

gas-liquid chromatography. In the fish removed within 24 hr of treatment, endothall concentration in the flesh ranged from 0.02 to 0.1 ppm.

After 30-min exposure to water containing 2 ppm of [ $^{14}\text{C}$ ]endothall, [ $^{14}\text{C}$ ]labeled residues (expressed as endothall) ranging from 0.02 to 0.04 ppm were detected in the blood. After 60 min, the concentration of [ $^{14}\text{C}$ ] in the blood increased up to 0.08 ppm. Longer exposure up to 4 hr did not significantly increase the level of radioactivity in the blood.

The concentration of [ $^{14}\text{C}$ ] in the various tissues, 48 hr after the fish were fed [ $^{14}\text{C}$ ]endothall through the digestive tract, is shown in Table III. The results show that the herbicide is absorbed by the intestinal tract though the fish were observed to eliminate 73% of the administered herbicide during this period. In the fish fed [ $^{14}\text{C}$ ]labeled herbicide through the digestive tract, like the bath-exposed fish, the concentration of radioactivity was highest in the viscera and lowest in the flesh. The pattern of distribution of radioactivity in the various tissues in the feed-exposed fish was similar to that observed for the bath-exposed fish, with the exception that the scales had a relatively lower proportion of the total radioactivity.

These findings have demonstrated that bluegills absorb endothall directly from water as well as through the intestinal tract. Adsorption of endothall on the scales also contributes to the herbicide residues in the fish. This is sup-

ported by the observation that the proportion of [ $^{14}\text{C}$ ] in the scales decreased when the fish were fed endothall through the digestive tract.

Thin-layer chromatographic analysis of the methanol extract from the fish treated with the herbicide for 48 hr showed that all the [ $^{14}\text{C}$ ] in the alcohol extractable fraction was present in the form of unchanged endothall ( $R_f$  0.48 and 0.75 on silica gel and cellulose plates, respectively). The [ $^{14}\text{C}$ ] in the extract co-chromatographed with authentic [ $^{14}\text{C}$ ]endothall. In contrast to aquatic microorganisms which were found to readily degrade endothall (Sikka and Saxena, 1973), bluegills do not appear to be capable of metabolizing the herbicide.

#### ACKNOWLEDGMENT

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## The Mechanism of Chlorobiphenyl Metabolism

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4-Chlorobiphenyl was administered to rabbits and the major urinary metabolites were identified as 4'-chloro-4-biphenylol and 4'-chloro-3,4-biphenyldiol. It was also shown that 4'-chloro-4-biphenylol was also converted into the diol as well as its monomethyl derivatives. The biohydroxylation pathway was further investigated using 4'-[ $^3\text{H}$ ]-4-chlorobiphenyl as a substrate. The 4'-chloro-4-biphenylol metabolite retained 79% of the deuterium and the results are consistent with the intermediacy of

an arene oxide in the first hydroxylation reaction. The diol metabolite retained ca. one-half the deuterium found in the phenol (39%) and it is therefore not formed directly from the arene oxide but by direct hydroxylation of the phenolic metabolite. The sequence of hydroxylation of chlorobiphenyl is, therefore, analogous to the stepwise hydroxylation of phenylalanine to give 3,4-dihydroxyphenylalanine and butamoxane to give 6,7-dihydroxybutamoxane.

Polychlorinated biphenyls (PCB) are industrial compounds which are now recognized as among the most widespread pollutants in the global ecosystem (Hutzinger et al., 1974; Riebeling et al., 1968; Fishbein, 1973). Residues have been identified in both the terrestrial and aquatic environments (Koeman et al., 1969; Harvey et al., 1974; Jensen et al., 1969; Horn et al., 1974) as well as in animals (Bagley et al., 1970; Butler, 1973; Presti et al., 1970) and humans (Biroo et al., 1970; Jamieson et al., 1973). Recent work has shown that both commercial PCB mixtures and pure isomeric chlorobiphenyls are metabolized by animals (Hutzinger et al., 1972, 1974a; Safe et al., 1974, 1975a,b; Buras et al., 1974; Greb et al., 1973; Yoshimura and Yamamoto, 1973; Block and Cornish, 1969; Goto et al., 1974;

