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PERSISTENCE OF PCB CONGENERS IN CAPACITOR WORKERS AND YUSHO PATIENTS

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ABSTRACT

The clearance rates of the major lower PCB congeners from the sera of a group of formerly PCB-exposed capacitor workers were estimated from levels measured 7.7 yr. apart, and compared with the corresponding rates reported for a group of yusho disease (chloracne) patients. It was found that the mono-ortho chlorinated congeners were cleared 3-7 times as fast in the yusho patients as in the capacitor workers, while the di-ortho chlorinated congeners were cleared 3-7 times more slowly. These alterations in the yusho patients' PCB metabolism were attributed to a PCDF-induced increase in the level of P-448 cytochromes and a corresponding depression in that of the ordinary (e.g., phenobarbital-induced) type of cytochrome P-450.

KEYWORDS

Polychlorinated biphenyls; PCBs; polychlorinated dibenzofurans; PCDFs; yusho disease; cytochrome P-448; cytochrome P-450

INTRODUCTION

The polychlorinated biphenyl (PCB) residues in the tissues of higher animals exhibit chromatograms that are much simpler than those of the commercial Aroclor mixtures, owing to metabolic clearance of most of the lower congeners originally present. Typically, even a capillary gas chromatogram shows no more than a few strong peaks below the heptachlorobiphenyl range, along with a scattering of much weaker ones. The resulting patterns of strong and weak peaks for the PCB residues normally observed in humans (Wolff *et al.*, 1982; Safe *et al.*, 1985; Lawton *et al.*, 1985b; Masuda *et al.*, 1974) most other warm-blooded animals (Hansen *et al.*, 1975; Miyata *et al.*, 1977; Hori *et al.*, 1979; Muir *et al.*, 1988) and some lower animals as well (Farrington *et al*, 1986) are quite similar.

In 1974, Masuda, Kagawa, and Kuratsune reported that the gas chromatographic patterns for the PCBs retained in the bodies of yusho disease patients, who had developed chloracne after consuming a mixture of PCBs and PCDFs, were abnormal. They called the most abnormal pattern observed Pattern A. Some patients showed less extensive alterations that were termed Patterns B and C. Subsequent work showed that the heavily altered Pattern A could also be produced in both rats (Miyata *et al.*, 1977) and mice (Hori *et al.*, 1979) if administered PCDFs in addition to PCBs. Human Pattern A PCB chromatograms were again seen following the second rice oil, or "yu-cheng," poisoning episode, which occurred in Taiwan in 1980 (Chen *et al.*, 1982).

The alteration in chromatographic pattern obviously indicates a change in the pattern of PCB congener clearance rates; however, it has not been known whether this change involved an increase in the clearance rates of one group of congeners, a decrease in that of another group, or both. Also unknown has been the chemical basis for the reactivity differences.

Chen *et al.*, (1982) reported clearance rates for two PCB congeners that were relatively rapidly cleared from the sera of a 19-member group of yusho patients, and also the raw data from which the clearance rates of several other (more persistent) congeners could be calculated. However, there have been no comparable reports for the rates of clearance of these congeners from normal human populations.

In order to develop such data, we undertook a detailed examination of the chromatographic record that had accumulated as one result of an ongoing medical surveillance of a group of capacitor workers that had previously had direct occupational exposure to PCBs. Despite the high levels of serum PCB observed in this group in early 1976, the group showed no significant PCB-related abnormalities in spirometric (Lawton *et al.*, 1986), biochemical, hematological, or other (Lawton *et al.*, 1985a) indicators of health status. No chloracne was observed in this study group (Lawton *et al.*, 1985a) and the gas chromatograms of the group all showed normal patterns (Lawton *et al.*, 1985b) of residual PCB congener distribution. Accordingly, it was concluded that the rates of PCB congener decay in this group could be taken as representative of those in normal, as opposed to chloracnegenic, human populations.

