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Elimination of Chlorinated Dibenzofurans Associated with Polychlorinated Biphenyls Fed to Mallards (*Anas platyrhynchos*)

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Elimination of Chlorinated Dibenzofurans Associated with Polychlorinated Biphenyls Fed to Mallards (*Anas platyrhynchos*). NORSTROM, R. J., RISEBROUGH, R. W., AND CARTWRIGHT, D. J. (1976). *Toxicol. Appl. Pharmacol.* 37, 217–228. A method which allows chlorinated dibenzofurans (CDFs) to be extracted from lipid and separated from interfering polychlorinated biphenyls (PCBs) was tested. Greater than 90% recovery of tetra- to hexa-CDFs was found. Starting with 40 g of lipid, the detection limit for individual CDFs in lipid was of the order of 0.01 μg/kg. Lipid from mallards which had been fed Aroclor 1254 for approximately 1 year at levels from 5 to 100 mg/kg in the diet was found to contain 3750 mg/kg of PCB. The minimum concentration of individual CDFs (nine tetra- to hexa-CDFs) potentially in the lipid from this PCB concentration was in the order of several tenths μg/kg. It was concluded that less than 3% of the dosed CDFs were accumulated by the mallards, since CDFs could not be detected by a comparison of lipid to Aroclor 1254 extracts containing the same amount of PCBs. It appears unlikely that CDFs are sufficiently persistent in avian species to enable their detection in environmental samples.

The chlorinated dibenzofurans (CDFs) are structurally similar to the chlorinated dibenzo-*p*-dioxins (CDDs), some of which are both highly toxic and teratogenic (Schwetz *et al.*, 1973; Verrett, 1971). The effectiveness of several tetra- and penta-CDFs, particularly 2,3,7,8-tetra-CDF, in inducing aryl hydrocarbon hydroxylase activity has been found to be nearly as high as that of 2,3,7,8-tetra-CDD (A. Poland and A. S. Kende, personal communication), the latter being the most toxic of the CDDs. The potency of aryl hydrocarbon hydroxylase induction by CDDs has been shown to be strongly correlated with toxicity (Poland and Golver, 1973). A dietary level of 5 μg/kg of 2,3,7,8-tetra CDF was found to be highly toxic to chicks (Goldstein *et al.*, 1974).

Embryotoxicity of the polychlorinated biphenyls (PCBs) Clophen A-60 and Phenoclor DP-6 has been attributed to CDFs present as trace contaminants in the commercial preparations (Vos and Koeman, 1970; Vos *et al.*, 1970). Subsequently, tetra-, penta-, and hexa-CDFs were detected in the American preparations of PCB,

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Aroclor 1248, 1254, and 1260; concentrations of the individual CDFs were in the order of 0.1 μg/kg of the PCB (Bowes *et al.*, 1973). Therefore, CDFs are possible causes of embryonic mortality and birth defects observed in PCB-feeding experiments (Tumasonis *et al.*, 1973), and in PCB-contaminated populations of Common Terns (*Sterna hirundo*) in Long Island Sound (Hays and Risebrough, 1972) or Herring Gulls (*Larus argentatus*) in Lake Ontario (Gilbertson and Hale, 1974).

Although little is known of their environmental persistence or accumulation in food webs, substantial amounts of CDFs have entered the global environment associated with PCBs as well as with chlorinated phenols (Buser *et al.*, 1975). Attempts to detect CDFs in eggs of Common Terns (*Sterna hirundo*) and Ospreys (*Pandion haliaetus*) from Long Island Sound, in eggs of Herring Gulls from Lake Ontario (R. W. Risebrough and G. W. Bowes, unpublished results; Bowes *et al.*, 1973), and in several species of birds and fish from the Bay of Fundy region (Zitko, 1972) have been unsuccessful. In some cases, amounts of material used for lipid extraction, cleanup, and subsequent analysis may have been too small. Before starting on a more exhaustive search for residues in birds whose lack of reproductive success may be attributable to CDFs, it was considered important to establish a method for quantitative recovery, and to obtain information on the persistence of CDFs in avian species.

A PCB-feeding experiment of mallards (*Anas platyrhynchos*) carried out in 1971 and 1972 showed both embryonic mortality and deformities among ducklings (D. Cartwright, unpublished results). Subsequently the Aroclor 1254 used in this experiment was examined for its CDF content, and the concentrations of tetra-, penta-, and hexa-CDFs were determined [preparation Aroclor 1254 (*1970*) of Bowes *et al.*, 1975a]. Total CDF concentration was 1.5 mg/kg of the PCB used. A high PCB concentration in the mallards at the end of the experiment and the ample amount of available material afforded an opportunity to determine the relative persistence of CDFs to PCBs in duck tissues during the feeding experiment.

METHODS

PCB Feeding Experiment

Aroclor 1254[3] was dissolved in corn oil and stirred into duck feed with a Hobart feed blender. Three groups of 22 birds, 11 females and 11 males, were fed diets containing 5, 50 and 100 mg/kg of Aroclor 1254 starting January 1, 1971. Progeny of these birds hatched in the spring of 1971 were fed on the same diet as their parents until June 22, 1972, when they were killed. The average dosage period of the progeny was 296 days.

