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METABOLISM OF 2,2′,3,3′,6,6′-HEXACHLOROBIPHENYL AND 2,2′,4,4′,5,5′-HEXACHLOROBIPHENYL BY HUMAN HEPATIC MICROSOMES*,†

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Abstract—Since the metabolism of polychlorinated biphenyls (PCBs) is the critical factor that determines whether or not they accumulate in adipose tissue, we have studied the metabolism of two hexachlorobiphenyls (HCBs), 2,2′,3,3′,6,6′-hexachlorobiphenyl (236-HCB) and 2,2′,4,4′,5,5′-hexachlorobiphenyl (245-HCB), by human hepatic microsomes. Human microsomes were isolated from patients undergoing liver resection and were found to have cytochrome P-450 levels (0.28 nmoles/mg microsomal protein) and cytochrome P-450-dependent enzymatic activities similar to those reported by other workers. 245-HCB was not metabolized by human microsomes under various conditions, while 236-HCB was metabolized with an apparent K_m of 8.8 μM and a V_{max} of 5.1 pmoles/mg microsomal protein/min. Two major metabolites were formed and identified by gas chromatography–mass spectrometry as 2,2′,3,3′,6,6′-hexachloro-4-biphenylol and 2,2′,3,3′,6,6′-hexachloro-5-biphenylol. [^{14}C]236-HCB equivalents were found to covalently bind to microsomal protein. Addition of 1 or 5 mM reduced glutathione decreased the degree of covalent binding. These data suggest that HCBs are metabolized through an arene oxide. The fact that 245-HCB was not metabolized explains why it is the predominant PCB found in human adipose tissue.

Polychlorinated biphenyls (PCBs) have been identified as ubiquitous environmental pollutants for over 10 years [1]. The chemical stability of these compounds has led to their persistence in the environment, while their lipid solubility has resulted in their accumulation in the food chain of higher animals [2]. PCBs have been detected in the adipose tissue of humans and animals [3, 4]. The toxic responses in humans and animals following exposure to PCBs have been studied extensively [5]. Some of the toxicities from PCB exposures may result from their accumulation in the body as a consequence of low rates of metabolism. However, other PCB associated toxicities, such as liver injury, may be related to the electrophilic products formed during their metabolism [6, 7].

The susceptibility of PCBs to metabolism to less lipophilic compounds determines whether they will be retained in the body or excreted. The rate of PCB metabolism is governed by both the number and position of the chlorines on the biphenyl ring [8, 9] as well as the ability of each animal species to metabolize a particular PCB congener [10, 11]. For example, the monkey, dog, and rat can metabolize 2,2′,3,3′,6,6′-hexachlorobiphenyl (236-HCB) and thus reduce their body burden of this congener [10, 12]. However, the rates of excretion show dramatic differences among the species. This difference appears to reflect differences in the rates of metabolism. In contrast, the 2,2′,4,4′,5,5′-hexachlorobiphenyl (245-HCB) is markedly resistant to metabolism by the rat, mouse, and monkey and, thus, a major portion of the administered dose is retained by these species [11–13]. The dog is unique among the species examined since it can metabolize 245-HCB and eliminate it, albeit, more slowly than 236-HCB [10, 11]. In an effort to determine how humans metabolize PCBs, we have studied the metabolism of two hexachlorobiphenyls, 236-HCB and 245-HCB, by human hepatic microsomes. We also report some preliminary findings on the covalent binding of [^{14}C]236-HCB equivalents to human hepatic microsomal protein.