METHODS

The study population (Lawton *et al.*, 1985a) originally consisted of 194 individuals who had direct occupational exposure to dielectric fluids in a pair of capacitor plants where the fluids used included substantial levels of Aroclor 1254 during the period 1946-1954, Aroclor 1242 during 1950-1971, and Aroclor 1016 during 1972-1977. Exposure occurred via both inhalation and dermal contact. Following discontinuance of all PCB usage in June, 1977, airborne PCB levels dropped 5-fold in the first year and opportunities for dermal contact by a presumably much larger factor. Complete medical examinations, including determinations of serum PCB levels, were performed on the group in 1976, 1979, and 1983. The serum PCB analyses were performed by WARF Laboratories (subsequently Hazleton Raltech Inc., now Hazleton Laboratories America) of Madison, WI as previously described (Lawton *et al.*, 1985b) and reported as levels of Aroclors 1242, 1254 and (after 1976) of 1260 as calculated by the sum of selected peak heights procedure (Lawton *et al.*, 1985b). The peaks used for the determination of the Aroclor 1242 and 1254 levels were those having relative retention times (on the EPA Method 608 mixed-phase packed column used) of 37, 70, and 84; and 125, 146, 160, and 184, respectively.

Since there were no records of the contributions of the individual peak heights to the sum that formed the basis for the reported "Aroclor" level, it was necessary to go back to the original analytical chromatograms and remeasure the peaks. Unfortunately, all of the 1979 and most of the 1976 chromatograms had been lost during the intervening years and changes in laboratory ownership. However, we were able to recover 39 usable 1976 chromatograms for individuals who were also examined in 1983, and to determine from them the relative levels of each of the seven major packed column peaks and their geometric means for the group at each of the two dates, and hence the geometric mean ratio of 1976 to 1983 peak levels for each peak.

Since Aroclor 1016 (which contains as much Peak 37 PCB as Aroclor 1242, but only a third as much Peak 70 and virtually no higher congeners) was still in use during the first 1.3 years after the first examination it was assumed that substantial reductions in body levels of these congeners did not commence until after the discontinuance of Aroclor 1016 use, but that reduction in the levels of the higher peaks was underway during the entire (7.7 yr) interval between the 1976 and 1983 samplings.

In order to identify the specific PCB congeners responsible for the Method 608 packed column peaks measured (and also those responsible for the SE-30 packed column peaks in Masuda's original examples of normal and abnormal chromatograms (Masuda *et al.*, 1974)) the packed column chromatograms were carefully compared with those determined on a 1981 subpopulation (Lawton *et al.*, 1985b) of the same group using a DB-1 capillary, for which congener assignments have been reported (Brown *et al.*, 1987) and with those for the sera of some other Aroclor 1254-exposed workers, which were additionally chromatographed (by L.S. Sheldon and E. Pellizzari, Triangle Research Inst., Research Triangle Park, NC) on an Apiezon M capillary, which permits resolution of some congeners that coelute on both DB-1 and DB-5.

RESULTS

Table 1. Identification of Lower PCB Congener Peaks Present in Normal and Pattern A Mammalian PCB Residues

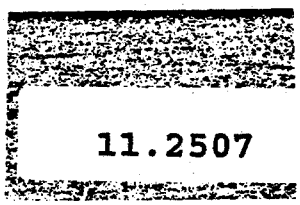
Packed Column Peak RRT[a]		DB-1 Capillary Peak Nos.		Chlorinated positions (•)		Reduced in Pattern A
				Major Peak Components	Minor Components	
SE-30	608	Ref. (•)	Ref. (••)			
28	28	14	-	-	4-4	x
37	37	24	4-3	24-4	-	x
47	47	31,32,33	6-4	-	25-25,24-25,24-24[c]	
70	70a	46	9-4-a	245-4	-	x
70	70b	48	9-4-c	-	24-34	x
78	78	50	10-4	-	234-4	x
84	84	53,54	11-5-c	245-24	245-25	
125	125	69	15-5-a	245-34	-	x
146	-	73,74,75	-	234-34,245-245	235-245	(x)
-	146	73,75	16-5	245-245	235-245	
	160	74	16-6	234-34	2345-25,2345-24	x
174	184	80,82	18-6	234-245,2356-34	234-235	

a. Retention times relative to DDE.
b. Major peaks of group underlined; ref. (••) peak numbers given only for major peak.
c. This peak minor in the human, but prominent in the rat.
• Brown *et al.*, 1987.
•• Chen *et al.*, 1982.

Our identifications of the congeners responsible for normal human and rat packed column gas chromatographic peaks are listed in Table 1. They correspond closely to those given by previous investigators, except that we found the human PCB residues responsible for the weak packed column peak at RRT 47 to contain somewhat more 2,2',4,4'-tetrachlorobiphenyl (PCB No. 47) than 2,2',5,5'-tetrachlorobiphenyl (PCB No. 52), and the major component of the strong peak at RRT 174 or 184 to be 2,3,3',4',5,6-hexachlorobiphenyl (No. 163) rather than 2,2',3,4,4',5'-hexachlorobiphenyl (No. 138), in extensively metabolized specimens. Thus, the generalization that the persistent congeners all show 4,4'-substitution is not strictly true; certain congeners carrying 2,3,5-trichlorophenyl or 2,3,5,6-tetrachlorophenyl groups are also highly persistent. In addition, the residues showed small quantities of congeners carrying 2,5-dichlorophenyl groups, probably because of their high levels in the original Aroclor mixtures.

