2,3',4,4',5-五塩素化ビフェニル (CB118) のモルモット肝ミクロゾームによる代謝

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Abstract The in vitro metabolism of 2,3',4,4',5-pentachlorobiphenyl (pentaCB) (CB118) was studied using liver microsomes of guinea pigs and the effect of cytochrome P450 inducers, phenobarbital (PB) and 3-methylcholanthrene (MC) on CB118 metabolism was also compared. After 30 min-incubation at 37°C with liver microsomes of guinea pigs, CB118 was hydroxylated to two metabolites (M-1 and M-2) with retention times of 15.84 min and 20.01 min in GC/ECD, respectively. GC/MS showed that the methylated derivative of a major metabolite M-2 had the molecular weight of 354 and an intense fragment ion of [M^+-50] which is a characteristic ion for PCBs possessing a methoxy-group at the 2 (2')- or the 6 (6')-position. By comparison of the mass fragmentation and the retention times in GC/MS with the synthetic authentic compounds, M-2 was identified as 2-hydroxy-3,3',4,4',5-pentaCB (CB126). On the other hand, the methylated derivative of a minor metabolite M-1 had the molecular weight of 320 and the similar fragment ion of [M^+-50] to the methylated M-2, assuming that M-1 was a dechlorinated monohydroxy-tetrachlorobiphenyl (tetraCB) possessing hydroxy-group at the 2 (2')- or the 6 (6')- position. However, the precise structure of M-1 could not be determined because its retention time in GC was in disagreement with that of the candidate 6-hydroxy-3,3',4,4'-tetraCB. PB-treatment increased the formation of M-1 and M-2 to 2.2- and 6.8-fold of untreated animals, whereas MC-treatment increased only M-2 to 2.6-fold of untreated ones. Addition of antiserum against a PB-inducible guinea pig cytochrome P450, CYP2B18, completely inhibited the formation of M-2. These results suggest that CB118 is principally metabolized by CYP2B18 to 2-hydroxy-CB126 which is formed via a 2,3-epoxide intermediate and the subsequent NIH-shift of a chlorine at the 2-position to the 3-position in guinea pig liver.

はじめに

2,3',4,4',5-Pentachlorobiphenyl (pentaCB) (CB 118) はカネミ油症の原因物質である PCB 製品 (Kanechlor 400) の主要な成分の1つである[28]とともに，油症患者血液中の PCB 成分のうちで，健常者よりも濃度が低い PCB 異性体として知られている[23]. PCB を含むダイオキシン類の毒性の強さを表す指標として毒性評価係数 (TEF) があるが，CB118 に対しては，2,3,7,8-tetrachlorodibenzo-*p*-dioxin を1.0とした場合，0.00001の強さを有するとされている[34].

PCB 異性体は毒性の1つとして，肝チトクロム P450 (P450) を中心とした肝薬物代謝酵素に対する強い誘導能を有しており，その誘導様式の違いから，phenobarbital(PB)型，3-methylcholanth-

rene (MC) 型および混合型に分類されている[32]. PB型には，mono-ortho-および di-ortho-PCB が，また，MC型には高毒性の coplanar PCB が含まれる. CB118 は mono-ortho-PCB であるが coplanar PCB の性質も有することから，2,3,3',4,4'-pentaCB (CB105) とともに混合型に分類されている[37].

一方，PCB 異性体のうち，2,4,5-三塩素置換 PCB，例えば CB118，2,2',3,4,4',5'-hexachlorobiphenyl (hexaCB) (CB138)，2,2',4,4',5,5'-hexaCB (CB 153)，2,2',3,4,4',5,5'-heptachlorobiphenyl (heptaCB) (CB180) および 2,2',3,4',5,5',6-heptaCB (CB187) は，肝，脂肪組織および血液などへの残留性が非常に高いことが知られている[5)9)11)33]. また，最近，ヒト血液中において，4-水酸化 (OH)-CB187 や 4-OH-2,2',3,4',5,5'-hexaCB (CB146) などの，特に PCB の 4 位が水酸化された代謝物が高濃度で検出されている[5)9)33]. さらに，これらの OH 体は血液中の甲状腺ホルモン輸送タンパク質である transthyretin と高い親和性を有していることから，結果的に血中甲状腺ホルモンの低下をもたらすといわれる[6)7]. これらの事実は，PCB によるヒトへの生体影響を明らかにするためには，2,4,5-三塩素置換 PCB の代謝研究が急務であることを示している.

モルモットはダイオキシン類などの毒性に対して，最も感受性の高い動物として知られているが[17]，一方，PCB に対する代謝活性に関してもラットとかなり異なっている. 例えば，3,3',4,4'-tetrachlorobiphenyl (tetraCB) (CB77) などの coplanar PCB に対する代謝能はラットに比べ，かなり低い[22]のに対し，2,4,5-三塩素置換 PCB に対する代謝能は逆にラットよりかなり高い[2)12]. 当研究室では，高蓄積性 PCB の CB153 や CB138 などの in vitro 代謝を調べ，その結果，モルモットではラットより数倍高い代謝活性を有すること，NIH 転位代謝物である 2-OH 体が主代謝物であること，さらに，これらの代謝に PB 誘導性 P450 である CYP2B18 が強く関与することを報告した[3)19].

しかしながら，2,4,5-三塩素置換 PCB の原型ともいえる CB118 の代謝に関する報告はほとんどない. Haraguchi ら[10]は CB118 投与ラットの糞中に，4 種類の OH 体，すなわち，4-OH-2,3',4',5-tetraCB (CB70)，4-OH-2,3,3',4',5-pentaCB (CB107)，4'-OH-2,3',4,5,5'-pentaCB (CB120) および 5'-OH-CB118 と 2 種類のメチルチオ (MeS) 体，すなわち 5'-および 6'-MeS-CB118 を検出した. また，4-OH-CB107 および 4'-OH-CB120 は特に血液中および肝，肺，腎などの臓器に高濃度分布していることも明らかにした. さらに，最近，Haraguchi ら[12]は Kanechlor 500 投与のラット，ハムスターおよびモルモットにおいて，代謝物の組織分布を比較したところ，ラットおよびハムスター血液中では CB118 由来と考えられる 4-OH-CB107 が高濃度検出されたが，モルモットではラットやハムスターとは全く異なり 3-OH-CB118 のみが検出されたと報告した. これらの結果は CB118 の in vivo 代謝において，大きな動物種差があることを示しているが，われわれがモルモット肝を用いて行った CB153 や CB138 の in vitro 代謝で報告した 2-OH 体について，CB118 では全く検出されていない. そこで本研究では，この点を明らかにするため，モルモット肝ミクロゾームを用いて CB118 の in vitro 代謝を調べた. また，代謝に関与する P 450 分子種を明らかにするため，代表的な P 450 誘導剤として知られている PB および MC で前処理した動物でも同様に検討した.

実験方法

1. 実験材料

（1）CB 118 の合成

CB 118 は Cadogan の方法[8]で合成した. まず，1,2-dichlorobenzene および 2,4,5-trichloroaniline を tetrachloroethylene で溶解し，さらに亜硝酸イソペンチルを加えて，110°Cで 24 時間反応させた. 反応物はアルミナカラム (100 g, Merck) およびシリカゲル 60 カラム (65 g, Merck) で精製した後，不純物を除くため，高速液体クロマトグラフィー (HPLC) に付した. HPLC 条件は次の通りである. カラム，ODS (250 × 20 mm i.d., 5 μm, YMC)；移動相，acetonitrile；流速，4 ml/min；検出波長，254 nm. CB118 の純度を電子捕獲型検出器付ガスクロマトグラフィー (GC/ECD) により調べた結果，最終的に 99.0 %以上であった.

CB 118：MS (EI) m/z (relative intensity) 324 (100) [M^+], 326 (163) [M^++2], 328 (99) [M^++4], 330(32) [M^++6], 254(40) [M^+- CH_3 Cl].

（2）代謝物の合成

2-OH-CB 126 の合成は，3,4-dichloroaniline と 2,3,4-trichlorophenol を合成原料として用い，Hutzinger らの方法[13]で行った．反応後，シリカゲル 60 カラム (65 g, Merck) および HPLC で精製した．なお，メチル化は，アルカリ性条件下，ジメチル硫酸の添加により行った[24]．一方，3-methoxy (MeO)-CB118 および 4-MeO-2,3,3',4',5-pentaCB (CB107) の合成は，Cadogan の方法[8]で行った．合成原料として 3,4-dichloroaniline と 2,3,6-trichloroaniline を用い，これらを tetrachloroethylene で溶解し，さらに亜硝酸イソペンチルを加えて，110°C で 24 時間反応させた．精製は上記と全く同様に，アルミナカラム (100 g, Merck)，シリカゲル 60 カラム (65 g, Merck) および HPLC で行った．また，6-MeO-CB77, 5-MeO-2,3,3',4'-tetraCB (CB56) および 6-MeO-CB56 の合成は，3,4-dichloroaniline と 3,4-dichlorophenol を合成原料として用い，Hutzinger らの方法[13]で行った．反応後，シリカゲル 60 カラム (65 g, Merck) および HPLC で精製した．なお，メチル化は，diazomethane により行った．

2-OH-CB126：^{1}H-NMR (500 MHz, chloroform-*d*) δ 7.52 (1H, d, J=8.3 Hz, 5'-H), 7.47 (1H, d, J=2.0 Hz, 2'-H), 7.28 (1H, s, 6-H), 7.22 (1 H, dd, J=8.3 Hz, 2.0 Hz, 6'-H), 3.95 (3H, s, 4-MeO).

2-MeO-CB126：MS (EI) m/z (relative intensity) 354(100) [M^+], 356(160) [M^++2], 358(109) [M^++4], 360 (38) [M^++6], 304 (103) [M^+-CH_3Cl], 284(26) [M^+-Cl_2], 241(35) [M^+-$COCH_3$ Cl_2].

