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THE HYDROXYLATION, DECHLORINATION, AND GLUCURONIDATION OF 4,4′-DICHLOROBIPHENYL (4-DCB) BY HUMAN HEPATIC MICROSOMES*,†

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Abstract—Since chlorine placement and the degree of chlorination of the biphenyl nucleus play an important role in the metabolism and ultimate elimination of polychlorinated biphenyls (PCBs), we have studied the metabolism of 4,4′-dichlorobiphenyl (4-DCB) by human hepatic microsomes. This low molecular weight PCB congener is substituted at the preferred site of metabolism (para-position). 4-DCB was metabolized by human microsomes with a K_m of 0.43 μM and a V_{max} of 1.2 pmoles/mg microsomal protein/min. Six metabolites were identified: 4,4′-dichloro-3,3′-biphenyldiol, 4′-chloro-3-biphenylol, 4′-chloro-4-biphenylol, 4,4′-dichloro-2-biphenylol, 4,4′-dichloro-3-biphenylol (most abundant), and 3,4′-dichloro-4-biphenylol. [^{14}C]-4-DCB equivalents were found to covalently bind to microsomal protein. Addition of a 1 mM concentration of reduced glutathione decreased the degree of covalent binding. These data suggest that human microsomes metabolize this PCB through an arene oxide and that an "NIH shift" occurs. When UDPGA was added to the incubation, human microsomal glucuronosyltransferase catalyzed the formation of the glucuronide of the major metabolite, 4,4′-dichloro-3-biphenylol. These and previous *in vitro* results show that the biotransformation of PCBs by humans is governed by the same principles established for the *in vivo* biotransformation of PCBs by the rat, mouse and monkey. That is, PCBs without two adjacent unsubstituted carbon atoms are poorly metabolized and that an unsubstituted para-position facilitates metabolism.

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants that have been implicated in a number of animal and human toxicities [1]. Some of these toxicities, such as liver injury and mutagenicity, appear to result from electrophilic intermediates formed during their metabolism [2–4]. The extent of PCB metabolism is dependent on the animal species [5–7], and on the number and position of the chlorines on the phenyl rings [8, 9]. Those PCB congeners that are resistant to metabolism to less lipophilic compounds are retained and accumulate in adipose tissue.

Previously, we examined the *in vitro* metabolism of two highly chlorinated PCB isomers by human hepatic microsomes [10]. The PCB congener, 2,2′,3,3′,6,6′-hexachlorobiphenyl (236-HCB), which contains two adjacent unsubstituted carbon atoms on each aromatic ring, was metabolized. In contrast, 2,2′,4,4′,5,5′-hexachlorobiphenyl (245-HCB), which does not contain adjacent unsubstituted carbon atoms, was not metabolized. These results were consistent with *in vivo* data obtained in the monkey and rat [5, 6, 9]. That is, hexachlorobiphenyls without two adjacent unsubstituted carbon atoms are poorly metabolized and accumulate in adipose tissue. To investigate the metabolism of a low molecular weight PCB by human liver microsomes, we examined the metabolism of 4,4′-dichlorobiphenyl (4-DCB). This congener was chosen because the preferred site of metabolism is substituted. In addition, 4-DCB has been found recently in the plasma and adipose tissue of workers in a capacitor manufacturing facility using PCBs [11] and is found in both Aroclor 1254 and Aroclor 1242 [12].