Gardner et al., 1973), plants (Mosa et al., 1973, 1974), and microorganisms (Wallnofer et al., 1973; Ahmed and Pocht, 1973; Maas et al., 1975) to give a range of hydroxylated metabolites. The major rabbit urinary metabolites of 2,2',5,5'-tetrachlorobiphenyl were 2,2',5,5'-tetrachloro-3-biphenylol, 2,2',5,5'-tetrachloro-4-biphenylol, and *trans*-3,4-dihydro-2,2',5,5'-tetrachloro-3,4-biphenyldiol and these all can conceivably be formed from the 3,4-epoxy intermediate. NIH rearrangement (Jerina, 1974; Jerina and Daly, 1974; Daly et al., 1972) of this intermediate (i.e., *trans*-3,4-dihydro-2,2',5,5'-tetrachloro-3,4-epoxybiphenyl) could yield the two phenolic metabolites and hydrolysis of the epoxide would yield the dihydrodiol product. The experiments described in this paper are concerned with the mechanism of PCB hydroxylation in the rabbit using 4-chlorobiphenyl, 4'-chloro-4-biphenylol, and 4'-[ $^3\text{H}$ ]-4-chlorobiphenyl as model substrates.

#### MATERIALS AND METHODS

**Chlorobiphenyl Substrates.** 4'-Chloro-4-biphenylol was synthesized as described (Savoy and Abernethy, 1942).

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Table 1. Identification and Recovery of the Rabbit Urinary Metabolites of 4-Chlorobiphenyl and 4'-Chloro-4-biphenylol

| Compd (M <sup>+</sup> ) <sup>a</sup>               | Substrate <sup>a</sup> |           |                        |           | NMR data for the metabolites, $\delta$ , ppm                                                                                                         |
|----------------------------------------------------|------------------------|-----------|------------------------|-----------|------------------------------------------------------------------------------------------------------------------------------------------------------|
|                                                    | 4-Chlorobiphenyl       |           | 4'-Chloro-4-biphenylol |           |                                                                                                                                                      |
|                                                    | Free, mg               | Bound, mg | Free, mg               | Bound, mg |                                                                                                                                                      |
| 4'-Chloro-4-biphenylol ( <i>m/e</i> 204)           | 80.4                   | 15.0      | 72.6                   | 12.9      | 7.55 (d, <i>J</i> = 8.2 Hz), 7.48 (d, <i>J</i> = 8.2 Hz), 7.42 (d, <i>J</i> = 8.2 Hz), 7.18 (d, <i>J</i> = 8.2 Hz)                                   |
| 4'-Chloro-3-methoxy-4-biphenylol <sup>b</sup>      |                        |           | 13.2                   | 3.0       | 8.0-7.0 (m), 3.94 (s), 3.91 (s)                                                                                                                      |
| 4'-Chloro-4-methoxy-3-biphenylol ( <i>m/e</i> 234) |                        |           |                        |           |                                                                                                                                                      |
| 4'-Chloro-3,4-biphenyldiol ( <i>m/e</i> 220)       | 10.5                   | 1.5       | 45.6                   | 7.5       | 7.43 (d, <i>J</i> = 8.2 Hz), 7.37 (d, <i>J</i> = 8.2 Hz), 7.06 (d, <i>J</i> = 1.9 Hz), 7.01 (q, <i>J</i> = 8.2, 1.9 Hz), 6.93 (d, <i>J</i> = 8.2 Hz) |

<sup>a</sup> M<sup>+</sup> = molecular ion. <sup>b</sup> The two methoxy isomers could not be separated by TLC and were analyzed by gas-liquid chromatography (GLC) as described (Safe et al., 1975b); the relative percentages of the methoxy isomers were determined by the GLC peak areas and the values given in the table were calculated from the yield of diol obtained after demethylation of the mixture. <sup>c</sup> The figures given are based on milligrams of compound recovered from the urine of a single rabbit; each rabbit (average wt, 5 kg) was given 300 mg of substrate by intraperitoneal injection in a vegetable oil solution.

4-Chloro-4'-iodobiphenyl (1.0 g, prepared from the corresponding 4-amino-4'-chlorobiphenyl) was dissolved in dry tetrahydrofuran (25 ml) and lithium aluminum deuteride (0.20 g) was carefully added. The mixture was then refluxed for 8 hr, cooled, and quenched by the addition of deuterium oxide (6 ml). The products were isolated with ether extraction and purified by preparative thin-layer chromatography (TLC). The major product was 4'-[<sup>3</sup>H]-4-chlorobiphenyl (0.280 g, 85% purity by mass spectrometry) and some 4,4'-[<sup>3</sup>H]<sub>2</sub>biphenyl (0.05 g) was also isolated. Unlabeled 4-chlorobiphenyl was obtained from commercial sources (Eastman) and used directly as a metabolic substrate.

