THE CONGENER SPECIFIC DETERMINATION OF PCBS: ANALYSES OF WHOLE BLOOD.

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ABSTRACT

A routine method for the determination of congener-specific PCBs in whole blood using GC/ECD dual-capillary column analysis, is under development. Recovery of 92 ± 8% S.D. for a 22.3 ppb standard has been obtained in thawed, whole blood extracted with ethyl ether/hexane. This method can be used in conjunction with carbon-fibre separation of suspected toxic congeners. Data generated by this method can be interpreted by multivariate statistical methods and be compared with enzyme-induction assays.

INTRODUCTION

The congener-specific determination of polychlorinated biphenyls (PCBs) in biological matrices is an active area of analytical method development. Data generated in these determinations are appropriate for: (i) the identification of environmentally exposed populations (1) (ii) the establishment of source Arochlors (2) and the grouping of exposed populations and subpopulations (2,3) and (iii) in the identification, in conjunction with enzyme-induction assays, of the congeners suspected to account for the toxicity of PCB mixtures (3,4). This laboratory is engaged in the development of routine PCB analysis methods in complex matrices which meet the demands of all three of these areas of concern. We present here progress in the development of a method for the determination of PCB congeners in whole blood which involves a straightforward extraction and a one-step column clean-up. A carbon/glass-fibre column clean-up for the separation of the potentially toxic non-ortho and mono-ortho substituted congeners has also been developed to be used in conjunction with the extraction of congener specific PCBs. The carbon/glass fibre separation procedure will be presented in poster form. The congener-specific PCB data generated in the analysis of whole blood as described here are suitable for interpretation by multivariate statistical analysis.

EXPERIMENTAL

Congener Specific PBCs in Whole Blood

The method of Burse et al. (5) has been modified for the extraction of PCBs in whole blood. An accurately weighed 5.0 g sample of thawed whole blood was placed in a centrifuge tube, a 2 mL portion of methanol added and the tube vortexed for about 1 min. The blood then was extracted with 3 x 5 mL 50:50 ethyl ether/hexane. The combined supernatant was gently evaporated to 1 mL under N_2 in a heated water bath. The concentrated supernatant was cleaned-up on anhydrous sodium sulphate, 40% H_2SO_4/silica gel and 5% H_2O deactivated Florisil packed sequentially in a large volume Pasteur pipette. The sample was added to the top of the column and eluted with 10 ml of hexane. The eluted sample was then evaporated under N_2 in a heated water bath to 1.0 mL or to 0.1 mL for capillary-column gas chromatographic analysis. All solvents used were distilled in glass (Caledon, Georgetown, Ontario).

Five whole blood samples from a single individual (courtesy Hamilton Area Red Cross) were spiked with a total PCB content of 2800 ppm and with 22.3 ppb PCB 65, in methanol, (final volume 1.0 mL) and carried through the method described above to assess extraction efficiencies. Three additional spiked samples (22.3 ppb PCB 65, in methanol, final volume of 0.100 mL) were extracted to assess recoveries in final volumes less than 1.0 mL. Unspiked blood from the same source was extracted for use as the blank. Well-characterized serum samples from the Center for Disease Control in Atlanta have been incorporated in this analysis scheme to assess accuracy.

Instrumental Analysis

Congener-specific PCBs were analyzed using a Hewlett-Packard model 5890 Gas Chromatograph with dual capillary columns and dual electron-capture detectors (GC/ECD). The GC/ECD was equipped with a 30 m DB-5 column and a 30 m DB-17 capillary column each with 0.25 mm inner diameter and 0.25 μm film thickness. The stationary phases of the columns used have substantially different polarities so that the possibility of an interference for a particular compound occurring on both columns is negligible. This system therefore allows peak confirmation. The injector temperature was set at 250 C, and the detector temperatures were set at 300 C. The injection port was operated in a splitless mode with a purge delay

of 1 minute. A 2 µL inject volume was used. The initial column temperature was 50 C (2 min. hold) followed by a 2 C/ min. ramp to 260 C (15 min. final hold) for a total run time of 122 minutes. The gas chromatograph was calibrated daily, using external standards. An in-house Arochlor standard made according to Swackhammer (6) (Cambridge Isotope Laboratories) was used for congener-specific analyses. Over seventy-five PCB congeners can be identified and quantitated by this method.

RESULTS AND DISCUSSION

Average recovery for PCB 65 in the spiked blood taken to a final volume of 1.0 mL was 92 ± 8 % (standard deviation, n=5). Average recovery for selected isomers for each homologue group was 83 ± 9% (pooled standard deviation, n=40, k=8). The whole blood extraction method also has been extended to final volumes of 0.100 mL. Preliminary results with the 0.100 mL final volumes indicate lower recoveries of 15%, 36%, and 51% for PCB 65. These low recoveries have been traced, at least in part, to evaporative losses. Preliminary limits of detection are estimated, based on three times background, to be 0.2 ppb for a sample size of 5.0 g and a final volume of 1.0 mL and 0.02 ppb for a sample size of 5.0 g and a final volume of 0.100 mL.

Analysis of control serum samples from the Atlanta Center for Disease Control indicates that the method described here is capable of detecting a full complement of PCB congeners. Additional work in progress includes the determination of statistical detection limits and the standardization of lipid determinations for results reporting on a lipid basis.

ACKNOWLEDGMENTS

We acknowledge the kind help of Dr. Anita Ali and Ms. Christina Spiak of the Hamilton Area Red Cross for its gift of whole blood discards. We thank Dr. D.Patterson, of the Atlanta Centre for Disease Control for providing the control samples.

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