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Disposition of Chlorinated Diphenyl Ethers Isolated from Technical Pentachlorophenol in the Rat

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Chlorinated phenols have widespread use as wood preservatives and have been shown to contain several impurities including chlorinated diphenyl ethers (FIRESTONE et al, 1972; BUSER, 1975) which have been estimated at levels up to 1000 ppm (NILSSON and RENBERG, 1974). Evidence of environmental contamination by these compounds is apparent in the detection of tri- and tetrachlorodiphenyl ethers in mussels from Narragansett Bay (LAKE et al, 1981) and in diphenyl ethers containing from 6 to 9 chlorine atoms in fish from Lake Ontario (LAU and NEWSOME, 1982). The total quantity of these chlorinated diphenyl ethers ranged from 0.7 ppb in eel to 73 ppb in catfish. Further contamination of the food supply is possible through the use of livestock bedding derived from treated wood (RYAN and PILON, 1982).

The persistence of lower chlorinated diphenyl ethers has been examined in fish, where the tri-, tetra-, and pentachlorinated congeners were found to have half-lives comparable to the corresponding chlorodiphenyls (ZITKO and CARSON, 1977). In trout, the bioconcentration factor for tetrachlorodiphenyl ether was somewhat higher than that for hexachlorobenzene (NEELY et al, 1974).

Little data is available on the metabolism or toxicity of diphenyl ethers in animals. Phenoxyphenol metabolites of di- and trichlorodiphenyl ethers have been identified in rat urine and feces after a single oral dose of 250 mg/kg^{-1} (TULP et al, 1979). The induction of liver enzymes in rats administered diphenyl ethers containing 2, 3, 4, 5 or 10 chlorine atoms has been reported by CARLSON et al, (1980) who concluded that the extent and type of induction was governed by the degree and position of substituents analogously to the polychlorinated biphenyls. Since the major chlorinated diphenyl ether congeners present in chlorophenol preparations consist of those with 7, 8 and 9 chlorine atoms (BUSER, 1975; FIRESTONE et al, 1972) the present study was conducted to determine the tissue distribution and relative rates of elimination of such a mixture of congeners in the rat.

MATERIALS AND METHODS

Diphenyl ethers were isolated from technical pentachlorophenol by suspending the phenol (10 g) in 1 N NaOH (150 ml) and extracting with toluene (100 ml). The toluene layer was then re-extracted with 1 N NaOH (3 x 150 ml), dried with sodium sulfate and taken to dryness on a rotary evaporator. The residue was dissolved in hexane and chromatographed on a 12 g column of 2% deactivated Florisil. The diphenyl ether fraction was eluted with 70 ml of hexane and the solvent removed by evaporation in vacuo at 20°C. The processing of two 10 g batches of phenol yielded a

total of 18.6 mg of diphenyl ether fraction which was used to prepare the dosing solution. A capillary gas-liquid chromatogram of the mixture is shown in Figure 1. Assignment of the degree of chlorination was made by single ion monitoring on a Varian MAT 311A, from which it was found that the mixture contained 2 hexa-, 5 hepta-, 4 octa-, and 2 nonachlorodiphenyl ethers. Some individual isomers were identified by coincidence of the retention times with those of synthetic standards prepared as described previously (NEWSOME and SHIELDS, 1982).

