Gas Chromatographic Characteristics of Authentic Chlorinated Dibenzofurans. Identification of Two Isomers in American and Japanese Polychlorinated Biphenyls

Retention times of 15 authentic standards of chlorinated dibenzofurans containing from three to six chlorine atoms were determined relative to dieldrin on gas chromatograph columns of four different polarities (3% OV-1; 5% SP-2401; 1.5% OV-17/1.95% QF-1; and 3% OV-1/5% OV-210/6% OV-225). On the basis of coinjections of the standards with extracts from American (Aroclor) and Japanese (Kanechlor) polychlorinated biphenyls (PCB), two of the compounds in those extracts have been identified as 2,3,7,8-tetra- and 2,3,4,7,8-pentachlorodibenzofuran. The concentrations of the compounds were estimated to range from 0.08 to 0.83 μg/g of PCB.

Vos et al. (1970) have identified toxic components of European polychlorinated biphenyls (PCB) as two chlorinated dibenzofurans. Subsequently, several additional chlorinated dibenzofurans were found in the German preparation examined by Vos et al. (Bowes et al., 1973). Further investigations have led to the detection by high-resolution mass spectrometry of those contaminants in American (Aroclor) PCB (Bowes et al., 1975). A recent study reports unidentified tri-, tetra-, and possibly pentachlorodibenzofuran components in Kanechlor 400 (Roach and Pomerantz, 1974). Kuratsune et al. (1972) have identified Kanechlor 400 as the agent responsible for Yusho, a toxic syndrome in humans who have ingested contaminated rice oil.

The reported high toxicity of the chlorinated dibenzofurans (Vos et al., 1970) and their presence in PCB have prompted synthesis of authentic standards and determination of their effects on induction of the enzyme aryl hydrocarbon hydroxylase (Kanda et al., 1974). The present paper reports gas chromatographic characteristics of those standards, and the identification of two isomers in American and Japanese PCB based on co-injections of the standards with extracts from the PCB.

EXPERIMENTAL SECTION

Synthesis of Chlorinated Dibenzofurans. Authentic dibenzofuran derivatives (Table I) were synthesized by regiospecific methods and characterized by proton magnetic resonance and mass spectrometry (Kanda et al., 1974).

Extraction of Chlorinated Dibenzofurans from PCB, and Quantification. Aroclor 1248 and 1254 preparations (obtained in 1969 from Monsanto) were placed on Florisil columns, eluted in solvents of increasing polarity, and partitioned over alumina as described elsewhere (Bowes et al., 1975). The latter separation yielded mixtures of chlorinated dibenzofurans essentially free of PCB interference. Chromatograms of extract fractions resulting from use of these techniques are illustrated in Bowes et al. (1975) for Aroclor 1254 and in Bowes et al. (1973) for Clophen A-60, a German PCB. Coinjections of authentic standards were made with one or more fractions recovered from the Florisil column (and passed over alumina) for each PCB. All chlorinated dibenzofurans usually were found in the first three fractions. Once coincidence of authentic dibenzofuran peak with extract peak was established on the four GC columns (described below) for one or more fractions from each PCB, isomer concentrations were estimated for each fraction by assuming a peak having the same retention time as the dibenzofuran isomer is that compound, and summing the concentrations for all fractions if the peak occurred on the chromatogram (the OV-1 column was used for this basis of comparison). Compounds were quantified with the use of standard curves based on injections of different amounts of the appropriate dibenzofuran isomer.

For the present study, the same extraction and partitioning techniques were applied to Kanechlor 200 and 500. Compounds having the same gas chromatographic retention times on the OV-1 column as compounds identified previously by mass spectrometry as chlorinated dibenzofurans in the Aroclor PCB were isolated as mixtures also from the Japanese PCB. The gas chromatographic techniques described for the Aroclor extracts (co-injection and quantification) were used for the Kanechlor extracts.

Gas Chromatography. Concurrent with the foregoing analyses, 15 chlorinated dibenzofurans containing from three to six chlorine atoms were injected into gas chromatographs having electron-capture detectors. The retention times relative to dieldrin were carefully determined (±1%) on four columns of different polarities (Thompson et al., 1969; Canada, Department of National Health and Welfare, 1973).