MATERIALS AND METHODS

The 245-HCB[U-^{14}C] (24 mCi/mmole) and 236-HCB[U-^{14}C] (24 mCi/nmole) were purchased from California Bionuclear (Sun Valley, CA). These compounds were determined to be greater than 98% radiochemically pure by high pressure liquid chromatography (HPLC). Benzphetamine was obtained from the Upjohn Co. (Kalamazoo, MI). The radiolabeled benzphetamine[^{14}C-*N*-methyl] (9.3 mCi/mmole) was purchased from the New England Nuclear Corp. (Boston, MA). NADP, glucose-6-phosphate (G6P), glucose-6-phosphate dehydrogenase (G6PD), Trizma base, CM cellulose (CMC),

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bovine serum albumin (BSA), Diazald (diazomethane) and reduced glutathione (GSH) were obtained from the Sigma Chemical Co. (St. Louis, MO). Dimethyl sulfoxide (DMSO), biphenyl and 4-biphenylol were purchased from the Aldrich Chemical Co. (Milwaukee, WI). Anhydrous ethanol was obtained from the USI Chemical Co. (New York, NY). HPLC-grade hexane and acetonitrile were obtained from the Burdick & Jackson Chemical Co. (Muskegon, MI). All other chemicals were of reagent grade quality.

Preparation of microsomes. Apparently healthy liver tissue was obtained from three patients (ages 83, 58 and 62) undergoing liver resection. The tissue was obtained within minutes after loss of total blood flow to that lobe. It was cut into 1 cm cubes and washed repeatedly in ice-cold 0.25 M sucrose to remove as much blood as possible. The tissue was then homogenized in 3 vol. (w/v) of Tris buffer (50 mM Tris buffer containing 1.15% KCl, pH 7.4) at 4° using a Potter–Elvehjem homogenizer fitted with a Teflon pestle. The homogenate was centrifuged at 4° for 10 min at 1000 *g*, 10 min at 12,000 *g*, and 10 min at 27,000 *g*. The resulting supernatant fraction was filtered through glass wool and subsequently centrifuged at 227,000 *g* for 40 min at 4° in a Beckman TI 50.2 rotor. The microsomal pellet was resuspended in the Tris buffer, rehomogenized using a Douce hand homogenizer, and centrifuged at 227,000 *g* as before. The microsomes were then stored at −70° at a concentration of 10–25 mg/ml microsomal protein in the same buffer containing 0.1 mM EDTA. Protein was determined by the method of Lowry *et al.* [14]. Bovine serum albumin was used as the standard.

Characterization of the mixed function oxidase system. Cytochrome P-450 and cytochrome b_5 content were determined by the method of Omura and Sato [15] and Strittmatter and Velich [16] respectively. NADPH–cytochrome P-450 reductase was determined by the method of Masters *et al.* [17]. To further characterize the human liver microsomes, benzphetamine-*N*-demethylase activities and biphenyl 4-hydroxylase activities were determined by the method of Thomas *et al.* [18] and Yamazoe *et al.* [19] respectively. Briefly, 1.2 mM benzphetamine was incubated with 1–2 mg microsomal protein in 2 ml of Tris–KCl buffer in the presence of an NADPH-generating system at 37° for 20 min. Activity was determined from the rate of [^{14}C]-formaldehyde formation. Biphenyl (1 mM) was incubated with 0.25 mg microsomal protein in 0.5 ml of Tris–KCl buffer in the presence of an NADPH-generating system at 37° for 20 min. Activity was determined from the rate of 4-biphenylol formation which was quantified by HPLC using an Ultrasphere-ODS C_{18} reverse phase column. The mobile phase was acetonitrile–water–acetic acid (45:55:1) at a flow rate of 1.5 ml/min.

Microsomal incubations with PCBs. Various concentrations of the ^{14}C-labeled hexachlorobiphenyls (HCBs) that had been dissolved in DMSO (DMSO concentration was less than 1% in the final incubation volume) were incubated with 2 mg of microsomal protein, 0.3 mM NADP, 2.5 mM G6P, 0.05 mM $MgCl_2$, 1 unit G6PD, 0.25 mM EDTA and Tris–KCl buffer in a final volume of 2 ml. After a 2-min preincubation, the reactions were initiated by the addition of the HCB. Incubations were performed at 37° under air in a Dubnoff metabolic shaking incubator (80 oscillations/min) for various times. The reactions were stopped by the addition of 2.5 ml of 0.5 N NaOH, and the mixture was heated at 70° for 10 min to aid in solubilization of the microsomal protein.