3-MeO-CB118：MS (EI) m/z (relative intensity) 354(100) [M^+], 356(156) [M^++2], 358(93) [M^++4], 360 (35) [M^++6], 311 (53) [M^+-$COCH_3$], 241 (40) [M^+-$COCH_3Cl_2$].

4-MeO-CB107：MS (EI) m/z (relative intensity) 354(100) [M^+], 356(120) [M^++2], 358(82) [M^++4], 360 (35) [M^++6], 339 (48) [M^+-CH_3], 311 (45) [M^+-$COCH_3$], 241 (21) [M^+-$COCH_3Cl_2$]. ^{1}H-NMR(500 MHz, chloroform-*d*) δ 7.52 (1H, d, J=8.1 Hz, 5'-H), 7.47 (1 H, d, J=2.2 Hz, 2'-H), 7.28 (1H, s, 6-H), 7.22 (1H, dd, J=8.3 Hz, 2.0 Hz, 6'-H), 3.95 (3H, s, 4-MeO).

5-MeO-CB 56：MS (EI) m/z (relative intensity) 320(100) [M^+], 322(127) [M^++2], 324(43) [M^++4], 326 (8) [M^++6], 277 (14) [M^+-$COCH_3$], 207 (16) [M^+-$COCH_3Cl_2$].

6-MeO-CB 56：MS (EI) m/z (relative intensity) 320(100) [M^+], 322(132) [M^++2], 324(63) [M^++4], 326(13) [M^++6], 270(103) [M^+-CH_3 Cl], 207 (37) [M^+-$COCH_3Cl_2$].

6-MeO-CB 77：MS (EI) m/z (relative intensity) 320(100) [M^+], 322(132) [M^++2], 324(67) [M^++4], 326(15) [M^++6], 270(120) [M^+-CH_3 Cl], 207 (34) [M^+-$COCH_3Cl_2$].

2．薬物投与

Hartley 系雄性モルモット（体重約 300 g）に，P 450 誘導剤として PB (80 mg/kg/day) あるいは MC (20 mg/kg/day) を 3 日間腹腔内に投与した．PB は生理食塩水に，MC はコーン油に溶解した．モルモット肝ミクロゾームは，PB および MC の最終投与日の翌日に屠殺した後，肝を摘出し，常法により調製した．

3．代謝物の分析

モルモット肝ミクロゾームによる CB118 の代謝は既報[20]に準じて行った．すなわち，40 μM CB118, NADPH 生成系 (0.33 mM NADP, 5 mM glucose-6-phosphate, glucose-6-phosphate dehydrogenase 1.0 unit)，6 mM $MgCl_2$ およびモルモット肝ミクロゾーム (1 mg protein) を 100 mM HEPES 緩衝液 (pH 7.4) とともに合計 1 ml として，37°C で 30 min インキュベート後，代謝物を chloroform-methanol（2：1）1 ml と *n*-hexane 3 ml でそれぞれ 3 回ずつ抽出した．抽出液は diazomethane でメチル化後，GC / ECD に付した．GC/ECD の条件は次の通りである．分析機器，ECD 付 HP 5890 Series II ガスクロマトグラフ (Hewlett-Packard 製)；カラム，DB-1 fused silica capillary column (30 m × 0.25 mm

i.d., 0.25 μm 膜厚, J & W Scientific 製) ; オーブン温度, 220°C ; 注入口温度, 250°C ; 検出器温度, 250°C ; キャリアーガス, N_2 (1 ml/min).

一方, 代謝物の質量分析 (GC/MS) は質量分析計付ガスクロマトグラフ (Agilent 5973 inert MSD, Agilent 製) を用いて, EI モードで行った. 分析条件は次の通りである. カラム, HP-5 fused silica capillary column (60 m × 0.25 mm i.d., 0.25 μm 膜厚, Agilent 製) ; オーブン温度, 70°C (1.5 min) - 20°C/min - 230°C (0.5 min) - 4°C/min - 280°C (5 min) ; 注入口温度, 250°C ; 検出器温度, 230°C ; キャリアーガス, He (1 ml/min).

^{1}H-NMR スペクトルの測定は日本電子製の JEOL GSX-500 (500 MHz) を用いて行った. 試料は, chloroform-*d* に溶解し, tetramethylsilane を内部標準物質として用いた.

4. 抗体阻害実験

モルモット肝 P450 (CYP2B18) に対する抗血清をウサギで調製した[21]. 抗血清を 100 mM HEPES 緩衝液 (pH 7.4) 中で, 肝ミクロゾームとともに, 室温で 30 min 放置した. 抗血清の添加量は, 50, 100 および 150 μl とした. 次に, CB118 および $MgCl_2$ を添加後, NADPH 生成系を加えることにより, インキュベートを開始した. 代謝物の分析は, 上記の通りである.

5. その他

肝ミクロゾームのタンパク質の定量は, Lowry らの方法[25] に従い行った. なお, 標準タンパク質としてウシ血清アルブミンを用いた.

実 験 結 果

1. 肝ミクロゾームによる CB118 の代謝

CB118 を, 未処理, PB 前処理あるいは MC 前処理モルモットから調製した肝ミクロゾームとともに, NADPH 存在下, 37°C, 30 min インキュベートした. 有機溶媒抽出物をメチル化後, GC/ECD に供した結果, いずれの肝ミクロゾームによっても, 未変化体 (保持時間 12.80 min) 以外に, 代謝物と思われる 2 本のピークが保持時間 15.84 min および 20.01 min に検出された (Fig. 1). これを以下, それぞれ M-1 および M-2 とする. PB 前処理ミクロゾームの場合, M-2 が未処理ミクロゾームの 6.8 倍に著しく増加した. また, M-1 も 2.2 倍に増加した. 一方, MC 前処理ミクロゾームの場合にも, M-2 は未処理ミクロゾームの 2.6 倍に増加したが, M-1 は逆に 50 %以下に減少した.

2. 代謝物の化学構造

両代謝物の化学構造を明らかにするために, PB 前処理ミクロゾームを用いて 100 倍量 (100 ml) でインキュベーションを行った後, 代謝物を抽出し, さらにメチル化後, GC/MS により, M-1 および M-2 の分子量を調べた. その結果, M-1 および M-2 はそれぞれ分子量 320 および 354 を有することが明らかとなった (Table 1). これらの結果から, M-1 は塩素が 1 個脱離した OH-tetraCB であること, 一方, M-2 は CB118 の OH 体であることが明らかとなった. さらに, 両代謝物のメチル誘導体のマススペクトルにおいて, いずれもフラグメントイオン [M^+-50] が非常に多いことから, ortho 位すなわち 2 (6) 位または 2' (6') 位に MeO 基を有していることが示唆された. これまでの報告[1)18)20)] によると, ビフェニル骨格の para 位すなわち 4 (4') 位に MeO 基が置換されている場合にはフラグメントイオン [M^+-15] が, また meta 位すなわち 3 (5) 位あるいは 3' (5') 位に MeO 基が置換されている場合にはフラグメントイオン [M^+-43] が多く検出されることが知られている. そこで, M-1 および M-2 がそれぞれ 6-OH-CB 77 と 2-OH-CB 126 であると予想し, これらを別途合成した. その結果, 主代謝物M-2 (メチル化体) の GC 保持時間 15.43 min は, 2-MeO-CB126 のそれと完全に一致した (Table 1). また, マスフラグメンテーションもほとんど一致した. なお, 合成標品 3-MeO-CB 118 および 4-MeO-CB107 は, GC 保持時間がそれぞれ 15.45 min と 15.51 min であり, また, マスフラグメンテーションも M-2 (メチル化体) と異なっていた. 次に, M-1 (メチル化体) について, 合成標品の 6-MeO-CB77 と比較したところ, 分子量およびマスフラグメンテーションはほぼ一致したが, GC 保持時間では 0.03 min 遅く検出され完全には一致しなかった. 以上の結果から, M-2 は 2 位の塩素が 3 位に NIH 転位をした 2-OH-CB 126

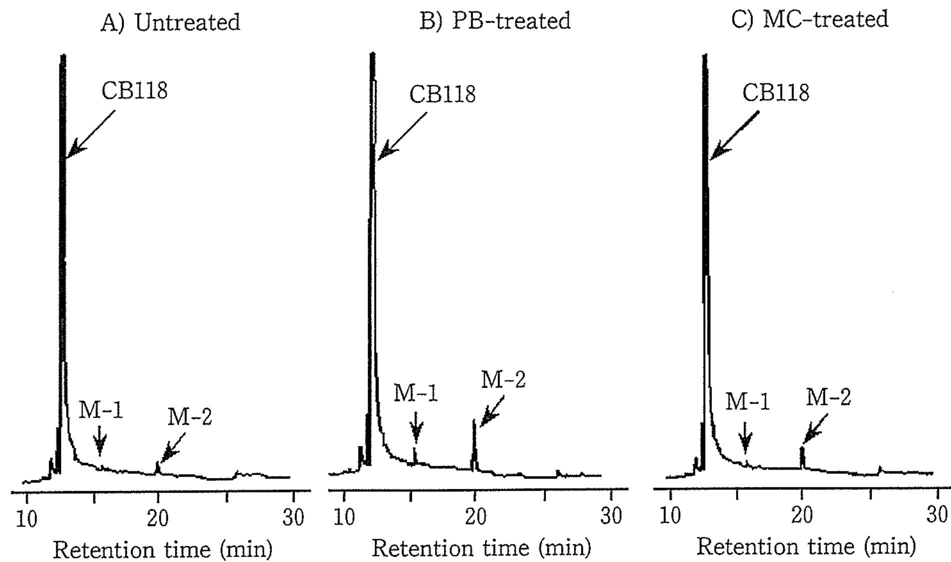

Fig. 1 Gas chromatograms of methylated derivatives of CB118 metabolites formed by liver microsomes of untreated (A), PB-treated (B) and MC-treated (C) guinea pigs.
Column for GC/ECD used was DB-1 capillary column (30 m long).