MATERIALS AND METHODS

The 4,4′-dichlorobiphenyl[U-^{14}C] (58 mCi/mmole) was purchased from California Bionuclear (Sun Valley, CA) and was determined to be greater than 98% radiochemically pure by high-performance liquid chromatography (HPLC). NADP, glucose-6-phosphate (G6P), glucose-6-phosphate dehydrogenase (G6PDH), Trizma base, CM cellulose (CMC), bovine serum albumin (BSA), Diazald (diazomethane), reduced glutathione (GSH), uridine diphosphoglucuronic acid (UDPGA), Brij 58, beta-glucuronidase (type H-1), saccharo-1,4-lactone, 4-nitrophenol and chloramphenicol were

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obtained from the Sigma Chemical Co. (St. Louis, MO). Dimethyl sulfoxide (DMSO), biphenyl, and 2- and 4-biphenylol were purchased from the Aldrich Chemical Co. (Milwaukee, WI). 4,4′-Dichloro-3-biphenylol, 4,4′-dichloro-3,3′-biphenyldiol, 4′-chloro-4-biphenylol and 3-biphenylol were obtained from Ultra Scientific (Hope, RI). Sodium dihydrobis(2-methoxyethoxy) aluminate was obtained from Pfaltz & Bauer (Stamford, CT). All other chemicals were of reagent grade quality.

Preparation of microsomes. Liver tissue was obtained from five patients (ages 83, 58, 62, 54 and 68) undergoing partial liver resection. The tissue was obtained within minutes after cessation of total blood flow to the lobe and cut into 1-cm cubes. The cubes were washed repeatedly in ice-cold 0.25 M sucrose and 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 or a Sorvall Omni-Mixer (2 min at full speed). Microsomes were obtained by differential centrifugation, resuspended, homogenized and centrifuged as previously described [10]. The microsomes were stored at −70° at a concentration of 10–25 mg microsomal protein/ml in the same buffer containing 0.1 mM EDTA. The method of Lowry *et al.* [13] was used to determine protein concentration. Standard curves were prepared using BSA. Cytochrome P-450 content was determined by the method of Omura and Sato [14].

Metabolism of 4-DCB. Microsomal incubation conditions were the same as previously described [10]. Briefly, microsomal protein (2 mg) was incubated with 0.3 mM NADP, 2.5 mM G6P, 0.05 mM $MgCl_2$, 1 unit G6PDH, 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 various concentrations of ^{14}C-labeled 4-DCB dissolved in DMSO (DMSO concentration was less than 1% in the final incubation mixture). Incubations were performed at 37° under air in a Dubnoff metabolic shaking incubator. Preliminary experiments were done to determine linearity of product formation with respect to protein concentration (0.25 to 1.0 mg/ml) and time (1 to 90 min). 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. The parent 4-DCB was removed from the samples by repeated extraction with 5-ml aliquots of hexane–ethanol (19:1) until no radioactivity appeared in the organic layer. The ^{14}C in the aqueous phase was determined by liquid scintillation spectrometry to quantify the total amount of aqueous soluble metabolites. Control incubations lacked the NADPH-generating system and never contained more than 30 cpm above background. The rate of metabolism was determined by subtracting the amount of product formed in the control incubations from the corresponding incubation containing the NADPH-generating system. A similar extraction procedure has been reported recently by Parkinson and Safe [15]. The results of this work were analyzed by the method of Hofstee [16]. The apparent K_m and V_{max} values were determined from the slope and y-intercept respectively.

The major PCB metabolites were obtained from the aqueous phase following acidification with 0.25 ml of 18 N H_2SO_4 and two extractions with 5 ml of hexane:ethyl ether:ethanol (H:E:E, 19:5:1). This procedure removed approximately 75% of the total metabolites. Following evaporation of the solvents under nitrogen, the H:E:E extractable metabolites were subjected to HPLC analysis. The remaining unextractable metabolites in the aqueous phase were not examined except those covalently bound to microsomal protein. Covalent binding was determined as previously described by extraction of the microsomal protein with organic solvents of various polarities [10].

HPLC analysis. Individual incubations were subjected to reverse phase HPLC analysis as previously described using a 5 μm Ultrasphere ODS column (Altex) [10]. The mobile phase was acetonitrile:-H_2O:ethanol:acetic acid (50:44:5:1) at a flow rate of 2 ml/min. Fractions were collected every 0.2 min, and the amount of ^{14}C in each fraction was determined by liquid scintillation spectrometry. Peaks were tentatively identified by co-elution using the u.v. absorbance (254 nm) of the synthetic standards: 4,4′-dichloro-3,3′-biphenyldiol, 4′-chloro-4-biphenylol, and 4,4′-dichloro-3-biphenylol.

