ABSTRACT

GARNER, C. EDWIN. Dermal Absorption of Polychlorinated Biphenyls (PCBs) and the Effect of PCB Metabolites on Estrogen Metabolism. (Performed under the direction of H.B. MATTHEWS, PhD)

The fate of selected mono-, di-, tetra- and hexachlorobiphenyls was investigated following single dermal administration (0.4 mg/kg) to determine the effects of chlorine substitution on the dermal absorption and disposition and excretion of polychlorinated biphenyls (PCBs). Single dermal doses of ^{14}C-labeled mono-, di-, tetra- and hexachlorobiphenyls were administered to 1 cm^2 areas on the backs of F-344 male rats. Distribution of radioactivity in the dose site and selected tissues was determined by serial sacrifice at time points up to 2 weeks. Dermal penetration varied inversely with degree of chlorination. Penetration rate constants correlated well with log K_{ow}. PCBs were retained in the epidermis for up to two weeks post-dose. The skin favors the rapid absorption of less chlorinated PCBs but the relatively rapid metabolism and elimination of these compounds would result in lower body burdens. More highly chlorinated PCBs penetrate less rapidly but remain in the site of exposure and slowly enter the systemic circulation. The time course of equivalents in the tissues showed a dependence on rate and extent of absorption. The most rapidly absorbed PCBs reached peak tissue concentrations at early times but the higher chlorinated PCBs were slowly absorbed and reached peak tissue at times after removal of unabsorbed dose. Excretion of absorbed radioactivity varied

with chlorine content and excretion profiles following dermal doses tended to differ from profiles following equivalent IV doses, as did the metabolite profiles in excreta. The data from these studies suggest that systemic absorption of PCBs involves a combination of sequential processes including penetration across the stratum corneum, possibly metabolism in the epidermis and/or dermis, adsorption to proteins, and finally absorption into the systemic circulation. The rate of absorption, and therefore the disposition of PCBs following dermal administration may be mediated, either in part or fully, by transdermal metabolism. These data further indicate that the half-life of PCBs absorbed dermally may be significantly longer than when introduced as a bolus by iv or po administration.

Transdermal PCB metabolism suggested that body burdens of phenolic and catechol metabolites may be elevated. Studies into the toxic effects of PCB metabolites were conducted to determine the effects of PCBs and catechol PCB metabolites on the catabolism of 4- and 2-hydroxyestradiol (4-OHE_2 and 2-OHE_2, respectively). Following treatment with Araclor 1254 for 21 days (5 mg/kg/day) female SD rats showed an elevation of catechol estrogens and a nearly complete reduction in CE O-methylation. In classical enzyme kinetics studies, the catechol PCBs were demonstrated to be good substrates for COMT and also mixed -type inhibitors of the O-methylation of CEs. The data suggest that PCBs significantly alter the clearance of catechol estrogens in vivo and that this

effect may be mediated by catechol PCB metabolites. Further, methyltransferase inhibition by PCB catechols may contribute to PCB-mediated endocrine effects and liver carcinogenesis.

The catechol PCBs were demonstrated to be agonists of the estrogen receptor in cultured HeLa cells. The responses elicited by the PCB catechols tested fell within the range of effect measured for the catechol estrogens and phenolic PCBs, and were within the range previously reported for other "environmental estrogens" such as nonylphenol and o,p'-DDT.. The observed estrogenicity of PCB catechols suggests that further oxidative metabolism of estrogenic PCB phenolic metabolites would not necessarily result in lowering the total estrogenic burden of a PCB exposed organism.

DERMAL ABSORPTION OF POLYCHLORINATED BIPHENYLS (PCBs) AND THE EFFECT OF PCB METABOLITES ON ESTROGEN METABOLISM.

by

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For Liz, Laura, and Paul.

BIOGRAPHY

The author was born in Humboldt, Tennessee on December 4, 1966. He graduated from E.T. Beddingfield High School, Wilson, North Carolina in 1985, and entered Barton College, Wilson, North Carolina in 1986. While attending Barton, he married Elizabeth Ann Bell in 1987. He received a B.S. in chemistry with a minor in mathematics in 1990. The author then worked as a chemist in the Center for Bioorganic Chemistry, Research Triangle Institute in Research Triangle Park under the supervision of James M. Mathews, Ph.D. His daughter, Laura Elizabeth , was born in 1992 and his son, John Paul was born in 1994. In 1995 he was accepted into the Department of Toxicology, North Carolina State University. Concurrent with his entry into graduate school was his acceptance of a National Institute of Environmental Health Sciences Predoctoral Fellowship. The author began his doctoral research while at the Laboratory of Pharmacology and Chemistry, National Institute of Environmental Health Sciences, Research Triangle Park, North Carolina under the supervision of H.B. Matthews, Ph.D.

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Polychlorinated Biphenyls (PCBs) and the Dermal Route of Exposure
C. Edwin Garner

PCBs in the Environment: Potential for Exposure

PCBs were produced commercially in the United States from 1929 until 1977 under trade names such as Aroclor, Askarel, and Therminol (WHO, 1993). The annual U.S. production peaked in 1970 with a total production volume of 85 million pounds (39 million kg) of Aroclors (WHO, 1993). Between 1957 and 1971, 12 different types of Aroclors, with chlorine contents ranging from 21 to 68% were produced in the United States (IARC 1978). The estimated cumulative production volumes of PCBs in the United States and western Europe between 1930 and 1975 was over 2 billion pounds (ca 800 million kg) (WHO, 1993).

Prior to 1974, PCBs were used both for closed applications (capacitor and transformers, and heat transfer and hydraulic fluids) and in open-end applications (plasticizers, surface coatings, inks, adhesives, electric wire insulation, flame retardants, pesticide extenders, paints, and microencapsulation of dyes for carbonless duplicating paper) (WHO, 1993). PCBs were used in metal coatings and in paints to impart qualities of heat resistance, plasticity, and antifouling (Welsh 1995). PCBs were also used as a slide-mounting medium for microscopes (Durfee 1976). In 1974, use of PCBs was restricted to closed applications such as production of capacitors and

transformers (WHO, 1993). By 1976, only 5% of the transformers produced in the United States were filled with PCBs, and by 1979 Aroclors were no longer used in the production of capacitors and transformers. However, the life expectancy of transformers containing PCBs is greater than 30 years, and the life expectancy of capacitors is up to 20 years (IARC 1978). In 1981, an estimated 131,000 transformers containing PCBs were in service in the United States. Explosions or overheating of transformers containing PCBs may release large amounts of these compounds into the environment.

PCBs are dispersed in the environment mostly through atmospheric deposition (WHO, 1993). Currently, the major source of PCB release to the atmosphere is the redistribution of the compounds already present in soil and water (Eisenreich et al. 1992; Murphy et al. 1985). PCBs may also be released to the atmosphere from other sources including disposal sites containing transformers, capacitors, and other PCB wastes; incomplete combustion during incineration of PCB-containing wastes; and improper (or illegal) disposal or spills of the compounds to open areas (Larsson 1985; Morselli et al. 1985, 1989; Murphy et al. 1985; Sawhney and Hankin 1985; Swackhamer and Armstrong 1986; Tiernan et al. 1983). PCBs were included among the contaminants at approximately 12% of Superfund sites, and PCBs were included in the contaminant list for 17% of all sites appearing on the National Priorities List (EPA 1990). Landfills and hazardous waste sites are expected to be continuing sources of PCB release into the atmosphere. The amount of PCBs released from landfills and

incinerators, 10–100 kg/year (22–220 pounds/year) and 0.25 kg/stack/year (0.55 pounds/stack/year), respectively, is small, compared to the quantity of PCBs that is estimated to be released into the atmosphere through cycling from environmental processes (900,000 kg/year) (2 million pounds/year) (Murphy et al. 1985).

PCBs are virtually insoluble in water, but are readily adsorbed onto soil, dust, and surfaces in homes and in factories (WHO, 1993). Thus, the dermal route represents a potential major route of human exposure to PCBs. The inhalation and dermal routes are considered the main exposure routes to PCBs in occupational settings (Wolff 1985). For the general population, the oral route is the major route for PCB exposure (Humphrey 1983). The dermal route of exposure has been recognized as a significant contributor to the accumulation of PCBs in adipose tissue of workers in the capacitor manufacturing industry (Maroni et al. 1981a, 1981b; Smith et al. 1982; Wolff 1985). For example, it was reported that the concentration of PCBs in wipe samples from the face and hands of employees at a utility company varied from 0.05 to 5 mg/cm^2 (Smith et al. 1982). Additionally, the dermal route, through skin contact with contaminated water or soil, represents a potential route of exposure to PCB mixtures for populations near hazardous waste sites. Damaged electrical components also represent a significant source of dermal PCB exposure. For example, children exposed to polychlorinated biphenyls (PCBs) while playing with parts of a lightning damaged capacitor had elevated serum concentrations of lower

chlorinated PCBs and later developed pruritis and rash (Wolff and Schecter 1991).

