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TRANSPORT AND CELLULAR UPTAKE OF POLYCHLORINATED BIPHENYLS (PCBs)—I

ASSOCIATION OF INDIVIDUAL PCB ISOMERS AND CONGENERS WITH PLASMA LIPOPROTEINS AND PROTEINS IN THE PIGEON

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Abstract—Polychlorinated biphenyls (PCBs) are abundant and persistent pollutants in the ecosystem. Commercial mixtures (e.g. Aroclor 1254) can contain up to 80 different isomers and congeners, many of which accumulate in biological systems by the ingestion of PCB-contaminated lipid components of food chains. PCBs are lipophilic and lipid-rich lipoproteins provide an excellent system to transport PCBs to tissues. We report here the distribution of PCBs between plasma fractions in the pigeon. Twenty-four hours after injection, [^{14}C]4-monochlorobiphenyl and [^{14}C]2,2′,5,5′-tetrachlorobiphenyl were associated with the protein-rich HDL fraction and the lipoprotein-poor fraction (predominantly albumin), rather than with the lipid-rich VLDL and LDL fractions. Five days after injection with the commercial PCB mixture Aroclor 1254, there was a distinctive distribution between the plasma fractions of the 41 congeners detected. Avian species have a poorly developed lymphatic system and dietary lipids are secreted into the portal vein. To emphasize this route of entry, the lipoprotein particles formed are termed portomicrons rather than chylomicrons. The most striking result was that the lipid-rich portomicron and the VLDL fraction was associated almost exclusively with only one congener (2,2′,4,4′,5,5′-hexachlorobiphenyl), whereas the other isomers and congeners were distributed amongst the LDL, HDL and the lipoprotein-poor (predominantly albumin) fractions. Thirteen of the congeners detected accounted for 74, 53 and 54%, respectively, of the total amount of PCBs in the LDL, HDL and lipoprotein-poor protein fractions. Five congeners that are highly toxic were enriched in the latter fraction. The distribution of PCBs is more complex than can be explained solely by their solubility in the lipid components of plasma fractions, and may suggest a complex association with apolipoproteins and plasma proteins that are important in transporting PCB to tissues. The identification of individual PCBs in lipoprotein fraction provides evidence for their role in the transport of lipophilic xenobiotics in blood and it is suggested that PCBs associated with lipoproteins are taken up by cells as lipoprotein–PCB complexes

PCBs are fat-soluble commercial products noted for their chemical stability, miscibility with organic solvents, non-flammability and excellent electrical insulation properties. This has led to their widespread use as industrial fluids, flame retardants, as diluents, and as hydrolic fluids for capacitors and transformers. Careless disposal practices, e.g. leakage from disused equipment and disposal into sewers, rivers and coastal waters, have led to PCBs becoming one of the most abundant and persistent industrial pollutants in the ecosystem (see Refs. 1–3 for reviews).

Commercial mixtures of PCBs (e.g. Aroclor 1254, see Materials section) can contain up to 80 different isomers and congeners (i.e. individual components), many of which accumulate in biological systems *via* the ingestion of PCB-contaminated lipid components of food chains, and PCBs are present in the fatty tissues and blood of species at the apex of these chains (see Refs. 4 and 5 for reviews). For example, we have routinely identified individual PCB isomers and congeners in human milk samples and in blood samples obtained from a number of species of fish-eating seabirds [6, 7].

A major role of the lipoproteins and certain other proteins (such as albumin) in blood is to transport lipids of dietary and endogenous origin to the tissues of the body. PCB isomers and congeners have a high affinity for lipophilic environments, and plasma lipoproteins and proteins could provide an excellent system to transport ingested PCBs to tissues. There appear to be no reports, however, on the association *in vivo* or *in vitro* between the components of commercial PCB mixtures and individual plasma fractions. Although there are theoretically 209 possible PCBs isomers and congeners, only six radiolabelled PCB congeners are available, and only the distribution *in vivo* and *in vitro* of the congener [^{14}C]2,2′,4,4′,5,5′-hexachlorobiphenyl between plasma fractions in rat and human blood has been

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‖ Abbreviations used: PCB, polychlorinated biphenyl; VLDL, very low density lipoprotein; LDL, low density lipoprotein; HDL, high density lipoprotein.

studied [8–10]. Similarly, the need for high-resolution capillary GLC-mass spectrometry to separate and identify unambiguously individual PCB isomers and congeners has hampered studies on the distribution between plasma fractions of the components of commercial mixtures of PCBs. For example, although a [^{14}C]-labelled commercial PCB mixture has been used to follow the digestion of PCBs and heir transport by lymph and plasma components in dogs [11], the distribution of individual PCB isomers and congeners between these components was not studied.

