Identification of Chlorinated Dibenzofurans in American Polychlorinated Biphenyls (Aroclor®)

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ABSTRACT

Chlorinated dibenzofurans, highly toxic compounds structurally related to the chlorinated dibenzodioxins, have been detected as contaminants in American preparations of polychlorinated biphenyls (PCB). They are assumed to account for some of the toxic properties of the crude PCB mixtures.

Polychlorinated biphenyls (PCB) are now ubiquitous on Earth. These industrial chemicals have been detected in polar bears (Ursus maritimus) from remote areas of the Canadian Arctic and in snow petrels (Pagodroma nivea) from the Antarctic (1). They are widespread in both marine and fresh-water ecosystems, with highest concentrations recorded in fish-eating birds and mammals from waters adjacent to industrialized areas (2). The reproductive failures of many of these populations, particularly of the fish-eating birds, are among the examples of environmental degradation to which PCB may contribute; a role in eggshell thinning, however, has been discounted (3). Embryonic mortality accounts for a substantial portion of the reproductive failures; embryos in 20 per cent of the eggs of the herring gulls (Larus argentatus) in Lake Ontario colonies in 1973 were found to die during the first week of incubation (4). Experimental work has demonstrated the embryotoxic effects of PCB (5); the active components in German and French PCB, however, have been identified as chlorinated dibenzofurans, present in trace amounts as contaminants in the preparations (6). Like the structurally related chlorinated dibenzodioxins (Figure 1), the chlorinated dibenzofurans are toxic to embryos in sub-microgram amounts (6, 7). They are therefore among the most toxic compounds known. Since the chlorinated dibenzofurans and dibenzodioxins share chemical properties with the dominant chlorinated hydrocarbons in environmental samples, principally the DDT and PCB compounds, they are difficult to detect in the small amounts at which they exert biological effects (8). Techniques which detected the chlorinated dibenzofurans in the French and German PCBs failed to demonstrate their presence in the American PCB Aroclor® 1260 (6).

In the present paper we report the presence of chlorinated dibenzofurans in the American PCBs Aroclor® 1248, 1254, and 1260, and their absence in detectable quantities in Aroclor® 1016.

Samples of PCB examined, obtained from the Monsanto Company in the years indicated, include: Aroclor® 1248, 1254, and 1260 (1969); Aroclor® 1254 (1970); and Aroclor® 1016 (1972). Amounts ranging from 1.0 to 2.0 g were dissolved in 400 ml hexane, placed on a Florisil column (180 g, column i.d. 31.5 mm), and eluted with: 1) an additional 1600 ml hexane, and successive 800 ml volumes each of 2) 5% ethyl-ether/hexane, 3) 25% ethyl-ether/hexane, and 4) acetone, at a rate of approximately 7 ml/min. The major portion of the PCB was eluted in the hexane fraction. This was discarded. Upon addition of the 5% mixture, the eluates were collected in 6 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 micro alumina column (9) 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 prior to gas chromatographic analysis. Aliquots of all fractions obtained before and after partitioning on alumina were injected into a 6 foot glass column containing 3% OV-1 on 100-120 mesh Supelcoport in a Tracor MT-220 gas chromatograph equipped with ^{63}Ni electron-capture detectors. PCB compounds 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.

Upon 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 with use of a 20:1 effluent splitter, and a trap consisting of a 1 mm i.d., 100 mm long capillary tube, bent to a U shape, immersed in a liquid nitrogen bath. Four microliters of 20% methylene chloride in hexane were injected into the capillary as a rinse, removed with a 1.0 µl micropipet, and placed directly upon the mass spectrometer probe. The probe was inserted into a GEC AEI MS-902 high resolution mass spectrometer and the solvent removed by the fore pump. The probe was rapidly inserted into the ion source and multiple scans were recorded in the on-line high resolution mode (10).

A gas chromatogram showing components derived from the Aroclor® 1254 obtained in 1969 is represented in Figure 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 above, and identified by mass spectrometric analysis. A nominal mass plot of the high resolution mass spectrum of peak 2 is shown in Figure 2. The plot includes all the ions with elemental compositions ranging to the maximum empirical formula $C_{13}H_6O^{35}Cl_5{}^{37}Cl_4{}^{13}C_2$. 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 steric isomer. The accurate mass measurements for the characteristic ions are within 2 ppm of the calculated exact masses. Peaks identified on this chromatogram and their retention times relative to dieldrin are as follows: (1) a mixture of tetra- and pentachlorobiphenyl (1.02); (2) tetrachlorodibenzofuran (1.30); (3) pentachlorobiphenyl (1.46); (4) tetrachlorodibenzofuran (1.57); (5) hexachloronaphthalene (1.75); (6) pentachlorobiphenyl (1.86); (7) hexachloronaphthalene (2.00); and (8) heptachloronaphthalene (3.46). In Aroclor® 1248, three tetrachloro- and four pentachlorodibenzofurans were found. Each of the Aroclor® 1254 preparations and Aroclor® 1260 contained eleven chlorinated dibenzofurans: two tetrachloro-, four pentachloro- and five hexachlorodibenzofurans.