A total of 23 progeny, about four males and four females from each of the dosage groups, was used for the current study. Beaks, long wing feathers, and feet were removed and discarded, and the remainder was thoroughly homogenized by three passes through a Hobart food chopper. After mixing and freeze drying, about 10% of the sample was placed in a preextracted paper thimble in a large Soxhlet apparatus and extracted with 5 liters of hexane/acetone azeotrope for 48 hr. The bulk of the solvent was removed in a large evaporating dish on a warm hot plate with successive additions of the extract to prevent oxidation of lipid, and the final solvent removed under a nitrogen stream, leaving 740 g of lipid.

[3] Canada Colours, Ltd., Toronto, Canada; purchased in 1970.

Two 0.5-g aliquots of the lipid were cleaned up for PCB analysis on a 2% water-deactivated Florisil column (McLeod and Ritcey, 1973) using 300 ml of hexane as the eluant. All analyses were performed on a Hewlett-Packard 5700 A gas chromatograph equipped with a linear Ni-63 detector and a 6-ft × 2-mm i.d. glass column at 190°C packed with 3% SP-2100 on Supelcoport 100/120 mesh.[3] Other conditions were: injection port at 250°C, detector at 300°C, and flow rate at 30 ml/min of 10% methane in argon. Chromatograms of the Aroclor 1254 standard and the lipid extract are compared in Fig. 1. Using the peak with retention time approximately 10 min as reference, the concentration of Aroclor 1254 in the lipid was calculated to be 3750 mg/kg.

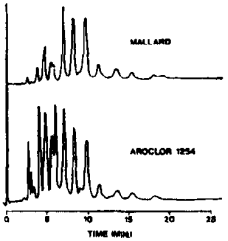

FIG. 1. Relative abundance of PCBs in Aroclor 1254 compared to those in mallard lipid after 296 days' administration of Aroclor 1254. The chromatograms have been normalized to the peak with retention time of 10 min.

Separation of CDFs from Lipids and PCBs

The method for separation of CDFs and PCBs from lipids (procedure 1, Table 1) was adapted from the column partitioning method of Porter and Burke (1973). The necessity of using large volumes of lipid ruled out adsorption column cleanup. The Florisil[4]/lipid column was prepared according to Porter and Burke (1973), but the eluting solvent was changed to 5% water/acetonitrile. The acetonitrile was shaken with 100 ml of hexane, then 50 ml of saturated sodium sulfate, 800 ml of water, and 15 ml of methylene chloride were added. After shaking again, the aqueous layer was discarded and the hexane layer was washed twice with 100 ml of water. The hexane layer was filtered through anhydrous sodium sulfate, evaporated to dryness in a rotary evaporator, and the residue was taken up in 5 ml of hexane. Previous experience in our laboratory had shown these modifications to give essentially 100% recovery of organochlorines such as Mirex which are poorly recovered by the original method, without significantly increasing the amount of co-eluting lipid.

The separation of PCBs from CDFs was the same as that of Bowes *et al.* (1975a). The bulk of the PCBs was removed on a column of 180 g of Florisil (deactivated with 2% water), as outlined in procedure 2, Table 1. Up to 2 g of PCBs may be placed on the column.

[3] Supelco, Inc., Bellefonte, Pa.
[4] Florisil PR grade; Floridin Corp., Pittsburgh, Pa.

TABLE 1

COLUMN CHROMATOGRAPHY PROCEDURES USED FOR THE SEPARATION OF CDFs FROM PCBs AND LIPIDS[a]

Procedure No.	Column type	Eluant	Eluant volume	Fraction no.	Content of fraction
1	Partition[b]	5% water/acetonitrile	200	—	Lipid, biogenic hydrocarbons, PCBs, PCNs and CDFs
2	Florisil[c]	hexane	1200	1	PCBs, PCNs
		5% diethyl ether/hexane	400	2	PCBs, PCNs, di- and tri-CDFs
		5% diethyl ether/hexane	400	3	PCBs, PCNs, tetra- and penta-CDFs
		25% diethyl ether/hexane	400	4	PCBs, PCNs, tetra-, penta-, and hexa-CDFs
		25% diethyl ether/hexane	400	5	PCBs, PCNs, hexa-CDFs, lipid, and biogenic hydrocarbons
		acetone	500	6	lipid, biogenic hydrocarbons
3	Alumina[d]	1% methyl chloride/hexane	10	—	PCBs, PCNs
		20% methylene chloride/hexane	10	—	tetra- to hexa-CDFs, some PCNs, lipid and hydrocarbons
		50% methylene chloride/hexane	10	—	lipid, biogenic hydrocarbons

[a] CDF = chlorinated dibenzofuran; PCB = polychlorinated biphenyl; PCN = polychlorinated naphthalene.

[b] Porter and Burke (1973). See text for modifications. For mallard lipid, five partition column eluates, concentrated to 10 ml, were combined for procedure 2.

[c] Vos *et al.* (1970); Bowes *et al.* (1975a). Fractions 1 and 2 contained the majority of the PCBs, and were discarded. Fractions 3, 4, and 5, which were highly enriched in CDFs and PCNs, were evaporated just to dryness and taken up in 1 ml of hexane for procedure 3. Fraction 6 did not contain any CDFs.