であると推定されたものの，M-1についての正確な化学構造は未定となった．

3．抗CYP2B18抗血清による代謝阻害

モルモット肝P450のCYP2B18は，PB誘導性P450としてだけでなく，未処理肝ミクロゾームにおいて主要なP450としても知られている[19)30)]．そこで，CB118代謝におけるCYP2B18の寄与を明らかにするために，このP 450に対する抗血清を用いて，主代謝物M-2の生成阻害を試みた．なお，肝ミクロゾームは最もM-2生成活性が高かったPB前処理モルモット肝ミクロゾームを用いた．その結果，抗血清150 μlの添加で，M-2の生成はほとんど完全に阻害された（Fig. 2)．これらの結果から，モルモット肝におけるM-2の生成にはCYP2B18が強く関与していることが明らかとなった．

考　　察

今回，CB118のモルモット肝ミクロゾームによるin vitro代謝を調べたところ，2種類の代謝物(M-1およびM-2）が生成された．さらに，GC/

Table 1 Mass spectral data and retention times of methylated derivatives of two metabolites and six synthetic compounds in GC/MS

Compound	Molecular weight	Mass spectral data				Retention time (min)
		[M⁺]	[M⁺-15]	[M⁺-43]	[M⁺-50]	
M-1	320	100	—	—	108	14.40
M-2	354	100	—	—	103	15.43
6-MeO-CB 56	320	100	—	—	103	13.72
5-MeO-CB 56	320	100	—	13	—	14.32
6-MeO-CB 77	320	100	—	—	120	14.37
2-MeO-CB 126	354	100	—	—	103	15.43
3-MeO-CB 118	354	100	—	53	—	15.45
4-MeO-CB 107	354	100	48	45	—	15.51

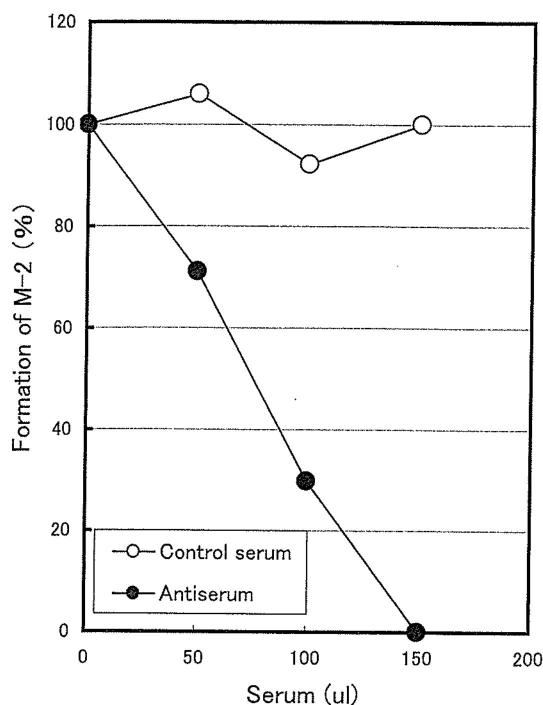

Fig. 2　Effect of antiserum against CYP2B18 on CB118 metabolism by liver microsomes from PB-treated guinea pigs.
Open and closed circles indicated control serum and antiserum against CYP2B18, respectively. Each point represents the mean of duplicate determinations.

MS により，M-1 は塩素が 1 個脱離し，かつ ortho 位に水酸基が置換された OH-tetraCB であること，また M-2 は 2-OH-CB126 であることが明らかとなった．これまでに，われわれは CB153 や CB138 などの 2,4,5-三塩素置換 PCB の代謝をモルモットで調べ，主代謝物として NIH 転位代謝物の 2-OH 体が生成されることを報告している[2)20)]．今回の CB118 の場合も，NIH 転位代謝物の 2-OH 体が主代謝物であったことから，モルモット肝における CB 118 の代謝は，CB153 や CB138 と同様に，まず中間体として 2,3-epoxide の生成，さらには 2 位の塩素の 3 位への NIH 転位を経て進行することが示唆された（Fig. 3）.

前述のように，Haraguchi らは CB 118 投与ラットにおいて，4-OH-CB107, 4'-OH-CB120 および 3-OH-CB118 が生成されること，このうち前 2 者は主に血液に局在することを報告している[10)]．さらに，Kanechlor 500 を腹腔内投与したモルモット血中では，3-OH-CB118 のみが局在していることも報告した[12)]．これらの代謝物のうち，4-OH-CB107 は，CB118 からだけではなく CB 105 からも生成されること[10)16)]，また血中での半減期が比較的長い主要な PCB 代謝物の 1 つであることが明らかにされている[26)29)]．さらには，ヒト

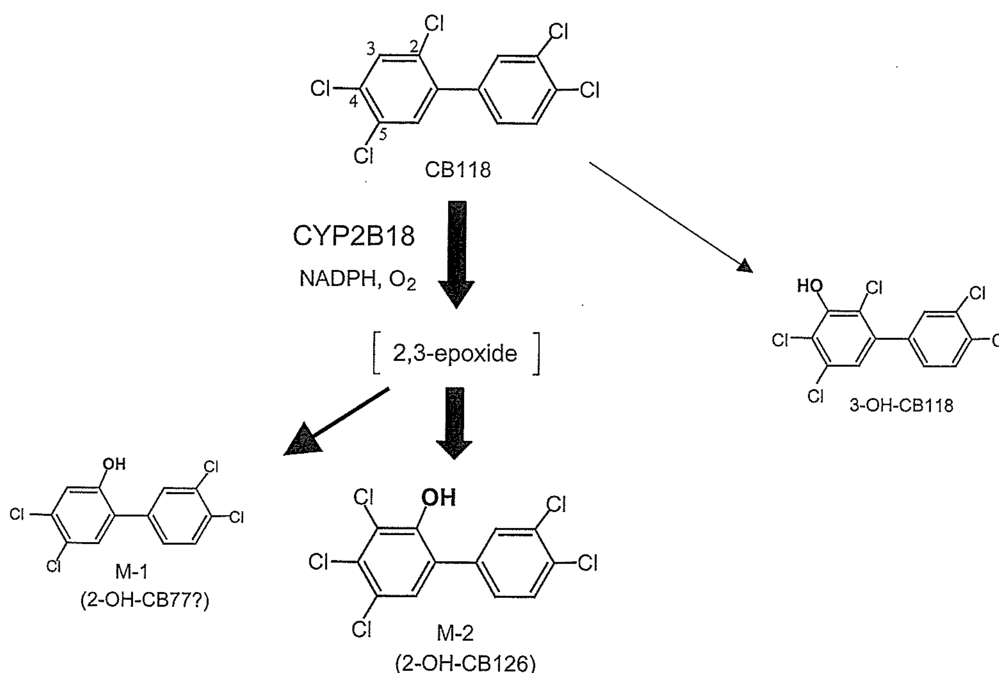

Fig. 3　Postulated metabolic pathways of CB118 in guinea pig liver.

エストロゲン硫酸転移酵素に対し強い阻害活性を有する[15]ことやラット胎児への神経毒性を示す[27]ことからも，注目されている．しかしながら，今回モルモット肝ミクロゾームを用いたin vitro代謝系では，4-OH-CB107および4'-OH-CB120は全く生成されなかった．ただ，3-OH-CB118についてはlarge scale（100 ml）で行ったときに，痕跡程度検出されたにすぎなかった（データ未掲載）．このように，in vitro代謝で生成された2-OH体がなぜin vivo代謝でみられなかったのか現在不明であるが，代謝物の局在性が異なっていることによるのかもしれない．すなわち，2-OH体はそのほとんどが血液以外の組織に分布したり，糞中へと排泄されることによるのかもしれない．今後，CB118投与モルモットにおける代謝物の生体内分布と糞中への排泄を調べることが必要であろう．

PCB代謝物として，脱塩素化されたOH体がしばしば報告されている．Di-ortho-PCBのCB 138[20]，CB 153[1)~3)]および2,2',4,4',6,6'-hexaCB（CB155）[4)14)]，またtri-ortho-PCBのCB187[31]だけではなく，coplanar PCBのCB77[35)36)]でもみられている．しかしながら，この生成機構に関しては，P450による酸化反応に伴い生成されるという事実を除き，ほとんど不明のままである．本研究のCB118代謝においても，同様に脱塩素化された代謝物M-1が生成された．マススペクトルより，ortho位にOH基が入ったtetraCBであることから，6-OH-CB77であろうと予想された．そこで，これを合成しM-1と比較したが，期待に反してGC保持時間が一致しなかった．以上のことから，M-1の正確な化学構造については現在不明であるが，モルモット肝ミクロゾームに存在する酵素系は，この酸化的脱塩素化機構を検討するのに有益であろう．

CB153やCB138の代謝に関与するP450分子種として，モルモット肝ではPB誘導性のCYP2B18が最も重要であることが明らかになっている[2)3)20)]が，抗2B18抗血清を用いてCB118の代謝阻害を試みたところ，M-2の生成がほぼ完全に阻害された．この結果から，CB118を含め2,4,5-三塩素置換PCBの代謝では，CYP2B18が強く関与していることが示唆された．一方，MC前処理ミクロゾームにより，M-2の生成が未処理ミクロゾームの約2倍に増加したが，この事実はMC誘導性のP450，特にCYP1A1あるいはCYP1A2の関与を示唆している．しかしながら，モルモット肝において，これらのMC誘導性P450がPCB代謝に関与するかどうかは全く報告がなく，今後の研究課題である．

総　　括

1．未処理，PBおよびMC前処理モルモット肝ミクロゾームによるCB118の代謝を調べた．その結果，2種類の代謝物（M-1およびM-2）が生成された．これらのうち，主代謝物のM-2は合成標品との比較により，2-OH-CB126であることが明らかとなった．また，M-1はマススペクトルより，ortho位置換OH- tetraCBであることが明らかとなった．

2．CB118代謝に及ぼすP450誘導剤の影響を調べたところ，PB前処理によりM-1は未処理の2.2倍，M-2は6.8倍に増加した．また，MC前処理でもM-2は2.6倍に増加したが，M-1は逆に50％以下に減少した．

3．抗モルモットCYP2B18抗血清を用いて，主代謝物M-2の代謝阻害を試みたところ，抗血清150 μlの添加で，M-2の生成はほとんど完全に阻害された．

以上の結果から，モルモットにおいて，CB118は主にCYP2B18によって代謝され，中間体として2，3-epoxideを経由した後，NIH転位を経て，主代謝物2-OH-CB126へと変換されることが示唆された．

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In utero and lactational exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) affects tooth development in rhesus monkeys

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Introduction

The current tolerable daily intake (TDI) of dioxin and dioxin related compounds has been set at 4 pg TEQ/kg/day in Japan. This value was calculated from the lowest-observed-adverse-effect level (LOAEL) in experimental animals, mostly rodents. Gray *et al.* reported that a single oral dose of 200 ng/kg of 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) to pregnant rats on day 15 of gestation resulted in abnormalities of reproductive organs in the offspring[1]. The maternal body burden at this dose was measured to be 86 ng/kg. To attain this body burden level, human daily intake was calculated to be 43.6 pg/kg/day. An uncertainty factor of 10 was applied to this value, and the human TDI was established. However, due to great differences in the biological half life of TCDD between human and rodents, the validity of this calculation is questioned. To obtain more reliable LOAEL in the second generation, we initiated a long-term study in rhesus monkeys in 1999.

