

Note

High resolution gas chromatographic analysis of cytochrome P-448 inducing polychlorinated biphenyl congeners in hazardous waste

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Polychlorinated biphenyls (PCBs), which have been commercially manufactured since 1929, are complex mixtures of many congeners and several homologues. They have been used in a variety of industrial and non-industrial applications which has allowed large quantities to be released into the workplace and environment¹⁻⁶. Based on a 1981 estimate, as many as 17 million pounds of PCBs were entering land disposal sites in the U.S.A. annually (for a total of 385 million pounds)^{5,7}. While their toxicity varies among isomer and toxicity test species, PCBs have been sufficiently implicated in animal and human toxicity to warrant their ban in the U.S.A.¹.

Most of the early methods of analysis of PCBs were directed toward commercial products (such as Aroclors). It is still common to report results as ppm PCB-as Aroclor 1242, 1254, etc.^{8,9}. Disposal of PCBs is based on a generic classification as PCB type and quantitated as > 50 or > 500 ppm. This is at best an approximation when the sample matches the standard, and is not applicable when microbial degradation and/or mammalian metabolism preclude visual matchings^{10,11}. The problem of PCB analysis has become more complex with the discovery of specific congener toxicities^{10,12-18}, with special concerns arising over the health effects of populations exposed to PCBs through contamination of the food chain¹⁹⁻²², in the workplace²³⁻²⁶, and most recently to the questions of by-product PCBs^{2,27} and incinerator and chemical destruction method residues²⁸⁻²⁹.

Until recently the results of body burden studies have been reported as ppm of Aroclor X with no mention made as to the presence of specific PCB congeners. A recent study has reported the results with quantitation of specific congeners³⁰. The importance of the quantitation of specific PCBs is underscored by studies that have demonstrated differences between mechanisms and degree of toxicity for individual PCB congeners^{6,10,12-18}. For example, induction of cytochrome P-448 hepatic mixed function oxidase is effected only by certain PCBs. The P-448 is a fraction of the native hepatic enzyme system that metabolizes polycyclic aromatic hydrocarbons, such as

benzo[*a*]pyrene, to reactive electrophiles. These electrophiles react with available tissue macromolecules, including DNA. Therefore, the induction of the P-448 fraction is thought to be of significance in the proposed mechanism of mutagenesis and carcinogenesis caused by xenobiotics. The most potent of these P-448 inducers are PCB No. 77 (3,4,3',4'-tetrachlorobiphenyl), 126 (3,4,3',4',5'-pentachlorobiphenyl), and 169 (3,4,5,3',4',5'-hexachlorobiphenyl), (see refs. 27 and 31 for a discussion of this system of nomenclature). Reports of isomer-specific biochemical reactions include the rapid metabolism of PCBs with unsubstituted adjacent carbons, which may be related to the potential for metabolism by an epoxide intermediate^{18,32}.

Because the focus of PCB toxicity research efforts has been centered on the cytochrome P-448 inducing effects of congeners 77, 126 and 169, it was decided that capillary chromatographic analysis of actual samples taken from an uncontrolled hazardous waste disposal facility could help to determine if further studies are necessary to formulate appropriate personal protection and waste disposal strategies. This study addresses the gas chromatographic (GC) examination of actual PCB contaminated samples obtained during the clean-up of an uncontrolled disposal facility.

EXPERIMENTAL

All samples were actual PCB-containing sludges from a hazardous waste facility. Sample preparation included: extraction in hexane, concentrated sulfuric acid clean-up and additional clean-up by florisil column as necessary as described by Erickson²⁷. The tetra- and hexachlorobiphenyl isomers were purchased from UltraScientific and the pentachlorobiphenyl isomer was supplied by the U.S. Army Corps of Engineers.