To conclusively identify the metabolites, the extractable radioactivity from many experiments was pooled and subjected to reverse phase HPLC analysis. The major radioactive peaks were collected, extracted with H:E:E, evaporated to dryness and reductively dechlorinated using sodium dihydro-bis(2-methoxyethoxy) aluminate as described by others [17, 18]. These dechlorinated monohydroxylated biphenyls were extracted with H:E:E, evaporated to dryness, and subjected to HPLC analysis. The monohydroxylated products were identified by co-elution with 2-, 3-, and 4-biphenylol standards on a Waters 10 μm NH_2 radial compression column (Z-module) as described by Burke and Prough [19]. Reductive dechlorination of the standard, 4,4′-dichlor-3-biphenylol, by this procedure yielded 3-biphenylol.

To further assist in the identification of the metabolites, a fraction of the major radioactive peaks was methylated by ethereal diazomethane and subjected to gas chromatography–mass spectrometry (GC–MS) using a Finnigan 3300 mass spectrometer. Because of the small amount of metabolite available, selective ion monitoring was used. The GC was interfaced to the MS with a glass jet separator with the electron impact mode at 70 eV. The GC was carried out using a 6 ft 2 mm column packed with 3% OV-17 on 100/120 Chromosorb Q packing. The carrier gas was helium at a flow rate of 30 ml/min. The injection port temperature was 300°, and the initial oven temperature was 160°. The oven temperature was increased at a rate of 10°/min to 260°. The method of Tulp *et al.* [20] and Jansson and Sundstrom [21] was used in the interpretation of the mass spectral data.

Glucuronidation of 4-DCB metabolites. The ability of human liver microsomes to glucuronidate 4-DCB metabolites was determined by including 3 mM UDPGA and 1 mg Brij 58 in the previously described microsomal incubations. The non-ionic detergent Brij 58 has been used by other investigators to acti-

vate glucuronosyltransferases [22, 23]. In our studies, addition of 0.5 mg of Brij 58/mg microsomal protein produced the greatest amount of glucuronidated product. To further demonstrate the glucuronidation of 4-DCB metabolites, various concentrations of the glucuronosyltransferase inhibitors 4-nitrophenol or chloramphenicol were added to the standard incubation mixtures [22].

The incubations were stopped, and the total amount of aqueous soluble metabolites was determined as described previously. The hydroxylated metabolites were extracted into H:E:E by adjusting the pH of the aqueous phase between 5 and 7 with approximately 0.3 ml of 4 N H_2SO_4. This pH range prevents the extraction of the glucuronides. The amount of ^{14}C remaining in the aqueous phase was determined to quantify conjugated (non-H:E:E extractable) metabolites. The aqueous phase (0.6 to 1 ml) was incubated in 0.1 M sodium acetate (pH 5) containing 275 Fishman units of beta-glucuronidase at 37° for 16–18 hr. Following three extractions with 6 ml H:E:E, the amount of ^{14}C remaining in the aqueous phase was determined. Parallel incubations were done containing 20 mM saccharo-1,4-lactone to inhibit the glucuronidase. The difference between the amount of ^{14}C found in the aqueous phases lacking saccharo-1,4-lactone and those containing it was defined as the amount of glucuronides formed.

To identify which primary metabolites were glucuronidated, HPLC analysis was performed on the aqueous phase preceding the glucuronidase treatment and on H:E:E extracts following glucuronidase treatment as described above for the phase I incubations. The resulting peak from the glucuronidase treatment was collected, dechlorinated and identified as described for the hydroxylated metabolites.