A comparison of the concentrations of the principal classes of plasma lipoproteins in different animals shows that there are considerable differences amongst species [12]. Avian species have a poorly developed lymphatic system and secrete dietary lipids into the portal system as portomicrons rather than into the lymphatic system as chylomicrons. However, the concentrations of both VLDLs and LDLs are surprisingly similar in the pigeon and in man [12]. Interestingly, the concentrations of these latter lipoprotein fractions are very much higher than those in rodents, though the concentration of lipid-poor HDLs in the pigeon is approximately five-fold higher than in man [12]. In addition, avian portomicrons and VLDLs contain an apolipoprotein that is very similar to the apolipoprotein B-100 of human LDLs [13].

Since the lipid component of triacylglycerol-rich VLDLs is taken up by adipose tissues and lipid-rich LDLs are taken up into tissues by receptor-mediated and receptor-independent mechanisms [14, 15], these plasma fractions are good candidates for transporting lipid-soluble PCBs to tissues for cellular uptake. The objective of the present study was therefore to investigate the distribution in blood of [^{14}C]4-monochlorobiphenyl and [^{14}C]2,2′,5,5′,-tetrachlorobiphenyl as well as that of the individual isomers and congeners present in the commercial PCB mixture Aroclor 1254 in order to understand the transport of PCBs in the blood and to assess the potential mechanisms involved in the uptake of PCBs by cells utilizing the models of the receptor-dependent endocytosis of lipoprotein–PCB complexes.

MATERIALS AND METHODS

Materials

Aroclor 1254, a commercial mixture of PCBs, was provided by Dr J. P. G. Wilkins, MAFF Harpenden Laboratory, Herts, U.K. This mixture of chlorinated biphenyls contains approximately 80 different isomers and congeners with M_r ranging from 118 to 430, and the average M_r for the mixture is 326. The IUPAC system of numbering individual PCB isomers and congeners (see Ref. 16) has been used where convenient. [^{14}C]-Labelled PCB congeners were obtained from the Sigma Chemical Co. (Poole, U.K.) (the position of labelling is not specified by the supplier). Twenty-three PCB congeners of >99% purity were synthesized via aryl–aryl coupling using an adaptation of the Cadogen method [17], and other congeners of >99% purity were gifts from Prof. L. W. Robertson, Graduate Center for Toxicology, Lexington, U.S.A. Capillary columns (DB-5) were obtained from Scientific Glass Engineering (Milton Keynes, U.K.). Chemicals were purchased from BDH (Poole, U.K.), heparin from Willingtons Medical Ltd (Bristol, U.K.) and Insta-Gel from Packard Instruments (Pangbourne, U.K.).

Animals. Adult female feral pigeons (*Columba livia*) were obtained from Lincolnshire Pheasantries (Boston, U.K.). They were fed a diet containing equal weights of barley, wheat and maize that was supplemented (10% w/w) with a high protein concentrate ('Chick Starter Crumbs'; T. Allsop & Sons, Ltd., Crowthorne, U.K.). Food and water were available *ad lib.* Routine *post mortem* examination at the end of each experiment showed that their ovaries were quiescent (i.e. they were not laying).

Methods

Pigeons of 300 ± 15 g body weight (mean value ± SD; N = 17) were given a single intraperitoneal injection (0.5 mL total volume) of 0.5 μCi (7.2 Ci/mol) [^{14}C]4-monochlorobiphenyl in ethanol per kg body weight (N = 3 pigeons), or of 0.5 μCi (10.2 Ci/mol) [^{14}C]2,2′,5,5′-tetrachlorobiphenyl in ethanol per kg body weight (N = 4 pigeons) at 10 a.m. They were killed 24 hr later and samples of blood taken. Pigeons (N = 10) were also given a single intraperitoneal injection (up to 1 mL total volume) of 500 mg Aroclor 1254 (1.5 mmol assuming an average M_r of 326) in corn oil per kg body weight at 10 a.m., and killed 120 hr later. Plasma was prepared by centrifugation of samples of blood in plastic tubes that contained approx. 0.3 mL heparin (5000 units/mL) for 15 min at 500 g_{av} at room temperature. Portions of plasma (4 mL) were stored at −20° and thawed once. The concentration of total protein in portions of plasma was measured using the 'Total Serum Protein' assay kit obtained from BCL (Lewes, U.K.).

