Bull. Environm. Contam. Toxicol. 23,051-056 (1979)

Separation and Quantitation of 3,3′,4,4′-Tetrachlorobiphenyl and 3,3′,4,4′,5,5′-Hexachlorobiphenyl in Aroclors Using Florisil Column Chromatography and Gas-Liquid Chromatography

LaVerne R. Kamops, William J. Trotter, Susan J. Young, Audrey C. Smith, John A. G. Roach, and Samuel W. Page

Division of Chemistry and Physics, Food and Drug Administration, U.S. Department of Health, Education, and Welfare, Washington, DC 20204

There has been considerable interest in those polychlorinated biphenyl (PCB) congeners which exhibit much greater specific biological activity and/or toxicity than the many other possible PCB congeners. POLAND and GLOVER (1977) examined 16 halogenated biphenyls for their ability to induce hepatic aryl hydrocarbon hydroxylase (AHH) activity in the chicken embryo. Of the 16 tested, only 3 congeners, 3,3',4,4'-tetrachlorobiphenyl (3,3',4,4'-TCB), 3,3',4,4',5,5'-hexachlorobiphenyl (3,3',4,4',5,5'-HCB), and 3,3',4,4',5,5'-hexabromobiphenyl, were found to be active. It was concluded that there are 2 structural requirements for halogenated biphenyls to induce AHH activity: (1) the presence of at least 2 adjacent halogen atoms in the lateral positions of each phenyl ring, and (2) the absence of halogens adjacent to the biphenyl bridge (positions 2,2',6, and 6'). In addition, GOLDSTEIN et al. (1977) found that 3,3',4,4'-TCB and 3,3',4,4',5,5'-HCB exhibit certain biological activity to a greater extent than the other PCB congeners examined. MCKINNEY et al. (1976) compared 5 HCB isomers in relation to several biological parameters including toxicity as measured by mortality; 3,3',4,4',5,5'-HCB was found to be the most biologically active.

EXPERIMENTAL

Reagents and Apparatus

(a) Solvents. Petroleum ether and ethyl ether suitable for use in pesticide residue analysis (available from Burdick & Jackson Laboratories, Inc., Muskegon, MI 49442) (OFFICIAL METHODS OF ANALYSIS 1975).

(b) Florisil. 60-100 mesh PR grade (Floridin Co., Berkeley Springs, WV 25411). If received in plastic containers or paperboard boxes, transfer Florisil to glass containers with glass-stoppered or foil-covered lids and store in the dark. Heat uncovered at 130°C for $\geq$5 hr but preferably overnight before use. Store at 130°C in foil-covered bottles.

(c) Aroclors 1242, 1248, 1254, and 1260. Obtained from FDA Industrial Chemicals Repository; Monsanto lot numbers of these

0007-4861/79/0023-0051 $01.20

Aroclors were AK-255, AL-3, AK-38, and AK-3, respectively. An additional sample of Aroclor 1248 was obtained from J. Allen, University of Wisconsin, Madison, WI; this Aroclor (lot number not traceable) was used in his PCB feeding studies with monkeys (ALLEN and BARSOTTI 1976).

(d) 3,3',4,4'-TCB and 3,3',4,4',5,5'-HCB. Obtained from J. McKinney, NIEHS, Research Triangle Park, NC.

(e) Gas-Liquid Chromatographs (GLCs). (1) Varian (Palo Alto, CA 94303) 3700; column, 6 meter x 0.27 mm id glass $C_{87}H_{176}$ WCOT capillary and ^{63}Ni-EC detector. Operating conditions: column, 170°C; detector, 320°C; injector, 300°C; column flow, 1 ml N_2/min; injection split 1/75 to detector. (2) Perkin-Elmer (Norwalk, CT 06856) 3920 with 6' x 4 mm id glass column packed with 5% OV-101 on 80-100 mesh Chromosorb W(HP) and ^{63}Ni-EC detector. Operating conditions: column, 200°C; detector 325°C; injector, 225°C; column flow, 60 ml (5 + 95) (CH_4 + Ar)/min. At these GLC conditions, 3,3',4,4'-TCB and 3,3',4,4',5,5'-HCB elute at retentions relative to aldrin of 1.88 and 5.67, respectively.