Partition coefficients. The partitioning of 4-DCB between the microsomes and a Tris–KCl buffer was determined in order to estimate the concentration of the PCB in the microsomes and thus near the active site [10, 17]. Briefly, tubes containing 4 μM 4-DCB (in DMSO), microsomal protein (1 mg/ml), 0.25 mM EDTA, and Tris–KCl buffer were incubated at 37° for 20 min, and an aliquot was taken to determine the total [^{14}C]4-DCB present in the suspension. To sediment the microsomes, 0.3 ml of a CMC suspension was then added to the tubes. The tubes were centrifuged for 10 min at 200 *g* and an aliquot was taken to determine the amount of [^{14}C]4-DCB present in the aqueous buffer. The amount of [^{14}C]4-DCB in the microsomes was obtained by subtracting the amount in the aqueous buffer from the amount in the suspension. Since it has been suggested that the partitioning of compounds into the microsomes is a function of their lipid content, we have expressed the partition coefficients 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 [25] and quantified by the methods of Chen *et al.* [26] and Lowry *et al.* [13] respectively. The method of Parry *et al.* [24] was used to calculate partition coefficients.

Statistics. Results are expressed as mean ± standard deviation unless otherwise stated. One-way analysis of variance was used to compare the partition coefficients and the rates of aqueous soluble metabolite formation. The Student–Newman–Keuls' test was used to determine differences in the means with $P \leq 0.05$ accepted as the level of significance.

RESULTS

Phase I metabolism of 4-DCB. The cytochrome P-450-mediated metabolism of 4-DCB to hydroxylated metabolites was investigated using liver microsomes from three humans. For each human liver preparation, preliminary studies were performed to establish linearity with respect to time and protein (Fig. 1). In all cases the formation of aqueous soluble metabolites was linear between 1 and 90 min and 0.5 to 2.0 mg protein. The K_m and V_{max} were calculated as described in Materials and Methods, and the results appear in Table 1. The mean V_{max} value obtained for the three preparations was 1.2 pmoles/mg microsomal protein/min. When the V_{max} was

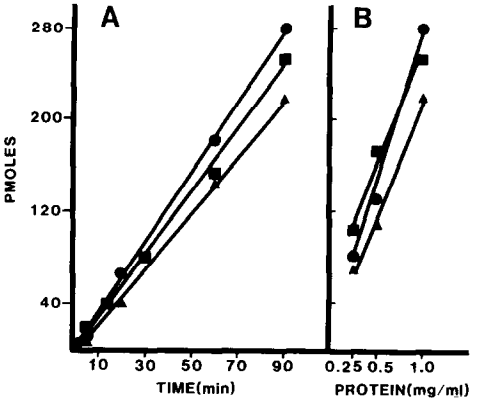

Fig. 1. Effect of time and protein concentrations on the rate of formation of aqueous soluble metabolites. 4-DCB (4 μM) was incubated with 1 mg/ml microsomal protein for various times (A) or for 90 min at several protein concentrations (B). The amount of 4-DCB metabolized at 1 mg/ml for 45 min is 1–2%. The rates of formation were determined using each of three human livers (●,■,▲). Each point represents the mean of at least triplicate determinations. Incubation conditions were the same as described under Materials and Methods.

Table 1. Kinetic constants for the metabolism of 4-DCB by human liver microsomes*

Enzyme constants	Mean ± S.D. (N = 3)
Apparent K_m (μM)	0.43 ± 0.13
Apparent K_m† (mM)	1.1 ± 0.33
V_{max} (pmoles/mg protein/min)	1.2 ± 0.08
V_{max} (pmoles/nmole P-450/min)	4.4 ± 1.4

* Various concentrations of 4-DCB were incubated with 1 mg/ml microsomal protein, an NADPH-generating system in a Tris–KCl buffer at 37° for 45 min. K_m and V_{max} were determined as described in Materials and Methods.

† K_m has been calculated to reflect the sequestration of 4-DCB into the microsomes which may reveal a more accurate K_m.