[d] Porter and Burke (1971). Fractions 3, 4, and 5 may be passed through procedure 3 either separately or combined. The first eluate was discarded. The second eluate was evaporated to dryness, then taken up in 1 ml of hexane and tested by GC for the presence of PCBs. The second eluate was passed through procedure 3 up to two more times. The third eluate potentially contained higher chlorinated CDFs, but no CDF present in PCB mixtures has been found in this eluate.

TABLE 2

RECOVERY OF TRI- TO HEXA-CDFs FROM THE CLEANUP AND PCB SEPARATION PROCEDURES[a]

Recovery test no.	Procedure no. from Table 1	Percent Recovery of: 2,3,8-tri-CDF	3,4,6,7-tetra-CDF	1,2,3,7,8-penta-CDF	1,2,3,6,7,8-hexa-CDF	2,3,4,6,7,8-hexa-CDF
1	2	92	95	95	40[b]	94
2	2 and 3	87	97	95	39	94[c]
3	1, 2, and 3	82	94	93	36	—

[a] The spiking levels and test solutions are described in the text. All recoveries are the mean of two results. The results were within ±2 percentage units from the mean in all cases.

[b] A large excess of acetone eluant did not recover any further amount.

[c] The recovery in the alumina procedure (No. 3, Table 1) was 39% in the 20% methylene chloride/hexane fraction. An additional 55% appeared in the 50% methylene chloride/hexane fraction.

Further separation to remove last traces of PCBs was accomplished by passing fractions 3, 4, and 5 from the Florisil column through an alumina[3] microcolumn, as outlined in procedure 3, Table 1. Each of the three 20% methylene chloride/hexane eluates was passed two more times through procedure 3.

In order to have as direct a comparison as possible between CDFs in Aroclor 1254 fed to mallards and the amount present in the lipid, a solution of Aroclor 1254 was made in corn oil so that the PCB concentration was the same as that in the mallard lipid (3750 mg/kg). A series of five 8-g aliquots of both the corn oil solution and the mallard lipid were then carried through procedure 1 (Table 1) and combined for separation of the PCBs and CDFs by procedures 2 and 3, for a total of 40 g of lipid and 150 mg of PCB per sample. The series was done in duplicate. The final volume was 1 ml for each of the three final fractions used for GC analysis.

Recovery Experiments

The recovery of CDFs was studied in three tests employing spikes of authentic CDF standards. A tri-, tetra-, penta- and two hexa-CDFs, as indicated in Table 2, were chosen because their retention times on the SP-2100 column employed for GC analysis did not overlap those of any CDFs present in Aroclor 1254 (Bowes *et al.*, 1975b). Aroclor 1254 was spiked at the 0.1-mg/kg level with the first four authentic CDFs for recovery tests 2 and 3. The solutions for each of the recovery tests were made up as follows: test 1, approximately 15 mg of each of the five authentic CDFs dissolved in 10 ml of hexane; test 2, 2 g of spiked Aroclor 1254 dissolved in 100 ml of hexane; and test 3, 150 mg of spiked Aroclor 1254 dissolved in 8 g of corn oil.

RESULTS

Table 2 indicates the results of the recovery tests. With the exception of 1,2,3,6,7,8-hexa-CDF, the recovery of all CDFs was generally greater than 90%. Neither the absolute amount of CDF present, nor the presence of PCBs and lipid appeared to affect the recoveries. The tri-CDF was partially lost in the alumina chromatography step. The methods outlined in Table 1 can therefore be recommended for CDFs with four to six chlorine atoms per molecule. It is not known whether hepta- and octa-CDFs would be recovered by the methods. Complete recovery of 2,3,4,6,7,8-hexa-CDF from the alumina column required a 50% methylene chloride/hexane eluant.

The low recovery of 1,2,3,6,7,8-hexa-CDF could be ascribed to partial decomposition during storage as a solution in hexane. GC analysis of the standard showed the parent and a decomposition peak at longer retention time. The second peak was not recovered in any of the tests, indicating that it was a polar compound. Since the parent CDF was recovered in fractions 4 and 5, Table 1, but no more could be eluted with acetone in fraction 6, it is probable that during decomposition a compound formed which could react in the GC inlet to reform the parent CDF, but could not be eluted from Florisil.

The pattern of PCB accumulation by the mallards at the end of the 296-day dosing period is shown in Fig. 1. All peaks with retention time less than approximately 6 min were significantly reduced relative to the later peaks. Peaks with retention time greater than approximately 10 min remained essentially unchanged in height relative to one

[3] Cat. No. A540; Fisher Scientific, Fair Lawn, N.J.

another when the mallard extract was compared to the Aroclor 1254 standard, as was found for White Carneau pigeons (deFreitas and Norstrom, 1974). This is strong indication that the later PCBs were not metabolized to any extent by the mallards.

Elimination of unmetabolized PCB did, however, occur in females, since egg production is an important route of excretion of unmetabolized PCBs from female birds (Dahlgren *et al.*, 1972). In the present study, the majority of females were producing eggs during the 3-month period prior to sacrifice. As a result, the PCB concentration in the fat of individual dosage groups, which ranged from a low of 300 mg/kg for 5 mg/kg-fed females to 9988 mg/kg for 100 mg/kg-fed males, was two to three times higher for males than females in each dosage group (D. Cartwright, unpublished results).