In rodents, teeth are known to be targets of developmental toxicity of dioxin. *In utero* and lactational TCDD exposure affects rat incisor and molar developmet[2,3]. In humans also tooth abnormalities were reported among populations exposed to dioxins[4]. In our monkey experiment, some young were stillborn or died neonatally. These animals provided us with a unique opportunity to study tooth development in primate young exposed to TCDD *in utero* and lactationally. By macroscopic observation we found some tooth abnormalities among died young exposed to TCDD[5]. This prompted us to examine surviving young by radiography. This is an interim report of our findings in these young.

Methods and Materials

Animals: Adult female rhesus monkeys at the age of 5-7 years and weighing 4-6 kg purchased from China National Scientific Instruments & Materials Import/Export Corporation (Beijin, China) were used. Details of breeding conditions were given elsewhere[6]. Female monkeys were allowed to cohabit with males for three days on days 12, 13, and 14 of the menstrual cycle. When copulation was confirmed visually, the median day of the mating period was disignated as day 0 of gestation (GD 0). On GD18 or 19, pregnancy was confirmed by an ultrasound device. Pregnant monkeys were divided into three groups each consisting of approximately 20 animals and allowed to deliver naturally. The day on which delivery was detected was designated as postnatal day 0 (PD0).

Administration of TCDD: TCDD was dissolved in a mixture of toluene/DMSO (1:2, v/v) at a concentration of 300 ng/ml. Pregnant females were given TCDD subcutaneously into the back region on day 20 of gestation at an initial dose level of 30 or 300 ng/kg. The control animals received the vehicle in a volume of 1 ml/kg. For maintenance of a certain body burden, 5% of the initial dose, i.e. 0.6 or 6 ng/kg, was given to dams every 30 days during pregnancy and lactation until day 90 after birth.

Measurement of TCDD in maternal serum: Approximately 20 ml of blood was taken from the femoral vein of the dams on day 80 of pregnancy, and centrifuged. The obtained serum was subjected to high resolution gas chromatography (HRGC)/high resolution mass spectrometry (HRMS) by the method of Patterson *et al.*[7]

Observation of teeth of the young: Stillborn and postnatally died young were autopsied, and the upper and lower jaws were dissected for detailed observation. Surviving young were anesthesized by intramuscular injection of ketamine at 10 mg/kg into the thigh before examination. Photographs were taken by an intraoral digital camera (Crystal Cam II, GC Co., Ltd., Tokyo). Conventional intraoral radiographs were taken by a portable X-ray apparatus (KX-60, Asahi Roentgen Ind. Co., Ltd., Kyoto) with a charge coupled device (CCD) (Gendex Visualix, Dentsply International Inc., York, PA, USA).

Results and Discussion

Pregnancy outcome and postnatal development of the young: Table 1 summarizes the pregnancy outcome and postnatal mortality of the young. Abortions, stillborns, and postnatal deaths occurred fairly frequently even in the control group. To increase the number of surviving young in the 300 ng/kg, we added 9 dams to the group approximately 2 years after the initiation of the experiment. However, only two young survived more than a year due to a high incidence of abortions. No significant differences were noted in the gestation length and birth weight among the control and TCDD-treated groups, indicating the body burden of TCDD at 300 ng/kg did not affected general growth of the young.

Table 1: Pregnancy outcome and postnatal mortality of rhesus monkeys exposed to TCDD.

Group	No. of dams	No. of abortions	No. of stillborns	No. of live borns	No. of postnatal deaths	Gestation length (days)	Birth weight (g)
Control	23	2	3	18	5	161.8±7.8	426.1±58.6
30 ng/kg	20	0	5	15	3	163.8±5.9	426.8±56.9
300 ng/kg	20	2	2	16	8	164.9±9.7	408.6±63.7
300 ng/kg[1)]	9	5	1	3	1	165.0±3.0	466.0±87.1

1) Newly added group

Tooth abnormalities in the young: The incidence of tooth abnormalities in the young was shown in Table 2. Tooth abnormalities in the stillborn and postnatally died young were described previously[5]. No abnormalities were detected in the control and 30 ng/kg groups, whereas more than half of the young in the 300 ng/kg had tooth abnormalities as listed in Table 3. The upper permanent lateral incisors were most frequently affected. In contrast, among the deciduous teeth, the central incisors seemed to be most sensitive targets of developmental toxicity of TCDD. The permanent premolars were also affected frequently, while the canine and the first molar were resistant to the adverse effect of TCDD. Probably these larger teeth have become resistant to odontotoxic chemicals during the course of evolution.

Table 2: Incidence of tooth abnormalities among F1 exposed to TCDD.

Group	Stillborns and postnatally died young			Surviving young		
	No. of specimens	No. of specimens with tooth abnormalities (%)		No. of young	No. of young with tooth abnormalities (%)	
Control	4	0	(0)	13	0	(0)
30 ng/kg	5	0	(0)	12	0	(0)
300 ng/kg	8	3	(38)	8	6	(75)
300 ng/kg[1)]	2	0	(0)	2	1	(50)

1) Newly added group

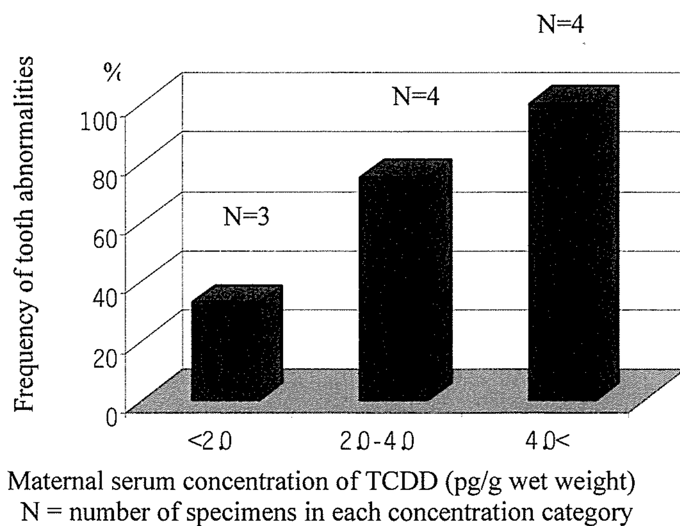
Relationship between maternal serum TCDD concentration and occurrence of tooth abnormalities: In the control maternal serum, the TCDD levels were below the detection limit. In the 30 ng/kg group, the levels were fairly constant, ranging from 0.19 to 0.21 pg/g wet weight. In contrast, the levels varied largely in the 300 ng/kg group, ranging from 1.1 to 8.9 pg/g wet weight. The average of those without tooth abnormalities in their young was 1.4 ± 0.6 pg/g wet weight, whereas that with tooth abnormalities was 4.3 ± 2.4 pg/g wet weight. The concentration-response relationship is shown in Fig. 1.

Table 3: Tooth abnormalities detected in the young exposed to TCDD at 300 ng/kg.

Young	Sex	Age (days) [2]	Abnormal Findings
31	♀	1430	542|24 missing |5 conical
39	♂	1410	542|245 missing
42	♀	1415	5|5 missing |4 conical
44	♂	1415	54|45 missing |5 conical
60	♂	1388	542|245 5|5 missing
66	♂	1338	52|2 1|1 missing
			54|45 malaligned |45 conical
106 [1]	♀	688	A|A 4|24 missing

1) Newly added group
2) Age at X-ray examination

Figure 1: Maternal serum concentration of TCDD and the incidence of tooth abnormalities.



Validity of the current TDI: The above results indicate that the LOAEL body burden for induction of tooth abnormalities in the rhesus monkey is at a certain level between 30 ng/kg and 300 ng/kg, probably not much different from the LOAEL body burden for rodents, 86 ng/kg. Hence it is reasonable to conclude that the current TDI of dioxins in Japan needs no immediate modification.

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Metabolism of Polychlorinated Biphenyls by Gunn Rats: Identification and Serum Retention of Catechol Metabolites

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The tissue distributions of persistent metabolites of polychlorinated biphenyls (PCBs) in Wistar rats and homozygous uridine diphosphate glucuronosyltransferase (UGT) deficient Gunn rats exposed to 2,4,5,2′,5′-pentachlorobiphenyl (CB101) and the commercial PCB mixture, Kanechlor-500 (KC500), were investigated. After exposure to CB101, four hydroxy and two methylsulfonyl ($MeSO_2$) metabolites were detected in liver, lung, kidney, blood, and adipose tissues. One was identified as 3′,4′-$(OH)_2$-2,4,5,2′,5′-pentaCB, which was retained selectively in the serum of Gunn rats. Comparative analysis of the metabolite profiles in both rat strains after exposure to KC500 showed higher formation ratios of several dihydroxy PCB metabolites in the liver of Gunn rats; major metabolites are the catechols from 2,5,3′,4′-tetraCB, CB101, 2,3,6,3′,4′-pentaCB, and 2,3,6,2′,4′,5′-pentaCB. Thus, Gunn rats effectively metabolized PCBs with 2,5- or 2,5,6-chlorine substitution to the 3,4-catechol, but less formed $MeSO_2$ metabolites in the liver. Although both rat strains retained 4-OH-2,3,5,3′,4′-pentaCB in serum, Gunn rats also retained the catechol PCBs, accounting for about 52% of the total phenolic PCBs. These results suggest that a lack of UGTs markedly alters the formation ratios and retention profiles of catechols and $MeSO_2$ metabolites of PCBs.