Preparation and chemical analysis of plasma fractions. Lipoprotein fractions were separated in a Beckman 25-65 Ultra centrifuge at 12°. Plasma (4 mL) was adjusted to a salt density of 1.21 g/mL by the addition of a solid KBr (1.3004 g) and analysed with 2 mL of a KBr solution of density 1.21 g/mL. The plasma was then centrifuged in a 70.1 Ti rota for 40 hr at 1.1×10^5 g_{av}. The total lipoprotein fraction in the upper 2 mL was removed by aspiration [18], and the infranatant taken as the lipoprotein-poor protein fraction. The total lipoprotein fraction was diluted to a salt density of 1.063 g/mL and centrifuged for 20 hr at 1.1×10^5 g_{av}. The infranatant was taken as the HDL fraction and the upper 2 mL was diluted to a salt density of 1.019 g/mL and centrifuged for 20 hr at 1.1×10^5 g_{av}. The supernatant (3 mL) was taken as the portomicron plus VLDL fraction and the infranatant as the LDL fraction. Fractions were dialysed versus 0.15 M NaCl/0.24 mM EDTA, made to a standard volume containing a 10 mM sodium azide and stored at 4°.

The purity of the lipoprotein fractions was checked by gradient polyacrylamide disc gel electrophoresis [18], except that a current of 2 mA/tube was passed for 45 min to effect separation.

The electrophoretic mobilities of the fractions were compared with those obtained when samples of plasma were stained and separated using the same

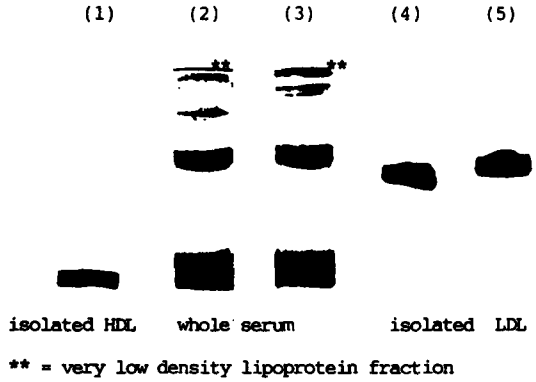

Fig. 1. Plasma (lanes 2 and 3), LDL (lanes 4 and 5) and HDL (lane 1) fractions prepared from control pigeons, stained with Sudan Black and separated by SDS–PAGE. The results are typical of those obtained from eight independent experiments.

conditions (Fig. 1), as well as with values reported for avian lipoproteins [19]. The lipoprotein-poor fraction contained predominantly albumin.

The concentrations of triacylglycerol and of total cholesterol in portions of each of these lipoprotein fractions were measured using 'Merkotest Triglycerides, enzymatic' and 'MERCK System Cholesterol Enzyme Mixture' kits obtained from BDH and the 'Cholesterol Calibrate' kit from the Sigma Chemical Co. The total apolipoprotein concentration measured using the 'Pierce Coomassie Protein Assay' reagent kit supplied by Pierce (U.K.) Ltd (Cambridge, U.K.), and by the method of Lowry *et al.* [20]. The concentration of protein in the lipoprotein-poor plasma protein fraction was measured using the 'Total Serum Protein' assay kit from BCL.

Radioactivity. The radioactivity associated with each of the plasma fractions was measured by liquid scintillation with a 10-fold excess (v/v) of Insta-Gel.

Extraction, separation and quantitation of PCB isomers and congeners. Portions of each plasma fraction obtained after treating pigeons with Aroclor 1254 were extracted with chloroform (1 : 10 v/v) three times, the pooled extracts dried over anhydrous sodium sulphate and reduced in volume to 0.5 mL at 70°. The PCBs in the extracts were separated by high-resolution capillary GC using a column (vitrous silica, of 25 or 50 m length and 0.33 mm internal and 0.42 mm external diameter) 5% chemically bonded with phenylmethylsilicone of 0.22 μm film thickness, attached to a Pye Unicam 204 gas chromatograph fitted with an electron capture detector (^{65}Ni). The flow rate of carrier gas to the detector was 1 mL/min. The temperature was programmed at 50° for 2 min, raised at 20°/min to 220° and maintained at 220°. The capillary GC was interfaced with a Hewlett Packard HP 3090A computer to quantitate the peaks corresponding to individual PCB isomers and congeners. In each analysis, their relative retention times were measured using heptachloronaphthalene and octachloronaphthalene as internal standards. The individual PCB isomers and congeners were identified unambiguously, using the operating conditions described above, by high-resolution capillary GC-mass spectrometry (Jeol JMS-DX 300 double-focussing mass spectrometer) by comparison with sixty synthetically pure (>99%) PCB isomers and congeners.