The PCB concentration in the lipid of the composite whole body sample used for CDF analysis therefore gave the minimum amount of CDFs potentially in the lipid, that is, assuming that no metabolism or excretion of PCBs or CDFs had occurred. Using values given by Bowes *et al.* (1975a) for the CDF content of Aroclor 1254 (*1970*), and 3750 mg/kg of Aroclor 1254 in lipid, the minimum potential concentrations in the lipid were approximately 0.8 μg/kg of tetra-CDFs, 1.5 μg/kg of penta-CDFs, and 0.9 μg/kg of hexa-CDFs.

Figure 2 compares the CDF content of 150 mg of Aroclor 1254 standard to the CDF content of 40 g of mallard lipid. The lipid also contained 150 mg of Aroclor 1254, based on the PCB reference peak with retention time 10 min (Fig. 1). For the individual CDFs

TABLE 3

IDENTITY OF THE CDFs AND PCNs PRESENT IN AROCLOR 1254

Peak designation[a]	Compound type	No. of chlorines	Relative retention time[b]	Reference[c]
A	CDF[d]	4	1.58	a
B	PCN	6	1.72	a
C	PCB	5	1.90	a
D	PCN	6	2.00	a
E	CDF	5	2.40	b
F	CDF	5	2.54	b
G	CDF	5	2.77	b
H	CDF[e]	5	2.95	a
I	PCN	7	3.44	a
J	CDF	6	4.11	c
K	CDF	6	4.25	c
L	CDF	6	4.68	b
M	CDF	6	4.98	b
N	PCN	8	5.68	c

[a] As shown on Fig. 2.
[b] Relative to dieldrin on a 6-ft., 3% SP-2100 (OV-1) column.
[c] a, Bowes *et al.* (1975a); b, Bowes *et al.* (1973); c, G. W. Bowes, personal communication.
[d] Positional isomer identified as 2,3,7,8-tetrachlorodibenzofuran.
[e] Positional isomer identified as 2,3,4,7,8-pentachlorodibenzofuran.

in Aroclor 1254, the peak height reproducibility between duplicates was within 10%. The lettered peaks and their retention times relative to dieldrin are listed in Table 3. Only two of the nine CDFs have been structurally identified (Bowes *et al.*, 1975b).

With minor exceptions, there were no detectable peaks in the mallard lipid extract that matched a CDF present in Aroclor 1254. Based on a 2-mm peak height, less than

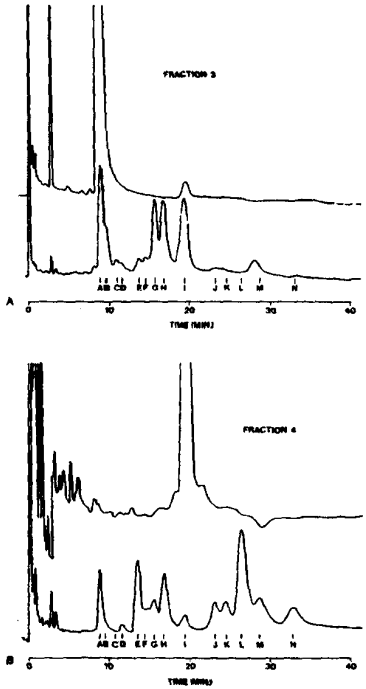

FIG. 2. Relative abundance of CDFs in Aroclor 1254 (lower chromatograms) compared to those in mallard lipid (upper chromatograms) after 296 days' oral administration of Aroclor 1254. Upper panel (A) is Fraction 3, Table 1; middle panel (B) is Fraction 4, Table 1; panel (C) is Fraction 5, Table 1. The amount of Aroclor 1254 represented is the same for all chromatograms, based on the peak with retention time 10-min, Fig. 1.

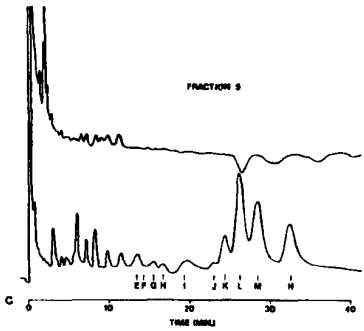

FIG. 2 *continued*

3% of the minimum potential concentration of CDFs was estimated to be present. The actual concentration may have been much lower. The detection limit for individual CDFs was estimated to be in the order of 0.01 μg/kg starting with a 40-g lipid sample.

The large peak in the mallard extract with a similar retention time to peak A (Fig. 2A) was shown not be 2,3,7,8-tetra-CDF by means of differing retention times on a 5-ft 3% OV-1/5% OV-210, 0.5-ft 3% OV-1/6% OV-225 column. The efficiency of PCB and polychlorinated naphthalene (PCN) separation from CDFs in mallard fraction 3 (Fig. 2A) was not as good as, and more variable than that of pure Aroclor 1254, probably due to the presence of biogenic hydrocarbon or lipid materials. The heptachloronaphthalene, peak I, derived from Aroclor 1254 was present in large quantities in the mallard lipid (Fig. 2B), indicating that it was not easily metabolized or excreted. The negative peaks in the chromatogram of mallard fraction 5 (Fig. 2C) were shown to be due to hydrocarbons when Florisil cleanup and treatment with concentrated sulfuric acid did not remove them. GC analysis using a flame ionization detector showed a continuous series of peaks with retention times from 5 min to 2 hr.