(f) Mass Spectrometer (MS). Finnigan (Sunnyvale, CA 94056) 3300F, coupled to Finnigan 9500 GLC via glass jet separator. GLC operating conditions: 5' x 2 mm id glass column packed with 3% OV-101 on 80-100 mesh Chromosorb W(HP): column flow, 25 ml He/min; injector, 230°; column, 200°C for TCB, 220°C for HCB; glass jet separator, 210°C; transfer line, 220°C. With this GLC column at 200°C, 3,3'5,5'-TCB, 3,3',4,5'-TCB, 3,3',4,4'-TCB, and 3,3',4,4',5,5'-HCB elute at retentions relative to p,p'-DDE of 0.68, 0.81, 1.04, and 1.75, respectively. MS operating conditions: full mass scans recorded at 2 sec intervals in electron impact mode with Finnigan 6100 data system; electron energy, 70 eV; filament emission current, 0.5 ma.

Syntheses of 3,3',4,4'-TCB, 3,3',5,5'-TCB, and a Mixture Containing 3,3',4,5'-TCB

These TCBs were prepared using palladium acetate coupling reaction (modification of method of CLARK et al., 1975) with (A) 1,2-dichloro-5-iodobenzene (3,3',4,4'-TCB); (B) 1,3-dichloro-5-iodobenzene (3,3',5,5'-TCB); (C) combination of (A) and (B) (mixture of 3,3',4,5'-TCB, 3,3',4,4'-TCB, and 3,3',5,5'-TCB). Heat 1.0 g (3.7 mmole) dichloroiodobenzene isomer and 0.42 g (1.9 mmole) palladium acetate in 35 ml anhydrous dimethylformamide at 140-150°, with stirring for about 4 hr under anhydrous conditions. Monitor the progress of the reaction by GLC analyses of aliquots from reaction mixture. Cool the reaction mixture and filter it through 25 g Celite 545, using a 4-inch Buchner funnel fitted with Whatman No. 598

filter paper. Add 500 ml water to the filtrate to precipitate the TCB or TCB mixture. Collect the precipitate by filtration through a 4-inch medium-frit, sintered-glass funnel. Wash the TCB(s) precipitate with 200 ml aqueous saturated sodium metabisulfite, then with 200 ml H_2O, and air-dry TCB(s) in the funnel. Purify TCB(s) by column chromatography as follows: Weigh 20 g alumina (ICN, basic, activity grade super 1) into a 400 ml beaker, and add sufficient hexane to make a slurry. Transfer the slurry to a 22 mm id x 300 mm glass column with medium porosity glass frit and a Teflon stopcock. Dissolve the TCB(s) precipitate in about 20 ml methylene chloride. Add about 2 g alumina and mix thoroughly. Remove the solvent on a rotary evaporator. Transfer the alumina containing the TCB(s) to a prepared alumina column. Elute the column (5 ml/min) with 200 ml hexane (discard). Elute the column with 300 ml (1 + 99 v/v) methylene chloride + hexane, collecting the eluate in a 500 ml round-bottom flask. Remove the solvent on a rotary evaporator.

The flask contains the purified TCB(s). The synthesis yields about 320 mg of product (yield about 60%) with a purity >98% (TLC, GLC, GLC/MS). Structure of the TCB(s) was confirmed by NMR, IR, and MS. Reaction conditions have not yet been optimized. The competing protio-deiodination reaction was increased markedly by the presence of water. 3,3',4,4',5,5'-HCB and a mixture containing 3,3',4,4',5-pentachlorobiphenyl have also been prepared by similar syntheses.

Method

Since the adsorptivity of activated Florisil may vary from lot to lot, the analyst may need to determine the exact amount of a particular lot of Florisil and/or petroleum ether eluant which yields optimum separation of most of the PCB congeners, 3,3',4,4'-TCB, and 3,3',4,4',5,5'-HCB. We used Florisil lot 702, which had a lauric acid value of 96.16 equivalent to 22.9 g Florisil (private communication, FDA Minneapolis District) (FDA Pesticide Analytical Manual 1973 (PAM) I,121.32).

Chromatograph the sample extract or Aroclor standard solution (we used about 100 µg PCBs) on a PAM I 211.14d Florisil column. To elute most of PCB congeners (Fraction A), substitute 250 ml petroleum ether for the first eluant. Elute 3,3',4,4'-TCB and 3,3',4,4',5,5'-HCB (Fraction B) with 200 ml (6 + 94) ethyl ether + petroleum ether. Concentrate Fraction B to a suitable volume for GLC analysis. Determine levels of 3,3',4,4'-TCB and 3,3',4,4',5,5'-HCB by comparing appropriate gas chromatogram peaks (area or height) of reference solutions and Fraction B.