RESULTS AND DISCUSSION

Distribution of [^{14}C]4-*monochlorobiphenyl and* [^{14}C]2,2′,5,5′-*tetrachlorobiphenyl in plasma fractions 24 hr after their injection into pigeons*

Vomachka *et al.* [8] and Gallenberg & Vodicnik

Table 1. [^{14}C]4-Monochlorobiphenyl and [^{14}C]2,2′,5,5′-tetrachlorobiphenyl associated with components of plasma lipoproteins and with lipoprotein-poor plasma proteins 24 hr after injection into pigeons

Plasma fraction	(a) Radioactivity recovered in each fraction (%)	(b) Concentration (mg/mL plasma)				(c) Calculated values assuming that the total radioactivity is associated exclusively with the component shown (dpm/mg)			
		Apolipoproteins	Triacylglycerol	Total cholesterol	Lipoprotein-poor proteins	Apolipoproteins	Triacylglycerol	Total cholesterol	Lipoprotein-poor proteins
Portomicron plus VLDL	4 ± 2	0.06 ± 0.02	0.42 ± 0.30	0.28 ± 0.08		129 ± 64	63 ± 30	86 ± 4	
LDL	6 ± 2	0.62 ± 0.08†	0.50 ± 0.37	0.36 ± 0.03		67 ± 23*	124 ± 42†	53 ± 15	
HDL	35 ± 14	9.37 ± 3.35†	0.81 ± 0.26	2.84 ± 0.59†		20 ± 6†	204 ± 67†	64 ± 20	
Lipoprotein-poor protein	55 ± 11				20 ± 2				10 ± 2

Pigeons were injected with [^{14}C]4-monochlorobiphenyl (N = 3) or with [^{14}C]2,2′,5,5′-tetrachlorobiphenyl (N = 4) and killed 24 hr later. The radioactivity associated with each of these plasma fractions (Table 1a) and the concentrations of total apolipoproteins, triacylglycerol and total cholesterol in preparations of the plasma lipoprotein fractions and of protein in the lipoprotein-poor fraction (Table 1b) were measured as described in Materials and Methods. Table 1c shows the values obtained if it is assumed that the total radioactivity present in each lipoprotein fraction is associated exclusively with the component shown. Values are means ± SD. Since there was no statistically significant difference in the distribution of the two congeners between the plasma fractions, the combined results are given for the seven independent experiments. Statistically significant differences from values for the portomicron plus VLDL fraction are shown, with *$P < 0.05$ and †$P < 0.001$.

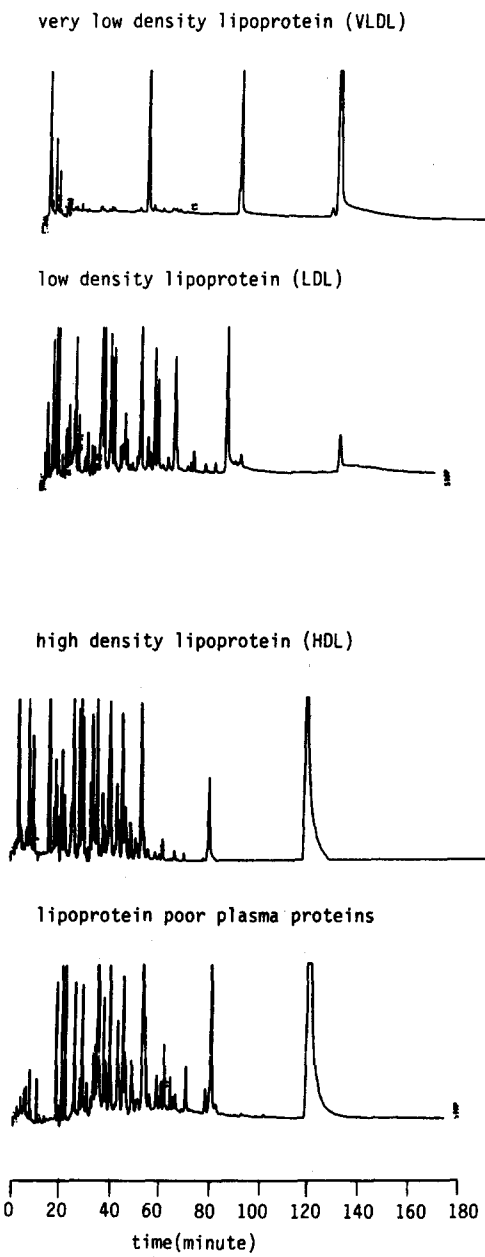

Fig. 2. High-resolution capillary GLC separation and quantitation of PCB isomers and congeners in plasma fractions 120 hr after injecting pigeons with 1.5 mmol Aroclor 1254/kg body weight. (A) Portomicron plus VLDL fraction; (B) LDL fraction; (C) HDL fraction; (D) lipoprotein-poor plasma protein fraction. Standards used to measure relative retention times are heptachloronaphthalene and octachloronaphthalene. The results are typical of those obtained from 10 independent experiments.