DISCUSSION

Little is known about the environmental persistence and bioaccumulation potential of CDFs. They are chemically stable (e.g., towards strong acids and bases) like many of the more persistent organochlorines, but also more photochemically labile than PCBs (Hutzinger *et al.*, 1973). There is some evidence that Atlantic salmon (*Salmo salar*) accumulated di-, tri-, and tetra-CDFs from food to increasingly higher levels as the number of chlorines/molecule increased (Zitko *et al.*, 1973). Explanations for the increase included easier metabolism, faster excretion and decreased efficiency of uptake from the GI tract of the lower chlorinated CDFs. The accumulated concentrations of all CDFs were very low. Curley *et al.* (1975) produced tentative mass spectral evidence that a

tetra-CDF was eliminated in the urine of rats fed Aroclor 1254. The presence of 2,3,7,8-tetra-CDF in Aroclor 1254 has been confirmed (Bowes *et al.*, 1975b). The ability to detect CDFs in urine suggests that they are more rapidly eliminated from the rat than PCBs, since the total tetra-CDF intake of all rats throughout the study was approximately 2.4 μg,[6] and the urine sample represented only a small time period in the PCB-ingestion experiments.

Studies with the chemically similar CDDs may be indicative of CDF bioaccumulation potential. Microbial metabolism and model ecosystem studies with 2,3,7,8-tetra-CDD (TCDD) indicated that it was more stable towards metabolic breakdown than most organochlorine compounds (Matsumura and Benezet, 1973; Isensee and Jones, 1975). However, unlike DDE and the more highly chlorinated PCBs, which are also relatively resistant to metabolism, TCDD appeared to be less efficiently absorbed from the GI tract, and was cleared from organisms more rapidly as the unchanged molecule (Vinopal and Casida, 1973; Piper *et al.*, 1973).

The evidence, therefore, seems to point to a faster elimination of CDFs than of PCBs, which is strongly reinforced for birds by the present study which has shown that mallards eliminated >97% of all CDFs associated with Aroclor 1254 in the long-term feeding experiment. The PCB concentrations reached were similar to those found in the most highly contaminated wild birds, such as Herring Gulls in Lake Ontario (Gilbertson, 1974; Frank *et al.*, 1975). A large body burden of PCBs may contribute to more rapid clearance of CDFs by inducing a higher level of hydroxylase activity.

Further attempts to find CDF residues in the environment, even in birds with exceptionally high PCB concentrations, appear destined to fail. The practical difficulties of environmental CDF analysis can be seen from the present study. To achieve the CDF detection limit of 0.01 μg/kg in lipid, approximately 500 g wet weight tissue, containing a potential CDF concentration of 0.8 ng/kg (ppt), was required for each analysis. The use of mass fragmentography may eliminate time-consuming cleanup steps (Buser, 1975), but is unlikely to give much better detection limits. The contribution of chlorinated phenols to environmental contamination by CDFs may be as high as that of PCBs (Firestone *et al.*, 1972), but given the apparent lack of CDF bioaccumulation, this contribution is also unlikely to result in measurable residues at the top of a food web.

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REFERENCES

Bowes, G. W., Mulvihill, M. J., Simoneit, B. R. T., Burlingame, A. L., and Risebrough, R. W. (1975a). Identification of chlorinated dibenzofurans in American polychlorinated biphenyls. *Nature (London)* **256**, 305–307.

Bowes, G. W., Mulvihill, J. J., DeCamp, M. R., and Kende, A. S. (1975b). Gas chromatographic characteristics of authentic chlorinated dibenzofurans; identification of two isomers in American and Japanese polychlorinated biphenyls. *J. Agr. Food Chem.* 23, 1222–1223.

[6] Three grams total Aroclor consumption times 0.8 mg/kg tetra-CDFs (Bowes *et al.*, 1975a).

Bowes, G. W., Simoneit, B. R., Burlingame, A. L., de Lappe, B. W., and Risebrough, R. W. (1973). The search for chlorinated dibenzofurans and chlorinated dibenzodioxins in wildlife populations showing elevated levels of embryonic death. *Environ. Health Perspect. No.* 5, 191–198.

Buser, H. R. (1975). Analysis of polychlorinated dibenzo-*p*-dioxins and dibenzofurans in chlorinated phenols by mass fragmentography. *J. Chromatog.* 107, 295–310.

Curley, A., Burse, V. W., Jennings, R. W., Villaneuva, E. C., and Kimbrough, R. D. (1975). Evidence of tetrachlorodibenzofuran (TCDF) in Aroclor 1254, and in the urine of rats following dietary exposure to Aroclor 1254. *Bull. Environ. Contam. Toxicol.* 14, 153–158.

Dahlgren, R. B., Linder, R. L. and Carlson, C. W. (1972). Polychlorinated biphenyls: Their effects on penned pheasants. *Environ. Health Perspect. No.* 1, 89–101.

deFreitas, A. S. and Norstrom, R. J. (1974). Turnover and metabolism of polychlorinated biphenyls in relation to their chemical structure and the movement of lipids in the pigeon. *Canad. J. Physiol. Pharmacol.* 52, 1080–1094.

Firestone, D., Ress, J., Brown, N. L., Barron, R. P. and Damico, J. N. (1972). Determination of polychlorodibenzo-*p*-dioxins and related compounds in commercial chlorophenols. *J. Ass. Offic. Anal. Chem.* 55, 85–93.