Introduction

Polychlorinated biphenyls (PCBs)[1] are ubiquitous environmental contaminants that exhibit specific toxicity in different animals (*1*). PCBs undergo biotransformation by cytochrome P450 (CYP) to hydroxylated metabolites (*2*). Further metabolism involves the formation of catechol and quinone products (*3*) or glucuronidation (*4*), conjugation of arene oxide intermediates with glutathione, and subsequent formation of methylsulfonyl PCBs ($MeSO_2$-CBs) (*5*).

Some of the phenolic PCBs and $MeSO_2$-CBs have been shown to persist in blood or tissues of laboratory animals (*6*) as well as in humans (*7–9*). Recent concerns have focused on the action of these metabolites as endocrine disrupters. The hormone disruption may be due to the induction or inhibition of enzymes responsible for the metabolism of PCBs or PCB metabolites (*10*, *11*). Phenolic PCBs with one or more chlorine atoms adjacent to the hydroxyl group bind competitively to the thyroxine (T4) transporting protein, transthyretin (TTR), with high affinity (*12*) and alter thyroid hormone metabolism (*13*). Some hydroxylated PCBs are known to interact with estrogen receptors (*14*) or with sulfotransferases that sulfate estrogen (*11*). Similarly, several $MeSO_2$-CB metabolites reduce thyroid hormone levels in rats (*15*) and also exhibit antiestrogenicity in vitro (*16*).

Catechol PCB metabolites have been reported to be formed in rodents exposed to PCBs (*3*, *17*). Garner et al. (*18*) demonstrated that in vitro estrogenicity of catechol PCB metabolites was within the range of effects observed for phenolic PCBs. In addition, catechols can potentially be metabolized by peroxidases to quinones that are responsible for the formation of DNA adducts and exhibit cytotoxicity (*19*, *20*).

Generally, phenolic PCBs undergo detoxification by glucuronidation catalyzed by the uridine diphosphate glucuronosyltransferases (UGTs). A number of PCBs and PCB metabolites have been shown to induce hepatic UGTs (*21–23*) to facilitate excretion of PCBs. Although the increased glucuronidation resulted in the decreased serum T4 levels because of the increased clearance (*24*), a decrease in serum T4 levels also occurred in PCB-exposed Gunn rats, a mutant strain of Wistar rats

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[1] Abbreviations: PCBs, polychlorinated biphenyl; CYP, cytochrome P450; $MeSO_2$-CBs, methylsulfonyl PCBs; T4, thyroxine; TTR, transthyretin; UGT, uridine diphosphate glucuronosyltransferase; CB101, 2,4,5,2′,5′-pentachlorobiphenyl; KC500, Kanechlor-500; CB, chlorobiphenyl; SIM, selected ion monitoring; ECD, electron capture detector; diOH-CB101, 3′,4′-$(OH)_2$-2,4,5,2′,5′-pentachlorobiphenyl; diOH-CB70, 3,4-$(OH)_2$-2,5,3′,4′-tetrachlorobiphenyl; diOH-CB87, 3′,4′-$(OH)_2$-2,3,4,2′,5′-pentachlorobiphenyl; diOH-CB110, 4,5-$(OH)_2$-2,3,6,3′,4′-pentachlorobiphenyl; diOH-CB132, 4′,5′-$(OH)_2$-2,3,4,2′,3′,6′-hexachlorobiphenyl; 3-OH-CB99, 3-OH-2,4,5,2′,4′-pentachlorobiphenyl; diOH-CB149, 4,5-$(OH)_2$-2,3,6,2′,4′,5′-hexachlorobiphenyl; 4-OH-CB107, 4-OH-2,3,5,3′,4′-pentachlorobiphenyl; 3′-OH-CB138, 3′-OH-2,3,4,2′,4′,5′-hexachlorobiphenyl; CB105, 2,3,4,3′,4′-pentachlorobiphenyl; CB118, 2,4,5,3′,4′-pentachlorobiphenyl; CB138, 2,3,4,2′,4′,5′-hexachlorobiphenyl; CB146, 2,3,5,2′,4′,5′-hexachlorobiphenyl; CB153, 2,4,5,2′,4′,5′-hexachlorobiphenyl; CB156, 2,3,4,5,3′,4′-hexachlorobiphenyl; CB167, 2,4,5,3′,4′,5′-hexachlorobiphenyl; CB170, 2,3,4,5,2′,3′,4′-heptachlorobiphenyl; CB180, 2,3,4,5,2′,4′,5′-heptachlorobiphenyl; CB187, 2,3,5,6,2′,4′,5′-heptachlorobiphenyl.

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deficient in several forms of UGTs (*25*). Tampal et al. (*4*) demonstrated that phenolic PCB metabolites that persist in the body are poor substrates for hepatic UGTs and resist conjugation. Therefore, it would be of interest to determine the persistent PCB metabolite profiles in relation to UGT deficiency.

The present study was performed to investigate whether Gunn rats (UGT1A deficient Wistar rats) exposed to PCBs show altered formation ratios of phenolic PCB metabolites as compared to Wistar controls. Several catechol PCB metabolites were isolated from Gunn rats dosed with PCBs. This paper describes the identification and characterization of three kinds of persistent metabolites (monohydroxy, dihydroxy, and methylsulfonyl) in the liver and blood of Gunn and Wistar rats exposed to a single dose of 2,4,5,2′,5′-pentachlorobiphenyl (CB101) and the commercial PCB mixture, Kanechlor-500 (KC500).

Materials and Methods

Caution: *Synthetic PCBs and their metabolites should be considered potentially toxic and hazardous and therefore should be handled in an appropriate manner.*

Chemicals. CB101 was synthesized using the Cadogan coupling reaction (*26*). Methoxy PCBs were synthesized according to the method reported by Bergman et al. (*27*). $MeSO_2$-CBs were synthesized as described previously (*28*). Methylated derivatives of catechols (veratrole PCBs) were prepared and characterized as follows. 4-Amino veratrole was treated with chlorobenzene to yield veratrole chlorobiphenyls (CBs), which were further chlorinated by sodium chlorate in hydrochloric acid. The products were separated on an ODS semipreparative column, eluted with acetonitrile/water (8:2, v/v). The purities of isomers were determined to be >99% by gas chromatography. The mass spectral and ^{1}H NMR data were characterized as follows.

3,4-Dimethoxy-2,5,3′,4′-tetrachlorobiphenyl (diOH-CB70 Derivative). ^{1}H NMR (500 MHz, chloroform-*d*): δ 3.95 (s, 3H, OCH_3), 3.97 (s, 3H, OCH_3), 7.12 (s, 1H, 6-H), 7.25 (dd, J = 2.0, 8.6 Hz, 1H, 6′-H), 7.28 (d, J = 2.0, 1H, 2′-H), 7.50 (d, J = 8.6 Hz, 1H, 5′-H). MS (EI) *m/z* (relative intensity): 350 (87) [M^+], 352 (100) [M^+ + 2], 374 (50) [M^+ + 4], 335 (45) [$M^+ - CH_3$], 307 (20) [$M^+ - COCH_3$], 292 (20) [$M^+ - COCH_3 - CH_3$], 272 (25) [$M^+ - COCH_3Cl$], 194 (25) [$M^+ - (COCH_3Cl)_2$].

3′,4′-Dimethoxy-2,3,4,2′,5′-pentachlorobiphenyl (diOH-CB87 Derivative). ^{1}H NMR (500 MHz, chloroform-*d*): δ 3.99 (s, 3H, OCH_3), 4.01 (s, 3H, OCH_3), 7.12 (s, 1H, 6-H), 7.47 (d, J = 8.1 Hz, 1H, 5′-H), 7.47 (d, J = 8.1 Hz). MS (EI) *m/z* (relative intensity): 384 (68) [M^+], 386 (100) [M^+ + 2], 388 (65) [M^+ + 4], 369 (25) [$M^+ - CH_3$], 341 (10) [$M^+ - COCH_3$], 326 (10) [$M^+ - COCH_3 - CH_3$], 306 (20) [$M^+ - COCH_3Cl$], 228 (15) [$M^+ - (COCH_3Cl)_2$].

3′,4′-Dimethoxy-2,4,5,2′,5′-pentachlorobiphenyl (diOH-CB101 Derivative). ^{1}H NMR (500 MHz, chloroform-*d*): δ 3.96 (s, 3H, OCH_3), 3.99 (s, 3H, OCH_3), 7.05 (s, 1H, 6-H), 7.35 (s, 1H, 6′-H), 7.59 (s, 1H, 3′-H). MS (EI) *m/z* (relative intensity): 384 (70) [M^+], 386 (100) [M^+ + 2], 388 (70) [M^+ + 4], 369 (20) [$M^+ - CH_3$], 341 (18) [$M^+ - COCH_3$], 326 (15) [$M^+ - COCH_3 - CH_3$], 306 (22) [$M^+ - COCH_3Cl$], 228 (20) [$M^+ - (COCH_3Cl)_2$].