Dermal Absorption of PCBs

Dermal absorption of commercial PCB mixtures has been investigated in only a few studies. Total absorption has been measured in monkeys and guinea pigs by comparing excretion following topical administration to that following parenteral administration. Single doses of ^{14}C-labeled PCBs (42% chlorine content) in benzene/hexane were applied to the abdominal skin of rhesus monkeys and to the skin behind the ear of guinea pigs (Wester et al. 1983). To an additional group of guinea pigs, PCB with 54% chlorine content was applied. The application amount ranged between 4.1 and 19.3 mg/cm^2 skin. The unabsorbed radioactivity was removed after 24 hours and radioactivity was monitored in the urine for several weeks post-dosing. Absorption efficiency ranged from ca. 15% to 34% of the applied radioactivity in the monkeys and in the guinea pigs averaged ca. 33% and 56% for the 42% chlorine and 54% chlorine mixtures. Washing the skin immediately after PCB application removed 59% of the applied dose. Wester et al. (1990) demonstrated that percutaneous absorption of Aroclor 1242 or Aroclor 1254 by monkeys was not significantly affected by vehicle. Total absorption remained ca. 20% of the applied dose (ca 4 mg/cm^2) whether the solvent was mineral oil or trichlorobenzene. Skin washing 24 hours after dosing removed only 25–45% of the applied dose indicating that removal is no longer possible after a certain period of PCB-skin contact. Recent data from the same group showed that, in monkeys,

percutaneous absorption of both Aroclors 1242 and 1254 from soil was ca. 14% of the applied dose (Wester et al. 1993). All of the above studies indicated the extent of absorption of the mixtures but made no attempt at estimating kinetic parameters of absorption.

Few studies have characterized the absorption of individual PCB congeners in animal species or humans. Fischer et al (1989) characterized the absorption of the PCB 2,4,5,2',4',5'-hexachlorobiphenyl in young and mature female rats. At 72 hr in vivo dermal penetration was 35% in young and 26% in adults. In vivo dermal penetration at 120 hr was ca 45% in both young and adults. These authors calculated a dermal penetration rate constant of 9 x 10^{-5} min^{-1}. Hughes et al (1992) compared the dermal penetration of equimolar doses of 2,4,5,2',4',5'-[14C]-hexachlorobiphenyl (HCB), and 3,4,3',4'-[14C]-tetrachlorobiphenyl (TCB) following application in several vehicles (solid, aqueous paste, suspension, or in volatile vehicle). Approximately 5-8% of both HCB and TCB penetrated into the skin within 24 hours. The major route of excretion was via the feces. At 5 days post application, 90% and 21% of the absorbed HCB and TCB derived radioactivity was retained in the body.

Disposition of PCBs Following Dermal Absorption

To date no studies have been performed regarding distribution in humans or animals following dermal exposure to PCBs. Additionally, there have been no studies investigating whether PCB distribution is route dependent.

Excretion Of PCBs Following Dermal Absorption

Limited data is available regarding the excretion of PCBs in experimental animals following dermal exposure. The urinary excretion half-life of an undefined PCB containing 42% chlorine applied to the abdominal skin was 6.9 days in monkeys (Wester et al. 1983). In guinea pigs in which the same mixture was applied to the back of the ear, a 2-phase urinary excretion process was observed. The first phase was rapid, with an elimination half-life of 2 days, and was followed by a slower phase, with an elimination half-life of 13 days. However, the elimination half-life of a PCB containing 54% chlorine was 3 days and was linear for the duration (16 days) of the urine collection (Wester et al. 1983). A recent study (Wester et al. 1990) reported that following percutaneous application of 4 mg ^{14}C-labeled Aroclor 1242/cm^2 to the abdominal skin of monkeys, ca 10% of the applied radioactivity was excreted over a 30-day period. Excretion had virtually stopped after the first 10 days. Urinary excretion of ^{14}C-derived radioactivity was twice fecal excretion. Following application of 4.8 mg ^{14}C-labeled Aroclor 1254/cm^2 in mineral oil or trichlorobenzene, 5.5% and 3.9% of the dose, respectively, were excreted over a 30-day period.

PCB Metabolism and Pharmacokinetics: Factors Affecting PCB Absorption, Distribution, and Excretion

The mechanism of absorption of PCBs by the dermal routes of exposure is not known. PCBs are well absorbed from the gastrointestinal tract. Because PCBs

are lipid-soluble, transfer from the aqueous environment of the intestine across cell membranes is a passive process (Albro and Fishbein 1972; Gage and Holm 1976; Matthews and Anderson 1975). The concentration gradient favors partitioning across the cells into blood. Absorption efficiency appears to increase with the degree of chlorination up to a certain point. In blood, PCBs are strongly associated with red blood cells, albumin, and lipoproteins (Matthews et al. 1984). Distribution in plasma is determined primarily by partition among the various proteins according to lipid solubility (Matthews and Dedrick 1984). As the degree of chlorination increased, the binding to lipoproteins also increased (Matthews et al. 1984). Partition of PCBs between blood and tissues also seems determined primarily by tissue lipid content and the concentration gradient. The fraction associated with red blood cells is more rapidly removed from the blood by the tissues than fractions associated with plasma proteins (Matthews et al. 1984).

Following IV or PO administration, PCBs are rapidly cleared from the blood and accumulate initially in the liver and muscle (Matthews and Dedrick 1984). This appears to be due to the high perfusion of the liver and the relatively large muscle volume. Due to their high affinity for lipophillic tissues, PCBs ultimately redistribute to adipose tissue and skin. Since elimination of PCBs is principally metabolism mediated (Matthews and Dedrick 1984), peripherally stored residues are less available for elimination in the liver. A dynamic equilibrium of PCB concentrations is established among all tissues for each PCB homologue (Matthews and Dedrick 1984). Pharmacokinetic modeling of PCB disposition

predicts that, at equilibrium, changes in the PCB concentration or changes in tissue volume of any tissue will lead to a corresponding change in all tissues (Matthews and Dedrick 1984). For instance, if the concentration of a PCB congener in the liver is reduced by metabolism or excretion, then the concentration of that PCB congener in all tissues will be reduced proportionally. Congeners that cannot be metabolized or excreted will concentrate in adipose tissue, but will still circulate to other tissues. Exposure to other tissues will be proportional to the respective tissue/blood ratios and the concentration in the main storage tissues. This dynamic distribution results in accumulation of persistent congeners in all tissues and depletion from all tissues of those congeners that can be cleared (Matthews and Dedrick 1984). Metabolites, however, may accumulate in specific tissues due to solubility differences as well as tissue binding.

Clearance of PCBs is essentially metabolism based; and nearly negligible amounts of parent are cleared via the feces or urine. The major routes of excretion of PCB metabolites are fecal and urinary. For higher chlorinated, poorly metabolized congeners such as penta- and hexachlorobiphenyls, the predominant route of metabolite excretion is via the feces. Lower chlorinated, more readily metabolized congeners are excreted in both urine and feces (Lutz and Dedrick 1987). Elimination kinetics tend to follow first order processes with elimination rates directly related to their metabolic rates (Gage and Holm 1976).

PCBs are metabolized by microsomal cytochrome P-450 to polar metabolites that may undergo conjugation with glutathione and/or glucuronic acid. The rate of metabolism of PCB congeners depends on 1) the degree of chlorination, 2) the chlorine substitution pattern, and 3) the pattern and levels of P-450 isozymes and other enzymes in the target tissue. PCBs with vicinal, unsubstituted carbons are readily metabolized to arene oxides that can undergo further metabolism (Matthews and Andersen, 1976). Those PCBs with 3,4-unsubstituted carbons are metabolically cleared at much faster rates than other PCBs (Kato et al., 1980; Matthews and Andersen, 1976). Examination of the individual congeners commonly present in the commercial Aroclors revealed that 53-80% of the congeners had such vicinal, unsubstituted 3,4 carbons (C. E. Garner, unpublished data). PCB congeners of low chlorine content are transformed into hydroxylated derivatives that are predominately eliminated in the urine. Highly chlorinated congeners with nonsusceptible substitution patterns are cleared slowly and tend to bioaccumulate.

Conclusions

The dermal route is recognized as a significant source of human exposure to PCBs. Unfortunately the dermal route of exposure has been poorly characterized. It is known that PCB mixtures are readily absorbed and that absorption extent differs between mixtures. Since the congener content is different between different Aroclors, it would follow that different congeners would have different absorption properties. However, none of the studies

performed to date have characterized the rate of dermal absorption of the Aroclors, making it nearly impossible to relate time of exposure and congener content to body burden following dermal exposure. Individual congener data is even more scarce. The mechanism of dermal absorption is unknown, therefore it is difficult to predict factors that may increase or decrease PCB absorption. Additionally it is unknown if there are any route dependent effects on disposition and excretion, either for mixtures or for individual congeners. In the chapters that follow, data will be forwarded that help to fill these data gaps. The first chapter addresses the absorption of individual PCB congeners, correlating chlorine content of individual PCBs to transdermal penetration and therefore allowing a congener-specific prediction of absorption kinetics. The next chapter investigates tissue disposition and excretion and relates these to the mechanism of transdermal absorption. In the final chapter in this section, the unusual dermal kinetics of an individual congener is related to an unusual absorption mechanism and the combination of these effects on disposition and excretion is explored.

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The Effect of Chlorine Substitution on the Dermal Absorption of Polychlorinated Biphenyls.

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ABSTRACT

The Effect of Chlorine Substitution on the Dermal Absorption of Polychlorinated Biphenyls. C. Edwin Garner and H.B. Matthews (1997). *Toxicol. Appl. Pharmacol*.

The fate of selected mono-, di-, tetra- and hexachlorobiphenyls was investigated following single dermal administration (0.4 mg/kg) to determine the effects of chlorine substitution on the dermal absorption and disposition of polychlorinated biphenyls (PCBs). Single dermal doses of ^{14}C-labeled mono-, di-, tetra- and hexachlorobiphenyls were administered to 1 cm^2 areas on the backs of F-344 male rats. Unabsorbed radioactivity was removed from the dose site either at sacrifice or 48 hr post-dose. Distribution of radioactivity in the dose site and selected tissues was determined by serial sacrifice at time points up to 2 weeks. Dermal penetration varied inversely with degree of chlorination and at 48 hr ranged from ca 100% for monochlorobiphenyl to ca 30% for the hexachlorobiphenyl. Penetration rate constants correlated well with log K_{ow}. PCBs were retained in the epidermis for up to two weeks post-dose. The data from these studies suggest that systemic absorption of PCBs involves a combination of sequential processes including penetration across the stratum corneum, possibly metabolism in the epidermis and/or dermis, adsorption to

proteins, and finally absorption into the systemic circulation. The skin favors the rapid absorption of less chlorinated PCBs but the relatively rapid metabolism and elimination of these compounds would result in lower body burdens. More highly chlorinated PCBs penetrate less rapidly but remain in the site of exposure and slowly enter the systemic circulation.. The dermal absorption of a commercial PCB mixture was modeled and the results suggest that the net result of the differences in absorbance rates would be a greater body burden of higher chlorinated PCBs relative to those which have a lower chlorine content.