Frank, R., Holdrinet, M. van H., and Rapley, W. A. (1975). Residue of organochlorine compounds and mercury in birds' eggs from Niagara Peninsula, Ontario. *Arch. Environ. Contam. Toxicol.* 3, 205–218.

Gilbertson, M. (1974). Pollutants in breeding Herring Gulls in the lower Great Lakes. *Canad. Field-Naturalist* 88, 273–279.

Gilbertson, M., and Hale, R. (1974). Early embryonic mortality in a Herring Gull colony in Lake Ontario. *Canad. Field-Naturalist* 88, 354–356.

Goldstein, J. A., McKinney, J. D., Lucier, G. W., More, J. A., Hickman, P., and Bergman, H. (1974). Effects of hexachlorobiphenyl isomers and 2,3,7,8-tetrachlorodibenzofuran (TCDF) on hepatic drug metabolism and porphyrin accumulation. *Pharmacologist* 16, 239.

Hays, H., and Risebrough, R. W. (1973). Pollutant concentrations in abnormal young terns from Long Island Sound. *The Auk* 89, 19–35.

Hutzinger, O., Safe, S., Wentzell, B. R., and Zitko, V. (1973). Photochemical degradation of di- and octochlorodibenzofuran. *Environ. Health Perspect. No.* 5, 267–271.

Isensee, A. R., and Jones, G. E. (1975). Distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) in aquatic model ecosystem. *Environ. Sci. Technol.* 9, 668–672.

Matsumura, F., and Benezet, H. J. (1973). Studies on the bioaccumulation and microbial degradation of 2,3,7,8-tetrachloro-*p*-dioxin. *Environ. Health Perspect. No.* 5, 253–258.

McLeod, H. A., and Ritcey, W. R. (1973). *Analytical Methods for Pesticide Residues in Foods*, Rev. Ed. 1973, Section 7. Health Protection Branch, Health and Welfare Canada. Ottawa.

Piper, W. N., Rose, J. Q., and Gehring, P. J. (1973). Excretion and tissue distribution of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in the rat. *Environ. Health Perspect. No.* 5, 241–244.

Poland, A., and Glover, E. (1973). Studies on the mechanism of toxicity of the chlorinated dibenzo-*p*-dioxins. *Environ. Health Perspect. No.* 5, 245–252.

Porter, M. L., and Burke, J. A. (1971). Separation of three chlorodibenzo-*p*-dioxins from some polychlorinated biphenyls by chromatography on an aluminum oxide column. *J. Ass. Offic. Anal. Chem.* 54, 1426–1428.

Porter, M. L., and Burke, J. A. (1973). An isolation and cleanup procedure for low levels of organochlorine pesticide residues in fats and oils. *J. Ass. Offic. Anal. Chem.* 56, 733–738.

Schwetz, B. A., Norris, J. M., Sparschu, G. L., Rowe, V. K., Gehring, P. J., Emerson, J. L., and Gerbig, C. G. (1973). Toxicology of chlorinated dibenzo-*p*-dioxins. *Environ. Health Perspect. No.* 5, 87–100.

Tumasonis, C. F., Bush, B., and Baker, F. D. (1973). PCB levels in egg yolks associated with embryonic mortality and deformity of hatched chicks. *Arch. Environ. Contam. Toxicol.* 1, 312–324.

Verrett, J. (1971). Report on 2,4,5,-T. (Executive Office of the President, Science Advisory Committee, Office of Science and Technology, March.)

VINOPAL, J. H., AND CASIDA, J. E. (1973). Metabolic stability of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin in mammalian liver microsomal systems and in living mice. *Arch. Environ. Contam. Toxicol.* **2**, 122–132.

VOS, J. G., AND KOEMAN, J. H. (1970). Comparative toxicologic study with polychlorinated biphenyls in chickens with special reference to porphyria, edema formation, liver necrosis, and tissue residues. *Toxicol. Appl. Pharmacol.* **17**, 656–668.

VOS, J. G., KOEMAN, J. H., VAN DER MAAS, H. L., TEN NOEVER DE BRAUW, M. C., AND DE VOS, R. H. (1970). Identification and toxicological evaluation of chlorinated dibenzofuran and chlorinated naphthalens in two commercial polychlorinated biphenyls. *Fd. Cosmet. Toxicol.* **8**, 625–633.

ZITKO, V. (1972). Absence of chlorinated dibenzodioxins and dibenzofurans from aquatic animals. *Bull. Environ. Contam. Toxicol.* **7**, 105–110.

ZITKO, V., WILDISH, D. J., HUTZINGER, O., AND CHOI, P. M. K. (1973). Acute and chronic oral toxicity of chlorinated dibenzofurans to salmonid fishes. *Environ. Health Perspect. No.* **5**, 187–190.

can be represented by a map composed of eigenvectors 2 and 8 as follows:

$$\text{map } a = 5.76\,(\text{eigenvector } 2) + 5.13\,(\text{eigenvector } 8) \quad (2)$$

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[6].