[10] have reported that [^{14}C]2,2',4,4',5,5'-hexachlorobiphenyl incubated with rat plasma for 24 hr distributed predominantly with the VLDL and the lipoprotein-poor (i.e. predominantly albumin) plasma protein fractions. This prompted us to measure the distribution amongst plasma fractions *in vivo* of [^{14}C]4-monochlorobiphenyl and [^{14}C]2,2',5,5'-tetrachlorobiphenyl. No statistically significant differences were found in the distribution of these two congeners between the plasma fractions, and both congeners were associated predominantly with the protein-rich HDL fraction (35 ± 14%) and with the lipoprotein-poor (i.e. predominantly albumin) protein fraction (55 ± 11%), whereas the triacylglycerol-rich portomicron plus VLDL fraction and the lipid-rich LDL fraction only accounted for 4 ± 2% and 6 ± 2%, respectively, of the total radioactivity recovered in the plasma fractions (combined results for the seven independent experiments; values are means ± SD).

An assumption was made, that individual PCBs may associate with different affinities towards the apolipoprotein, the triacylglycerol, or the total cholesterol component of that fraction. We therefore expressed the total radioactivity in each fraction on the basis of the apolipoprotein, lipid and cholesterol components of lipoproteins. The results in Table 1 suggest that the lipid-rich portomicron plus VLDL fraction had the highest radioactivity per mg total apolipoprotein and the lowest per mg triacylglycerol, the protein-rich HDL fraction had the lowest radioactivity per mg total apolipoprotein and the highest per mg triacylglycerol, and the radioactivity per mg total cholesterol was similar for the three fractions. These results indicate that the association of these two congeners with the total cholesterol content of lipoproteins may follow a mass relationship, so that with increased cholesterol concentration the association with PCBs increased concomitantly. This would explain the lack of significant difference between the cholesterol component of the lipoprotein fractions in their ability to associate with PCBs. The significant difference in the association of PCBs with the apolipoprotein components of lipoprotein fractions apparently does not follow a simple mass relationship, thus indicating the importance of differences between the apolipoproteins of the plasma fractions. In addition, significant differences between the lipoprotein fractions in their ability to associate with PCBs was observed when expressed on the triacylglycerol concentrations. Unfortunately, the method employed to measure triacylglycerol does not distinguish between the fatty acids esterified to glycerol in these fractions. However, differences in the fatty acids esterified to glycerol may account for the differences calculated in Table 1c.

The distribution of these radiolabelled PCB congeners appears therefore to be more complex than can be explained solely on the basis of their solubility in the lipid components of plasma lipoproteins. We investigated this further by measuring the association between plasma fractions and individual PCB isomers and congeners after pigeons had been injected with the commercial PCB mixture Aroclor 1254.

Distribution of individual PCB isomers and congeners in plasma fractions 120 hr after injecting pigeons with Aroclor 1254

The absorption of the dose of Aroclor 1254 in corn oil injected intraperitoneally was complete within 36 to 48 hr, as judged by measuring PCBs in plasma obtained from the branchial vein of the pigeons.

Table 2. Distribution of PCB isomers and congeners in plasma fractions 120 hr after injecting pigeons with Aroclor 1254*