To remove the parent HCB, the samples were extracted repeatedly with 5-ml aliquots of hexane–ethanol (19:1) for 15 min each until no radioactivity appeared in the organic layer (5–6 times). The ^{14}C in the aqueous phase was determined by liquid scintillation spectrometry and used to quantify the amount of aqueous soluble metabolites. Activity was determined by subtracting the product formed in the control incubations (without NADPH-generating system) from the corresponding incubation with the NADPH-generating system. The amount of ^{14}C in the control tubes was never more than 20 cpm above background. Parkinson and Safe [20] have reported recently a similar extraction procedure to determine the extent of PCB metabolism. The results of this work were analyzed by the method of Hofstee [21]. The apparent K_m and V_{max} were determined from the slope and *y*-intercept respectively.

To obtain the major metabolites, the aqueous phase was acidified with 0.25 ml of 18 N H_2SO_4 and extracted twice with 5 ml of hexane–ethyl ether–ethanol (76:20:4) for 15 min each. This procedure removed approximately 80% of the metabolites. The remaining unextractable metabolites in the aqueous phase were not examined except for those that were covalently bound to microsomal protein. The organic layer containing the extractable metabolites was evaporated to dryness under nitrogen and subjected to HPLC analysis.

HPLC analysis. Individual incubations were subjected to HPLC analysis to separate possible metabolites. A Beckman 110A pump fitted with an Altex 210 injector using an 0.25 ml loop was used in all analyses. A Whatman guard column was used with Co:Pell-ODS packing. The analytical column was an Ultrasphere-ODS (25 cm × 4.6 mm). The mobile phase consisted of acetonitrile–H_2O–ethanol–acetic acid (50:44:5:1) at a flow rate of 1.5 ml/min. Fractions (0.5 min) were collected using a Gilson FC-100 fraction collector, and the amount of ^{14}C in each fraction was determined in a Beckman LS 8100 liquid scintillation spectrometer.

Gas chromatography–mass spectrometry (GC–MS) analysis. To identify the isolated metabolites the aqueous extractable radioactivity from many experiments was pooled and subjected to HPLC analysis. The major radioactive peaks were collected, extracted into hexane–ethanol (19:1), evaporated to dryness, and methylated by etheral diazomethane. Each peak was subjected to gas chromatography–mass spectrometry using a Finnigan model 3300 GC–MS. The GC–MS was interfaced with a glass jet separator, with the electron impact mode at 70 eV. The GC was carried out using a 0.5 m × 2 mm column, and 3% OV-17 on 100/120 chromosorb Q packing. The carrier gas was helium at a flow rate of 20 ml/min. The injection port tem-

perature was 300° and the initial oven temperature was 160°. This temperature was held at 160° for 2 min and then increased at a rate of 10°/min to 290°.

Covalent binding of HCBs to microsomal protein. After the extractable metabolites were removed from the aqueous buffer, the microsomal protein remaining in the incubation tube was removed by aspiration and placed in another tube. Cold ethanol (5 ml) was added to the microsomal protein and the tube was centrifuged at 1500 *g* for 30 min at 4°. Covalently bound [^{14}C]HCB was determined by exhaustive extraction of the microsomal protein with organic solvents of various polarity as described by Sipes and Gandolfi [22]. In those experiments in which reduced glutathione was added to the incubation, the reaction was stopped by the addition of 5 ml of cold ethanol, and the covalently bound [^{14}C]HCB was isolated by the same method.

Partition coefficients. The partitioning of 245-HCB and 236-HCB between the microsomes and a Tris–KCl buffer was determined using the procedure described by Kennedy *et al.* [23] with the following modifications. Each tube contained either 15 μM 236-HCB or 19 μM 245-HCB (in DMSO), microsomal protein (1 mg/ml), 0.25 mM EDTA, and Tris–KCl buffer in a final volume of 2 ml. These tubes were incubated at 37° for 20 min and then mixed, and an aliquot was taken to determine the total [^{14}C]PCB present in the suspension. To sediment the microsomes, 0.3 ml of a CMC suspension (2.5% in Tris–KCl buffer) was then added to the remaining microsomal suspension. The tube was centrifuged at 200 *g* for 10 min, and an aliquot (0.2 ml) of the resulting supernatant fraction was removed to determine the amount of [^{14}C]HCB present in the aqueous buffer. By subtracting the amount of [^{14}C]HCB present in the aqueous buffer from the total [^{14}C]HCB present in the microsomal suspension, the amount of [^{14}C]HCB in the microsomes was determined.