INTRODUCTION

Polychlorinated biphenyls (PCBs) were widely used in a variety of industrial and consumer products before their production was banned in the 1970s. Over one million tons of PCBs were produced (WHO, 1993). Due to their chemical stability, PCBs are still ubiquitous environmental contaminants and are frequently found as complex mixtures of isomers in soil, dust, and on surfaces in homes and in factories (WHO, 1993).

A number of authors have reported that PCBs are absorbed into the skin of animals (Vos and Beems, 1971; Wester et al. , 1983; Fischer et al., 1989; Hughes, et al., 1992) and humans (Surber et al., 1990, Fischer et al., 1989). Wester et al. (1993) demonstrated that human skin will readily absorb PCBs from soil. Thus, a major potential source of human exposure is via the skin, making characterization of the dermal penetration of PCBs valuable for predicting the risk posed to humans by these exposures. However, the fact that not all PCBs are equally absorbed or metabolized and the fact that they exist in the environment as complex mixtures greatly complicates estimation of risks associated with environmental exposure (Tanabe et al., 1981). The objective of the present study has been to determine the absorption kinetics of individual PCB congeners in order to provide data on which predictions of the behavior of more complex PCB mixtures might be based. Studies described here were designed to determine the effects of the degree and position of chlorination on the absorption, disposition, and elimination of selected PCBs following dermal exposure at environmentally relevant concentrations.

MATERIALS AND METHODS

Animals.

Adult male F-344 rats (175-275 g) were purchased from Taconic (Germantown, NY) and acclimated at least one week prior to use in experiments. Animals were furnished food (NIH 31 rat chow) and tap water ad libitum and maintained on a 12 hour light/dark cycle. Rats used in experiments were housed in glass metabolism cages that provided for the separate collection of urine and feces.

Xenobiotic.

The chemical structures used in these studies are shown in Figure 1. The U-[^{14}C]-2,2',4,4'-tetrachlorobiphenyl (TCB) (specific activity 27.3 mCi/mmol) was purchased from New England Nuclear (Wilmington, DE). The U-[^{14}C]-4-chlorobiphenyl (MCB), (specific activity 18.8 mCi/mmol) and U-[^{14}C]-2,2',4,4', 6,6-hexachlorobiphenyl (HCB) (specific activity 14.7 mCi/mmol) were purchased from Sigma Chemical Co. (St. Louis, MO). The U-[^{14}C]-4,4'-dichlorobiphenyl (DCB) (specific activity 58 mCi/mmol) was previously prepared at NIEHS (Matthews and Anderson, 1975). Each was determined to be at least 95% radiochemically pure by HPLC. Non-radiolabelled material was not used in these experiments.

Preparation and Delivery of Dose Forms.

Dermal dose formulations were prepared by dissolving sufficient radiolabelled PCB in acetone for delivery of 0.4 mg PCB/kg in a dose volume of 0.15 ml/kg. Approximately 24 hours prior to dosing, animals were anesthetized with ketamine:xylazine (60:9 mg/kg IM) and the fur on a 10 x 10 cm area was clipped from each animal's back with electric clippers (Oster Corporation, Milwaukee, WI). Any animal with nicked skin was excluded from use. Dermal doses were administered onto a 1 cm^2 area on the backs of the animals using a 50 ml Hamilton HPLC syringe (Hamilton Co., Reno, NV) with a blunt-tipped needle and the solvent evaporated under a gentle stream of air. A 1 cm^2 dose site was chosen to provide for a standardized area to make absorption comparisons. A stainless steel wire mesh shield (Shandon-Lipshaw Co., NY,NY) was then glued over the dose site with cyanoacrylate adhesive (BONDINI-2, PRO-TEL Inc., Santa Monica, CA) to prevent grooming.

The applied dose was left on the dose site for up to 48 hours post-dose. Radioactivity remaining on the skin was removed at 48 hr post-application because we believe this time period was a reasonable estimate of the maximum time that contamination might be allowed to remain in contact with human skin. Disappearance of PCB from the dose site by evaporation was not likely since these PCBs are solids at 37°C and have negligible vapor pressure at that temperature (CRC, 1983). Control experiments were performed by applying the radiolabelled dose and washing the site as above within 1 minute post-application. The recovery of radiolabel in the swabs was in all cases greater

than 95%. Recovery approached 99.9% when dose site skin was included. Serial sacrifices were performed, with three animals sacrificed at 1, 4, 8, 12, 24, 48, 72, 96, and 336 hr post-dosing. At each timepoint up to and including 48 hr, the animals were anesthetized with ketamine/xylazine, exsanguinated by cardiac puncture and sacrificed by opening of the thoracic cavity. The dose site shield was removed and unabsorbed radioactivity was then washed from the dose site with 5 acetone-soaked cotton gauze swabs. Radioactivity present on the shield was determined by extracting the shield with 25 ml acetone and assaying the extract by liquid scintillation spectrophotometry (LSP) (Beckman LS9800, Irvine, CA). Swabs were placed in Ultima Gold (Packard Instrument Co., Meriden, CT) and analyzed directly for radiochemical content by LSP. The washed dose site was then excised, solubilized in 2N ethanolic NaOH, and triplicate aliquots of the solution assayed by LSP.

Animals representing time points later than 48 hr were anesthetized at 48 hr post-dose with Ketamine:Xylazine (60:9 mg/kg), the shield removed, and the dose site washed with 5 acetone-soaked swabs. A new shield was cemented in place and the animal was returned to the metabolism cage. The cyanoacrylate adhesive used did not produce visible irritation, redness, or swelling. Swabs and the shield were treated as above. At sacrifice animals were handled in a manner similar to that described above, and the dose site was excised, solubilized, and analyzed for total radioactive content.

Analysis of biological samples.

At sacrifice blood, adipose tissue, muscle, skin (ears) and the entire kidney and liver were removed for assay of radiochemical content. Small tissue samples were oxidized to [^{14}C]-CO_2 in a Packard Oxidizer (Packard Instrument Co., Downer's Grove, IL). Livers were homogenized with a Polytron (Kinematica AG, Switzerland) and the homogenates oxidized. Oxidized samples were then assayed by LSP.

Administered dose was defined as total radioactivity administered less the amount recovered from the dose site shield. The amount of material penetrating into and through the dose site skin was defined as the administered dose less the amount recovered in the dose swabs. Systemically absorbed dose was calculated as administered dose less the amount recovered in the dose site swabs and the amount in the dose site skin (viable epidermis and dermis). Total recovery was calculated for each sample and the mass balance for these studies ranged from 83 to 98% (Data not shown).

RESULTS

All four PCBs studied penetrated from the site of application into the skin (Figure 2). This figure represents penetration of compound into and through the stratum corneum into the viable epidermis since the amount of radioactivity removed by swabbing is actually a measurement of both unabsorbed material and material in the stratum corneum (Banks and Birnbaum, 1991). Both the rate and degree of

penetration into skin were inverse to the degree of PCB chlorination. Nearly all of the administered MCB penetrated into the skin by 24hr post-administration and greater than 90% of the DCB entered the epidermis in the same period. By 48 hr post-administration, 77 and 33% of the administered TCB and HCB, respectively, had penetrated. Penetration into the skin appeared to roughly follow first order kinetics (Figure 3). Penetration rate constants are shown in Table 1. These penetration rate constants correlated with logarithm octanol-water partition coefficients (log K_{ow}) (Figure 4) for the respective chlorinated biphenyls (Rappaport and Eisenreich, 1984) to fit the linear equation

(Eq. 1) $k_{pen} = -0.052 (\log K_{ow}) + 0.380$ ($r^2 = 0.95$).

This curve appeared to be heavily biased by the high value of the MCB constant and estimated negative penetration rates for log Kow values greater than ca 7.2. Calculating a regression curve that excluded the MCB penetration rate constant yielded a curve:

(Eq. 2) $k_{pen} = -0.0399 (\log K_{ow}) + 0.295$ ($r^2 = 0.99$).

The penetration rate constant for 2,2'4,4',5,5'-hexachlorobiphenyl calculated from the data of Fisher et al. (1989) was well predicted by the latter equation (0.003 h^{-1} predicted vs. 0.004 h^{-1} measured).

Retention of PCBs in the dose site skin (viable epidermis and dermis) , determined at each timepoint, is shown in Figure 5. Though TCB and HCB are absorbed more slowly across the stratum corneum, they showed a greater

tendency to remain in the dose site skin. Peak concentrations of TCB and HCB retained in the dose site skin reached ca 17% of the applied dose. Significantly lower peak concentrations of the more rapidly penetrating mono- and dichlorobiphenyls, 3 and ca 8% respectively, were observed in skin. The times at which peak concentrations occurred in the viable epidermis and dermis reflected the penetration rates. Peak times became shorter as rate of penetration increased.