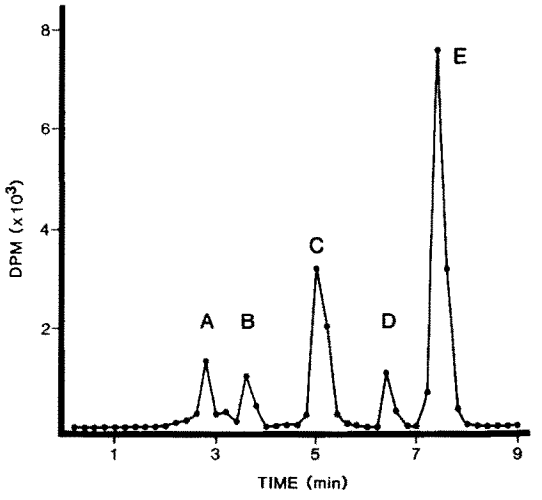

Fig. 2. HPLC radiochromatogram of 4-DCB aqueous soluble extractable metabolites. A C_{18} reverse phase column was used with a mobile phase of acetonitrile: water: ethanol: acetic acid (50:44:5:1) at a flow rate of 2 ml/min. Fractions were collected every 0.2 min, and the amount of ^{14}C in each fraction was determined by liquid scintillation spectometry.

expressed per nmole of cytochrome P-450, the rate was 4.4 pmoles/nmole P-450/min. The closeness of the V_{max} values, as reflected by the small standard deviation, may be the result of similar cytochrome P-450 concentrations in all the livers (0.28 ± 0.12 nmoles/mg microsomal protein; mean ± S.D.).

The apparent K_m calculated from the Eadie–Hofstee plot was 0.43 μM. However, it is known that PCBs readily partition into the microsomal membrane. Since it is not known whether the PCB interacts with cytochrome P-450 in the aqueous phase or within the lipid matrix of the microsomal membrane, we have also calculated a K_m based on the concentration of 4-DCB in the microsomes. Partition coefficients were determined to estimate the concentration of 4-DCB in the microsomes. The partition coefficient for 4-DCB between the microsomes and the aqueous buffer was $2.52 \pm 0.39 \times 10^3$ (N = 3). When the partitioning of 4-DCB into the microsomes is taken into consideration, a K_m of 1.1 mM is obtained.

4-DCB metabolites. The H:E:E extractable radioactivity accounted for approximately 75% of the metabolites. A typical HPLC radiochromatogram for a microsomal incubation subjected to C_{18} reverse phase HPLC analysis is depicted in Fig. 2. Five major peaks of radioactivity were apparent in a ratio of 0.12:0.07:0.3:0.06:1.0 (peaks A:B:C:D:E). Peaks A and D were not identified. Peaks B, C and E co-eluted with the synthetic standards 4,4′-dichloro-3,3′-biphenyldiol, 4′-chloro-4-biphenylol and 4,4′-dichloro-3-biphenylol respectively. Peak B was methylated and subjected to selective ion monitoring GC–MS analysis. The main molecular ion (M^+) was present at *m/e* 282 which indicates a dimethoxy-4-DCB. Strong molecular ions were seen at *m/e* 239 and *m/e* 126. This suggests that the hydroxyl group is either in the 3- or 4-position [20, 21]. Since 4,4′-dichloro-3-biphenylol is the major metabolite (see below) and peak B co-eluted with the standard 4,4′-dichloro-3,3′-biphenyldiol on reverse phase HPLC, we have assigned peak B the structure 4,4′-dichloro-3,3′-biphenyldiol. Although peaks C and E co-eluted with synthetic standards, a C_{18} reverse phase column cannot readily separate 4′-chloro-4-biphenylol from 4′-chloro-3-biphenylol nor 4,4′-dichloro-3-biphenylol from 3,4′-dichloro-4-biphenylol [17]. Therefore, to conclusively identify the metabolites, peaks C and E were reductively dechlorinated and subjected to normal phase HPLC. Two peaks of radioactivity were obtained when peak C was chromatographed. They co-eluted with the 3-biphenylol and 4-biphenylol standards. Thus, peak C actually contained 4′-chloro-3-biphenylol and 4′-chloro-4-biphenylol in approximately a 2:1 ratio. When peak E was subjected to dechlorination and normal phase HPLC, three peaks of radioactivity were obtained. They co-eluted with the 2-, 3-, and 4-biphenylol standards. Thus, the actual metabolites were 4,4′-dichloro-2-biphenylol, 4,4′-dichloro-3-biphenylol and 3,4′-dichloro-4-biphenylol in approximately a 1:9:3 ratio. The main molecular ion (M^+) of peak E was present at *m/e* 252 and was consistent with a dichlorinated-monohydroxylated-PCB. The structures assigned to the 4-DCB metabolites are shown in Table 2.

