since the total spike was 25,000 pg, a recovery of 1 pg for anthracene indicated an incorporation of only 0.004%. These data indicated that chlorpyrifos was incorporated into the hair 15 times more efficiently than anthracene. Despite the differences in incorporation rates, spiking of hair has displayed its utility in providing hair samples amenable to mass spectral analysis. For chlorpyrifos, 2.0 ppb sensitivity (30 pg chlorpyrifos/15 mg of hair) was demonstrated in this study, assuming 100% isolation efficiency, thus demonstrating the feasibility of this approach for conducting trace biological monitoring.

In this study, we demonstrated the feasibility of using hair as a matrix for detection for some environmentally hazardous compounds in conjunction with ion trap mass spectrometry. However, this research is in its preliminary stages and more work is required before hair becomes an accepted sample media. The main source of concern and future work with this technique involves determining the relationship between levels detected in hair and exposure. This method, if proven effective for other compounds, would be an excellent and easy way to analyze wildlife species and humans for exposure to environmental pollutants.

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