Systemic absorption (Figure 6) was greatly affected by the combination of rates of penetration and reservoir effect arising from retention of PCBs in skin. Systemic absorption was greatest for MCB and DCB, which penetrate into and through the viable epidermis/dermis rapidly and are retained very little. Absorption of MCB was nearly complete by 24 hr post-administration. DCB absorption slowed to essentially zero after the dose was removed at 48 h, supporting the observation of minimal retention in the skin. Nearly all of the TCB that penetrated into the skin reached the systemic circulation but, due to retention in the epidermis and dermis, at a much slower rate. Nearly 15% of the HCB dose that penetrated into the epidermis remained in the skin by 2 weeks post-administration.

Disposition of PCBs following IV and oral administration is known to be affected by degree and position of chlorination (Matthews and Anderson, 1975; Matthews and Tuey, 1980). PCBs of low chlorination rapidly distribute to the tissues and

then are quickly metabolized and eliminated, whereas, most higher chlorinated PCBs are more slowly distributed, metabolized, and eliminated. PCBs tend to distribute first into highly perfused tissues such as liver and muscle and then are redistributed to tissues with high lipid content and low perfusion such as adipose and skin. PCB distribution to tissues following dermal exposure was also influenced by relative rates of systemic absorption. Peak tissue burdens occurred at progressively later times, with the latest times for the most slowly absorbed HCB (Figure 7). The very rapidly absorbed MCB reached maximal concentrations in blood and other tissues by 4hr post-administration. The maximum internal exposure to MCB was at 4h, with 37% of the dose present in tissues but the fraction of absorbed dose remaining in the tissues at 2 weeks post-dose was 0.2% (Figure 7). DCB distribution was similarly influenced by rapid absorption, but tissue burden was much lower, the maximum internal dose being only ca. 8% of the total dose (Figure 7). The slowly absorbed PCBs did not reach elevated concentrations in tissues until well after the unabsorbed dose had been removed from the site of application. TCB and HCB tissue levels remained fairly level after peak concentrations because of slow absorption from the dose site reservoir. The effect was similar to that of a slow-release drug formulation, but was magnified because of the slow metabolism and elimination of the higher chlorinated PCBs. TCB internal exposure was the greatest , with ca 85% of the total absorbed dose present in tissues 72 hr post-administration. HCB equivalents in tissues continued to rise through 2 weeks post-dose since systemic absorption was still incomplete when the study was terminated.

DISCUSSION

Each of the PCBs studied here were absorbed into the skin. The rate and therefore the extent of dermal absorption of PCBs varied greatly over the range of chlorination. Penetration through the stratum corneum into the viable epidermis was apparently a first-order process and the rate constants appeared to be a function of K_{ow}. Within a group of structurally related molecules, K_{ow} is a measure more of a compound's tendency to partition out of water (hydrophobicity) than its absolute affinity for lipid (lipophilicity) (Leo et al., 1971). Therefore, the rate of penetration into the viable epidermis is probably limited more by penetration of the PCB into the relatively water-rich viable epidermis than by penetration through the lipid rich stratum corneum. MCB penetrated into the viable epidermis at a rate faster than its Kow would predict. However, metabolism at the stratum corneum /epidermal interface may have contributed to MCB penetration. The skin possesses cytochrome P450 activities in the epidermis that are approximately 80% of hepatic activities (Noonan and Wester, 1989). MCB is known to be rapidly and extensively metabolized in the rat liver by cytochromes P450 (Hass et al., 1977; Parkinson and Safe, 1981) and this laboratory has preliminary evidence that this PCB is metabolized in skin slices (data not shown). This data supports the suggestions of Noonan and Wester (1989) and Jackson et al. (1993) that penetration of highly lipophillic compounds into viable epidermis and subsequently into the systemic circulation may be rate limited by metabolism. The work of Holland et al (1984) and Kao et al (1985)

further suggest that the absorption of lipophillic compounds such as benzo(a)pyrene is increased following induction of metabolic enzymes

PCB retention in the viable epidermis was greatest with the more highly chlorinated TCB and HCB. MCB and DCB equivalents peaked very quickly in the viable epidermis and were very rapidly removed to the systemic circulation. This retention of the highly chlorinated PCBs may be a function of partitioning into adipose and/or adsorption to epithelial proteins. Albumin is the major binding constituent in skin and other tissues (Menczel et al. 1985) and PCBs adsorb tightly to such proteins (Matthews, et al., 1984). Bidmon et al. (1990) has reported that the hair follicles and sebaceous glands of rat skin serve as a reservoir for the hormone estradiol following penetration. Regardless of the mechanism, the viable epidermis/dermis served as a slow release reservoir for systemic absorption of the higher chlorinated PCBs. In the case of HCB ca 15% of the absorbed dose was still present in the dose site skin 12 days after the dose was removed from the dose site.

The rate of systemic absorption of PCBs was not characteristic of a simple first order process but is kinetically complex. This suggests that systemic absorption of PCBs involves a combination of sequential processes, including penetration across the stratum corneum, possibly metabolism in the epidermis and/or dermis, affinity for lipophillic sites on proteins and/or partition into sebum, and finally absorption into the systemic circulation. The skin favored the rapid

absorption of less chlorinated PCBs which, following absorption, are quickly metabolized in the liver and, presumably, in skin to more polar compounds that are quickly eliminated. The relatively rapid metabolism and elimination of these compounds would result in lower body burdens. Higher chlorinated PCBs do not penetrate into the skin as rapidly but are retained in the site of exposure and are very slowly absorbed systemically. The combination of slow systemic absorption with slow elimination from the tissues results in higher body burdens of the higher chlorinated PCBs.

The relationship between dermal penetration rates and Kow developed here (Equation 2) was used to predict the extent of dermal penetration of Aroclor 1254, a once commonly used commercial mixture of PCBs. Penetration rates of the PCB components of Aroclor 1254 (WHO, 1993) were estimated from log K_{ow} (Rappaport and Eisenreich, 1984). Approximately 20% of Aroclor 1254 is composed of PCBs with four or less chlorines, half are pentachloro-biphenyls, and 23% contain six or greater chlorine atoms. According to our model, within forty eight hours following application of Aroclor 1254 to the skin, approximately half of the mass of PCBs would penetrate the stratum corneum into the viable epidermis (Table 2). Greater than 85% of the components with 4 or less chlorines, ca 60% of pentachlorobiphenyls, and only 18% of hexachlorobiphenyls are predicted to penetrate the skin in 48 hr. Penetration of PCBs with 7 or more chlorines is predicted to be less than 1%. Sixty percent of the total absorbed PCBs will be pentachlorobiphenyls and less than 5% will be

hexachlorobiphenyls. The high mass contribution (62%) of the pentachlorobiphenyls in the penetrated PCBs is a function of their large mass proportion in Aroclor 1254 (ca 50%). The physical act of dermal penetration is predicted to slightly enrich the profile of systemically absorbed PCBs with lower chlorinated species. However, the more rapidly absorbed PCBs are also more rapidly cleared, resulting in lower contribution to total body burden. Penta- and hexachlorobiphenyls, which are the predominant species environmentally (WHO, 1993) are predicted to be slowly released from the epidermis/dermis reservoir, and because of their slow clearance, account for most of the body burden resulting from a hypothetical exposure.

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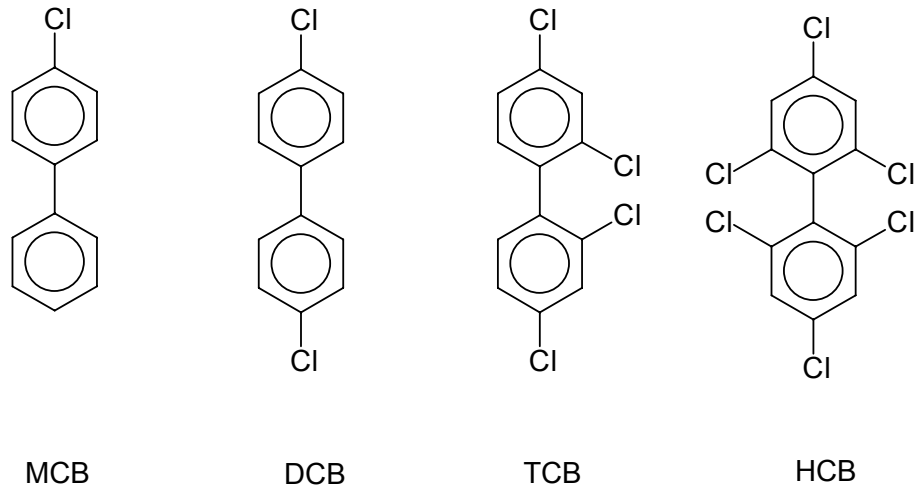


Figure 1. Chemical structures of PCBs used in dermal studies.

Abbreviations: MCB, 4-chlorobiphenyl; DCB, 4,4'-dichlorobiphenyl; TCB, 2,2',4,4'-tetrachlorobiphenyl; and HCB, 2,2',4,4',6,6'-hexachlorobiphenyl.