Covalent binding. ^{14}C-Equivalents of 4-DCB covalently bound to human liver microsomal protein. This binding was NADPH dependent and increased with longer times of incubation and increased concentrations of microsomal protein (Table 3). When 1 mM reduced glutathione was added to the incubation, the amount of covalent binding of 4-DCB to the microsomal protein was decreased by 60% (Table 3).

Glucuronidation of 4-DCB metabolites. The addition of UDPGA (3 mM) to microsomal incubations resulted in the formation of glucuronidated 4-DCB metabolites. In some experiments, microsomal samples were allowed to incubate for 90 min before the addition of UDPGA. Following the

Table 2. Metabolites of 4,4′-dichlorobiphenyl catalyzed by human liver microsomes.*

Peak†	Identified compounds
A	Not identified
B	4,4′-Dichloro-3,3′-biphenyldiol
C	4′-Chloro-3-biphenylol
C	4′-Chloro-4-biphenylol
D	Not identified
E	4,4′-Dichloro-2-biphenylol
E	4,4′-Dichloro-3-biphenylol
E	3,4′-Dichloro-4-biphenylol

* Identification of hexane:ethyl ether:ethanol extractable metabolites from human liver microsomal incubations with 4,4′-dichlorobiphenyl. The ratio of 4′-chloro-3-biphenylol to 4′-chloro-4-biphenylol was 2:1. The ratio of 4,4′-dichloro-3-biphenylol to 3,4′-dichloro-4-biphenylol to 4,4′-dichloro-2-biphenylol was 9:3:1.

† Peaks identified from C_{18} reverse phase HPLC (Fig. 2).

Table 3. Covalent binding of 4-DCB to microsomal protein

Incubation time (min)	Protein concn (mg/ml)	NADPH*	GSH (1 mM)	Covalent binding† (pmoles/mg protein)
90	1	+	−	8.7, 9.2
		−	−	2.4, 1.5
40	1	+	−	3.9, 4.4
		−	−	0.2, 0.9
		+	+	1.6, 1.8
90	0.5	+	−	4.0, 4.5
		−	−	0.3, 1.3

* Incubation was carried out in the presence (+) or absence (−) of an NADPH-generating system and 4 μM 4-DCB.
† Covalent binding was determined as previously described in the text. N = 2.

addition of UDPGA, there was a time-dependent decrease in H:E:E extractable metabolites and an increase in aqueous (i.e. non-H:E:E extractable) metabolites (Fig. 3).

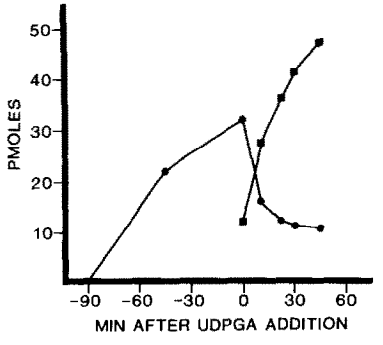

Fig. 3. Effect of UDPGA addition on the formation of phenolic and conjugated metabolites of 4-DCB. 4-DCB (4 μM) was incubated with microsomal protein (2 mg), an NADPH-generating system, and Brij 58 (1 mg) for 90 min. At time zero, UDPGA (3 mM) was added and the reaction was allowed to continue for up to 45 min. The amount of phenolic (H:E:E extractable, ●) and conjugated (non-H:E:E extractable, ■) metabolites was determined over time as described in Materials and Methods.