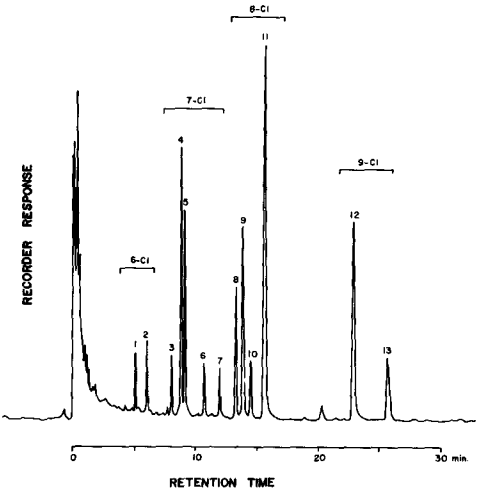

Figure 1. Capillary gas-liquid chromatogram of chlorinated diphenyl ethers isolated from technical pentachlorophenol. Peaks 4,6,7,10,11 and 13 were identified as the 2,2'3,4,4'5,6' 2,2'3,4,4'5,5' 2,3,3'4,4'5,5' 2,2'3,4,4'5,5'6 2,2'3,3'4,4'5,6' and 2,2'3,3'4,4'5,5'6 and 2,2'3,3'4,4'5,5'6 isomers respectively. Detection was by electron capture.

Male Sprague Dawley rats, body weight 220 g, were obtained from Canadian Breeding Farm and Laboratory Ltd., St. Constant, Quebec. They were treated with 2.1 mg kg^{-1} body weight of the diphenyl ether mixture in corn oil by gastric intubation and were sacrificed in triplicate by exsanguination under ether anesthesia. Blood, liver, adipose, muscle and skin tissues were removed and frozen pending analysis. Urine and feces were collected from three separate animals maintained in metabolism cages for 7 days.

Tissues were analyzed by a modification of the method described previously (NEWSOME and SHIELDS, 1982). Samples of blood or muscle (1.0 g), liver or skin (0.5 g), fat (50 mg), feces (0.5 g), or urine (5.0 g) were extracted by homogenizing with 2:1 acetone:hexane (30 ml). After removal of solids by filtration through Whatman No. 1 paper on a Buchner funnel, the extract was partitioned with water (10 ml) and the hexane layer recovered. Solvent was removed on a rotary evaporator, the residue taken up in hexane (0.5 ml) and chromatographed on a 1 g column of 2% deactivated Florisil. The diphenyl ether fraction was eluted with hexane (7.0 ml) and analyzed by capillary gas-liquid chromatography with electron capture detection. The fused silica 30 m x 0.25 mm column was coated

with SE-54 and run at 240°C with a helium carrier velocity of 33 cm $sec.^{-1}$ Components were quantitated by comparison of the peak heights to those of single hepta-, octa- or nonachlorodiphenyl ether standards. The reference standard for quantitating heptachloro-isomers was 2,2',3,4'4',5,5'-heptachlorodiphenyl ether, while the 2,2',3,4,4',5,5',6 octa- and 2,2',3,3',4,4',5,5',6 nona- were used to quantitate the octa- and nonchlorodiphenyl ethers, respectively.

RESULTS AND DISCUSSION

The uptake of total chlorinated diphenyl ether was rapid in blood, liver, and muscle reaching peak values after approximately 8 h (Table I-III). A somewhat slower incorporation was observed with adipose tissue and skin (Tables IV, V).

Table I

Concentration (ng/g) of Chlorinated Diphenyl Ether Congeners in Rat Blood at Various Intervals after Dosing

Time	7-Cl	8-Cl	9-Cl
1 h	18.5 ± 6.9	17.5 ± 5.4	10.4 ± 3.2
2 h	26.9 ± 8.2	27.3 ± 6.8	20.4 ± 5.5
4 h	48.2 ± 30.2	47.4 ± 24.9	36.1 ± 13.1
8 h	102 ± 23.4	93.7 ± 19.8	63.2 ± 31.2
1 day	12.5 ± 1.9	13.7 ± 1.5	12.9 ± 1.8
2 days	15.6 ± 7.0	12.4 ± 7.7	13.1 ± 6.6
3 days	10.6 ± 0.9	6.1 ± 0.7	5.6 ± 1.6
7 days	5.8 ± 0.8	3.4 ± 0.5	2.1 ± 0.2
14 days	8.2 ± 4.0	4.8 ± 2.9	3.3 ± 2.5
28 days	1.6 ± 0.4	1.4 ± 0.2	1.0 ± 0.2

Values are the means ± S.D. of determinations on three animals.