Instruments and chromatographic conditions are listed below: (1, Micro Tek 220): (a) column 3% OV-1 on 100–120 mesh Supelcoport, 6 ft × 4 mm i.d., glass; (b) detector: ^{63}Ni; (c) detector, inlet, and oven temperatures, 273, 225, and 195°, respectively; (d) column flow 50 cm^3 of N_2/min; (e) purge flow 55 cm^3 of N_2/min; (2, Micro Tek 220): (a) column: 5% SP-2401 (= QF-1, Supelco, Inc., Bellefonte, Pa.) on 100–120 mesh Supelcoport, 6 ft × 4 mm i.d., glass; (b) detector: ^{63}Ni; (c) detector, inlet, and oven temperatures: 273, 225, and 200°, respectively; (d) column flow 55 cm^3 of N_2/min; (e) purge flow: 55 cm^3 of N_2/min; (3, Varian 1440): (a) column: 1.5% OV-17/1.95% QF-1 on 100–120 mesh Chromosorb W, acid washed, DMCS treated, 6 ft × 2 mm i.d., glass, (b) detector: ^{3}H; (c) detector, inlet, and oven temperatures: 225, 230, and 192°, respectively; (d) column flow 35 cm^3 of N_2/min; (4, Varian 1440): (a) column: 3% OV-1/5% OV-210, 5 ft + 3% OV-1/6% OV-225, 0.5 ft on 60–80 mesh Chromosorb W, acid washed, 4 mm i.d., glass, (b) detector: ^{3}H; (c) detector, inlet, and oven temperatures: 232, 220, and 213°, respectively; (d) column flow 40 cm^3 of N_2/min.

RESULTS AND DISCUSSION

Retention times of the 15 authentic dibenzofurans relative to dieldrin ranged from 0.68 to 5.37 (Table I), these are within the range of retention times of the common chlorinated pesticides and PCB. The gas chromatographic parameters are the same as those used for the pesticides and PCB.

Individual coinjections of 2,3,7,8-tetra- and 2,3,4,7,8-pentachlorodibenzofuran with fractions isolated from Aroclor 1248 and 1254 and Kanechlor 200 and 500 showed coincidence of isomer retention time with peaks in the fractions on all four columns, with the exception that on OV-17/QF-1 the 2,3,7,8 compound eluted in an unresolved region between two large peaks in the Aroclor 1254 fraction. The retention time of 1,3,6,7-tetrachlorodibenzofuran also coincided upon coinjection with that of a peak in the isolates from all four PCB on the OV-1 and SP-2401 columns, but definitive peak matches on the other two column sys-

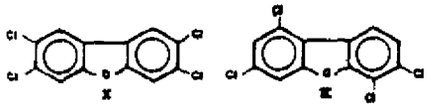
MONS 214402

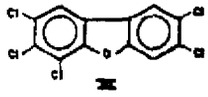
Table I. Gas Chromatograph Retention Times of Authentic Chlorinated Dibenzofurans Relative to Dieldrin

Structure of compd	Column: OV-1	SP-2401	OV-17/QF-1	OV-1/210/225
$2,3,8\text{-}Cl_3$	0.82	0.66	0.83	0.77
$2,3,6\text{-}Cl_3$	0.85	0.72	0.88	0.80
$2,3,4\text{-}Cl_3$	0.87	0.71	0.90	0.81
$1,3,7,8\text{-}Cl_4$	1.26	0.89	1.21	1.02
$1,3,6,7\text{-}Cl_4$	1.29	0.92	1.26	1.09
$2,3,6,8\text{-}Cl_4$	1.43	1.10	1.74	1.28
$2,4,6,7\text{-}Cl_4$	1.42	1.16	1.59	1.32
$2,3,7,8\text{-}Cl_4$	1.57	1.28	1.70	1.30
$2,3,6,7\text{-}Cl_4$	1.64	1.34	1.93	1.56
$3,4,6,7\text{-}Cl_4$	1.68	1.47	1.96	1.64
$1,2,3,7,8\text{-}Cl_5$	2.70	1.84	3.08	2.15
$1,2,3,6,7\text{-}Cl_5$	2.74	1.87	3.16	2.20
$2,3,4,7,8\text{-}Cl_5$	2.98	2.29	3.68	2.68
$2,3,4,6,7\text{-}Cl_5$	3.04	2.39	3.80	3.01
$1,2,3,6,7,8\text{-}Cl_6$	4.13	3.11	5.37	3.71

teme were not possible because of interference from overlapping peaks.