Although the packed column peaks for the original Aroclors generally included several individual congeners at significant levels, this was not the case for the packed column peaks of the human PCB residues. The capillary gas chromatograms showed that the strong Method 608 packed column peaks at RRT's 37, 70a, 84, 125, and 146 each contained well over 90% of the congener listed as "major peak component," and hence that the disappearance rate of the packed column peak could be taken as a reasonable measure of the clearance rate of that congener. For the peak at RRT 160, the content of the major congener was a little below 90%; however, the other components were roughly equally divided between more and less persistent species, so that they would not be expected to introduce serious error into the estimate of clearance rate. Only in the case of the strong peak at RRT 184, which includes major components not resolved by either packed column, or by either DB-1 or DB-5 capillaries, was there evidence that the packed column chromatographic data alone could not be used to estimate the clearance rate of a specific congener.

The identities of the SE-30 packed column peaks that appeared to be reduced relative to the others in the older human (Masuda *et al.*, 1974), rat (Miyata *et al.*, 1977), and mouse (Hori *et al.*, 1979) chromatograms



showing the PCDF-induced Pattern A are listed in the last column of Table 1. This shows that each of the peaks so indicated arises from a congener carrying no more than one chlorine atom in an ortho (2,2',6 or 6') position, whereas all of the other peaks arise from PCB congeners carrying two ortho chlorine atoms.

Table 2. Estimation of the Rates of PCB Congener Loss from the Sera of 39 Occupationally Exposed Capacitor Workers

RRT (608)	PCB Congener	PCB No.	Obs. Geom. Mean Loss (%)	Est. Net Clearance Time (yr)	Mean k(yr^{-1})	Mean t1/2(yr)
37	2,4,4'	28	95	6.3	0.49	1.4
70a	2,4,4',5	74	76	6.5	0.22	3.2
84	2,2',4,4',5	99	81	7.7	0.21	3.3
125	2,3',4,4',5	118	58	7.7	0.12	5.8
146	2,2',4,4',5,5'	153	35	7.7	0.056	12.4
160	2,3,3',4,4'	105	75	7.7	0.18	3.9
184	2,2',3,4,4',5 / 2,3,3',4',5,6	138 / 163	49	7.7	(0.089)[a]	(7.8)[a]

a. Composite clearance rate for both congeners over the interval studied. From partially resolved individual congener peaks on an Apiezon M chromatogram the half-lives of congeners 138 and 163 were estimated at 6-7 yr. and >20 yr., respectively.

The estimated rates of clearance of the major lower PCB congeners from the capacitor workers' sera are presented in Table 2. We observed the mean half lives of the major PCB congeners in this population to range between 1.4 and 12.4 years, with still longer values suggested for congener No. 163. These values are much greater than those recently suggested by Bühler (Bühler *et al.*, 1988) on the basis of isotope dilution measurements, and suggest that the latter observations may be measuring a redistribution of the PCBs among body compartments rather than actual elimination from the body.

Table 3 presents data on the mean rates of PCB congener loss in the yusho patient group studied by Chen (Chen *et al.*, 1982) and compares them with those observed in the capacitor workers. It shows that the mono-ortho chlorinated congeners were cleared 3-7 times as fast in the yusho patients as in the capacitor workers, while the di-ortho chlorinated congeners were cleared 3-7 times more slowly.

Table 3. Comparison of Estimated PCB Clearance Rates in Yusho Patients with those in Capacitor Workers

RRT (608)	PCB No.	Substitution Type	Mean Rate of Loss (yr^{-1})		
			Yusho Pts.[a]	Capac. Wkrs.	Ratio
70	74	monoortho	0.62[b]	0.22	2.9
84	99	diortho	0.03[c]	0.21	0.14
125	118	monoortho	0.85	0.12	7.3
146	153	diortho	0.014[c]	0.056	0.25
160	105	monortho	1.24	0.18	7.0
184	138 / 163	diortho	0.025[c]	0.089	0.28

(a) From data of Chen *et al.*, 1982.
(b) Value estimated from peak height on the single illustrative chromatogram shown.
(c) Value not significantly different from zero.