**Animal Feeding Experiments.** The chlorobiphenyl substrate (300 mg) was dissolved in vegetable oil (5-10 ml) and administered intraperitoneally to a male rabbit (ca. 5 kg) which was housed in a metabolic cage. Urine and feces were collected for 10 days after administration of the xenobiotic.

**Extraction and Analysis.** The urine samples were acidified to pH 4-5 with the addition of glacial acetic acid and extracted with ether to give the free metabolite extract. The urine samples were then carefully acidified with concentrated sulfuric acid to give a 2 N sulfuric acid solution which was then heated on a steam bath for 2 hr. The solution was cooled and then extracted with ether to give the bound metabolite extract.

The 4-chlorobiphenyl and 4'-[<sup>3</sup>H]-4-chlorobiphenyl metabolites were isolated by preparative TLC as described (Safe et al., 1975b) and the 4'-chloro-4-biphenylol metabolites were also obtained by TLC (Safe et al., 1975a). The crude hydroxy fractions were purified by their acetate derivatives and the corresponding hydroxylated metabolites were obtained by subsequent basic hydrolyses of the acetates. Demethylations were carried out by treatment of the metabolites with boron tribromide (0.1 ml) in methylene chloride (2 ml) for 9 hr at 25°. The solution was diluted with water (15 ml) and methylene chloride (20 ml). The demethylation product was then obtained by evaporation of the dried methylene chloride extract.

**Spectroscopic Methods.** Mass spectra were obtained on a Varian MAT CH-7 spectrometer and nuclear magnetic resonance (NMR) spectra were recorded on a Varian HR220 spectrometer using deuteriochloroform as solvent.

## RESULTS

A summary of the major rabbit urinary metabolites of 4-chlorobiphenyl and 4'-chloro-4-biphenylol is given in Table 1. The former substrate was converted into two metabolites, 4'-chloro-4-biphenylol and 4'-chloro-3,4-biphenyldiol; 4'-chloro-4-biphenylol was metabolized to give 4'-chloro-3,4-biphenyldiol as the major product and smaller amounts of a mixture of 4'-chloro-3-methoxy-4-biphenylol and 4'-chloro-4-methoxy-3-biphenylol. The latter two compounds could not be separated by TLC; however, the NMR spectrum of this fraction gave two methoxy signals at  $\delta$  3.94 and 3.91 ppm. Treatment of the mixture with boron tribromide in methylene chloride gave 4'-chloro-3,4-biphenyldiol as the sole product. These metabolism results with 4-chlorobiphenyl and 4'-chloro-4-biphenylol were similar to those obtained from the metabolism of these substrates in the rat (Safe et al., 1974; Safe et al., 1975a).

Administration of 4'-[<sup>3</sup>H]-4-chlorobiphenyl also gave 4'-chloro-4-biphenylol and 4'-chloro-3,4-biphenyldiol as metabolites and these were examined by mass spectrometry. The phenol gave molecular ion species at *m/e* 205 and 204 and 79% of the deuterium was retained in this metabolite. Examination of the NMR spectrum showed that the signal at 7.18 ppm was diminished in its expected intensity and confirmed the shift of the 4'-H atom to the 3 position. The mass spectrum of the 4'-chloro-3,4-biphenyldiol isolated from this experiment gave molecular ion peaks at *m/e* 221 and 220 and 39% of the deuterium was retained in this metabolite. The mass spectral data were obtained using the metabolites isolated from the free urine extract. Treatment of the deuterated chlorobiphenyls with 2 N sulfuric acid results in some H-<sup>3</sup>H exchange and for this reason the mass spectral data for the metabolites isolated from the bound fraction were not used for calculation of the <sup>3</sup>H retention.