PCB isomer or congener		Isomer or congener (%)			
IUPAC Number	Structure	Portomicrons plus VLDL (Fraction 1)	LDL (Fraction 2)	HDL (Fraction 3)	Lipoprotein-poor proteins (Fraction 4)
1	2-Monochloro-	—	0.44 ± 0.12 4§	2.54 ± 2.13	1.55 ± 0.21
8	2,4'-Dichloro-	—	0.60 ± 0.32 4‡	1.53 ± 1.01 4‡	0.01 ± 0.00
10	2,6-Dichloro-	—	2.21 ± 1.07 4§	2.53 ± 2.00 4‡	0.08 ± 0.08
12	3,4-Dichloro-	—	0.11 ± 0.19	—	—
15	4,4'-Dichloro-	—	0.94 ± 0.90	1.09 ± 0.14	0.68 ± 0.50
18	2,2',5-Trichloro-	—	—	2.13 ± 1.23	—
21	2,3,4-Trichloro-	—	2.39 ± 0.45 3§ 4‡	4.80 ± 0.95 4‖	0.82 ± 0.10
24	2,3,6-Trichloro-	—	2.54 ± 0.83 4‖	2.30 ± 1.33 4†	0.41 ± 0.20
26	2,3',5-Trichloro-	—	1.76 ± 1.00	2.78 ± 3.06	0.87 ± 0.39
28	2,4,4'-Trichloro-	—	1.19 ± 1.09	0.36 ± 0.07	0.50 ± 0.40
30	2,4,6-Trichloro-	—	0.48 ± 0.43 4†	0.66 ± 0.18 4†	5.05 ± 3.61
31	2,4',5-Trichloro-	—	1.90 ± 1.48	0.73 ± 0.23	1.20 ± 1.07
44	2,2',3,5'-Tetrachloro-	—	1.04 ± 0.06 3§	0.80 ± 0.09	1.17 ± 1.13
47	2,2',4,4'-Tetrachloro-	—	—	3.04 ± 0.96 4‡	7.77 ± 2.82
49	2,2',4,5'-Tetrachloro-	—	1.27 ± 1.21	1.20 ± 0.17 4‖	0.10 ± 0.08
52	2,2,5,5'-Tetrachloro-	—	3.08 ± 2.41	1.85 ± 0.47	1.27 ± 1.10
65	2,3,5,6-Tetrachloro-	—	3.10 ± 1.41 4§	3.73 ± 0.33 4‖	0.18 ± 0.10
66	2,3',4,4'-Tetrachloro-	—	0.43 ± 0.07	1.04 ± 0.07	1.57 ± 0.97
67	2,3',4,5-Tetrachloro-	—	5.93 ± 2.93 3† 4‡	1.93 ± 0.50 4§	0.68 ± 0.27
77	3,3',4,4'-Tetrachloro-	—	3.81 ± 1.27 3† 4‡	2.29 ± 0.24	1.45 ± 1.02
82	2,2',3,3',4-Pentachloro-	—	0.78 ± 0.40	—	0.41 ± 0.20
91	2,2',3,4',6-Pentachloro-	—	1.38 ± 0.05 3§	6.72 ± 3.04	3.57 ± 3.50
93	2,2',3,5,6-Pentachloro-	—	—	1.40 ± 0.99	1.07 ± 0.75
97	2,2',3',4,5-Pentachloro-	—	0.25 ± 0.38	0.60 ± 0.43 4†	0.04 ± 0.03
100	2,2',4,4',6-Pentachloro-	—	1.01 ± 0.52 4†	1.15 ± 0.58 4†	0.48 ± 0.08
101	2,2',4,5,5'-Pentachloro-	—	2.93 ± 2.04	2.53 ± 0.93	1.65 ± 0.27
110	2,3,3',4',6-Pentachloro-	—	2.08 ± 0.09 4§	2.19 ± 1.06	1.91 ± 0.32
114	2,3,4,4',5-Pentachloro-	—	—	0.61 ± 0.20	0.69 ± 0.48
118	2,3',4,4',5-Pentachloro-	—	1.06 ± 0.92 4§	1.59 ± 0.10 4‖	4.02 ± 0.66
119	2,3',4,4',6-Pentachloro-	—	12.35 ± 2.75 3§ 4‖	5.40 ± 0.26 4‖	3.03 ± 0.80
138	2,2',3,4,4',5-Hexachloro-	—	5.50 ± 0.55	4.80 ± 0.68	10.89 ± 8.09
139	2,2',3,4,4',6-Hexachloro-	—	1.17 ± 0.47 3† 4§	0.60 ± 0.19 4‖	2.61 ± 0.43
151	2,2',3,5,5',6-Hexachloro-	—	5.42 ± 4.21	3.92 ± 1.85	5.82 ± 1.35
153	2,2',4,4',5,5'-Hexachloro-	100 ± 5	11.95 ± 6.71	15.68 ± 3.04 4†	9.67 ± 5.64
155	2,2',4,4',6,6'-Hexachloro-	—	5.85 ± 0.77 3‡ 4‖	1.98 ± 0.87	1.45 ± 0.07
156	2,3,3',4,4',5-Hexachloro-	—	—	2.40 ± 1.47	4.14 ± 0.51
157	2,3,3',4,4',5'-Hexachloro-	—	0.85 ± 0.18 4‖	—	3.77 ± 0.60
168	2,3',4,4',5',6-Hexachloro-	—	0.63 ± 1.09 3†	1.99 ± 0.42	1.43 ± 1.01
170	2,2',3,3',4,4',5-Heptachloro-	—	4.42 ± 1.94	2.81 ± 1.08 4§	6.33 ± 1.02
180	2,2',3,4,4',5,5'-Heptachloro-	—	5.67 ± 2.84	4.25 ± 2.62	7.25 ± 1.59
183	2,2',3,4,4',5',6-Heptachloro-	—	3.50 ± 1.22	2.04 ± 0.98 4‡	4.42 ± 0.82

* The '%' values refer to the distribution of PCB isomers and congeners within each of the four fractions. Only PCB isomers or congeners present in sufficient amounts to be identified unambiguously are included. Values are means ± SD (N = 10).

Statistically significant differences between fractions 2, 3 and 4 are shown; †$P < 0.05$; ‡$P < 0.02$; §$P < 0.01$; ‖$P < 0.001$.

— Not detected (<0.01%).