RESULTS AND DISCUSSION

When Aroclor standard solutions were chromatographed on the Florisil column, about 95% of the PCBs were eluted in Fraction A. 3,3',4,4'-TCB and 3,3',4,4',5,5'-HCB, however, eluted mostly in Fraction B. Four recovery determinations through the method, each using 600 ng 3,3',4,4'-TCB, averaged 81% recovery (standard deviation, 5%) in Fraction B. Four recovery determinations through the method, each using 100 µg 3,3',4,4',5,5'-HCB, averaged 67% recovery (standard deviation, 18%) in Fraction B. We attribute the greater adsorptivity on Florisil of 3,3',4,4'-TCB and 3,3',4,4',5,5'-HCB to the fact that these molecules present greater and more polarizable π electron density to an adsorbent for interaction than most ortho-substituted PCBs. This is due to the greater planar character of nonortho-substituted PCBs like 3,3',4,4'-TCB and 3,3',4,4',5,5'-HCB compared to ortho-substituted PCBs. Ortho chlorines can hinder the free rotation of the phenyl groups in PCBs (SISSONS and WELTI 1971) and are thought to twist the phenyl groups out of coplanarity (MCKINNEY et al. 1976). Therefore, the planes of the π electron clouds for the phenyl groups are effectively skewed for PCBs with ortho chlorines. Ortho-substituted PCBs account for a large portion of the PCB congeners in many Aroclors (SISSONS and WELTI 1971).

TABLE 1

Analysis of Aroclors for 3,3',4,4'-TCB (ppm)
(ND = not detected)

Aroclor	3,3',4,4'-TCB	No. of determinations	Standard deviation
1242	2300	4	280
1248 (FDA)	2400	3	250
1248 (Allen)	3300	2	0
1254	ND	2	-
1260	ND	2	-

Table 1 presents the results of the GLC analyses of Aroclor standards for 3,3',4,4'-TCB. The values in Table 1 were not corrected for the recovery of 3,3',4,4'-TCB through the method. We also determined 3,3',4,4',5,5'-HCB and did not detect any of this component in these Aroclors. This is consistent with the low probability of producing an HCB with 4 meta-chlorines by chlorinating biphenyl. The analyses for 3,3',4,4'-TCB and 3,3',4,4',5,5'-HCB were performed by EC-GLC, using a capillary and a packed column, respectively. Our limits of detection of 3,3',4,4'-TCB and 3,3',4,4',5,5'-HCB were both about 200 ppm due to the amounts of Aroclors analyzed.

Figures 1 and 2 are gas chromatograms of Fraction B from the Florisil column chromatography of Aroclors 1248 (capillary column GLC) and 1242 (packed column GLC), respectively. The highest level of 3,3',4,4'-TCB in the Aroclors was found in Aroclor 1248. This is consistent with the fact that Aroclor 1248 should contain more TCB than the other Aroclors; Aroclor 1248 has a chlorine content (48%) similar to that of TCB (49%). SAEKI et al. (1971) have identified 3,3',4,4'-TCB as a component of Kanechlor 400; we found that Kanechlor 400 is similar in GLC response to Aroclor 1248. SISSONS and WELTI (1971) report that 3,3',4,4'-TCB would have a GLC retention (SCOT Apiezon L GLC column) later than expected for a TCB; they report that 3,3',4,4'-TCB would elute in the same region as penta- and hexa-chlorobiphenyls. Our MS analysis using a packed GLC column confirmed that the component in Aroclor 1242 at the retention time of 3,3',4,4'-TCB was a TCB. Using this same packed GLC column, MS analysis showed that a TCB in Aroclor 1242, a TCB and a pentachlorobiphenyl in Aroclor 1248, a pentachlorobiphenyl in Aroclor 1254, and a pentachlorobiphenyl and an HCB in Aroclor 1260