Since it has been suggested that the partitioning of compounds into microsomes is a function of their lipid content, we have expressed the partition coefficients of the HCBs on the basis of the phospholipid content of the microsomes [24]. Phospholipids and proteins from human microsomes were isolated as described by Sipes and Gandolfi [22], and the quantities of phospholipids and proteins present were determined by the method of Chen *et al.* [25] and Lowry *et al.* [14], respectively. The method of Parry *et al.* [24] was used to calculate partition coefficients. The K_m was corrected to reflect HCB concentration in the microsomes [24].

RESULTS

Characteristics of the human liver MFO system. Cytochrome P-450 content, cytochrome b_5 content, and NADPH-cytochrome P-450 reductase activity are presented in Table 1. Our values agree with those previously reported for human liver microsomes by Bosterling and Trudell [26], Kremers *et al.* [27], and Pelkonen *et al.* [28]. These microsomes were also characterized for their activity towards two microsomal substrates (Table 1). Benzphetamine-*N*-demethylase activity (0.57 nmole/mg protein/min) was similar to that reported by Kremers *et al.* [27]. When benzphetamine-*N*-demethylase activity is expressed as nmoles of product formed/nmole cytochrome P-450/min instead of nmoles/mg microsomal protein/min, the variability in activity among the livers decreased. This is shown by a smaller standard deviation. Biphenyl-4-hydroxylase (0.27 nmole/mg microsomal protein/min) activity was measured to determine the ability of human microsomes to hydroxylate a biphenyl ring. As with benzphetamine-*N*-demethylase, the variability between the livers decreased when the activity was expressed as nmoles/nmole cytochrome P-450/min instead of nmoles/mg microsomal protein/min.

Table 1. Characteristics of the human liver mixed function oxidase system

Component or enzyme activity	Amount or activity
Cytochrome P-450*	0.28 ± 0.08†
Cytochrome b_5*	0.35 ± 0.06
NADPH-cytochrome P-450 reductase‡	110 ± 29
Benzphetamine-*N*-demethylase‡	0.57 ± 0.18
Benzphetamine-*N*-demethylase§	2.03 ± 0.07
Biphenyl-4-hydroxylase‡	0.27 ± 0.11
Biphenyl-4-hydroxylase§	1.07 ± 0.08

* Expressed in nmoles/mg microsomal protein.

† Mean ± S.D., N = 3.

‡ Expressed in nmoles product formed/mg microsomal protein/min.

§ Expressed in nmoles product formed/nmole cytochrome P-450/min.

Partition coefficients. Because microsomal suspensions are heterogeneous and since PCBs are highly lipophilic, partition coefficients were determined in order to more accurately reflect the concentration of the HCBs available to the enzyme. The partition coefficients of 236-HCB and 245-HCB are depicted in Table 2. There was no significant difference (Student's *t*-test, P < 0.05) in the partition coefficients between 236-HCB and 245-HCB at a microsomal protein concentration of 1 mg/ml. Thus, any differences in the metabolism of these HCBs cannot be due to a difference in the substrate concentration in the microsomal membrane. It is interesting to note that the phospholipid–protein ratio is higher in

Table 2. Partition coefficients of 236-HCB and 245-HCB between human microsomes and an aqueous buffer*

Phospholipid : Protein ratio	Partition coefficients ($\times 10^{-3}$)	
	236-HCB	245-HCB
0.65 ± 0.13	2.29 ± 1.11	2.70 ± 0.32

* Microsomes (1 mg protein/ml) were incubated in the presence of 15 μM 236-HCB or 19 μM 245-HCB at 37° for 20 min in the absence of an NADPH-generating system. Partition coefficients were calculated as the ratio of PCB in the CMC precipitated microsomes to that of the aqueous buffer, as described in Materials and Methods. Values are mean ± S.D., N = 3. The partition coefficeints of 236-HCB and 245-HCB are not significantly different (Student's *t*-test, P < 0.05).

humans than that previously reported in rats by Borgese [29].