Avian plasma lipoproteins have very different half-lives (3–4 min for portomicrons, 7–8 min for VLDLs, 2–3 hr for LDLs [19] and probably in the order of days for HDLs), but 120 hr after its injection into pigeons, more than 95% of the dose of Aroclor 1254 given could be recovered from adipose tissues, and approx. 5% recovered from other tissues, with blood only containing 0.11% of the dose. It is well documented that blood is an unfavourable storage tissue for PCBs and studies on the pharmacokinetics using individual PCBs have shown that only approx. 0.1% of the original dose can be recovered in blood at 120 hr post-treatment [21]. Thus there is good agreement between our own findings and those reported by Matthews and Dedrik [21]. The distribution of PCBs amongst plasma fractions was therefore measured 120 hr after injecting Aroclor 1254 so as to approximate to a constant distribution of PCBs amongst plasma fractions and between these fractions and the tissues of the body. This is a much more realistic situation with a slow tissue release of PCBs into blood and the renewed uptake into tissues.

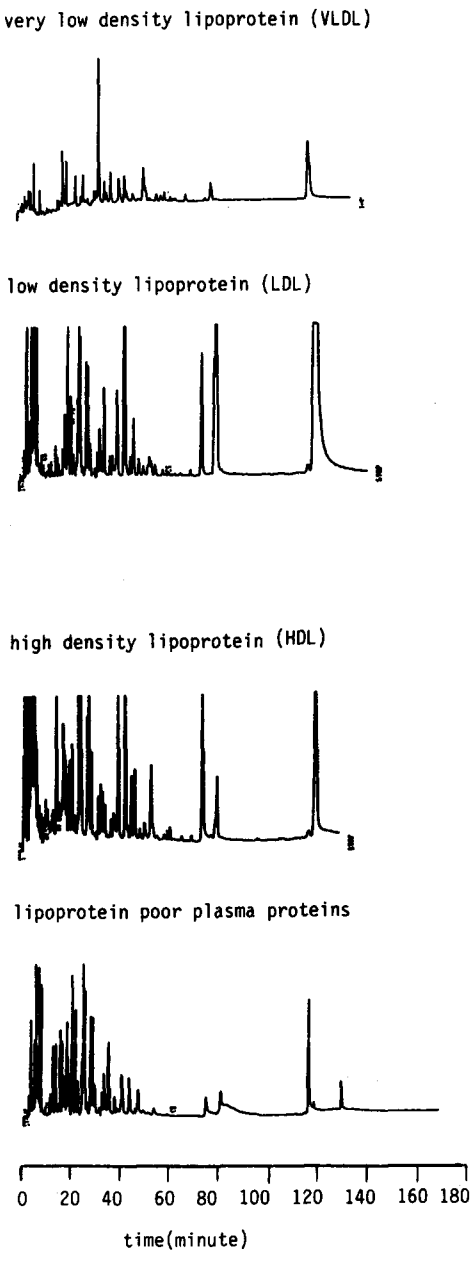

Fig. 3. Distribution of PCB isomers and congeners amongst plasma fractions 20 min after incubation with Aroclor 1254. Plasma (4 mL) from control pigeons was incubated for 20 min at 37° or 42° with 4 μmol Aroclor 1254 in ethanol (up to 40 μL). (A) Portomicron plus VLDL fraction; (B) LDL fraction; (C) HDL fraction; (D) lipoprotein-poor plasma protein fraction. Standards used to measure relative retention times are H, heptachloronapthalene and O, octachloronapthalene. The results are typical of those obtained from three independent experiments.

Figure 2 illustrates the separation and quantitation obtained by high-resolution capillary GLC of PCB isomers and congeners in the plasma fractions, and Table 2 documents the distribution of 41 PCB isomers and congeners that were detected in the plasma fractions. For convenience, these are referred to by their IUPAC number rather than by their chemical structure.

Statistically significant differences compared to the lipoprotein-poor plasma fraction were: (i) increased proportions in the LDL fraction of PCBs 8, 10, 21, 24, 65, 77, 100, 110, 119 and 155 but decreased proportions of 1, 30, 67, 118, 139 and 157; (ii) increased proportions in both the LDL and the HDL fractions of PCBs 8, 10, 21, 24, 65, 67, 100 and 119 but decreased proportions of 30, 118, and 139; and (iii) increased proportions in the HDL fraction only of PCBs 49, 97 and 153 but decreased proportions of 47, 170 and 183. Compared to the HDL fraction, there were increased proportions in the LDL fraction of PCBs 44, 67, 77, 119, 139 and 155 but decreased proportions of 21, 91 and 168.