## DISCUSSION

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The results obtained for the metabolism of 4-chlorobiphenyl and 4'-chloro-4-biphenylol in the rabbit were similar to the experiments already described for rats (Safe et al., 1974, 1975a). In addition, the metabolism of a series of chlorobiphenyls including 2,3-dichlorobiphenyl, 2,4,6-trichlorobiphenyl, 2,3,5,6-tetrachlorobiphenyl, and 2,3,4,5,6-pentachlorobiphenyl also gave the corresponding phenols,

catechols, and methoxyphenols as the major metabolites (Goto et al., 1974). It is conceivable that the formation of these products could occur via an arene oxide which on rearrangement would give the phenol and on hydrolysis followed by dehydrogenation would give the catechol (Daly et al., 1972). The methoxyphenols could then be formed by methylation of the catechols. The conversion of 4'-chloro-4-biphenyl into the corresponding diol presents a second alternative pathway for the formation of this metabolite. Analysis of the two urinary metabolites of 4'-[<sup>3</sup>H]-4-chlorobiphenyl clearly resolves this problem. The mass spectrum of 4'-chloro-4-biphenyl shows that 79% of the deuterium is retained in the metabolite and this is consistent with an arene oxide intermediate in which the hydrogen has migrated from the site of hydroxylation to a neighboring carbon atom (the NIH shift). This result is supported by the NMR spectrum of the metabolite in which the signal at 7.18 ppm is of diminished intensity due to the presence of deuterium. The NIH shift has also previously been reported for the oxidation of biphenyl to give biphenylol (Daly et al., 1968) and 4-fluorobiphenyl to give 4'-fluoro-4-biphenylol (Daly et al., 1969).

The mass spectrum of the 4'-chloro-3,4-biphenyldiol metabolite gave molecular ions at *m/e* 220 and 221 and 50% of the deuterium from the original 4'-[<sup>3</sup>H]-4-chlorobiphenyl substrate was retained in the metabolite. This result supports the formation of the diol by two independent hydroxylation reactions in which the first step occurs via an arene oxide followed by a second biological hydroxylation reaction. The monooxygenase enzymes responsible for the insertion of the second hydroxyl group thus remove one-half of the deuterium present at the 3 position in 4'-chloro-4-biphenyl. This sequence of events is analogous to the stepwise hydroxylation of phenylalanine to give tyrosine which is in turn hydroxylated yielding 3,4-dihydroxyphenylalanine (Guroff et al., 1966). Similarly, it has also been shown that the oxidation of butamoxane to give 6,7-dihydroxybutamoxane proceeds via two consecutive hydroxylation reactions rather than by the more direct route through an arene oxide intermediate (Murphy et al., 1974).

The arene oxides of several polycyclic aromatic hydrocarbons are known to be more active as carcinogens than either the parent hydrocarbon, the corresponding phenols, or dihydrodiols (Grover et al., 1971). The results reported in this paper show that a lower chlorinated PCB isomer, 4-chlorobiphenyl, is also metabolized via an arene oxide intermediate. Since the biological properties of PCB and their metabolites are not fully understood this is, therefore, an area of environmental concern. The possible toxicological properties of PCBs and their metabolites are currently under investigation in our laboratory.

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where the numerical coefficients are taken from equation (1). Similarly, the tree-growth anomaly pattern corresponding to the year following a high percentage of albacore caught north of San Francisco can be represented as:

$$\text{map } b = 8.56 (\text{eigenvector } 4) - 6.17 (\text{eigenvector } 9) + 11.54 (\text{eigenvector } 10) \quad (3)$$

These maps are presented in Fig. 4. The ring-width data were mostly from trees sited in arid localities, so that a wide ring would generally be associated with anomalously cool, cloudy weather and above normal precipitation whereas a narrow ring would reflect warm, sunny and dry conditions.

Below normal tree growth in the Pacific North-west (Fig. 4) is indicative of dry conditions associated with below normal cyclonic activity during the fishing season. Sunny and mild weather would favour albacore fishing in adjacent waters, as would above normal insolation regardless of weather. The resulting excess of stored heat in the ocean would be given up through evaporation during the following autumn and winter and lead to increased cyclonic activity and precipitation along the coast north of San Francisco. These conditions would lead to increased tree growth during the following growing season (Fig. 4).

Autumn and winter climatic anomaly features, combined with spring climate and the year-to-year autocorrelation of tree-ring widths, produce the other ring-width anomaly features in Fig. 4 for the following growing season. Narrow ring widths south of San Francisco, for example, imply below normal precipitation—an expected feature since winter precipitation in the Pacific North-west is negatively correlated with winter precipitation in southern California.

The reconstructed values of albacore catch distribution data (Fig. 3) and inferred population distribution also seem to exhibit long term changes over intervals of 100 yr or more, which suggest the possibility that long term fluctuations in the ocean-atmosphere system may be involved.