245-*HCB metabolism.* Under the incubation conditions, 245-HCB (19 μM) was not converted to aqueous soluble products by human microsomes. Even when the incubations were performed for 90 min the radioactivity remaining in the aqueous fraction following parent removal was less than 1.2 times above background. To further ensure that no 245-HCB metabolites were formed, the aqueous extracts of several incubations were pooled, extracted, and subjected to HPLC analysis. No significant peaks of radioactivity were detected.

236-*HCB metabolism.* Human liver microsomes did metabolize 236-HCB. The reaction was linear with microsomal protein concentration over a range of 0.25 to 1.0 mg/ml and was also linear with respect to time over a range of 1–90 min (Fig. 1). In a 90-min incubation, 263 pmoles/mg (mean) of aqueous soluble metabolites was formed (Fig. 1). The K_m and V_{max} were calculated as described in Materials and Methods, and the results appear in Table 3. Since it is not known whether the PCB interacts with cytochrome P-450 in the aqueous phase or within the microsomal membrane, we have stated the K_m under both conditions. The apparent K_m was 8.8 μM. However, when the apparent K_m was corrected for the partitioning of the parent 236-HCB into the microsomal membranes, a value of 20 mM was obtained [24]. The V_{max} was 5.1 pmoles/mg microsomal protein/min. When expressed as P-450 content, the V_{max} was 19.3 pmoles/nmole P-450/min.

236-*HCB metabolites.* To identify and confirm the nature of the 236-HCB metabolites, the aqueous soluble extractable radioactivity from several incubations was pooled and then subjected to HPLC analysis and mass spectrometry. A typical HPLC radiochromatogram is depicted in Fig. 2. Two major peaks of radioactivity were apparent. Peaks A and B represented approximately 38 and 31% of the extractable metabolites. The mass spectra of the two methylated metabolites are illustrated in Fig. 3. The main molecular ion (M^+) was present at *m/e* 388 for both metabolites. The highly isotopic distribution of polychlorinated biphenyls makes these spectra unambiguous. The characteristic fragmentation of methyl ether derivatives of hydroxylated PCB metabolites has been identified and validated by Tulp *et al.* [30] and Jansson and Sundstrom [31] and was used in the interpretation of the mass spectral data. The cleavage of —$COCH_3$ (M − 43) and Cl_2 (M − 70) groups from 2,2′,3,3′,6,6′-hexachloro-5-biphenylol yields *m/e* 345 and *m/e* 275 respectively

Table 3. Kinetic constants for the metabolism of 236-HCB by human liver microsomes*

Enzyme constants	Mean ± S.D. (N = 3)
Apparent K_m (μM)	8.8 ± 2.1
Apparent K_m† (mM)	20.2 ± 4.8
V_{max} (pmoles/mg/min)	5.1 ± 0.3
V_{max} (pmoles/nmole P-450/min)	19.3 ± 4.0

* Various concentrations of 236-HCB were incubated with 1 mg/ml microsomal protein and an NADPH-generating system in Tris–KCl buffer at 37° for 45 min. K_m and V_{max} were determined as described in Materials and Methods.

† K_m calculation was based on the concentration of 236-HCB in the microsomes as calculated from the partition coefficient in Table 2.