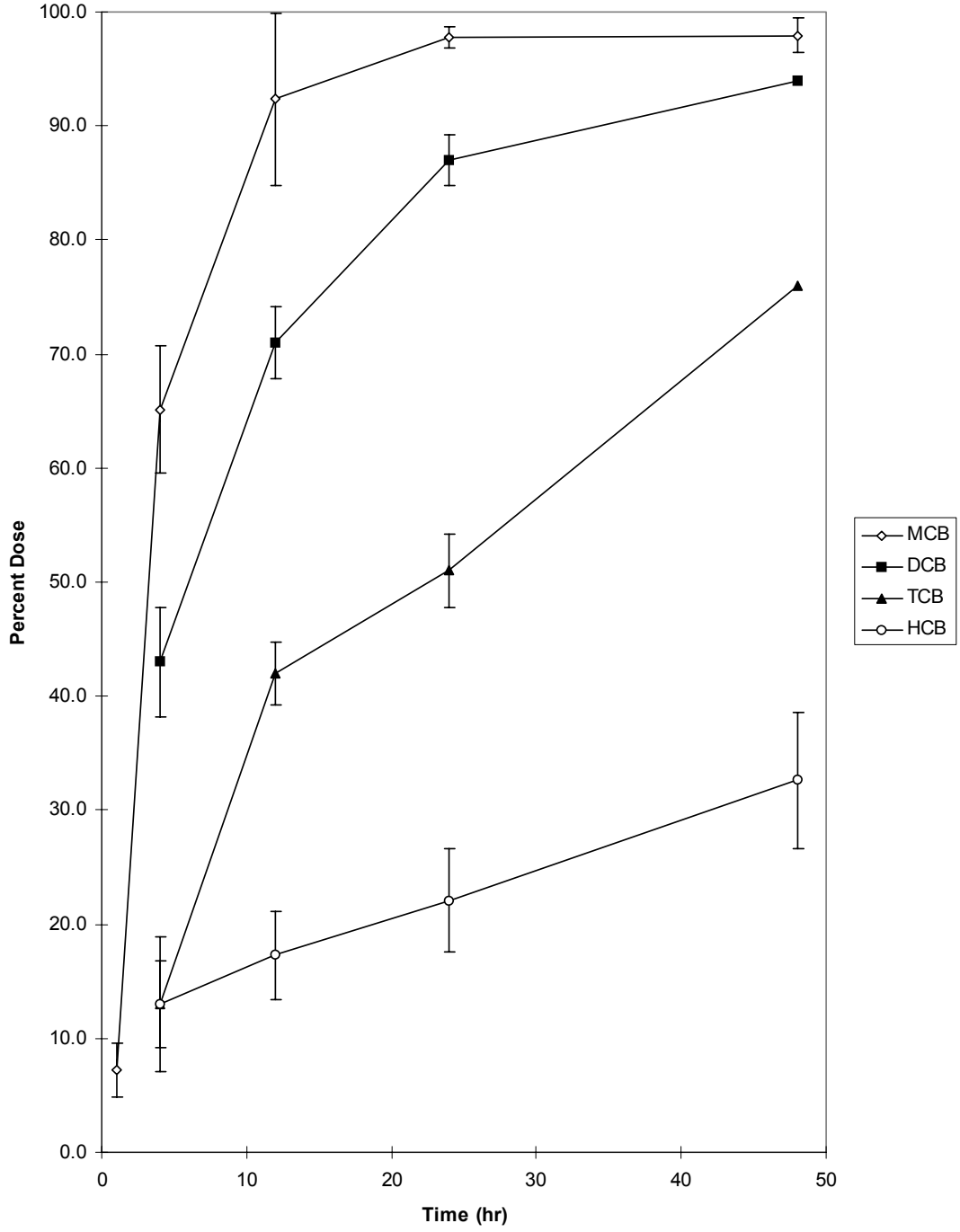


Figure 2. Cumulative penetration of selected PCBs into the dose site following dermal application (0.4 mg/cm^2) to F-344 rats.

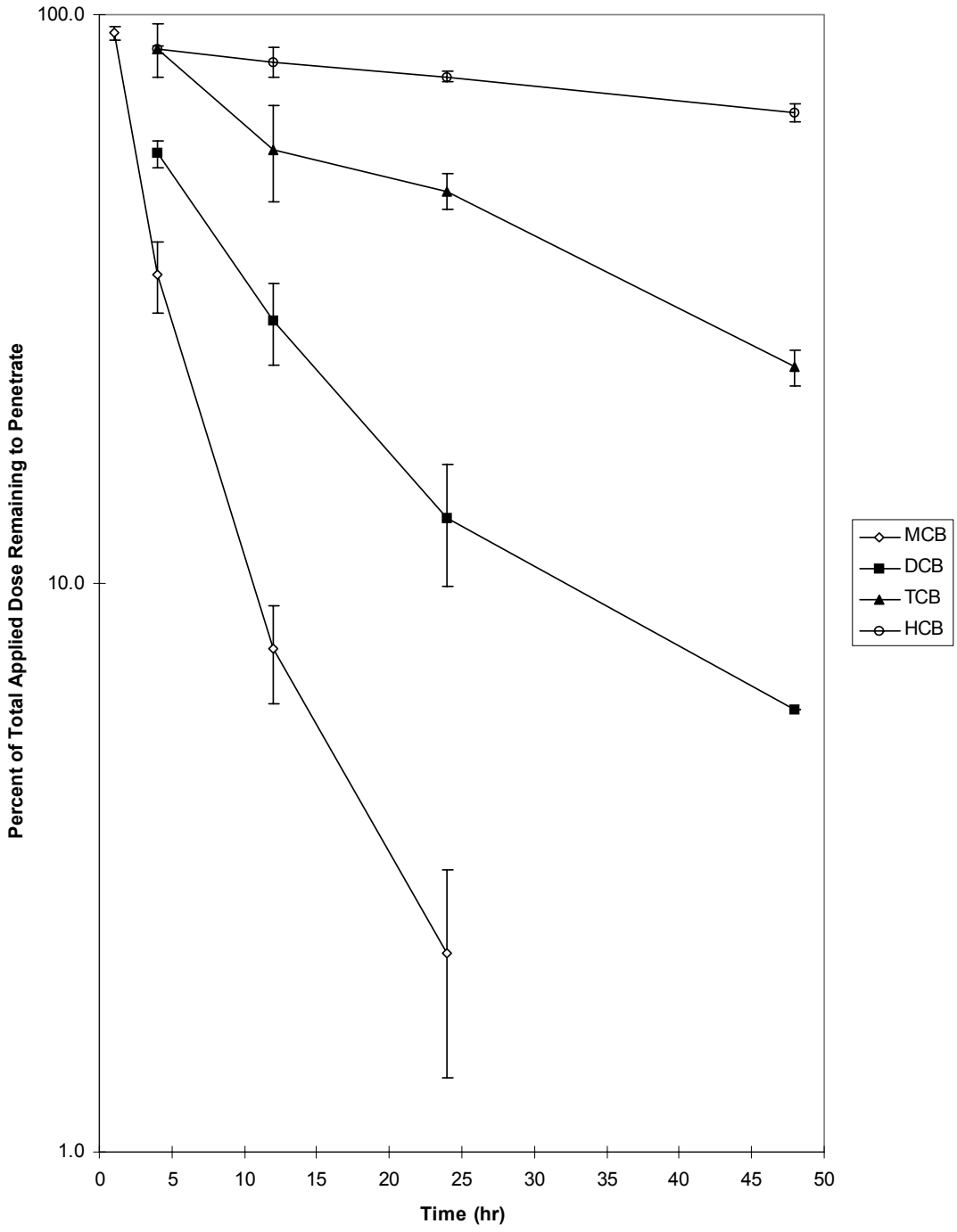
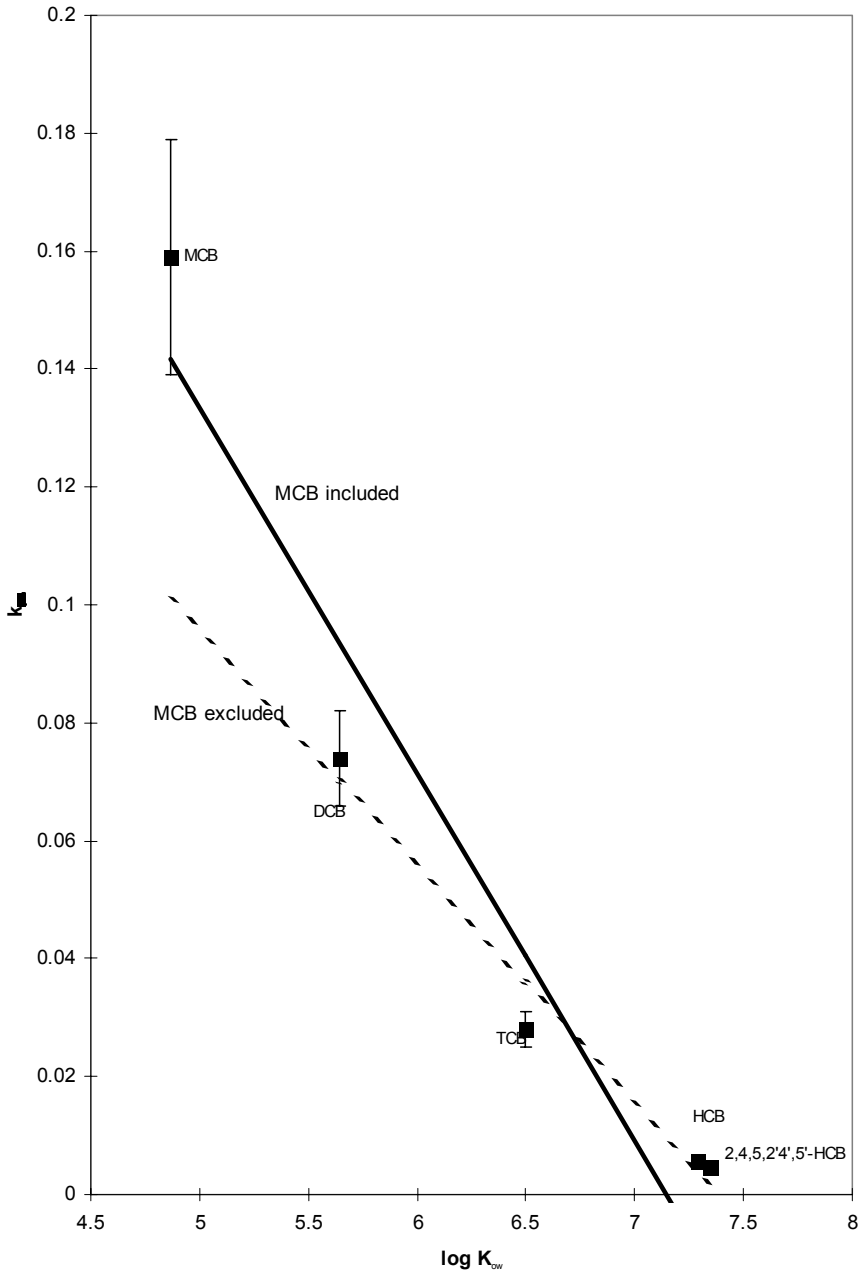


Figure 3. Amount of applied dose remaining to penetrate into the dose site following dermal application of PCBs (0.4 mg/cm^2) to F-344 rats.