For each of the three human livers used in these experiments, the formation of glucuronidated metabolites was linear over time (15–60 min). However, there was considerable variation in the rate of glucuronidation (range 0.3 to 3.2 pmoles/mg protein/mg). The inclusion of UDPGA in the microsomal incubations resulted in an increase in the rate of total metabolite formation as compared to those containing Brij 58 (Table 4). Because of the variation in glucuronidation rates, the data in Table 4 are expressed as a relative rate. The data in Table 4 also reveal that Brij 58 inhibited the hydroxylation of 4-DCB compared to complete incubations.

Following the removal of H:E:E extractable metabolites, treatment of incubation mixtures with beta-glucuronidase resulted in the hydrolysis of the conjugated aqueous soluble metabolites. To determine what percentage of the observed hydrolysis was enzymatically mediated, the glucuronidase inhibitor saccharo-1,4-lactone was added to parallel incubations with the beta-glucuronidase. These experiments showed that 78% of the hydrolysis products was enzymatically mediated. HPLC analysis of extracts from the glucuronidase-treated samples resulted in three peaks, co-eluting with peaks A (4%), C (4%) and E (92%) (Fig. 2). Reductive de-

Table 4. Effect of Brij 58 and UDPGA on the aqueous soluble metabolite formation of 4-DCB by human liver microsomes

Condition	Relative rate of aqueous soluble metabolite formation (pmoles/mg microsomal protein/min)
Complete*	1.00†
+ 0.5 mg Brij 58/mg microsomal protein	0.56‡
+ 0.5 mg Brij 58/mg microsomal protein and 3 mM UDPGA	1.36†

* Complete incubations contained 4 μM 4-DCB, an NADPH-generating system, microsomes (1 mg/ml) in a Tris–KCl buffer. Tubes were incubated for 45 min at 37°.
†‡ Rates with different superscripts are significantly different from one another; one-way analysis of variance and Student–Newman–Keuls' test, $P \leq 0.05$.

chlorination of the metabolites in peak E and subsequently analysis by HPLC using the NH_2 column resulted in a single peak that co-eluted with 3-biphenylol. Thus, the hydroxylated PCB, 4,4'-dichloro-3-biphenylol, is the major metabolite that is glucuronidated by human liver microsomes.

Competitive inhibitors of the glucuronosyltransferases, 4-nitrophenol and chloramphenicol, at concentrations that did not change primary metabolite formation, decreased glucuronide formation. A 30% decrease in glucuronide formation occurred in the presence of 45 μM nitrophenol, whereas 2.3 mM chloramphenicol was required to produce a comparable decrease. At concentrations of 3 mM or greater, chloramphenicol inhibited phase I metabolism. Thus, a wide concentration range of inhibitors could not be used in this coupled system.

DISCUSSION

The *in vitro* data obtained in this study show that human liver microsomes convert 4-DCB to less lipophilic metabolites. Such data were expected since many sites are available for hydroxylation by the cytochrome P-450 system. What is interesting is that the rate of metabolism of 4-DCB was about four times slower than that observed for the highly chlorinated PCB congener, 236-HCB (1.2 vs 5.1 pmoles/mg microsomal protein/min). These data clearly demonstrate the importance of chlorine placement on the phenyl rings as a factor in determining the rate of PCB metabolism. Furthermore, these and previous *in vitro* results show that the biotransformation of PCBs by humans is governed by the same principles established for the *in vivo* biotransformation of PCBs by the rat, mouse and monkey [5–9, 27]. These are PCBs without two adjacent unsubstituted carbon atoms are virtually unmetabolizable and that an unsubstituted para-position facilitates biotransformation. Although 4-DCB contains four areas with two adjacent unsubstituted carbon atoms, the chlorines in the para-positions retard its metabolism, presumably by retarding the formation of the 3,4-epoxide. Furthermore, these principles of PCB metabolism may explain why 245-HCB accumulates in the body and is the most abundant PCB congener found in human adipose tissue [28]. It lacks the structural requirements that facilitate PCB metabolism. The differences in the rates of 236-HCB and 4-DCB metabolism and the lack of 245-HCB metabolism were not the result of differing concentrations of the substrate in the microsomes, since there is no difference in the partition coefficients of these PCBs in the microsomal membrane [10].