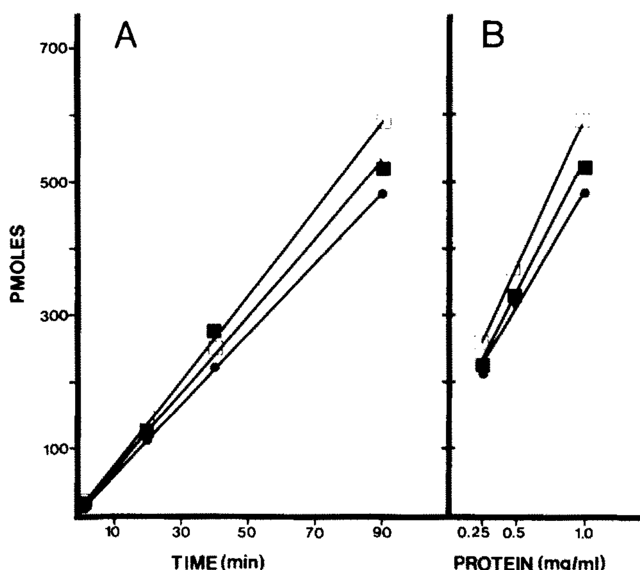

Fig. 1. Effect of time and protein concentration on the rate of aqueous soluble metabolite formation. 236-HCB (15 μM) was incubated with 1 mg/ml microsomal protein for various times (A) or for 90 min at several protein concentrations (B). The amount of 236-HCB metabolized at 1 mg/ml for 90 min was 1–2%. The rates of formation were determined using each of the three human livers (●, ■, □). Each point represents the mean of at least triplicate determinations. Incubation conditions were the same as described under Materials and Methods.

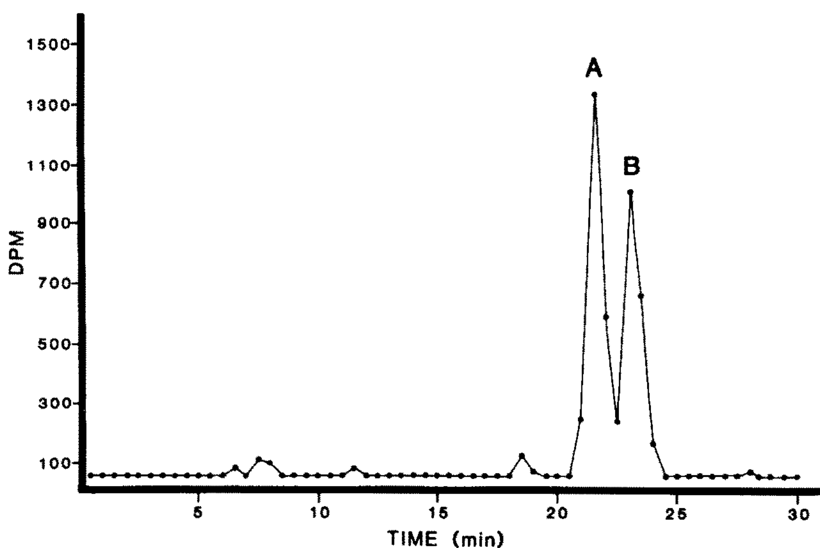

Fig. 2. HPLC radiochromatogram of 236-HCB aqueous soluble metabolites. HPLC conditions were the same as described under Materials and Methods.

(peak B). The cleavage of $—CH_3$ (M − 15), $—COCH_3$ (M − 43), and $—Cl_2$ (M − 70) from 2,2′,3,3′,6,6′-hexachloro-4-biphenylol yields *m/e* 373, 345 and 275 respectively (peak A). Thus, the two metabolites are 2,2′,3,3′,6,6′-hexachloro-4-biphenylol and 2,2′,3,3′,6,6′-hexachloro-5-biphenylol.