4,5-Dimethoxy-2,3,6,3′,4′-pentachlorobiphenyl (diOH-CB110 Derivative). ^{1}H NMR (500 MHz, chloroform-*d*): δ 3.94 (s, 3H, OCH_3), 3.98 (s, 3H, OCH_3), 7.07 (dd, J = 8.3, 2.0 Hz, 1H, 6′-H), 7.33 (d, J = 2.0 Hz, 1H, 2′-H), 7.55 (d, J = 8.3 Hz, 1H, 5′-H). MS (EI) *m/z* (relative intensity): 384 (70) [M^+], 386 (100) [M^+ + 2], 388 (68) [M^+ + 4], 369 (25) [$M^+ - CH_3$], 341 (20) [$M^+ - COCH_3$], 326 (18) [$M^+ - COCH_3 - CH_3$], 306 (15) [$M^+ - COCH_3Cl$], 228 (22) [$M^+ - (COCH_3Cl)_2$].

4′,5′-Dimethoxy-2,3,4,2′,3′,6′-hexachlorobiphenyl (diOH-CB132 Derivative). ^{1}H NMR (500 MHz, chloroform-*d*): δ 3.95 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 7.04 (d, J = 8.4 Hz, 1H, 6′-H), 7.50 (d, J = 8.4 Hz, 1H, 5′-H). MS (EI) *m/z* (relative intensity): 418 (50) [M^+], 420 (100) [M^+ + 2], 422 (80) [M^+ + 4], 403 (20) [$M^+ - CH_3$], 375 (15) [$M^+ - COCH_3$], 360 (15) [$M^+ - COCH_3 - CH_3$], 340 (18) [$M^+ - COCH_3Cl$], 262 (22) [$M^+ - (COCH_3Cl)_2$].

4,5-Dimethoxy-2,3,6,2′,4′,5′-hexachlorobiphenyl (diOH-CB149 Derivative). ^{1}H NMR (500 MHz, chloroform-*d*): δ 3.95 (s, 3H, OCH_3), 4.00 (s, 3H, OCH_3), 7.30 (s, 1H, 6′-H), 7.63 (s, 1H, 3′-H). MS (EI) *m/z* (relative intensity): 418 (50) [M^+], 420 (100) [M^+ + 2], 422 (82) [M^+ + 4], 403 (25) [$M^+ - CH_3$], 375 (12) [$M^+ - COCH_3$], 360 (20) [$M^+ - COCH_3 - CH_3$], 340 (22) [$M^+ - COCH_3Cl$], 262 (35) [$M^+ - (COCH_3Cl)_2$].

Animal Treatments. Male Wistar rats (160−200 g) and homozygous Gunn rats (190−260 g) were obtained from Japan SLC., Inc. (Shizuoka, Japan). Animals were housed three or four per cage with free access to commercial chow and tap water and maintained under a 12 h dark/light cycle (lights on, 08:00−20:00) in an air-controlled room (temperature, 24.5 ± 1 °C; humidity, 55 ± 5%). Groups of six rats received an intraperitoneal injection of CB101 (112 mg/kg) and KC500 (100 mg/kg each) dissolved in Panacete 810, a mixture of glycerides of medium chain fatty acids (Nippon Oils and Fats Co., Tokyo) (5 mL/kg). All rats were killed by decapitation on day 4 after the dosing, and the liver, kidney, brain, lung, blood, and adipose tissues were removed and weighed. After the blood clotted at room temperature, serum was separated by centrifugation and stored at −20 °C prior to analysis of PCB metabolites.

Isolation of Metabolites. Sample cleanup and quantification were carried out according to our methods reported previously (*29*). Briefly, tissue samples were homogenized with acetone/*n*-hexane (2:1, v/v). Three internal standards (2,3,4,5,6,3′,4′,5′-octaCB, 70 ng; 4-hydroxy-2,3,5,6,2′,3′,4′,5′-octachloro[^{13}C]-biphenyl, 50 ng; and 3-methylsulfonyl-4-methyl-5,2′,3′,4′,5′-pentaCB, 28 ng) were added to each extract, which was subjected to a gel permeation column packed with Bio-Beads S-X3 (50 g, Bio-Rad Laboratories, Hercules, CA). Dichloromethane/*n*-hexane (1:1, v/v) was used as a mobile phase at a flow rate of 4 mL/min. The metabolite fraction (120−200 mL) was partitioned between *n*-hexane and a 1 M KOH/ethanol (5:2, v/v) solution. The aqueous solution was acidified with HCl and then extracted with *n*-hexane/diethyl ether (9:1) for acidic metabolites, which were subsequently methylated by an excess of diazomethane. The neutral fraction was applied to a silica gel mini-column (1 g, Wakogel S-1, Wako Pure, Co. Ltd., Japan), eluted with *n*-hexane (10 mL) for PCBs and successively with dichloromethane (10 mL) for $MeSO_2$-CBs. Mean recoveries (n = 3) of three internal standards spiked into control liver tissues at 50 ng each were 91% for PCBs, 85% for phenolic PCBs, and 88% for $MeSO_2$-CBs.

Identification and Quantification. For CB101 metabolites, quantification was performed on a GC-14A (Shimadzu Co., Kyoto, Japan) instrument equipped with a ^{63}Ni electron capture detector (ECD) and a DB-5 capillary column (60 m × 0.25 mm, i.d. J&W Scientific, United States). Injection was carried out in the splitless mode. Temperature program: 100 °C (2 min), 100−250 °C at 20 °C/min, 250−280 °C at 2 °C/min. Composition analyses of KC500 and its metabolites were carried out on a GC/MS system (AOC-17, GC-17A, QP-5000, Shimadzu, Co., Ltd.) in electron ionization−selected ion monitoring (SIM) mode, with column conditions similar to those described above. The monols and catechol PCBs were monitored at molecular ion (M^+) and M^+ + 2 for methylated derivatives of tetra-, penta-, and hexachlorinated congeners. Individual metabolites were quantified by a standard curve for GC peak area ratios relative to the internal satandard vs the concentration of metabolites.

Results

CB101 Metabolism. Figure 1 shows the GC/ECD chromatograms of combined fractions of neutral and phenolic PCBs (after methylation by diazomethane) in liver and serum of Gunn rats 4 days after exposure to CB101. On GC and GC/MS analyses, four hydroxylated

Table 1. Concentrations of Unchanged PCB, Phenolic, and Methylsulfonyl Metabolites in the Liver and Serum of Gunn and Wistar Rats Dosed with CB101 (112 mg/kg, ip)[a]

	concentration (ng/g wet)			
	Gunn rat		Wistar rat	
congener	liver	serum	liver	serum
unchanged CB101	2428 ± 405*	128 ± 9	1254 ± 207	109 ± 23
3-OH-CB101 (M-1)	56 ± 27	17 ± 4	27 ± 10	33 ± 8
3′-OH-CB101 (M-2)	238 ± 50	90 ± 21	223 ± 70	87 ± 26
4′-OH-CB101 (M-3)	22 ± 7	12 ± 2	94 ± 42	13 ± 3
diOH-CB101(M-4)	328 ± 60**	612 ± 73**	91 ± 41	273 ± 65
sum of (monols + diol)	644 ± 102	731 ± 84	434 ± 153	406 ± 105
3′-$MeSO_2$-CB101 (M-5)	49 ± 17**	17 ± 1**	337 ± 63	90 ± 2
4′-$MeSO_2$-CB101 (M-6)	70 ± 15**	14 ± 4**	589 ± 109	52 ± 10
sum of $MeSO_2$-CBs	119 ± 31**	31 ± 3**	926 ± 171	142 ± 12
		ratios		
diol/monols	1.04	5.14	0.26	2.05
diol/CB101	0.14	4.78	0.07	2.50
3′-/4′-$MeSO_2$-CBs	0.70	1.21	0.57	0.17
$MeSO_2$-CBs/CB101	0.05	0.24	0.74	0.56

[a] Values represent means ± standard errors for $n = 4-5$. The statistical difference of changes were analyzed by Student's t-test, $p < 0.05$ (*) and $p < 0.001$ (**), as compared with Wistar controls.

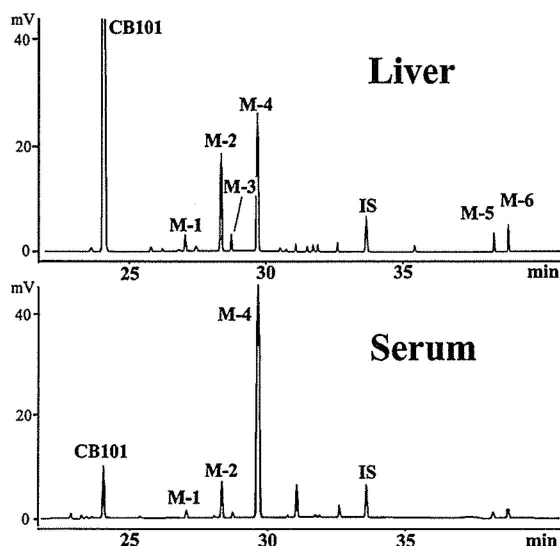

Figure 1. GC/ECD profiles of unchanged PCB and PCB metabolites in liver and serum of Gunn rats after exposure to CB101. The neutral and phenolic fractions were combined and methylated by diazomethane.

metabolites (M-1 to M-4) and two $MeSO_2$ metabolites (M-5 and M-6) were detected in Gunn rats. The metabolite structures were identified as 3-OH-2,4,5,2′,5′-pentaCB (M-1), 3′-OH-2,4,5,2′,5′-pentaCB (M-2), 4′-OH-2,4,5,2′,5′-pentaCB (M-3), 3′,4′-$(OH)_2$-2,4,5,2′,5′-pentaCB (diOH-CB101, M-4), 3′-$MeSO_2$-2,4,5,2′,5′-pentaCB (M-5), and 4′-$MeSO_2$-2,4,5,2′,5′-pentaCB (M-6) by GC/MS comparison with authentic standards. The levels of unchanged CB101, phenolic PCBs, and $MeSO_2$-CBs in liver and serum of both strains are shown in Table 1. More than 90% of the dose was eliminated from the body of both strains at 4 days after exposure to CB101. Unchanged CB101 was abundant in adipose tissue (approximately 7.5% of the dose), whereas OH-PCBs and $MeSO_2$-CBs were distributed in lung, kidney, and brain (data not shown) as well as liver and serum.