Initially, because authentic standards were not available, the tetrachlorodibenzofurans were quantitated against 2,4,5,2',4',5'-hexachlorobiphenyl; pentachloro- and hexachlorodibenzofurans were quantitated against 2,3,5,6,2',3',5',6'-octachlorobiphenyl. Later an authentic standard of 2,3,7,8-tetrachlorodibenzofuran was obtained and its electron capture response was compared to that of the hexachlorobiphenyl. This comparison showed that use of the hexachlorobiphenyl had yielded estimates of the tetrachlorodibenzofurans that were high by a factor of approximately 2. In the absence of authentic standards of the penta- and hexachlorodibenzofurans it is assumed that the original estimates of their concentrations based upon the octachlorobiphenyl are also high by the same factor. Corrected estimates of the dibenzofuran concentrations in Aroclors® are presented in Table 1.

No chlorinated dibenzofurans were found in Aroclor® 1016, a PCB mixture containing approximately 42% chlorine which has replaced Aroclor® 1242 in many applications, principally as the dielectric fluid in capacitors (11).

Among the PCB preparations obtained in 1969, 1) the total dibenzofuran concentration decreases with increasing chlorine content of the PCB; 2) the total concentration of the tetrachlorodibenzofurans decreases by a factor of ten from Aroclor® 1248 to Aroclor® 1260; 3) hexachlorodibenzofurans could not be detected in the Aroclor® 1248 preparation, but constituted 17% and 57% respectively of Aroclor® 1254 and 1260. The relative proportions of the dibenzofuran components in the Aroclor® 1254 obtained in 1970 were similar to those of the Aroclor® 1254 obtained in 1969, but the total concentration was 74% lower.

PCB residues in the North American environment and in human food have been the subject of extensive scientific investigations, have prompted governmental action and have resulted in substantial changes in many industrial processes which formerly used PCB. Toxic and sublethal effects of crude PCB mixtures include, in addition to embryotoxicity, the production of subcutaneous and abdominal edema, hydropericardium, liver necrosis, porphyria, chloracne, immunosuppression, and the induction of hepatic enzymes that cause elevated levels of steroid metabolism (7). Edema production and embryotoxicity, and the major portion of the acnegenic and hepatotoxic effects of European PCB were attributed to contaminant dibenzofurans. Our results suggest that many of the toxic effects of PCB in North America, such as the low hatchability of eggs from chickens on PCB-contaminated diets (12), were caused by dibenzofurans in the PCB preparations. The possibility therefore exists that chlorinated dibenzofurans are present in

the environment and contribute to the reproductive failures and occasional birth defects observed in populations of fish-eating species of birds, including the herring gulls of Lake Ontario (4), and the ospreys (Pandion haliaetus) and common terns (Sterna hirundo) of Long Island Sound (13).

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Table 1. Chlorinated dibenzofuran concentrations in Aroclor®*

PCB	4-Cl	5-Cl	6-Cl	Total
1248	0.24(20.9)	0.91(79.1)	nd	1.15
1254 (1969)	0.12(13.5)	0.62(69.7)	0.15(16.9)	0.89
1260	0.017(2.5)	0.27(40.3)	0.38(56.7)	0.67
1016	nd	nd	nd	----
1254 (1970)	0.012(5.2)	0.14(60.9)	0.08(34.8)	0.23

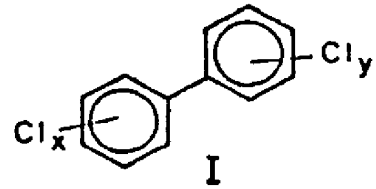
* Quantities are expressed as µg/g Aroclor®. Values in parentheses represent quantity as % of total dibenzofuran.

nd - not detected (< 0.001 µg/g)

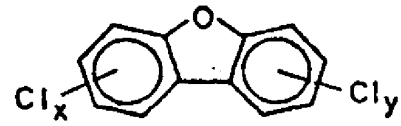
Figure Captions

Figure 1. Skeletal structures of I: chlorinated biphenyl; x + y = 1 to 10; II: chlorinated dibenzofurans, x + y = 1 to 8; III: chlorinated dibenzodioxins, x + y = 1 to 8.