Covalent binding. Only 236-HCB was found to be covalently bound to microsomal protein. Binding increased with increased times of incubation and

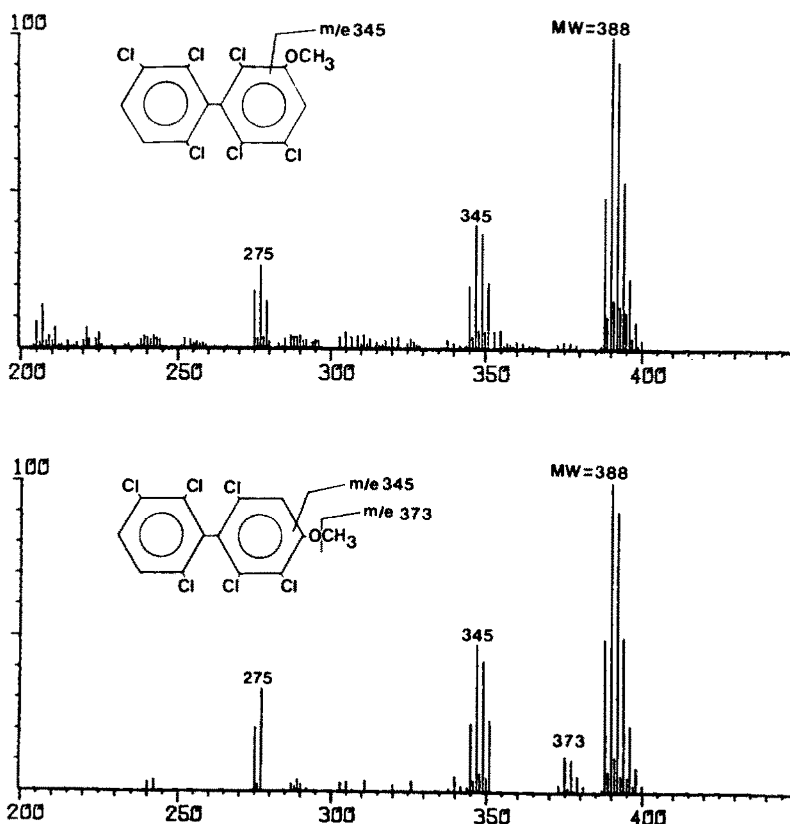

Fig. 3. Partial mass spectra of 2,2′,3,3′,6,6′-hexachloro-5-methoxy-biphenyl (top panel) and 2,2′,3,3′,6,6′-hexachloro-4-methoxy-biphenyl (bottom panel). GC–MS conditions are described in Materials and Methods. Prominent ions are identified.

Table 4. Covalent binding of PCBs to microsomal protein

PCB	Incubation time (min)	Protein concn (mg/ml)	NADPH*	Covalent binding† (pmoles/mg)
245-HCB	90	1	+	1.8‡
			−	1.6
236-HCB	90	1	+	28.8
			−	3.5
236-HCB	40	1	+	15.1
			−	4.1
236-HCB	90	0.25	+	14.1
		0.5	+	24.5
		1.0	+	28.8

* Incubation was carried out in the presence (+) or absence (−) of an NADPH-generating system. The concentrations of 236-HCB and 245-HCB were 15 and 19 μM respectively.

† Covalent binding was determined as previously described in the text.

‡ Mean, N = 2.

increased concentrations of microsomal protein (Table 4). The absence of NADPH markedly decreased the amount of covalent binding. Addition of 1 or 5 mM reduced glutathione also decreased the degree of covalent binding (Table 5).

DISCUSSION

In this study, we have isolated the microsomes from liver samples of three humans undergoing resection. The drug metabolism characteristics of these microsomes (Table 1) were comparable to those previously reported [26–28]. The average cytochrome P-450 content (0.28 nmole/mg microsomal protein) present in human liver microsomes was less than that present in Sprague–Dawley rat liver microsomes (0.62 nmole/mg microsomal protein) prepared in our laboratory [32]. We found that human liver microsomes metabolize benzphetamine and biphenyl at approximately 1/20 the rate reported by Haugen [33] for hepatic microsomes obtained from male, Sprague–Dawley rats. If we express these rates per nmole cytochrome P-450 (assuming P-450 content is approximately 0.6 nmole P-450/mg microsomal protein for non-induced rats), the catalytic activity of rat cytochrome P-450 for these compounds is approximately five times faster than that of human cytochrome P-450. The reason(s) for these differences in activities between rat and human microsomes is unclear but may result from differences in the concentration of specific cytochrome P-450 isozymes between these two species. However, other factors such as microsome preparation and storage, cytochrome P-450 reductase activity, etc., should also be considered.