Table II

Concentration (ng/g) of Chlorinated Diphenyl Ether Congeners in Rat Liver at Various Intervals after Dosing

Time	7-Cl	8-Cl	9-Cl
1 h	100 ± 26	60.8 ± 5.8	24.2 ± 4.1
2 h	206 ± 45	189 ± 63.4	116 ± 49.6
4 h	446 ± 230	408 ± 206	294 ± 143
8 h	2318 ± 190	2370 ± 162	1758 ± 152
1 day	154 ± 48	166 ± 48	226 ± 70
2 days	53.2 ± 12.4	59.6 ± 9.6	100 ± 18.8
3 days	41.4 ± 4.7	34.4 ± 3.5	80.4 ± 16.4
7 days	29.6 ± 4.8	20.0 ± 4.4	32.2 ± 10.4
14 days	13.6 ± 3.0	9.8 ± 2.0	14.5 ± 2.6
28 days	4.5 ± 1.4	3.4 ± 1.3	4.1 ± 1.8

Values are the means ± S.D. of determinations on three animals.

Table III

Concentration (ng/g) of Chlorinated Diphenyl Ether Congeners in Rat Muscle at Various Intervals after Dosing

Time	7-Cl	8-Cl	9-Cl
1 h	8.8 ± 3.3	3.4 ± 1.2	1.4 ± 0.6
2 h	4.3 ± 1.5	2.6 ± 0.	1.7 ± 0.5
4 h	16.4 ± 5.2	8.8 ± 3.4	3.2 ± 1.4
8 h	279 ± 29	175 ± 21	67.4 ± 7.5
1 day	49.4 ± 2.6	55.1 ± 5.9	49.8 ± 7.6
2 days	22.9 ± 4.1	15.9 ± 1.5	29.1 ± 7.1
3 days	16.6 ± 2.9	13.8 ± 1.3	25.1 ± 5.5
7 days	15.5 ± 3.5	11.6 ± 2.2	13.4 ± 1.7
14 days	9.7 ± 1.4	6.7 ± 0.8	6.2 ± 0.9
28 days	1.9 ± 0.5	1.2 ± 0.4	1.4 ± 0.9

Values are means ± S.D. of determinations on three animals.

Table IV

Concentration (ng/g) of Chlorinated Diphenyl Ether Congeners in Rat Adipose at Various Intervals after Dosing

Time	7-Cl	8-Cl	9-Cl
1 h	15.4 ± 5.5	8.8 ± 5.9	4.2 ± 3.2
2 h	21.8 ± 6.4	10.0 ± 3.7	5.6 ± 3.8
4 h	43.8 ± 12.4	17.0 ± 9.4	6.2 ± 5.0
8 h	508 ± 123	253 ± 67	88.0 ± 25.3
1 day	499 ± 198	264 ± 123	117 ± 56
2 days	551 ± 166	302 ± 88	153 ± 55
3 days	451 ± 153	262 ± 89	149 ± 43
7 days	434 ± 126	264 ± 85	164 ± 43
14 days	471 ± 115	290 ± 73	208 ± 47
28 days	229 ± 65	148 ± 75	105 ± 21

Values are the means ± S.D. of determinations on three animals.

Table V

Concentration (ng/g) of Chlorinated Diphenyl Ether Congeners in Rat Skin at Various Intervals after Dosing

Time	7-Cl	8-Cl	9-Cl
1 h	9.58 ± 1.9	6.0 ± 0.9	3.3 ± 0.4
2 h	11.1 ± 4.5	6.7 ± 2.6	3.2 ± 1.3
4 h	27.0 ± 11.6	14.2 ± 6.8	7.1 ± 3.4
8 h	187 ± 34.2	109 ± 23	39.8 ± 17.6
1 day	208 ± 60.4	102 ± 29	62.0 ± 19.4
2 days	182 ± 22.8	118 ± 15.9	73.2 ± 13.1
3 days	111 ± 15.2	82.8 ± 16.5	61.6 ± 20.8
7 days	65.0 ± 3.9	49.2 ± 5.4	44.0 ± 3.6
14 days	48.0 ± 9.2	31.2 ± 6.2	28.6 ± 5.5
28 days	18.3 ± 6.0	17.3 ± 5.7	22.8 ± 7.4

Values are the means ± S.D. of determination on three animals.