[a]Penetration rate constants correlated with log K_{ow} (Rappaport and Eisenreich 1984) to fit the linear equation: $k_{pen} = -0.052 (\log K_{ow}) + 0.380$ ($r^2 = 0.95$). A regression curve that excluded the MCB penetration rate constant (dashed line) yielded a curve:
$k_{pen} = -0.0399 (\log K_{ow}) + 0.295$ ($r^2 = 0.99$).

Figure 4. Dermal penetration rate constants of selected PCBs vs. log K_{ow}.

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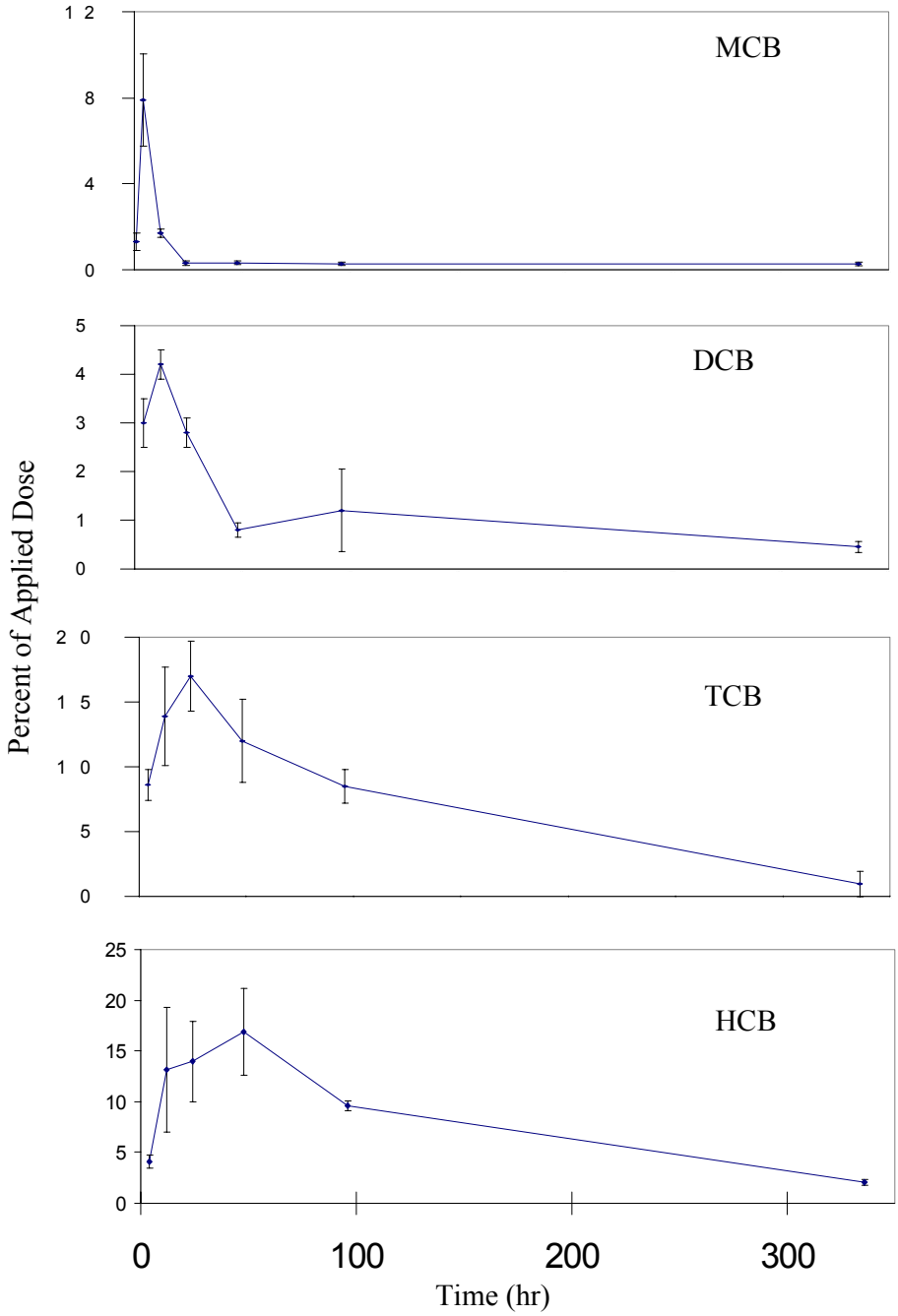

Figure 5. Percent of applied dose remaining in dose site skin following dermal application of PCBs (0.4 mg/cm^2) to F-344 rats.

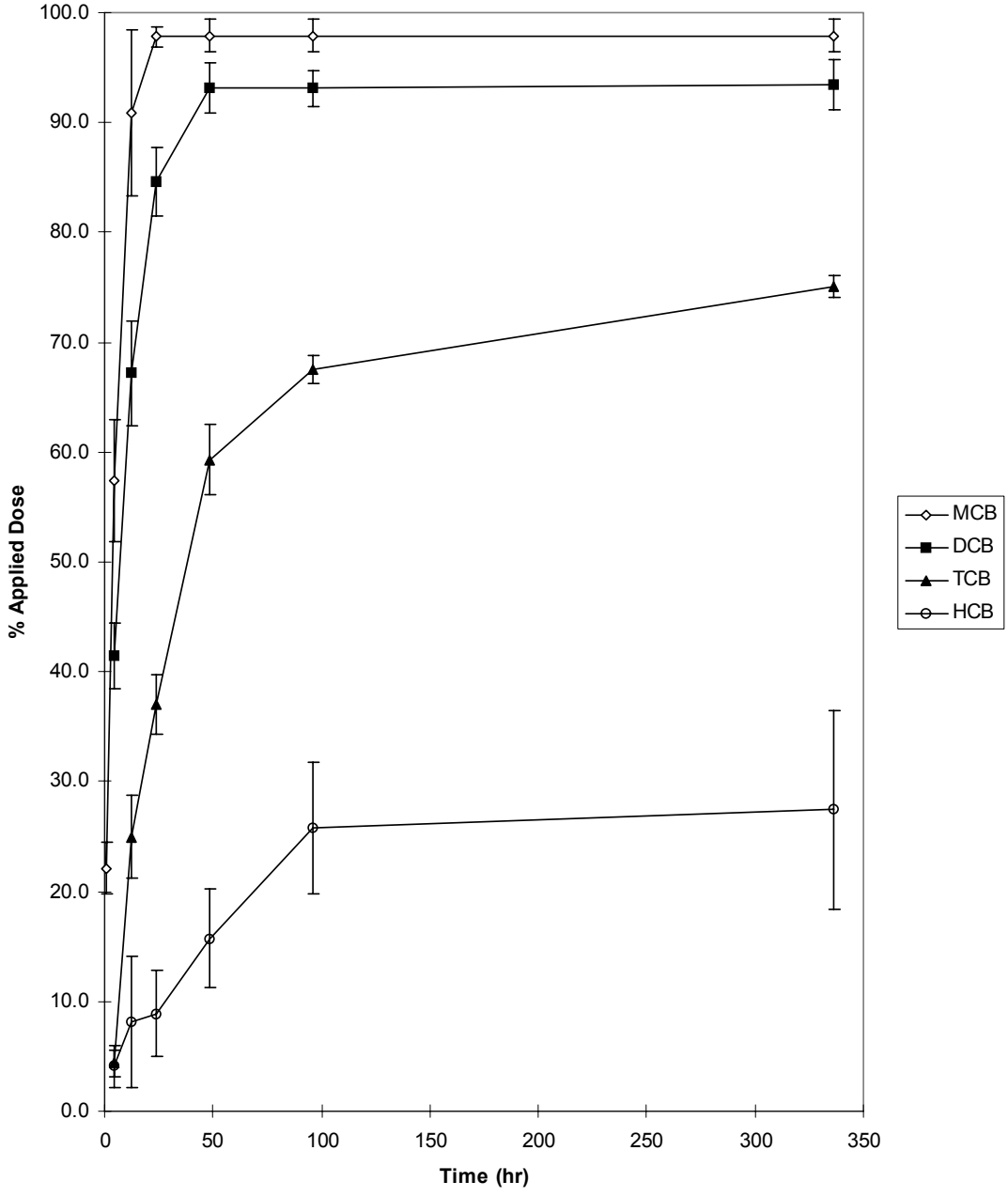

Figure 6. Percent of applied dose systemically absorbed following dermal application (0.4 mg/ cm^2) of selected PCBs to F-344 rats.