The hepatic concentration of residual CB101 in Gunn rats was significantly higher ($p < 0.05$) than that in Wistar rats. For hydroxylated PCBs, M-2 was most abundant in the liver of Wistar rats, whereas the catechol (M-4) was dominant in the serum of Gunn rats. The concentration ratios of catechol/monols in Gunn and Wistar rats were 1.04 and 0.26, whereas the ratios of catechol/parent CB101 were 0.14 and 0.07, respectively. In contrast, hepatic $MeSO_2$-CB levels in Gunn rats were 7.8-fold lower than those in Wistar controls. Thus, the concentration ratios of $MeSO_2$-CBs/CB101 in livers of Gunn and Wistar rats were 0.05 and 0.74, respectively.

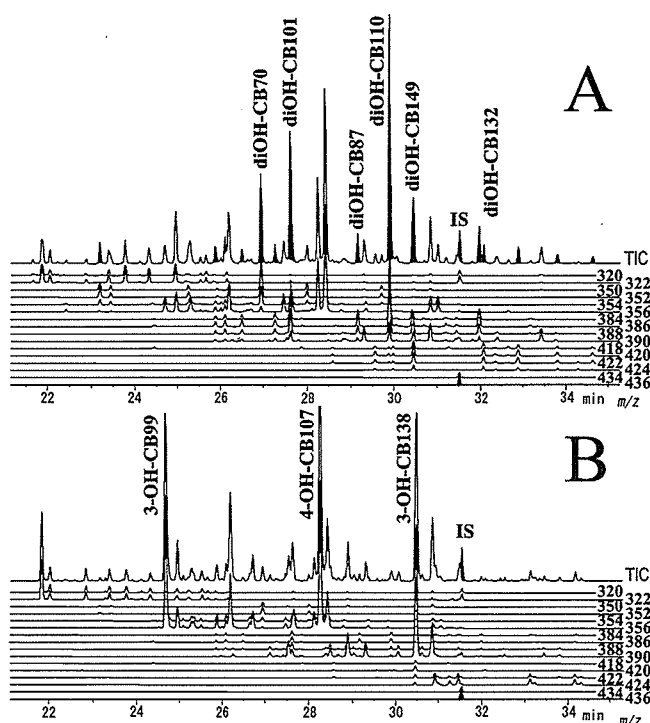

Figure 2. GC/MS/SIM profiles of phenolic PCBs (as methyl derivatives of monols and diols) in liver of Gunn (A) and Wistar (B) rats after exposure to KC500. Monitoring ions are M^+ and $M^+ + 2$ for methylated derivatives of monols and diols from tetra-, penta-, and hexaCBs.

KC500 Metabolism. Figure 2 shows the GC/MS/SIM profiles for monols and diols of tetra- to hexachlorinated congeners in the phenolic PCB fraction (methylated) of the livers of Wistar and Gunn rats after exposure to KC500. Phenolic PCB metabolites in Wistar rats were dominated by three monols, 3-OH-2,4,5,2′,4′-pentaCB (3-OH-CB99), 4-OH-2,3,5,3′,4′-pentaCB (4-OH-CB107), and 3-OH-2,4,5,2′,3′,4′-hexaCB (3-OH-CB138). On the other hand, phenolic PCB metabolites in Gunn rats were

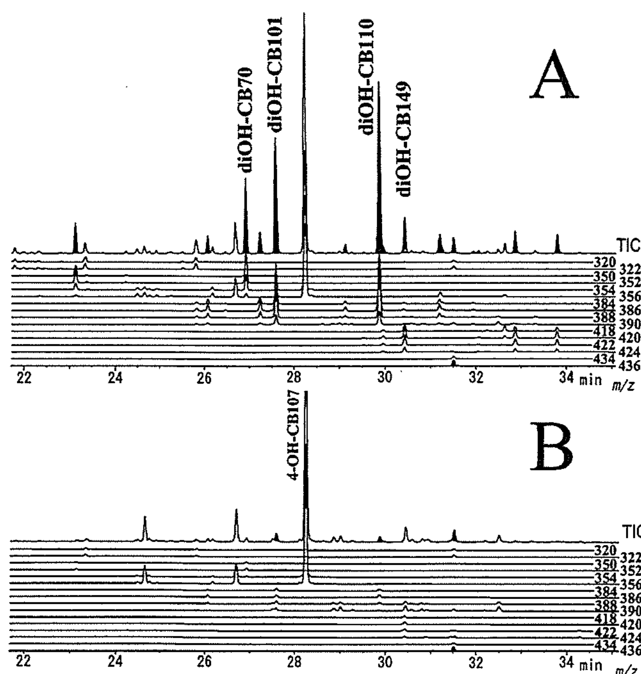

Figure 3. GC/MS/SIM profiles of phenolic PCB metabolites (as methyl derivatives of monols and diols) in serum of Gunn (A) and Wistar (B) rats after exposure to KC500. Monitoring ions are M^+ and $M^+ + 2$ for methylated derivatives of monols and diols from tetra-, penta-, and hexaCBs.

dominated by diols derived from tetraCBs (m/z = 350, M^+ for methylated derivatives), pentaCBs (m/z = 384), and hexaCBs (m/z = 418), some of which were identified as diOH-CB70, diOH-CB87, diOH-CB101, diOH-CB110, diOH-CB132, and 4,5-$(OH)_2$-2,3,6,2′,4′,5′-hexaCB (diOH-CB149) by comparison of mass spectra and relative GC retention times with reference standards.

Figure 3 shows the GC/MS/SIM profiles of phenolic PCB fraction (as methylated derivatives) for monols and diols of tetra- to hexaCB congeners in the serum of Wistar and Gunn rats after exposure to KC500. Metabolite profiles in the serum of Wistar rats were dominated by 4-OH-CB107. On the other hand, the metabolite profiles in serum of Gunn rats were dominated by four catechols, diOH-CB70, diOH-CB101, diOH-CB110, and diOH-CB149, in addition to 4-OH-CB107.

The levels of unchanged PCBs and phenolic PCB metabolites (monols and diols) in liver and serum of both rat strains are shown in Table 2. The total levels of major residual PCBs in liver of Gunn rats were 2.2-fold lower, but the levels of CB101 and CB149 were 2.5- and 6.0-fold higher, respectively, as compared to Wistar rats. For monol metabolites, 4-OH-CB107 was present at the highest level in all tissues of both strains. Thus, the concentration ratios of 4-OH-CB107 relative to the possible parent CBs, 2,3,4,3′,4′-pentachlorobiphenyl (CB105) and 2,4,5,3′,4′-pentachlorobiphenyl (CB118), in Gunn and Wistar rats were 0.12 and 0.08 in liver, whereas they were 9.2 and 6.4 in serum, respectively. For catechol metabolites, diOH-CB110 was present at the highest levels in serum of Gunn rats, whereas the level in Wistar rats was less than one-tenth. Thus, the levels of six catechol PCBs accounted for about 52% of total phenolic PCBs. The ratios of diOH-CB101/CB101 and diOH-CB149/CB149 were higher in serum than in liver for both rat strains.

Table 2. Congener Specific Determination of Unchanged and Phenolic PCBs in the Liver and Serum of Gunn and Wistar Rats Dosed with KC500 (100 mg/kg, ip)[a]

	concentration (ng/g wet)			
	Gunn rat		Wistar rat	
congener	liver	serum	liver	serum
	PCB			
CB99	313 ± 82*	21 ± 5*	1018 ± 14	47 ± 5
CB101	300 ± 61*	14 ± 3	121 ± 3	13 ± 2
CB105	383 ± 78*	25 ± 5*	664 ± 24	56 ± 4
CB118	680 ± 135*	47 ± 10*	1532 ± 26	95 ± 6
CB138	750 ± 169*	48 ± 10*	1571 ± 52	151 ± 9.2
CB146	139 ± 40*	11 ± 2*	289 ± 17	33 ± 3
CB149	155 ± 52*	3.6 ± 0.9	26 ± 2	4.1 ± 0.8
CB153	438 ± 114	38 ± 9*	1095 ± 57	129 ± 7.8
CB156	199 ± 48*	29 ± 12*	753 ± 30	84 ± 17
CB167	234 ± 59*	14 ± 3*	307 ± 17	27 ± 2
CB170	122 ± 37*	6.4 ± 1.4*	464 ± 25	49 ± 3
CB180	184 ± 55*	11 ± 2*	659 ± 50	84 ± 5
CB187	66 ± 21*	4.6 ± 1.2*	219 ± 16	25 ± 2
sum of PCBs	3964 ± 792*	270 ± 65*	8715 ± 283	798 ± 58
	phenolic PCB			
3-OH-CB99	32 ± 6*	14 ± 4	84 ± 7	14 ± 5
4-OH-CB107	127 ± 34	567 ± 42*	186 ± 13	905 ± 121
3-OH-CB138	28 ± 3*	82 ± 8*	141 ± 10	45 ± 8
sum of monols	186 ± 32*	663 ± 40	411 ± 22	964 ± 104
diOH-CB70	37 ± 14*	109 ± 31*	ND	5 ± 1
diOH-CB87	22 ± 5*	69 ± 25*	ND	ND
diOH-CB101	65 ± 19*	109 ± 41*	6 ± 2	6 ± 1
diOH-CB110	98 ± 32*	313 ± 87*	8 ± 2	10 ± 1
diOH-CB132	11 ± 3*	31 ± 9*	ND	ND
diOH-CB149	33 ± 5*	98 ± 24*	4 ± 2	5 ± 2
sum of diols	267 ± 43*	729 ± 141*	18 ± 2	26 ± 3
	ratio			
4-OH-CB107/ (CB105 + CB118)	0.12	9.2	0.08	6.4
diOH-CB101/CB101	0.22	7.8	0.05	0.5
diOH-CB149/CB149	0.21	55	0.15	1.2

[a] Values represent means ± standard errors for n = 4–5. *Significantly different from Wistar controls (Student's t-test, $p < 0.001$); ND = not detected (<0.1 ng/g).