The success of the calibration of tree rings with albacore catch indicates the possibility of relating tree-ring variations to any type of biological variations which are affected by large scale climatic fluctuations. Such relationships may be quantified and used to reconstruct objectively other climatically-caused biotic variations in the past.

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## Oats may grow better in water depleted in oxygen 18 and deuterium

While growing oats at different temperatures in water of different  $^{18}\text{O}$  and deuterium ( $\text{D}$ ) abundances, we noticed that oats grown in Antarctic water in which is depleted in  $^{18}\text{O}$  and  $\text{D}$  by  $-49\%$  and  $-400\%$ , relative to standard mean ocean water (SMOW) used as a comparative reference in hydrogen and oxygen isotope studies, showed initial growth 1-2 weeks sooner than did oats grown in water containing greater  $^{18}\text{O}$  and  $\text{D}$  concentrations. The oats seemed to grow better in water which was most depleted in the stable isotopes throughout the growth period.

The oats were grown from the same batch of seeds in two sealed glass-covered glass jars (approximately 10 l). Twenty-five oat seeds were added to each jar, containing the same amount of vermiculite and 500 ml water to which 5.0 g Rapid-Gro, a commercial fertilizer, had been added. One jar contained melted glacial ice from the Antarctic with isotope concentrations of  $-49\%$   $^{18}\text{O}$  (SMOW) and  $-400\%$   $\text{D}$  (SMOW). The other jar contained distilled ocean water with  $+1.0\%$   $^{18}\text{O}$  (SMOW) and  $+17\%$   $\text{D}$  (SMOW). Both jars were placed in the chamber at the same time.

The experiment was repeated three times with new materials: once the growth chamber was maintained between  $1.7$  and  $3.3^\circ\text{C}$ ; once between  $24$  and  $26.6^\circ\text{C}$ ; and once the temperature fluctuated between  $1.7$  and  $26.6^\circ\text{C}$ . Each time the oats in the jar containing water depleted in the heavy isotopes showed germination 1-2 weeks earlier and seemed to grow better throughout the growth period, than oats grown in distilled ocean water.

Using oats grown at  $15^\circ\text{C}$ , the first sign of germination in the jar containing water depleted in the heavy isotopes was 4 d after planting. On the day 6, eight plants (out of 25) had attained a height of 6 cm. The first sign of germination in the jar with water containing the heavier isotope concentration, was after 17 d. By the time five plants had attained a height of 6 cm in this jar, in that with water depleted in the isotopes, 23 plants had reached the top of the jar (approximately 25 cm).

Kashutin<sup>1</sup> observed that snow-water depleted in  $\text{D}$  increases the yield of cucumbers, radishes and spring wheat compared with controls grown in ordinary water of unspecified isotopic composition. He cites experiments on the egg productivity of hens and the weight gain of suckling pigs. In both cases water depleted in  $\text{D}$  was especially efficient in promoting productivity.

Although much has been done on the effect of D-enriched water on biological systems, we suggest that research on the effect of D-depleted water on plant and animal growth may prove fruitful. A major source of water depleted in  $\text{D}$  by over  $400\%$  ( $40\%$ ) compared with SMOW is snow and ice from the Antarctic polar plateau. Water depleted by 150-180%, is readily available in the USA from Rocky Mountain snow precipitating above 10,000 feet elevation.

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<sup>1</sup> Kashutin, K., *Prirada* (USSR), 58, 107 (1959).

## Identification of chlorinated dibenzofurans in American polychlorinated biphenyls

MORTALITY of embryos has contributed to the reproductive failures of several bird species, including the sparrowhawks (*Accipiter nisus*) of southern Scotland<sup>1</sup>, the white-tailed eagles (*Haliaeetus albicollis*) of Schleswig Holstein<sup>2</sup>, and the herring

<sup>1</sup> LaMarche, V. C., *Science*, 183, 1043-1048 (1974).

<sup>2</sup> Namias, J., *Mon. Weath. Rev.*, U.S. Dep. Agric., 97, 173-192 (1969).

<sup>3</sup> Lauer, R. N., et al., *Report of Joint National Marine Fisheries Service-American Fisheries Research Foundation Albacore Studies Conducted During 1972* (National Marine Fisheries Service, Southwest Fisheries Center, La Jolla, 1973).

<sup>4</sup> Clemens, H. B., and Craig, W. L., *Calif. Dept. Fish and Game, Fish Bull.*, 128 (1965).