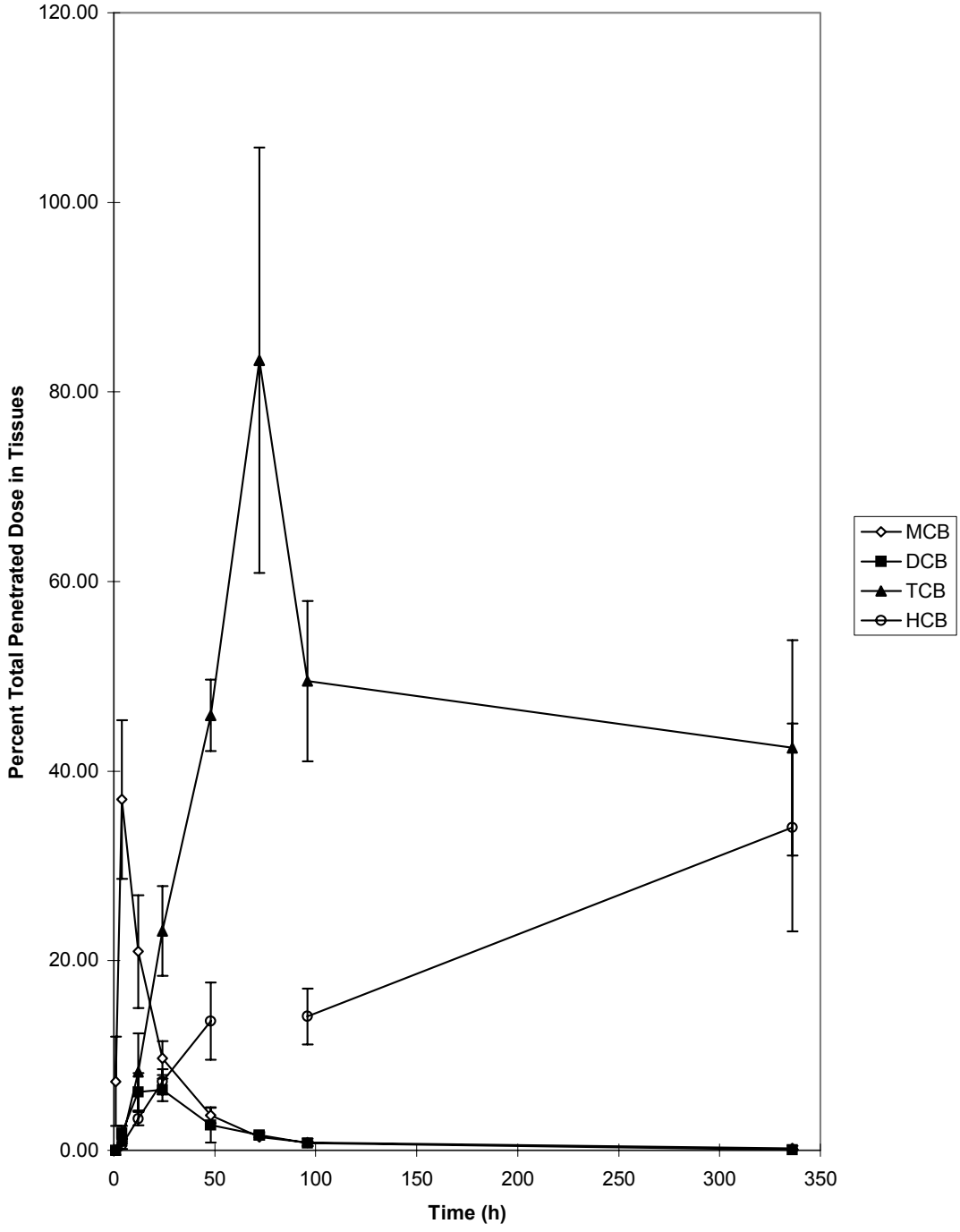

Figure 7. Percent of total penetrated dose present in tissues following dermal application (0.4 mg/ cm^2) of selected PCBs to F-344 rats.

Table 1. *Dermal Penetration Rate Constants for Selected PCBs.*

Compound	k_{abs} (hr^{-1})
MCB	0.14
DCB	0.074
TCB	0.028
HCB	0.0058

Table 2. *Predicted[a] Absorption of Aroclor 1254 48 hr Following Application to the Skin of F-344 Rats*

Number of Chlorine Atoms	*Percent Composition in Araclor 1254*	*Predicted Percent Absorption*	*Percent of PCBs Systemically Absorbed*
<4	1.0	93.5	1.8
4	18.7	84.8	31.0
5	50.7	62.8	62.3
6	13.8	18.4	5.0
7	9.3	0.0	<1
8	3.3	0.0	<1
>9	0.0	0.0	<1
Araclor 1254	----	52.8	----

[a]Fraction (F_p) of PCB mixture penetrating into the skin predicted by:

$F_{p(x)} = (m_{(x)}) \exp(0.0399 (\log K_{ow(x)})*t - 0.295 * t)$

Where:
$F_{p(x)}$ = Fraction of congener x penetrating into the skin.
$m_{(x)}$ = Mol % Fraction of PCB congener x in Araclor 1254.
$k_{pen(x)} = -0.0399 (\log K_{ow(x)}) + 0.295$ (Eq. 2).
t = 48 hr.

Penetration fraction was calculated for each individual congener and then mole fraction penetrating values summed for each group of congeners. See Materials and Methods for derivation of Equation 2.

The Effect of Chlorine Substitution on the Disposition of Polychlorinated Biphenyls Following Dermal Administration.

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ABSTRACT

The fate of selected polychlorobiphenyls (PCBs) was investigated following single dermal administration (0.4 mg/kg) to determine the effects of chlorine position on the disposition of PCBs following dermal absorption. Single dermal doses of ^{14}C-labeled mono-, di-, tetra- and hexachlorobiphenyls were administered to 1 cm^2 areas on the backs of F-344 male rats. Distribution of radioactivity in selected tissues and excreta were determined by serial sacrifice at time points up to 2 weeks. Unabsorbed radioactivity was removed from the dose site at either sacrifice or 48h post-dose. The time course of equivalents in the tissues showed a dependence on rate and extent of absorption. The most rapidly absorbed PCBs reached peak tissue concentrations at early times and were cleared from the tissues rapidly. The higher chlorinated PCBs were slowly absorbed and tended to accumulate in the adipose and skin even after removal of unabsorbed dose. Excretion of absorbed radioactivity varied with chlorine content ranging from 27 to ca 100% at two weeks post-dose. Excretion profiles following dermal doses tended to differ from profiles following equivalent IV doses, as did the metabolite profiles in excreta. Skin slice incubation experiments suggested that first pass metabolism in the dermal dose site was responsible for metabolism and disposition differences between routes of administration. The data further suggest that the rate of absorption, and

therefore the disposition of PCBs following dermal administration may be mediated, either in part or fully, by transdermal metabolism.

INTRODUCTION

Polychlorinated biphenyls (PCBs) were widely used in a variety of industrial and consumer products. Over one million tons of PCBs were produced before their production was banned in the 1970s. Due to their chemical stability, these compounds are still ubiquitous environmental contaminants (WHO, 1993). PCBs are virtually insoluble in water, but are readily adsorbed onto soil, dust, and surfaces in homes and in factories (WHO, 1993). Thus, the dermal route represents a major route of human exposure to PCBs. Consequently, development of data to characterize structure/activity relationships that determine PCB penetration of skin is a critical part of any assessment of human health risks associated with exposure.

A number of authors have reported that PCBs are absorbed into the skin of animals (Vos and Beems, 1971; Wester et al, 1983; Fischer et al, 1989; Hughes, et al, 1992) and humans (Surber et al, 1990; Fischer et al, 1989; Wester et al, 1993). There is evidence that dermal exposure may have been the major route of exposure to PCBs rather than inhalation in the workplace (Lees et al, 1987; Acquavella et al., 1986). Dermal exposure of animals to high PCB levels has been reported to result in gross toxicities such as liver necrosis, renal tubular degeneration, and dermal hyperplasia (Vos and Beems, 1971). Exposure to much smaller amounts of PCB mixtures through the skin has been demonstrated

to effect hepatic cytochrome P450s, thyroid function, and to alter thyroid hormone metabolism (Batomsky and Murthy, 1976; Batomsky and Wyse, 1975; Bickers et al, 1975). The present study of distribution kinetics of individual PCB congeners has been designed to provide kinetic data on which predictions of the behavior of more complex PCB mixtures will be based. Specifically, these studies were designed to determine the effects of degree and position of chlorination on dermal penetration, systemic absorption and distribution and elimination of selected PCBs following dermal administration.

MATERIALS AND METHODS

Animals

Adult male F-344 rats (175-275 g) were purchased from Taconic (Germantown, NY) and acclimated at least one week prior to use in experiments. Animals were furnished food (NIH 31 rat chow) and tap water ad libitum and maintained on a 12 hour light/dark cycle. Rats used in experiments were housed in glass metabolism cages that provided for the separate collection of urine and feces.

Xenobiotic

The structures of the chemicals used in these studies are shown in Figure 1. The U-[^{14}C]-2,2',4,4'-tetrachlorobiphenyl (24-TCB) (specific activity 27.3 mCi/mmol) was purchased from New England Nuclear (Wilmington, DE). The U-[^{14}C]-4-chlorobiphenyl (MCB), (specific activity 18.8 mCi/mmol), U-[^{14}C]-3,3',4,4'-tetrachlorobiphenyl (34-TCB)), (specific activity 27.3 mCi/mmol), and U-[^{14}C]-2,2',4,4', 6,6-hexachlorobiphenyl (246-HCB) (specific activity 14.7 mCi/mmol) were purchased from Sigma Chemical Co. (St. Louis, MO). The U-[^{14}C]-4,4'-dichlorobiphenyl (4-DCB) (specific activity 58 mCi/mmol) was previously prepared at NIEHS (Matthews and Anderson, 1975). The U-[^{14}C]-2,2',3,3',6,6'-hexachlorbiphenyl (236-HCB) (specific activity 18.1 mCi/mmol) was purchased from Pathfinder Laboratories (St. Louis, MO). Each was determined to be at least 95% radiochemically pure by HPLC. Non-radiolabelled material was not used in these experiments.