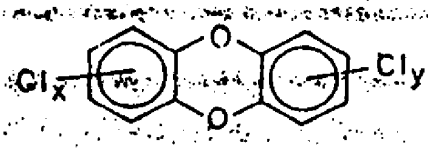
Figure 2. Top: Gas chromatogram of a fraction of Aroclor® 1254 containing a mixture of chlorinated biphenyls, dibenzofurans, and naphthalenes. Conditions and identity of peaks are given in the text. Bottom: Mass spectrum of peak 2, a tetrachlorodibenzofuran.



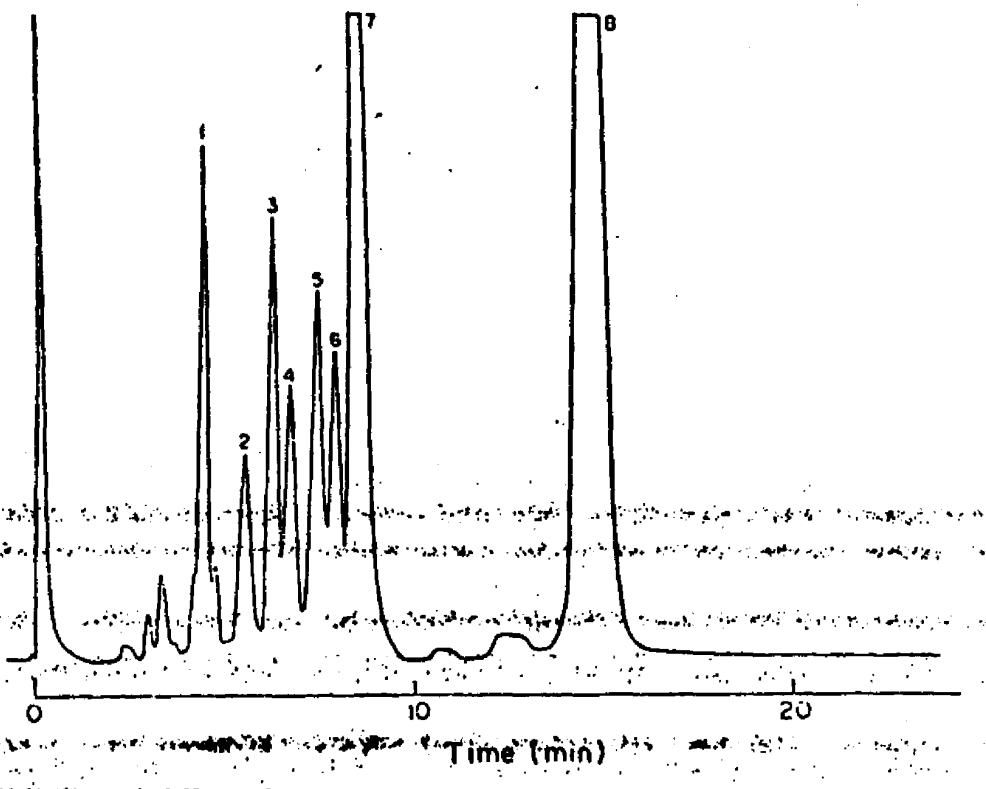
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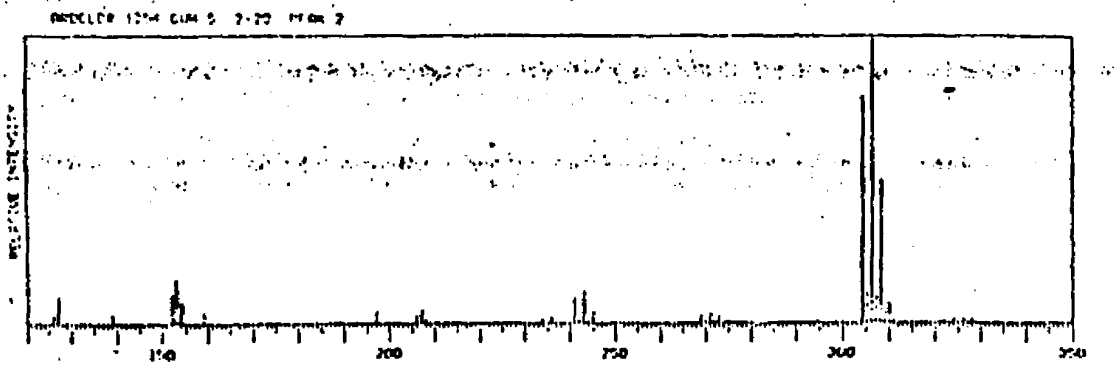


II



III


1
2
3
4
5
6
7
8
0
10
20
Time (min)



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