<sup>5</sup> Sette, O. L., *Calif. Coop. Oceanic Fish. Invest. Rept.*, 7, 181-194 (1960).

<sup>6</sup> Pyke, C. B., *An investigation of some precipitation patterns in California and adjacent regions* (University of California Water Resources Center, 1966).

gulls (*Larus argentatus*) of Lake Ontario<sup>8</sup>. Suspected causes include *p,p'*-DDE (2,2-bis-(*p*-chlorophenyl)-1,1-dichloroethylene), other chlorinated biocides and/or their derivatives, and the polychlorinated biphenyls (PCBs), all of which are present as contaminants in the eggs<sup>9-12</sup>. PCBs are present in high concentrations in the bird populations which suffer embryonic mortality<sup>13</sup>. Other organochlorine compounds which may be present in food webs include the chlorinated dibenzodioxins and the chlorinated dibenzofurans (Fig. 1), which are toxic to embryos in amounts<sup>14</sup> less than 1 µg. They are therefore among the most toxic substances known and are possible causes of the observed mortality.

The chlorinated dibenzodioxins and chlorinated dibenzofurans, however, have proved exceedingly difficult to detect in environmental samples in the concentrations at which they are expected to be embryotoxic<sup>15,16</sup>. The chlorinated dibenzodioxins enter the environment as contaminants in preparations of the herbicide 2,4,5-T (refs 5 and 11) and the fungicide pentachlorophenol<sup>17,18</sup>. Chlorinated dibenzofurans have been found in a French (Phenoclor DP6) and a German (Clophephen A60) PCB and were shown to be the active embryotoxic agent in these preparations<sup>9</sup>. The techniques used, however, did not detect chlorinated dibenzofurans in an American PCB, Aroclor 1260. We report here the presence of chlorinated dibenzofurans in Aroclor PCB, widely used in North America and Great Britain, and in the same Aroclor 1260 preparation examined previously with negative findings<sup>9</sup>.

Samples of PCB examined include: Aroclor 1248, 1254, and 1260 (1969); Aroclor 1254 (1970); Aroclor 1016 (1972); and the same three preparations studied by Vos *et al.*<sup>9</sup>: Aroclor 1260, lot No. AK-3; Clophen A-60, lot No. 912434; and Phenoclor DP-6, lot not specified. The latter three PCBs were obtained from Dr Vos, the others from the Monsanto Company in the years indicated in parentheses.

PCBs extracted from environmental samples most often have gas chromatographic profiles similar to those of PCB formulations containing approximately 48, 54 or 60% chlorine. In the Aroclor series, the former two PCBs are equivalent to Aroclor 1248 and Aroclor 1254, respectively. Aroclor 1260, Phenoclor DP6, and Clophen A60 all contain approximately 60% chlorine.

Chlorinated dibenzofurans were identified in all Aroclor preparations except Aroclor 1016, as well as in Clophen A60 and Phenoclor DP6. Aroclor 1016 is a PCB mixture containing

Table 1 Chlorinated dibenzofuran concentrations\* in Aroclor, Clophen and Phenoclor

|                         | PCB | 4-Cl     | 5-Cl      | 6-Cl     | Total |
|-------------------------|-----|----------|-----------|----------|-------|
| Aroclor 1248 (1969)     |     | 0.5 (25) | 1.2 (60)  | 0.3 (15) | 2.0   |
| Aroclor 1254 (1969)     |     | 0.1 (6)  | 0.2 (12)  | 1.4 (82) | 1.7   |
| Aroclor 1254 (1970)     |     | 0.2 (13) | 0.4 (27)  | 0.9 (60) | 1.5   |
| Aroclor 1260 (1969)     |     | 0.1 (10) | 0.4 (40)  | 0.5 (50) | 1.0   |
| Aroclor 1260 (lot AK-3) |     | 0.2 (25) | 0.3 (38)  | 0.3 (38) | 0.8   |
| Aroclor 1016 (1972)     |     | ND       | ND        | ND       |       |
| Clophen A-60            |     | 1.4 (17) | 5.0 (59)  | 2.2 (26) | 8.4   |
| Phenoclor DP-6          |     | 0.7 (5)  | 10.0 (74) | 2.9 (21) | 13.6  |

\*Expressed as µg g<sup>-1</sup> PCB. Values in parentheses represent quantity as percentage total dibenzofuran.