Table 5. Inhibition of 236-HCB covalent binding to microsomal protein by glutathione

	Covalent binding* (pmoles/mg)
Complete incubation†	22.3‡
+1 mM GSH	11.2
+5 mM GSH	0.7

* Covalent binding was determined as previously described in the text.

† Complete incubation consisted of 15 μM 236-HCB, 1 mg/ml microsomal protein, NADPH-generating system in Tris–KCl buffer for 45 min.

‡ Mean of triplicate determinations, N = 1.

The major findings of this study relative to the hexachlorobiphenyls are 2-fold: (1) that 245-HCB was not metabolized by human liver microsomes and, (2) that the 236-HCB congener was metabolized via a reactive intermediate (arene oxide) capable of binding to microsomal protein.

The 245-HCB congener accounts for 21.5% of the PCBs present in human adipose tissue [34]. Since this congener is found in the various PCB mixtures at lower percentages than that found in the adipose tissue, it appears that 245-HCB selectively accumulates in adipose tissue relative to those PCBs which can be metabolized and excreted. Despite this apparent lack of metabolism, it should be emphasized that humans *in vivo* may have a limited capacity to metabolize 245-HCB. However, the rate may be so slow that we were unable to detect formation of metabolites *in vitro* under the incubation conditions that were used in this study.

We were able to detect the formation of metabolites of 236-HCB by human liver microsomes. A calculated V_{max} of 5.1 pmoles/mg microsomal protein/min was obtained. The ability of humans to metabolize this PCB probably explains why this congener has not been detected in human adipose tissue in contrast to those PCBs which are not metabolized [34]. This congener has been reported to be present in a concentration of up to 1% in PCB mixtures [35].

Our results suggest that 236-HCB was metabolized through an arene oxide intermediate. The two major metabolites that were identified, the 4-hydroxy and 5-hydroxy substituted derivatives of 236-HCB, could likely result from the rearrangement of the 4,5-epoxide. Secondly, the NADPH-dependent covalent binding of [^{14}C]236-HCB equivalents and its inhibition by glutathione provide additional evidence for

the formation of a reactive epoxide. The results of *in vivo* studies by Kato *et al.* [12] have suggested that the rat metabolized 236-HCB to the 4,5-epoxide. It could be argued that 236-HCB is metabolized because the preferred site of hydroxylation, the *para*-position, is unsubstituted [12]. However, preliminary evidence obtained in our laboratory indicates that human liver microsomes metabolize 4,4′-dichlorobiphenyl (4-DCB), a PCB which has the *para*-position blocked [36]. The calculated V_{max} was 1.2 pmoles/mg microsomal protein/min, about one-fourth the activity obtained for 236-HCB. Thus, an unsubstituted *para*-position is not required for metabolism of PCBs by human microsomes. However, it apparently facilitates the metabolism of these compounds particularly when adjacent to an unsubstituted *meta*-position. Therefore, our results on the structural features that determine the metabolism of PCBs by human hepatic microsomes are consistent with those demonstrated for other species [5].

In conclusion, the lack of 245-HCB metabolism by human liver microsomes suggests that two adjacent unsubstituted carbon atoms are necessary for PCB metabolism by this species. When adjacent unsubstituted carbon atoms are not present, the PCB is more resistant to metabolism and will accumulate in adipose tissue. When these *in vitro* data and human epidemiological data are compared to the disposition studies for 236-HCB and 245-HCB in the monkey, rat, and dog, it can be concluded that humans metabolize these compounds qualitatively in a manner more closely related to that of the rat and monkey and not the dog. It appears that PCB metabolism by human microsomes is through an arene oxide intermediate as occurs in other species [12, 37]. The epoxide is reactive and thus capable of binding to tissue macromolecules. This covalent interaction may lead to some of the toxic results of PCB exposure.

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