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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1 LaMarche, V. C., *Science*, 183, 1043–1048 (1974).
2 Namias, J., *Mon. Weath. Rev. U.S. Dep. Agric.*, 97, 173–192 (1969).
3 Laurs, R. M., *et al.*, *Report of Joint National Marine Fisheries Service–American Fishermen's Research Foundation Albacore Studies Conducted During 1973* (National Marine Fisheries Service, Southwest Fisheries Center, La Jolla, 1974).
4 Clemens, H. B., and Craig, W. L., *Calif. Dept Fish and Game, Fish Bull.*, 128 (1965).
5 Sette, O. E., *Calif. Coop. Oceanic Fish. Invest. Rept*, 7, 181–194 (1960).
6 Pyke, C. B., *An Investigation of some precipitation patterns in California and adjacent regions* (University of California Water Resources Center, 1966).

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

While growing oats at different temperatures in water of different ¹⁸O and deuterium (D) abundances, we noticed that oats grown in Antarctic water in which is depleted in ¹⁸O and 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 ¹⁸O and 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 30 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 fertiliser, had been added. One jar contained melted glacial ice from the Antarctic with isotope concentrations of −49‰ $\delta^{18}O$ (SMOW) and −400‰ δD (SMOW). The other jar contained distilled ocean water with −1.0‰ $\delta^{18}O$ (SMOW) and +17‰ δD (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 °C, once between 24 and 26.6 °C; and once the temperature fluctuated between 1.7 and 26.6 °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 °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 that had reached the top of the jar (approximately 25 cm).

Kashutin[1] observed that snow-water depleted in 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 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 D-depleted water on plant and animal growth may prove fruitful. A major source of water depleted in D by over 400–440‰ 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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1 Kashutin, K., *Proc. [illegible] USSR*, 88, [illegible]

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[1], the white-tailed eagles (*Haliaeetus albicilla*) of Schleswig-Holstein[2] and the herring

gulls (*Larus argentatus*) of Lake Ontario[1]. 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[1-5]. PCBs are present in high concentrations in the bird populations which suffer embryonic mortality[1,2]. 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[6,7] 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[6-10]. 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[12-14]. Chlorinated dibenzofurans have been found in a French (Phenoclor DP6) and a German (Clophen A60) PCB and were shown to be the active embryotoxic agent in these preparations[6]. 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[6].

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.*[6]: 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

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.

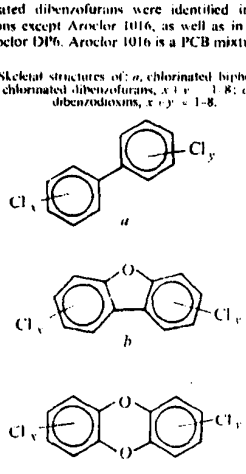

Table 1 Chlorinated dibenzofuran concentrations* in Aroclor, Clophen and Phenoclor

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)	1.3 (82)	1.7
Aroclor 1254 (1970)	0.2 (13)	0.1 (7)	0.9 (60)	1.5
Aroclor 1260 (1969)	0.1 (10)	0.1 (10)	0.8 (80)	1.0
Aroclor 1260 (lot AK-3)	0.2 (25)	0.1 (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 ng g^{-1} PCB. Values in parentheses represent quantity as percentage total dibenzofuran.

ND, not detected (< 0.001 ng g^{-1}).

Amounts of PCB ranging from 1.0 to 2.0 g were dissolved in 400 ml hexane, placed on a Florisil column (380 g, internal diameter 41.5 mm), and eluted with: an additional 1,000 ml hexane, and successive 800 ml volumes each of 5%, diethylether hexane, 25%, diethylether hexane and acetone, at a rate of approximately 7 ml min^{-1}. The major portion of the PCBs was eluted in the hexane fraction, which was discarded. On addition of the 5% mixture, the eluates were collected in six successive 400 ml volumes. To eliminate the polar solvents, 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[15] and eluted with 10 ml each of 1%, and 20%, methylene chloride in hexane. These were also taken twice just to dryness and made to up 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 Supelcoport in Fracer M720 and Hewlett Packard 5700 gas chromatographs equipped with ^{63}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%, 4 μl) in hexane was injected into the capillary as a rinse, removed with a 1.0 μl microsyringe, and placed directly on the mass spectrometer probe. The probe was inserted into a GEC AEI MS902 high 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[15].

approximately 42% chlorine and has replaced Aroclor 1242 in many applications, principally as the dielectric fluid in capacitors[16]. 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[6]; our first analyses of the Clophen revealed an additional five chlorinated dibenzofurans[9]. 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.*[6] detected no chlorinated dibenzofurans in an Aroclor 1260 preparation at a detection limit of 5 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^{-1} 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

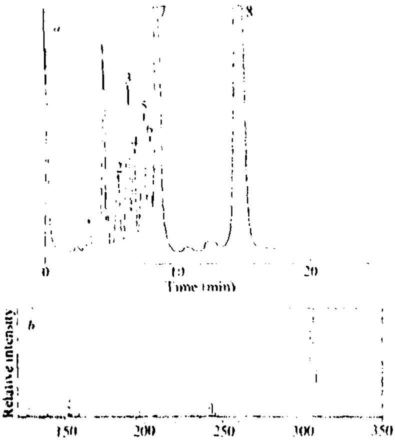

Fig. 2 *a*, Gas chromatogram of a fraction of Aroclor 1254 containing a mixture of chlorinated biphenyls, dibenzofurans, and naphthalenes. Identities of peaks are given in the text. *b*, Mass spectrum of peak 2, a tetrachlorodibenzofuran.