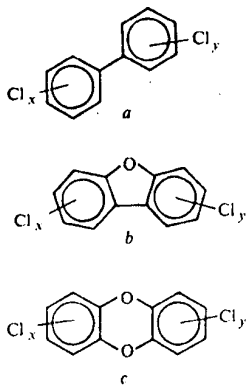
ND, not detected (<0.001 µg g<sup>-1</sup>).

Amounts of PCB ranging from 1.0 to 2.0 g were dissolved in 400 ml hexane, placed on a Florisil column (180 cm internal diameter 31.5 mm), and eluted with: an additional 1,600 ml hexane, and successive 800 ml volumes each of 5% diethyl-ether-hexane, 25% diethyl-ether-hexane and acetone, at a rate of approximately 7 ml min<sup>-1</sup>. The hexane portion of the PCB was eluted in the hexane fraction, which was discarded. On addition of the 5% mixture, the eluates were collected in six successive 40 ml volumes. To eliminate the solvent, each eluate was evaporated twice just to dryness and taken up each time in a minimal amount of hexane. Each fraction, in 1 ml hexane, was placed on a microalumina column<sup>11</sup> and eluted with 10 ml each of 1% and 20% methylene chloride in hexane. These were also taken twice just to dryness and made up to a volume of 1 ml in hexane to eliminate the methylene chloride before gas chromatographic analysis. Aliquots of all fractions obtained before and after partitioning on alumina were injected into a six foot glass column containing 3% OV1 on 100-120 mesh Supelcoplex in Tracor MT220 and Hewlett-Packard 5700 gas chromatographs equipped with <sup>63</sup>Ni electron-capture detectors. PCBs were found to be present in each fraction eluted from the Florisil column in amounts sufficient to interfere with the detection of trace contaminants. Partitioning on the alumina columns separated most of the PCB interference into the 1% methylene chloride fractions. On removal of this interference, different peak patterns appeared in the chromatograms of the 20% methylene chloride fractions. Compounds eluting in the 20% methylene chloride fraction were collected for mass spectrometric analysis using a 20:1 effluent splitter, and a trap consisting of a capillary tube (1 mm internal diameter, 100 mm long) bent to a U-shape, immersed in a liquid nitrogen bath. Methylene chloride (20% µl) in hexane was injected into the capillary as a rinse, removed with a 1.0 µl micropipette, and placed directly on the mass spectrometer probe. The probe was inserted into a GEC AEL 44502 low resolution mass spectrometer and the solvent removed by the force pump. The probe was rapidly inserted into the ion source and multiple scans were recorded in the on-line high resolution mode<sup>11</sup>.

approximately 42% chlorine and has replaced Aroclor 1242 in many applications, principally as the dielectric fluid in capacitors<sup>19</sup>. Values reported in Table 1 represent the total of those compounds found in 400 ml Florisil fractions 2-6. A total of 10-12 isomers was identified in each PCB. Two chlorinated dibenzofuran contaminants have been reported for the Clophen and Phenoclor previously<sup>9</sup>; our first analyses of the Clophen revealed an additional five chlorinated dibenzofurans<sup>9</sup>. The structures contained four to six chlorine atoms. Other dibenzofurans including those chlorinated to a lesser extent may have been present in the first 400 ml fraction but this was not examined in detail as it contained substantial PCB interference. Recently synthesised 2,3,7,8-tetra-, 2,3,4,7,8-penta- and 2,3,4,6,7,8-hexachlorodibenzofuran were used to quantify tetra-, penta-, and hexachlorodibenzofurans, respectively. The former two authentic standards had retention times on the OV1 column the same as those of two dibenzofurans isolated from the PCB.

Vos *et al.*<sup>9</sup> detected no chlorinated dibenzofurans in an Aroclor 1260 preparation at a detection limit of 1 p.p.m. Fractionation and examination of the identical Aroclor 1260 in our study confirm their findings based on the stated limit, but reveal the presence of 11 chlorinated dibenzofurans in the preparation, having a total concentration of 0.8 µg g<sup>-1</sup> PCB (Table 1). The same workers also found diethyl ether extracts of the Clophen A60 and Phenoclor DP6 to be much more toxic to chick embryos than diethyl ether extracts of Aroclor 1260. Our study confirms those findings on the basis of chlorinated

Fig. 1 Skeletal structures of: a, chlorinated biphenyl,  $x+y=1-10$ ; b, chlorinated dibenzofurans,  $x+y=1-8$ ; c, chlorinated dibenzodioxins,  $x+y=1-8$ .