The GC analyses were performed using a Varian Model 4600 gas chromatograph equipped with a ⁶³Ni electron-capture detector, a J&W Scientific on-column, splitless injector and a Vista 401 data system with dual disk drives. The column used was a 30 m × 0.25 mm I.D. fused silica, DB-5 liquid phase, prepared by J&W Scientific. The carrier gas was helium at a constant head pressure of 21 p.s.i. with a resultant linear flow-rate of 0.86 ml/min (27 cm/sec) at 235°C (the temperature at the approximate retention time of 3,4,5,3',4',5'-hexachlorobiphenyl). The injector purge flow was kept at 12 ml/min. The detector make-up gas was nitrogen with a flow-rate of 60 ml/min. The detector was maintained at 280°C and injections were performed at room temperature. The temperature program used was 100–150°C at 10°C/min and 150–235°C at 2°C/min. Injection volumes ranged from 0.5–2.0 μl.

The packed column analyses were also performed on a Varian Model 4600 gas chromatograph equipped with a ⁶³Ni electron-capture detector. The column was a 6 ft. × 1/8 in. I.D. Supelco Tight Spec with a 3% SP-2100 liquid phase on a 100–120-mesh Supelcoport. The carrier gas was nitrogen with a flow-rate of 30 ml/min. The injector was maintained at 225°C and the detector at 300°C. All runs were performed isothermally at 190°C.

RESULTS AND DISCUSSION

The chromatographic separation achieved using both packed and capillary GC procedures are illustrated in Figs. 1 and 2. Figs. 1a and 2a illustrate the analysis of