These distributions showed a number of interesting features. For example, of the 41 PCBs detected, 13 (PCBs 52, 65, 67, 77, 101, 119, 138, 151, 153, 155, 170, 180 and 183) accounted for 74, 53, and 54%, respectively, of the total amount of PCBs found in the LDL, HDL and lipoprotein-poor protein fractions. It is of interest that five congeners (PCBs 77, 114, 118, 156 and 157) which are known to bind with high affinity to an intracellular receptor protein (Ah-receptor) implicated in the regulation of hepatic drug metabolism and which produce different patterns of hepatotoxic and immunosupressive effects [1, 22–24] were enriched (14.1%) in the lipoprotein-poor plasma protein fraction when compared with the LDL (5.7%; $P < 0.01$) and the HDL (6.9%; $P < 0.02$) lipoprotein fractions (values are means ± SD of the total proportion of the five congeners in each fraction). These results indicate a non-uniform distribution of toxic PCBs amongst plasma fractions and evidence is presented that highlights the importance of plasma proteins in the transport of highly toxic coplanar and monoortho-substituted PCB congeners in blood.

However, the most striking feature of the distribution shown in Table 2 was that the portomicron plus VLDL fraction was associated almost entirely with congener 153 (i.e. 2,2′,4,4′,5,5′-hexachlorobiphenyl). This unusual distribution was also observed (Fig. 3) when plasma from control pigeons was incubated for 20 min with Aroclor 1254 and the plasma fractions isolated, i.e. 80 ± 10% of congener 153 was associated with the portomicron plus VLDL fraction (mean ± SD of three independent experiments), whereas the majority of isomers and congeners were distributed amongst LDL, HDL and lipoprotein-poor plasma protein fraction. The results shown for VLDL conflicts with the view that the lipid content of lipoproteins is the determining factor in their association with PCBs, especially when taking account that VLDL is the most lipid-rich lipoprotein particle in blood. In addition, LDL that is principally derived from VLDL via IDL contains many of the individual isomers and congeners as shown in Table 2, and therefore differs dramatically. The reasons for the inability of VLDL to associate with several PCBs are not understood. However, the results exemplify the complexity in the kinetics of PCB transport in blood and the difficulty in predicting the association of individual PCBs with lipoprotein particles. It is well established that an

extensive transfer of lipid and protein components between lipoprotein particles takes place and it is likely that PCBs could be exchanged among lipoproteins during this process. This transfer could explain, at least in part, the significant differences observed for PCB–lipoprotein complexes (see Table 2). This further complicates an assessment of the kinetics of PCB transfer amongst lipoproteins to be made, especially when dealing with a mixture of >80 different isomers and congeners. Factors that also needed to be assessed are the tissue kinetics of PCBs and in particular a comparison of PCB transport in blood and their uptake in tissues. However, an assessment of PCBs in tissues is further complicated due to the fact, that different tissues have varying abilities in the metabolism of PCBs by cytochrome P450-dependent monooxygenases. We have therefore analysed organs and tissues of treated animals and found that >95% of the total dose recovered was found in adipose tissue, suggesting that this tissue is the favoured storage site for PCBs. An assessment of the tissue kinetics of individual isomers and congeners suggests that certain PCB structures, e.g. highly chlorinated PCBs and PCBs that lack vicinal meta-para unsubstituted carbon atoms readily accumulate in all tissue and organs studied and that the proportional distribution of these PCBs recalcitrant to metabolism did not differ amongst all tissues investigated. By contrast, PCBs that were readily metabolized, differed dramatically when assessing the tissue kinetics, with the liver having the lowest concentration of metabolizable PCBs and this result correlates well with the high cytochrome P450-dependent monooxygenase concentrations found in this tissue (unpublished results).

Since the distribution of PCB isomers and congeners amongst the plasma fractions is more complex than can be explained solely by their solubility in the lipid components of plasma lipoproteins (see Table 2) measurements such as octanol–water partition coefficients of xenobiotics do not provide reliable predictions of distribution of individual PCBs amongst these particles. The results shown in Table 2 do not indicate clear patterns of distributions of PCBs based on factors such as their molecular weight, molecular configuration (e.g. coplanarity vs 'out of plane' configuration), specific pattern of chlorine substituents, or the presence of vicinal protons in either *meta–para* or *ortho–meta* positions. For example, the coplanar PCB congener 77 was enriched in the LDL fraction whereas the other coplanar PCB congener 15 was not (Table 1).

The precise mechanisms by which PCBs associate with lipoproteins is unknown, but the results presented suggest that lipoprotein and plasma proteins are important in these processes and that the latter fraction could be of particular significance in transporting toxic PCB to the tissues of the body. The receptor-mediated uptake of lipoprotein PCB particles could, however, provide an excellent model to permit the cellular uptake of PCBs.

The toxic effect of PCBs on changes in plasma concentrations of the apolipoproteins, triacylglycerol and cholesterol components of lipoproteins and of the lipoprotein-poor protein fraction in response to Aroclor 1254 *in vivo*, are discussed in the following paper so as to explain the effects of PCBs on the accumulation of lipids in hepatocytes ('fatty liver syndrome'). These toxic effects of PCBs will then be discussed with the proposal of a model that suggests a lipoprotein-dependent uptake of PCBs by cells.

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