The highest post absorptive levels were found in adipose tissue followed by skin > liver > muscle > blood, in an order similar to those reported for chlorinated biphenyls (MATHEWS and ANDERSON, 1975). After the initial uptake, dissipation rates of hepta-, octa- or nona-chlorophenyl ether congeners were similar in all tissues (Table VI) with the exception of adipose where the slow removal of all isomers precluded the calculation of half-lives.

Table VI

Half-lives of Chlorinated Diphenyl Ethers in Various Tissues.

Congener	Liver	Skin	Blood	Muscle
7-Cl	7.4	9.8	9.3	7.6
8-Cl	6.8	10.2	10.4	7.0
9-Cl	5.7	13.4	8.9	5.9

Half-lives, reported in days, were obtained using data from the terminal decline phase (days 2-28 inclusive). The SE of the half-lives is $\pm$ 16%.

A comparison of the isomer distribution determined 14 days after dosing, with that of the original dosing solution (Table VII) reveals a similar pattern in all tissues, with a marked decrease in the heptachloro-(peak 5) and octachloro-(peak 11). Concomitantly, heptachloro isomers 6 and 7, octachloro isomer 10, and nonachloro isomer 13 are increased relative to the proportion in the dosing solution, indicating that they are more persistent. The proportion of total hepta-, octa-, or nonachloro isomers remained relatively constant for all tissues except liver, where the nonachloro isomers are increased. This increase of the nonachlorodiphenyl ether congener is a reflection of an increased uptake by the liver rather than a decreased elimination rate, since the half-lives of the hepta- octa- and nonachloro isomers are similar.

Fecal excretion accounted for 20% of the initial dose within the first 7 days, with only 0.04% being eliminated in the urine over the same interval. Further studies are in progress to identify phenoxyphenol metabolites expected to occur in feces and urine (TULP et al, 1979).

The results indicate that the higher chlorinated diphenyl ethers can be expected to show an accumulation and tissue distribution pattern similar to that reported for the PCB's. Like the chlorinated biphenyls, it can be expected that environmental and food samples would retain these compounds if there was previous exposure to diphenyl ether contamination.

Table VII

Percentage Composition of Isomers in Tissues Fourteen Days after the Administration of the Diphenyl Ether Fraction from Pentachlorophenol

Peak No.	Solution Administered	Adipose	Liver	Skin	Blood	Muscle
3	4.6	3.3	1.1	3.2	2.4	2.0
4	20.4	20.2	11.4	18.8	19.1	15.8
5	15.4	7.0	4.0	6.3	8.2	4.4
6	3.9	11.6	8.8	9.6	10.4	11.4
7	3.5	6.4	7.6	6.6	10.4	9.2
$\sum_{3}^{7}$	47.8	48.5	35.8	44.5	50.5	42.8
8	6.1	5.1	3.1	4.1	3.1	3.3
9	8.3	9.0	8.4	8.8	9.9	9.5
10	2.6	6.6	11.2	6.9	6.2	8.1
11	16.3	9.3	4.3	9.1	10.4	8.8
$\sum_{8}^{11}$	33.3	30.0	25.8	28.9	29.6	29.7
12	14.4	13.8	22.0	16.7	12.5	16.4
13	4.5	7.6	18.1	9.9	7.5	11.1
$\sum_{12}^{13}$	18.9	21.4	38.4	26.6	20.0	27.5

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