The metabolite profile and covalent binding of 4-DCB equivalents to microsomal protein suggest that the biotransformation of 4-DCB proceeds through an arene oxide intermediate. The major metabolite, 4,4'-dichloro-3-biphenylol, could result from the rearrangement of a 2,3-epoxide or a 3,4-epoxide. The rearrangement of a 3,4-epoxide with the migration of the chlorine to the meta-position (NIH shift) results in the metabolite 3,4'-dichloro-4-biphenylol [29]. A 2,3-epoxide could rearrange to form the minor metabolite 4,4'-dichloro-2-biphenylol. The dechlorinated metabolites 4'-chloro-3-biphenylol and 4'-chloro-4-biphenylol could both result from the 3,4-epoxide and the loss of the chlorine. All of these metabolites have been formed and identified in the rat with the exception of 4,4'-dichloro-2-biphenylol [17, 30–33]. Additional evidence for an arene oxide intermediate is that 4-DCB equivalents covalently bind to microsomal protein and that glutathione inhibits this binding. Glutathione is known to conjugate a variety of arene oxides and reduce their covalent binding [29].

However, Preston *et al.* [34] recently found that phenobarbital-induced rat liver microsomes metabolized 2,2',5,5'-tetrachlorobiphenyl to 2,2',5,5'-tetrachloro-3-biphenylol by a non-arene oxide pathway. While our data suggest an arene oxide, it is possible that additional reaction mechanisms may be operative in the biotransformation of 4-DCB by human liver microsomes.

The major metabolite of 4-DCB, 4,4'-dichloro-3-biphenylol, was conjugated with glucuronic acid by human liver microsomes supplied with UDPGA. The evidence for the formation of this glucuronide is (1) a decrease in H:E:E extractable metabolites and an increase in aqueous (non-H:E:E extractable) metabolites when UDPGA was included in the incubation mixture, (2) the extraction of radiolabel from the aqueous phase following its treatment with beta-glucuronidase, (3) the identification of the aglycone 4,4'-dichloro-3-biphenylol, and (4) the inhibition of glucuronide formation by known glucuronosyltransferase inhibitors.

There was a 2.4-fold increase in the rate of 4-DCB metabolism when UDPGA was included in the incubations (Table 4). Bock [35] and Fahl *et al.* [36] have shown that, in a coupled system such as that used in this study, glucuronidation increases the phase I metabolism of benzo[*a*]pyrene. They suggested that the increase in phase I metabolism when UDPGA is included may be the result of removing inhibitory or competing primary metabolites. Our results with 4-DCB are consistent with this hypothesis.

Little evidence is available in the literature regarding the nature of the conjugates of hydroxylated PCBs that are excreted. Block and Cornish [37], Norback *et al.* [38] and Bakke *et al.* [39] have reported the identification of glucuronidated PCB metabolites in the excreta of the rabbit, rhesus monkey and rat. Our results suggest that humans may also form glucuronides of PCB metabolites.

In summary, the structural features that determine the metabolism of PCBs by human hepatic microsomes are consistent with those demonstrated for most other species [1]. When adjacent unsubstituted carbons are not present, the PCB is more resistant to metabolism. Furthermore, unsubstituted meta- and para-positions facilitate the metabolism of these compounds. The metabolite profile as well as the covalent binding of 4-DCB equivalents to microsomal protein and our previous work with 236-HCB suggest that PCB metabolism by human microsomes involves an arene oxide intermediate as occurs in other species [40, 41].

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