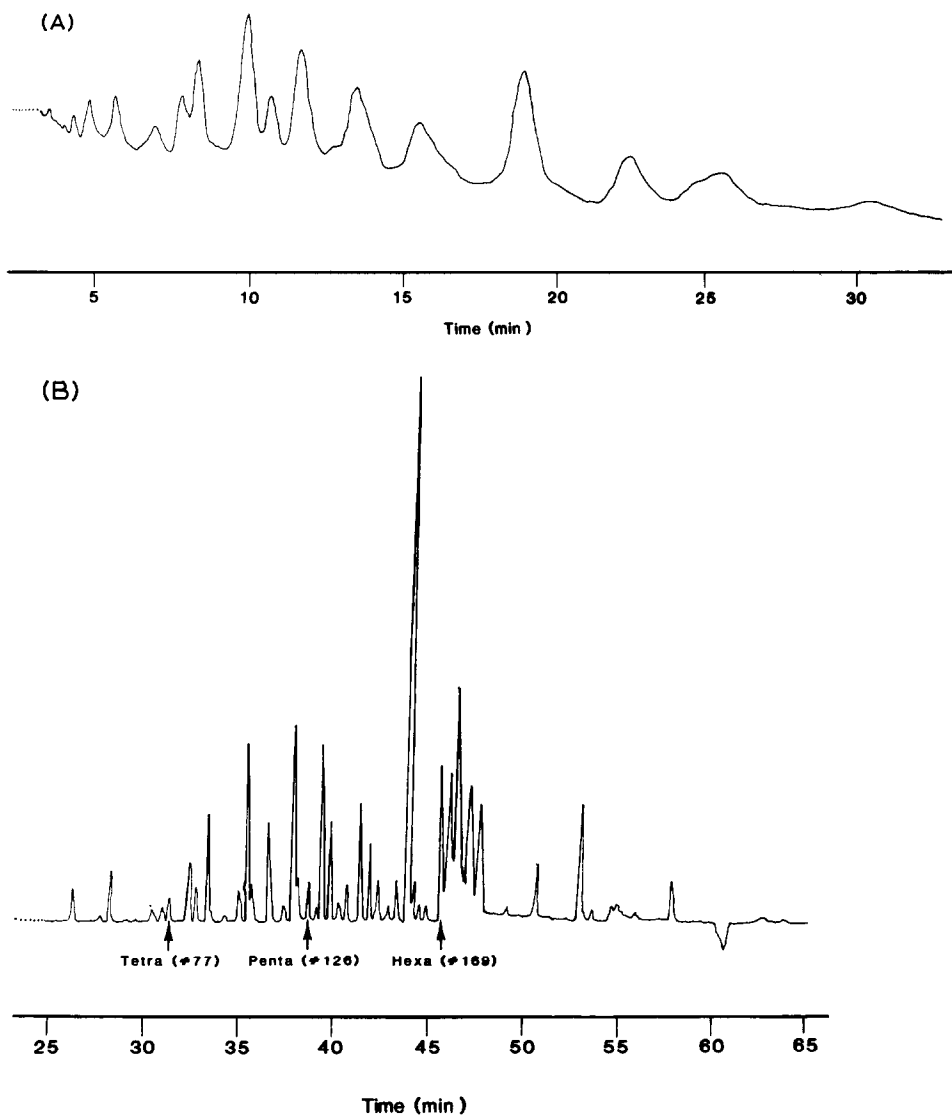


Fig. 1. Chromatograms of Aroclor 1260 using packed column isothermal (A) and temperature programmed capillary (B) techniques.

Aroclor 1260. Figs. 1b and 2b illustrate the analysis sample No. 9, which is a typical sample of hazardous waste sludge obtained from the remedial action site. Prior to chromatographic analysis, this sample had been subject to clean-up using florisil.

The presence of the tetrachlorobiphenyl congener No. 77, the pentachlorobiphenyl congener No. 26, and the hexachlorobiphenyl congener No. 169 are indicated by standard addition of those pure congeners to the sample prior to the high-resolution chromatographic separation. In each instance, the retention time and chromatographic peak shapes were unchanged. A separation number of 43 was measured