The hepatic concentrations of $MeSO_2$-CBs in both rat strains are shown in Table 3. Eighteen $MeSO_2$-CBs congeners were identified in the liver, consisting of *meta*- and *para*-$MeSO_2$-CBs derived from nine parent CB congeners with 2,5- or 2,3,6-chlorine substitution. When classified according to the chlorination pattern, $MeSO_2$-CBs with 2,5-chlorine substitution were dominated by *meta*-$MeSO_2$-CBs (*meta*/*para* concentration ratios = 0.64–11.3), whereas $MeSO_2$-CBs with 2,3,6-chlorine substitution were dominated by *para*-$MeSO_2$-CBs (the ratios = 0.07–0.25). Although there were no differences in the congener profiles of $MeSO_2$-CBs between both rat strains, the levels of each congener in Gunn rats were significantly low ($p < 0.001$) as compared to Wistar rats.

The levels of unchanged PCBs, phenolic PCBs, and $MeSO_2$-CB metabolites in the liver of both strains exposed to KC500 are summarized in Table 4. In comparison with Wistar rats, Gunn rats exhibited lower levels of total $MeSO_2$-CBs, but higher levels of catechol PCBs in the liver (statistical significance; Student's t-test, $p < 0.001$).

Discussion

To our knowledge, this is the first report of the identification and tissue retention of catechol PCB metabolites of tetra- to hexachlorinated biphenyls in rats exposed to KC500. To date, the formation of catechol PCBs has been demonstrated only from selected CB

Table 3. Concentrations of $MeSO_2$-CBs in the Liver of Gunn and Wistar Rats Dosed with KC500 (100 mg/kg, ip)[a]

congener	concentration (ng/g wet) and ratio: Gunn rat	*meta*/*para* ratio	Wistar rat	*meta*/*para* ratio
2,5-chlorine substitution				
3-$MeSO_2$-2,5,4′-triCB	9.2 ± 2.2*	11.3	32 ± 6	11.2
4-$MeSO_2$-2,5,4′-triCB	0.8 ± 0.2*		2.7 ± 0.3	
3-$MeSO_2$-2,5,2′,4′-tetraCB	10 ± 2*	7.76	59 ± 5	7.55
4-$MeSO_2$-2,5,2′,4′-tetraCB	1.3 ± 0.4*		7.7 ± 0.5	
3-$MeSO_2$-2,5,3′,4′-tetraCB	18 ± 4*	0.97	81 ± 8	1.54
4-$MeSO_2$-2,5,3′,4′-tetraCB	20 ± 3*		53 ± 5	
3′-$MeSO_2$-2,3,4,2′,5′-pentaCB	6.4 ± 4.3*	1.05	22 ± 2	1.09
4′-$MeSO_2$-2,3,4,2′,5′-pentaCB	6.1 ± 4.2*		20 ± 2	
3′-$MeSO_2$-2,4,5,2′,5′-pentaCB	19 ± 7*	0.64	129 ± 15	1.17
4′-$MeSO_2$-2,4,5,2′,5′-pentaCB	29 ± 8*		110 ± 6	
sum	119 ± 26*		515 ± 34	
2,3,6-chlorine substitution				
5-$MeSO_2$-2,3,6,2′,4′-pentaCB	3.1 ± 0.6*	0.25	40 ± 7	0.28
4-$MeSO_2$-2,3,6,2′,4′-pentaCB	12 ± 2*		128 ± 20	
5-$MeSO_2$-2,3,6,3′,4′-pentaCB	1.7 ± 0.5	0.07	4.6 ± 2.1	0.09
4-$MeSO_2$-2,3,6,3′,4′-pentaCB	24 ± 6		53 ± 6	
5′-$MeSO_2$-2,3,4,2′,3′,6′-hexaCB	1.3 ± 0.6*	0.21	9.1 ± 2.0	0.98
4′-$MeSO_2$-2,3,4,2′,3′,6′-hexaCB	6.1 ± 2.7		9.4 ± 2.0	
5-$MeSO_2$-2,3,6,2′,4′,5′-hexaCB	0.8 ± 0.5	0.16	2.0 ± 0.9	0.21
4-$MeSO_2$-2,3,6,2′,4′,5′-hexaCB	5.2 ± 2.2*		9.8 ± 2.1	
sum	55 ± 9.3*		256 ± 28	
sum of *meta*-$MeSO_2$-CBs	70 ± 16*	0.67	401 ± 20	1.08
sum of *para*-$MeSO_2$-CBs	104 ± 27*		370 ± 32	
total $MeSO_2$-CBs	174 ± 39*		771 ± 40	

[a] Values represent means ± standard errors for n = 4–5. *Significantly different from Wistar controls (Student's t-test, p < 0.001).

Table 4. Concentrations of PCBs and Their Metabolites in the Liver of Wistar and Gunn Rats Exposed to KC500 (100 mg/kg, ip)[a]

congener	concentration (ng/g wet weight): Gunn rat	Wistar rat	ratio Gunn/Wistar
PCBs	3964 ± 884*	8715 ± 283	0.45
OH-PCBs	186 ± 32*	411 ± 22	0.45
diOH-PCBs	267 ± 43*	18 ± 2	14.8
$MeSO_2$-PCBs	174 ± 39*	771 ± 40	0.23
ratio			
OH-PCBs/PCBs	0.05	0.05	1.0
diOH-PCBs/PCBs	0.07	0.002	35
$MeSO_2$-CBs/PCBs	0.04	0.09	0.4
PCB metabolites/PCBs	0.16	0.14	1.1

[a] Values represent means ± standard errors for n = 4–5. *Significantly different from Wistar controls (Student's t-test, p < 0.001).

congeners that are not relevant in the environment except for CB101 (*17*). The present study demonstrated that catechols could be readily formed from CBs with 2,5- or 2,3,6-chlorine substitution in UGT1A deficient Gunn rats treated with KC500.

In CB101-treated Gunn rats, we isolated three monol and one diol PCB metabolites (as methylated derivatives) from the phenolic fraction, as well as two $MeSO_2$ metabolites from the neutral fraction of tissues analyzed, where a catechol PCB and two $MeSO_2$-CBs were distributed at higher levels than monol metabolites, especially in serum. The metabolic pathway of CB101 also included 3-hydroxylation on the 2,4,5-trichlorinated phenyl ring. Such hydroxylation has been observed in minks exposed to CB101 (*30*).

In KC500-treated Gunn rats, we identified six catechols derived from CB70, CB87, CB101, CB110, CB132, and CB149 in all tissues and blood. These parent CBs are the major components (38% in total) of KC500 that have chlorine atoms at the 2,5- or 2,3,6-position of one ring and at the 3,4-, 2,3,4-, or 2,4,5-position of the other (*31*). The percentage compositions of catechol PCBs were estimated to be more than 60% in total of phenolic PCBs detected in the liver. Although we could not identify catechols from 2,5,2′,5′-tetraCB or 2,3,6,2′,5′-pentaCB (15% in total of KC500) due to lack of authentic standards, we hypothesized that all PCB components with 2,5- or 2,5,6-chlorine substitution were likely to be substrates for 3,4-catechol formation in Gunn rats. The possibility of the formation of 2,3-catechols from CBs with *meta*- and *para*-substituted chlorine atoms should not be excluded, as previous reports indicated that 3,4,3′,4′-tetraCB could be metabolized to the 2,3-catechol in rats (*32*) and 2,4,5,2′,4′,5′-hexaCB could be metabolized via 2,3-epoxide in guinea pigs (*33*). In some cases, the catechol may be formed through dechlorination from an NIH shift metabolite, such as 4-OH-CB107 in rats (*29*).

P450-catalyzed hydroxylation of biphenyl rings has been observed in vitro by use of bioactivation systems based on hepatic microsomes from rodents treated with various enzyme inducers (*34*) and human hepatic microsomes (*35*). It is therefore hypothesized that treatment of Gunn rats with KC500 may activate an alternative pathway of PCB metabolism involving the induced form of CYP. The possible metabolic pathways for monol or catechol formation from CB118 and CB110 (major components in KC500) are illustrated in Scheme 1. Metabolism of CB118 may involve 4-hydroxylation via 3,4-epoxide and NIH shift to form 4-OH-CB107. Aromatic hydroxylation of CBs with 2,3,6-chlorine substitution such as CB110 in Gunn rats is likely to involve the direct insertion of a hydroxyl group on the *meta*-position and/or isomerization or hydration of an arene oxide intermediate to form monol or dihydrodiol, followed by further oxidation of monols or dehydration of a dihydrodiol (*3, 18, 36*).

The present study also demonstrated marked differences in the tissue levels of parent PCBs, catechol PCBs, and $MeSO_2$-CBs between Gunn and Wistar rats (Table 4). CB101 metabolism by Gunn rats resulted in higher levels of residual CB101 as compared to Wistar controls (Table 1). Similarly, the concentration ratios of both CB101 and CB149 relative to other residual PCBs were higher in KC500-treated Gunn rats (Table 2). This may be explained by the observation that Gunn rats are less susceptible to induction of CYP2B1/2 that catalyzes the metabolism of CB101 and CB149 as compared to Wistar rats (*37*). On the other hand, the levels of catechols were higher in Gunn rats, whereas the levels of $MeSO_2$-CBs were lower in Gunn rats as compared to Wistar rats. Thus, the hepatic concentration ratio of ΣdiOH-PCB/Σ$MeSO_2$-CB was 1.5 in Gunn rats, while it was 0.02 in Wistar rats (Table 4). These results suggest that a lack of UGTs markedly alters the formation ratios of catechol PCBs and $MeSO_2$-CBs.

Marniemi et al. (*39*) have reported that although the activities of bilirubin UGTs and UDP-glucosyltransferase are significantly low in Gunn rats as compared to Wistar rats, microsomal epoxide hydrase and soluble gluthatione-epoxide transferase activities are normal. In our recent study (*25*), UGT deficient Gunn rats exhibited no activity of UGT1A and a lower activity of UGT2B as compared to Wistar rats. These observations suggest that the formation ratios of catechol and $MeSO_2$ metabolites may