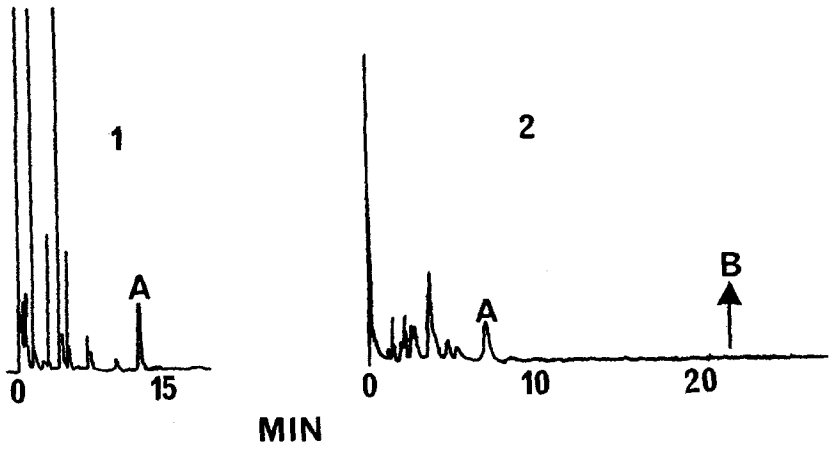

FIG. 1 - Capillary column EC-GLC curve of Fraction B from Florisil column chromatography of 100 µg Aroclor 1248. 1.5 µg equivalent Aroclor 1248 injected. Peak A: GLC peak due to 3,3',4,4'-TCB component of Aroclor 1248.

FIG. 2 - Packed column EC-GLC curve of Fraction B from Florisil column chromatography of 100 µg Aroclor 1242. 230 µg equivalent Aroclor 1242 injected. Peak A: GLC peak due to 3,3',4,4'-TCB component of Aroclor 1242. B: retention time of 3,3',4,4',5,5'-HCB reference standard.

elute at about the GLC retention time of 3,3 4,4'-TCB. MS analyses estimated the quantity of TCB eluting at the GLC packed column retention time of 3,3',4,4'-TCB as 2700 ppm, Aroclor 1242; 3500 ppm, Aroclor 1248 (FDA); and 3600 ppm, Aroclor 1248 (J. Allen).

Since we attribute the Florisil column chromatographic separation of 3,3',4,4'-TCB and 3,3',4,4',5,5'-HCB from most of the PCBs in an Aroclor to be due to the lack of ortho substitution in 3,3',4,4'-TCB and 3,3',4,4',5,5'-HCB, we synthesized and gas-chromatographed 3,3',5,5'-TCB, 3,3',4,5'-TCB, and 3,3',4,4'-TCB. This was done to eliminate the possibility that the TCB congener in an Aroclor eluting at the GLC retention time of the 3,3',4,4'-TCB reference standard might be 3,3',5,5'-TCB or 3,3',4,5'-TCB. 3,3',4,4'-TCB, 3,3'5,5'-TCB, and 3,3',4,5'-TCB are the only nonortho-substituted TCBs with 2 chlorines in each phenyl ring. We found that 3,3',5,5'-TCB and 3,3',4,5'-TCB are readily resolvable by GLC from the component eluting at the retention time of 3,3',4,4'-TCB in Aroclors.

At the present time, we are analyzing food samples containing high PCB residues for the presence of 3,3',4,4'-TCB and 3,3',4,4', 5,5'-HCB (50-800 ppm).

ACKNOWLEDGMENTS

We thank the following of the Division of Chemical Technology, Food and Drug Administration: D. Phillipson for determining the capillary GLC conditions, T. Farrell for the use of his capillary GLC, and K. White and R. Davis for their assistance in the TCB synthesis.

REFERENCES

ALLEN, J. and D. BARSOTTI: Toxicology 6, 331 (1976).
CLARK, F., R. NORMAN and C. THOMAS: J. Chem. Soc. Perkin Trans. I 1975, 121.
GOLDSTEIN, J., P. HICKMAN, H. BERGMAN, J. MCKINNEY and M. WALKER: Chem.-Biol. Interact. 17, 69 (1977).
MCKINNEY, J., K. CHAE, B. GUPTA, J. MOORE and J. GOLDSTEIN: Toxicol. Appl. Pharmacol. 36, 65 (1976).
OFFICIAL METHODS OF ANALYSIS, 12th Ed., secs. 29.001-29.015, Association of Official Analytical Chemists, Washington, DC (1975).
PESTICIDE ANALYTICAL MANUAL. 2nd Ed., Vol. 1. Food and Drug Administration, Washington, DC (1973 revision).
POLAND, A. and E. GLOVER: Mol. Pharmacol. 13, 924 (1977).
SAEKI, S., A. TSUTSUI, K. OGURI, H. YOSHIMURA and M. HOMANA: Fukuoka Acta Medica 62, 20 (1971).
SISSONS, D. and D. WELTI: J. Chromatogr. 60, 15 (1971).