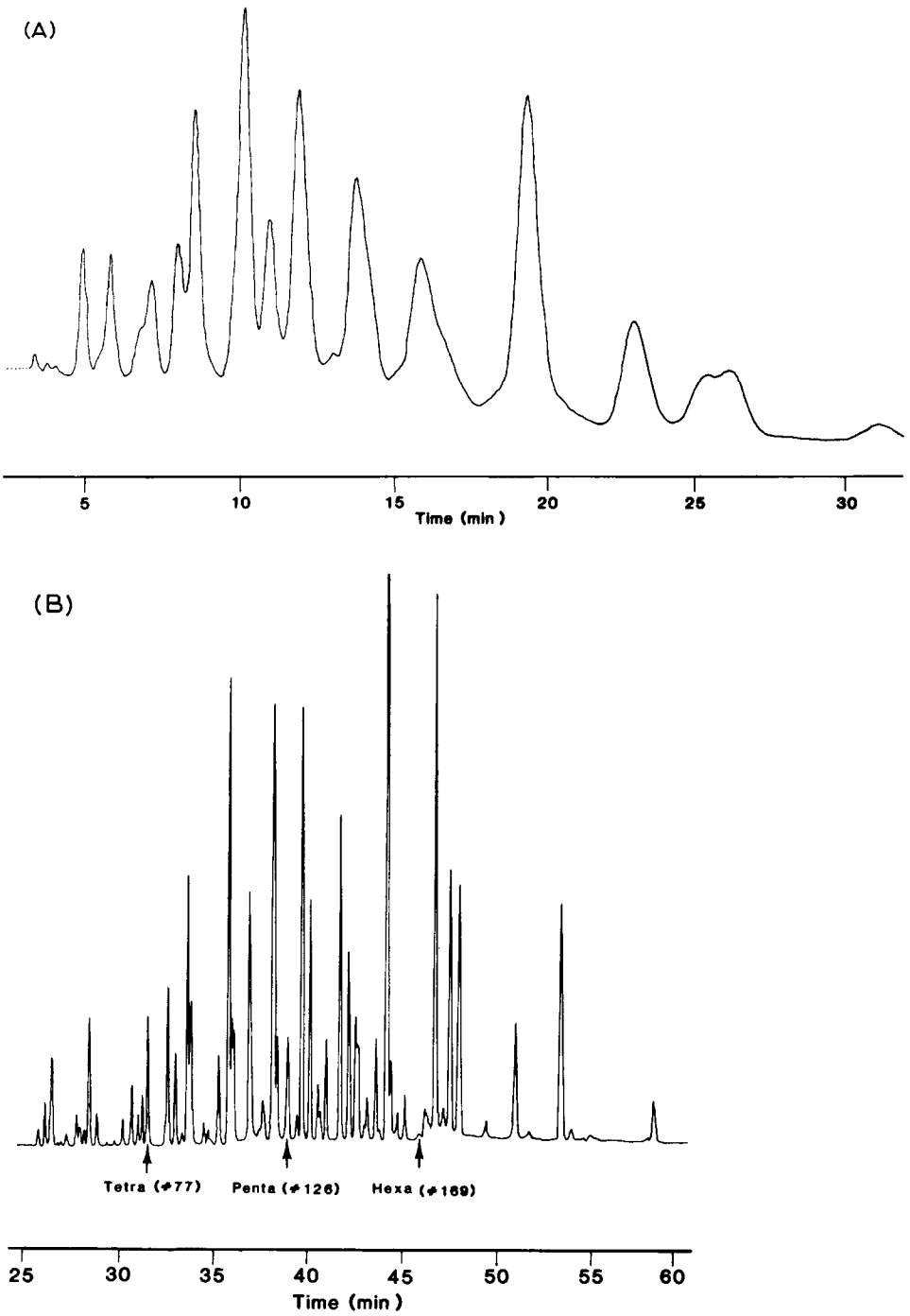


Fig. 2. Chromatograms of hazardous waste extract sample 9 using packed column isothermal (A) and temperature programmed capillary (B) techniques.

TABLE I

TOTAL PCB AND PCB-CONGENER CONTENT (ppm, w/v) OF SAMPLES FROM A HAZARDOUS WASTE REMEDIAL ACTION SITE

ND = Not detected.

Sample	Total*	Tetra** (No. 77)***	Penta** (No. 126)***	Hexa** (No. 169)***
2	141	0.6	0.07	0.3
4	400	0.4	0.2	0.5
5	1618	3	2	2
6	1136	2	1	ND
9	8346	8	10	Trace
10	1444	6	3	ND
11	16	0.03	0.01	Trace

* Determined as Aroclor 1260 by packed column GC.

** Determined by capillary column GC.

*** 3,4,3',4'-Tetrachlorobiphenyl (congener 77); 3,4,3',4',5'-Pentachlorobiphenyl (congener 126); 3,4,5,3',4',5'-Hexachlorobiphenyl (congener 169).

for congeners No. 77 and No. 126, which differ by one chlorine. It is recognized that separation numbers are usually applied to homologous series differing by one methylene group³². In this case, the application of the separation number to two compounds structurally identical except for one additional chlorine can be useful in indicating the column performance³³.

The results of the analysis of the hazardous waste sample extracts for the P-448 inducing isomers are shown in Table I along with the results obtained for total "Aroclor" concentration by the low resolution (packed column, isothermal) analysis of the same samples. The ranges of concentrations, given in ppm (w/v), found for the isomers are 0.03–8 for tetra (No. 77), 0.01–10 for penta (No. 126), and not detected (*ca.* 0.05)–2 for hexa (No. 169). There is no apparent correlation between the total Aroclor concentrations and the concentrations of the specific P-448 inducing congeners.

Although this study did not include a health hazard evaluation, the presence of the P-448 inducing congeners in the ppm range, coupled with the abundance of literature dealing with the significance of P-448 induction, indicate that a re-evaluation of the methods of disposal and personal protection at hazardous waste sites may be necessary.

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