DISCUSSION

The most striking feature of mammalian PCB residues is the relative persistence of congeners that are chlorine-substituted in both 4 and 4' positions. The same pattern of persistence was observed *in vitro* (Saeki *et al.*, 1983) when mixed Kanechlors were metabolized with rat liver homogenates that had been induced with phenobarbital (PB), or when various dichlorobiphenyls were incubated with purified cytochrome P-450 PB-B, the major isozyme isolatable from PB-induced rat liver (Kaminsky *et al.*, 1981). Accordingly it would appear that the process responsible for most PCB clearance in normal animals, including man, is metabolism by cytochrome P-450 of the PB-induced subtype(s).

This metabolism is well known (Sundström *et al.*, 1976) to yield chlorobiphenols that are hydroxylated at either the 3- or 4- positions, and to procede via an initial 3,4-epoxidation. Presumably, this 3,4-epoxidation is inhibited when chlorine atoms are present on either the 3- or 4-positions, thus accounting for the enhanced persistence of congeners where either the 4- or 3,5-positions are substituted on both rings.

When animals are treated with agents that bind to the Ah receptor, such as a laterally substituted polychlorinated dibenzofuran (PCDF) or tetrachlorodibenzodioxin (TCDD), or 3-methylcholathrene (3-MC), β-naphtoflavone (BNF), or certain PCB congeners, induction of a different group of cytochrome P-450 isozymes occurs (Saeki *et al.*, 1983; Kaminsky *et al.*, 1981; Parkinson *et al.*, 1983). These show a CO-difference spectral peak at 448 nm, and hence have been referred to as cytochrome P-448. They are also active at hydroxylating polycyclic aromatic hydrocarbons, and hence are often measured by their aromatic hydrocarbon hydroxylase (AHH) activity.

When mixed Kanechlors were treated *in vitro* with homogenates of rat livers that had been induced with 3-MC overall PCB metabolism was sharply reduced, but that of 2,4,4'-trichlorobiphenyl was increased (Saeki *et al.*, 1983) The major isozyme isolatable from BNF-induced rat livers, cytochrome P-450 BNF-B, was found to be much more active than P-450 PB-B in attacking 4,4'-dichlorobiphenyl, but much less so in attacking the 2,2'-dichlorinated isomer (Kaminsky *et al.*, 1981) Accordingly, it would appear that P-448 should have the ability to attack 4,4'-disubstituted PCB congeners, as long as they are not 2,2'-disubstituted, and hence that the increased clearance of mono-ortho 4,4'-disubstituted PCBs observed in the Pattern A subjects can be attributed to the action of cytochrome P-448.

The data of Table 3 show, however, that P-448 induction is not the sole basis for the development of PCB congener persistence "Pattern A" in the yusho patients. Not only is the clearance rate for the mono-ortho PCB congeners increased 3- to 7-fold, reflecting P-448 induction, but that of the di-ortho congeners is suppressed 3- to 7-fold, indicating a corresponding suppression of the normal P-450 PB type of activity. This surprising result contrasts with that in the mouse, where PCDF treatment leads to accelerated loss of mono-ortho PCB congeners without suppression of di-ortho congener loss (Hori *et al.*, 1982) and in the polar bear, which shows near total clearance of mono-ortho congeners even in the normal state (Muir *et al.*, 1988). It suggests that the toxicological effects of PCDF and other chloroacnegens on the human may not consist solely of turning on new enzyme systems and other processes controlled by the Ah receptor--they may also be associated with the marked inhibition of at least one normal drug-metabolizing enzyme activity that also occurs.

The complementary effects of P-448 induction and P-450 PB suppression upon the relative persistence of the major PCB congeners, along with the rather long half-lives of those congeners in the body, suggest a diagnostic test for past episodes of chloracne. Table 3 shows that in normal individuals the pentachlorobiphenyl peak at RRT 84 is slightly more rapidly metabolized, and hence slightly less persistent, than those of the other two major pentachlorobiphenyls, which give peaks at RRTs 125 and 160. However, in the chloroacne patients it is 30-40 times more persistent, meaning that it can be used as an indicator of the original pentachlorobiphenyl level. Hence, the observation of Peaks 125 or 160 at depressed levels relative to Peak 84, which was a feature of all the yusho patients' chromotograms, regardless of whether Pattern A, B, or C, should indicate whether the subject had previously been exposed to chloracnegens at levels sufficient to cause induction/suppression of P-450 cytochromes.

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