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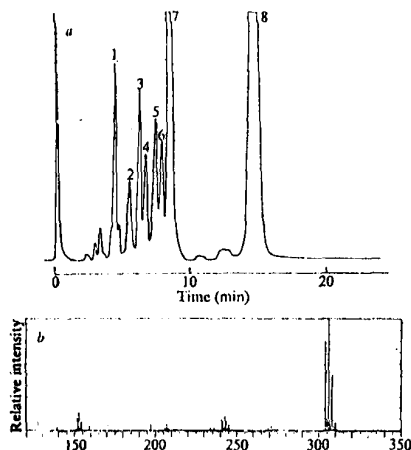


Fig. 2 a, Gas chromatogram of a fraction of Aroclor 1254 containing a mixture of chlorinated biphenyls, dibenzofurans, and naphthalenes. Identities of peaks are given in the text. b, Mass spectrum of peak 2, a tetrachlorodibenzofuran.

dibenzofuran content: the identical Clophen and Phenoclor contain 11 and 17 times more total chlorinated dibenzofurans, respectively, than the Aroclor 1260.

A gas chromatogram showing components derived from the Aroclor 1254 obtained in 1969 is represented in Fig. 2. The components were eluted in the second 400 ml Florisil fraction and recovered from the alumina column in 20% methylene chloride-hexane. Each of the numbered peaks was trapped as described here, and identified by mass spectrometric analysis. A nominal mass plot of the high resolution mass spectrum of peak 2 is shown in Fig. 2. The plot includes all the ions with elemental compositions ranging to the maximum empirical formula  $C_{13}H_4O^{12}Cl^{37}Cl^{37}Cl^{37}Cl^{37}$ . The molecular ion cluster at nominal *m/e* 304-310 fragments by successive losses of Cl to yield the ions at *m/e* 269-275 and CO to the ions at *m/e* 241-245. A minor loss of Cl from the peaks at *m/e* 269-275 also occurs to yield the ions at *m/e* 234-238, followed by CO elimination to *m/e* 206-210.

The group of peaks at *m/e* 152-154 are the doubly charged molecular ions. An identical spectrum was obtained from an authentic standard of 2,3,7,8-tetrachlorodibenzofuran. This latter compound has a retention time identical to that of peak 4. Peak 2 is, therefore, a positional isomer. The accurate mass measurements for the characteristic ions are within 2 p.p.m. of the calculated exact masses. Peaks identified on this chromatogram and their retention times relative to dieldrin are as follows: a mixture of tetra- and pentachlorobiphenyl (1.02); tetrachlorodibenzofuran (1.30); pentachlorobiphenyl (1.46); tetrachlorodibenzofuran (1.57); hexachloronaphthalene (1.75); pentachlorobiphenyl (1.86); hexachloronaphthalene (2.00); and heptachloronaphthalene (3.46). An aliquot of combined fractions derived from the same Aroclor 1254 was treated with diazomethane to assess whether any chlorinated ortho-hydroxybiphenyls (pre-furans) were present. Gas chromatographic analysis of the sample before and after treatment resulted in identical chromatograms.

As large quantities of PCBs have entered the global environment<sup>11-29</sup>, it may be assumed that the contaminant dibenzofurans

also have been released in proportional amounts. Their persistence, effects, and significance remain to be determined.

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## Niche breadth in Bryozoa as a test of competition theory

COMPETITION theory predicts that intraspecific and interspecific competition should often have opposite effects on the use of resources by a population, the former increasing, the latter decreasing, the range of resource actually used<sup>1,2</sup>. Field data supporting these predictions are well known for the interspecific case<sup>3-6</sup> but are scarce for the intraspecific condition, and we have been unable to find any reference demonstrating both effects within a single species. We therefore report here the verification of both predictions in respect of competition for space by the epiphytic bryozoan *Alcyonidium hirsutum*; less extensive data suggesting the same effects within other bryozoans are also reported.

Intraspecific competition should result in an increase in the range of a resource spectrum used by a species, as at high population levels the advantages to any individual of being at the competition-free optimum of a resource gradient are offset by the intense intraspecific competition found there (Fig. 1a); this is the 'principle of equal opportunity' of MacArthur<sup>7</sup>. Interspecific competition, on the other hand, should tend to restrict the range of the resource spectrum used by a species, as individuals attempting to exploit marginal resources cannot do so as efficiently as