On the basis of the procedures described, 2,3,7,8-tetrachlorodibenzofuran (I) and 2,3,4,7,8-pentachlorodibenzofuran (III) appear to be present in Aroclor 1248 and 1254 and Kanechlor 200 and 500. The presence of the 1,3,6,7 isomer (II) in the PCB cannot be considered established to the same extent. The retention times relative to dieldrin on an OV-1 column for a tetra- and a pentachlorinated dibenzofuran identified in Clophen A-60 (Bowes et al., 1973; note Figure 1, peaks B and E) are the same as those reported here for the 2,3,7,8- and 2,3,4,7,8-dibenzofuran isomers (Table I).





Amounts of chlorinated dibenzofuran isomers calculated to be present in the PCB were estimated to range from 0.06 to 0.83 μg/g (Table II). Although there is no evidence that chlorinated dibenzofurans are present in wildlife, these data allow one to estimate their potential concentrations. If the dibenzofurans were present in tissue in the same amounts relative to the PCB, the range of concentrations of the dibenzofuran isomers identified here would be approximately 25 to 250 pg/g fresh weight in Great Lakes herring gull (*Larus argentatus*) livers found to contain 300 ppm of PCB (Bowes et al., 1973).

Chicks fed 5 μg/kg per day of 2,3,7,8-tetrachlorodibenzofuran died within 8–16 days (Goldstein et al., 1974). The toxicity to chicks is therefore similar to that reported by Schwetz et al. (1973) for 2,3,7,8-tetrachlorodibenzo-*p*-dioxin. The latter compound is known to be teratogenic (Schwetz et al., 1973), the teratogenic potential of the chlorinated dibenzofurans has not been established. Chick embryo bioassays have demonstrated that the ability of 2,3,7,8-tetrachloro- and 2,3,4,7,8-pentachlorodibenzofuran to effect induction of aryl hydrocarbon hydroxylase is comparable to that of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (Kende et al., 1974); this property was characteristic of all polychlorodioxins tested (Poland and Glover, 1973). Our findings point to compounds I and III as possible sources of toxicity of commercial PCB.

Table II. Concentration of Chlorinated Dibenzofuran Isomers in PCB (μg/g of PCB)

Structure of compd	PCB: Aroclor 1248	Aroclor 1254	Kanechlor 200	Kanechlor 500
$2,3,7,8\text{-}Cl_4$ (I)	0.33	0.11	0.10	0.19
$2,3,4,7,8\text{-}Cl_5$ (III)	0.83	0.12	0.10	0.08

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LITERATURE CITED

Bowes, G. W., Mulvihill, M. J., Simoneit, B. R., Burlingame, A. L., Risebrough, R. W., *Nature* (London) **256**, 305 (1975).

Bowes, G. W., Simoneit, B. R., Burlingame, A. L., de Lappe, B. W., Risebrough, R. W., *Environ. Health Perspect.* No. 5, 191 (1973).

Canada, Department of National Health and Welfare, "Analytical Methods for Pesticide Residues in Food", 2nd ed., Queens' Printer, Ottawa, Canada, Catalog No. H44-2868, revised, 1973.

Goldstein, J. A., McKinney, J. D., Lucier, G. W., Moore, J. A., Hickman, P., Bergman, H., *Pharmacologist* **16**, 239 (Abstract no. 278) (1974).

Kende, A. S., Wade, J. J., DeCamp, M. R., Ridge, D., Poland, A., 167th National Meeting of the American Chemical Society, Los Angeles, Calif., April 4, 1974, paper ORGN 130, manuscript in preparation.

Kuratsune, M., Yoshimura, T., Matsuzaka, J., Yamaguchi, A., *Environ. Health Perspect.* No. 1, 119 (1972).

Poland, A., Glover, E., *Mol. Pharmacol.* **9**, 736 (1973).

Roach, J. A. G., Pomerantz, I. H., *Bull. Environ. Contam. Toxicol.* **12**, 336 (1974).

Schwetz, B. A., Norris, J. M., Sparschu, G. L., Rowe, V. K., Gehring, P. J., Emerson, J. L., Gerbig, C. G., *Environ. Health Perspect.* No. 5, 87 (1973).

Thompson, J. F., Walker, A. C., Mossman, R. F., *J. Assoc. Off. Anal. Chem.* **52**, 1263 (1969).

Vos, J. G., Koeman, J. H., van der Maas, H. L., ten Noever de Brauw, M. C., de Vos, R. H., *Food Cosmet. Toxicol.* **8**, 625 (1970).

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