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 $C_{12}H_4O^{35}Cl_3{}^{37}Cl_1{}^{13}C_2$. 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[17–20], 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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1 Newton, I., and Bogan, J., *Nature*, 249, 582–583 (1974).
2 Koeman, J. H., Hadderingh, R. H., and Bijleveld, M. F. I. J., *Biol. Conserv.*, 4, 373–377 (1972).
3 Culbertson, M., and Hale, R., *Can. Fld Nat.*, 88, 354–356 (1974).
4 Hegginbotham, G. R., *et al.*, *Nature*, 220, 702–703 (1968).
5 Sparschu, G. L., Dunn, F. L., and Rowe, V. K., *Food Cosmet. Toxic.*, 9, 405–412 (1971).
6 Vos, J. G., Koeman, J. H., Van der Maas, H. L., ten Noever de Brauw, M. C., and de Vos, R. H., *Food Cosmet. Toxic.*, 8, 625–633 (1970).
7 Vos, J. G., *Environ. Hlth Persp.*, 1, 105–117 (1972).
8 Bowes, G. W., Simoneit, B. R., Burlingame, A. L., de Lappe, B. W., and Risebrough, R. W., *Environ. Hlth Persp.*, 5, 191–198 (1973).
9 Baughman, R. and Meselson, M., *Environ. Hlth Persp.*, 5, 27–35 (1973).
10 Baughman, R., and Meselson, M., *Adv. Chem.*, 120, 92–104 (1973).
11 Report on 2,4,5-T (Executive Office of the President, Science Advisory Committee, Office of Science and Technology, March, 1971).
12 Jensen, S., and Renberg, L., *Ambio*, 1, 62–65 (1972).
13 Firestone, D., Ress, J., Brown, N. L., Barron, R. P., and Damico, J. N., *J. Ass. Off. Analyt. Chem.*, 55, 85–92 (1972).
14 Porter, M. L., and Burke, J. A., *J. Ass. Off. Analyt. Chem.*, 54, 1426–1428 (1971).
15 Burlingame, A. L., Olsen, R. W., and McPherson, R. V., *Adv. Mass Spectr.*, 6, 1053–1059 (1974).
16 Nisbet, I. C. T., and Sarofim, A. F., *Environ. Hlth Persp.*, 1, 21–38 (1972).
17 Bowes, G. W., and Jonkel, C. J., *J. Fish. Res. Bd Can.* (in the press).
18 Jensen, S., Johnels, A. G., Olsson, M., and Otterlind, G., *Nature*, 224, 247–250 (1969).
19 Koeman, J. H., ten Noever de Brauw, M. C., and de Vos, R. H., *Nature*, 221, 1126–1128 (1969).
20 Risebrough, R. W., Reiche, P., Peakall, D. B., Herman, S. G., and Kirven, M. N., *Nature*, 220, 1098–1102 (1968).

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[1,2]. Field data supporting these predictions are well known for the interspecific case[3,4] 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[1]. 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

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The issue is raised again!
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PCB's: How Toxic?

Polychlorinated biphenyls (PCB's) are recognized as ubiquitous environmental contaminants. In 1973, this worldwide problem resulted in a decision by the Organization for Economic Cooperation and Development to control the use and disposal of PCB's (*1*). At present, both the U.S. and Canadian governments are preparing legislation for the control of toxic substances.

The concern over PCB's is based on two factors, namely their environmental persistence and their toxicity. Recent results, however, cast doubts on the latter. Bowes *et al.* (*2*) observed concentrations of highly toxic polychlorinated dibenzofurans (PCDF's) ranging from 0.1 to 0.5 microgram per gram in all but one of the North American PCB's (Aroclor). Earlier studies by Vos *et al.* (*3*) had indicated that only PCB's manufactured in Japan (Kanechlor) and Europe (Clophen, Phenochlor) contained such impurities. In addition, PCDF's and other by-products were recently found in "pure" PCB isomers (*4*).

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The toxicity of PCDF's exceeds that of PCB's by approximately four to six orders of magnitude. Their presence in PCB's has, therefore, significant bearing on toxicity studies on PCB's, commercial mixtures, and isomer preparations alike. Yet, in only a small proportion of the scientific reports on this subject is the problem of PCDF impurities in PCB's discussed. Obviously, the degree of this contamination is variable with the origin and probably also with other details of the manufacturing processes.

I strongly recommend, therefore, that in all future toxicity studies and for as many past studies as can be documented, precise information on the PCB's used (source, date of manufacture, lot number, and so forth) be recorded. I further recommend that past experiments for which such information is available be reevaluated in view of the strong possibility of the presence of PCDF's and their overriding toxic effects.

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References

1. Organization for Economic Cooperation and Development, press release (OECD Press No. 7303, Paris, 14 February 1973), pp. 1–2.
2. G. W. Bowes, M. J. Mulvihill, M. R. DeCamp, A. S. Kende, *J. Agric. Food Chem.* **23**, 1222 (1975).
3. J. G. Vos, H. L. Van der Maas, M. C. Ten Noever De Brauw, R. H. De Vos, *Food Cosmet. Toxicol.* **8**, 625 (1970); J. G. Vos, *Environ. Health Perspect.* **1**, 105 (1972).
4. M. Morton, G. Sundström, C. A. Wachtmeister, *Acta Chem. Scand.* **27**, 3121 (1973); D. C. Ayres, *Nature (London)* **240**, 161 (1972).