Preparation and Delivery of Dose Forms

Dermal dose formulations were prepared by dissolving sufficient radiolabelled PCB in acetone for delivery of 0.4 mg PCB/kg in a dose volume of 0.15 ml/kg. Approximately 24 hours prior to dosing, animals were anesthetized with ketamine:xylazine (60:9 mg/kg IM) and the fur on a 5 x 5 cm area was clipped from each animal's back. Any animal with nicked skin was excluded from use. Dermal doses were administered onto a 1 cm^2 area on the backs of the animals using a 50 mL Hamilton HPLC syringe (Hamilton Co., Reno, NV) with a blunt-tipped needle and the solvent evaporated under a gentle stream of air. A 1 cm^2 dose site was chosen to provide for a standardized area to make absorption comparisons. A stainless steel wire mesh shield (Shandon-Lipshaw Co., Pittsburgh, PA) was then glued over the dose site with cyanoacrylate adhesive (BONDINI-2, PRO-TEL Inc., Santa Monica, CA) to prevent grooming.

The applied dose was left on the dose site for up to 48 hours post-dose. Radioactivity remaining on the skin was removed at sacrifice or 48 hr post-application (see below), whichever was longer. It was reasoned that 48 hours was a reasonable estimate of the maximum time that contamination might be allowed to remain in contact with human skin. Disappearance of PCB from the dose site by evaporation was not likely since the PCBs studied are solids at 37° and have negligible vapor pressure at that temperature (Weast and Astle, 1983). Serial sacrifices were performed, with three animals per time-point at 1, 4, 8, 12,

24, 48, 72, 96, and 336 hr post-dosing. At each timepoint up to and including 48 hr, the animals were anesthetized with ketamine/xylazine, exsanguinated by cardiac puncture and sacrificed by opening of the thoracic cavity. The dose site shield was removed and unabsorbed radioactivity was then washed from the dose site with 5 acetone-soaked cotton gauze swabs. Radioactivity present on the shield was determined by extracting the shield with 25 ml acetone and assaying the extract by liquid scintillation spectrometry (LSS) (Beckman LS9800, Irvine, CA). Administered dose was calculated as applied radioactivity minus the amount of radiolabelled material recovered from the dose-site shield. Swabs were placed in Ultima Gold (Packard Instrument Co., Meriden, CT) and analyzed directly for radiochemical content by LSS. The washed dose site was then excised, solubilized in 2N ethanolic NaOH, and triplicate aliquots of the solution assayed by LSS.

Animals representing time points later than 48 hr were anesthetized at 48 hr post-dose with Ketamine:Xylazine (60:9 mg/kg IM), the shield removed, and the dose site washed with 5 acetone-soaked swabs. A new shield was cemented in place and the animal was returned to the metabolism cage. The cyanoacrylate adhesive used did not produce visible irritation, redness, or swelling. Swabs and the shield were treated as above. At sacrifice animals were handled in a manner similar to that described above, and the dose site was excised, solubilized, and analyzed for total radioactive content.

Intravenous dose formulations were prepared in Emulphor:ethanol:H_2O (1:1:8, v:v:v) for delivery of 0.4 mg/kg PCB/kg in a volume of 1 mL/kg and were injected into a lateral tail vein.

Analysis of Biological Samples

At sacrifice in the dermal studies blood, adipose tissue, muscle, skin (ears) and the entire kidney and liver were removed for assay of radiochemical content. Small tissue samples were oxidized to CO_2 with a Packard Oxidizer (Packard Instrument Co., Downer's Grove, IL). Livers were homogenized with a Polytron (Kinematica AG, Switzerland) and the homogenates oxidized. Oxidized samples were then assayed by LSS. Urine and feces excreted by each animal during the dermal and IV studies were collected separately and total radioactivity determined. Aliquots of urine were added directly to Ultima Gold. Feces were homogenized in an equal weight of water, triplicate aliquots were taken and allowed to air-dry. The dried samples were then oxidized and radiochemical content determined by LSS.

Profiles of radioactivity in excreta samples were determined by HPLC. Urine samples were filtered through a 0.45mm filter (Waters, Milford, MA) and injected directly onto an HPLC column. Feces samples were homogenized in an equal volume of H_2O and the homogenate extracted twice with 10 volumes of $CHCl_3$:Methanol (8:2, v:v). The extract was filtered through a 0.45mm filter (Waters, Milford, MA) and reduced to dryness under a gentle stream of N_2. The

extract was reconstituted in acetone and analyzed by HPLC. The HPLC system (System A) consisted of Waters model 510 pumps, a Rainin Microsorb MV C8 column with a IN/US b-RAM flow-through radioactivity detector (250mL lithium glass cell) and a gradient mobile phase flow of 2 mL/min. The gradient mobile phase conditions were: 100% H_20 initially, ramping linearly to 50% acetonitrile (MeCN) and holding for 10 minutes, then ramping to 100% MeCN over 5 minutes. Column eluent was collected in 1 minute fractions and counted by LSC to determine column recovery. Column recovery was quantitative.

Incubation of PCBs With Rat Skin Samples

Rats were anesthetized with ketamine (60 mg/kg) and hair was removed from their backs with animal clippers. Areas of 1 X 2 cm area were inscribed onto the clipped skin with a permanent felt-tipped marking pen. Cork rings 2mm thick with a 1 cm^2 annular space were cemented onto the skin surrounding the marked areas with cyanoacrylate adhesive (BONDINI-2, PRO-TEL Inc., Santa Monica, CA). The skin and cork ring were excised with scissors, removed, and placed in 4 mL of Waymouth's complete medium in a 6-well tissue culture dish and the animals were sacrificed by CO_2 asphyxiation. The cork ring prevented the upper surface of the slice from touching the medium or inverting. The dose (15 mL acetone containing PCB) was then applied to the top of each skin slice floating in the medium. The PCBs were applied at concentrations necessary to represent a 0.4 mg/kg dose applied to 1 cm^2 area of skin on a 250 g rat. The

slices were incubated at 37° C while atop a rotary shaker rotating at 90 RPM. Slices were removed from the medium at 8 h, separated from the cork rings, placed in 2 mL of acetone and shaken. The slices were washed with acetone 5 more times and then frozen at -20 C. The medium was removed from the culture dish wells, the well was rinsed with ca 2 mL of MeOH, and the rinse combined with the medium. Sample weights were taken by difference, and total radioactivity determined in each fraction by LSS. Skin slices were minced with scissors, frozen in liquid nitrogen and ground into a fine powder with a mortar and pestle. The skin powder was then extracted twice with 1 mL of $CHCl_3$. The $CHCl_3$ was filtered with a 0.45mm filter and evaporated under a gentle stream of nitrogen. The extract was then reconstituted in 1 mL of acetone and the radioactivity profile determined by HPLC.

The HPLC system (System B) consisted of Waters model 510 pumps, a Rainin Microsorb MV C8 column with a IN/US b-RAM flow-through radioactivity detector and a gradient mobile phase flow of 2 mL/min. The gradient mobile phase conditions were: 100% H_20 initially, ramping linearly to 100% acetonitrile (MeCN) over 15 minutes and holding for the remainder of the run. Column eluent was collected in 1 minute fractions and counted by LSC to determine column recovery. Column recovery was quantitative.

Definitions

Administered dose was defined as the total radioactivity applied less the amount of radioactivity recovered from the dose site shield. The amount of PCB

penetrating into the dose site skin was defined as the administered dose less the amount recovered in the dose site swabs. Systemically absorbed dose was calculated as administered dose less the amount recovered in the dose site swabs and the amount in the dose site skin (viable epidermis and dermis). Total internal dose was calculated as the sum of total radioactivity in all tissues measured plus that excreted. Total recovery was calculated and the mass balance for these studies ranged from 83 to 98% (Data not shown).

RESULTS

PCB-derived radioactivity in individual tissues of rats following a single dermal 0.4 mg/kg dose of MCB, 4-DCB, 24-TCB, or 246-HCB is shown in Tables 1-6.

Concentrations of MCB equivalents in all tissues reached a peak at 4 h post-dose. Maximum concentrations were found in skin, adipose, and kidney (Tables 3,4, and 6, respectively) but elimination from these and the other tissues was rapid. Radioactivity fell to undetectable levels in blood, muscle, and adipose by two weeks post-dose (Tables 1,5, and 6, respectively). Radioactivity in liver, kidney and skin declined at the same rate as in blood, being reduced to less than 1% of peak radioactivity by two weeks.

4-DCB was more slowly absorbed than MCB (Garner and Matthews, 1997, 1998) and this was reflected by the later peak in blood levels at 12 h and tissue concentrations at 12 -24h. Elimination of 4-DCB from blood was very rapid though, and concentrations fell below detection limits by 96h post-dose. Adipose equivalents reached a peak level approximately 10 times that of blood and concentrations remained elevated relative to blood through 96h.

24-TCB and 246-HCB were more slowly absorbed and tissue concentrations peaked at much later times. Blood concentrations reached a maximum at 24 and 48h post-dose for the 24-TCB and 246-HCB, respectively. There was not a consistent time of peak tissue concentrations, as with the lower chlorinated PCBs. However, maximum concentrations in most tissues occurred much later than in blood. Tissue concentrations were elevated considerably

relative to blood and remained so through 2 weeks post-dose as elimination was very slow for these two compounds. 24-TCB equivalents reached a plateau in adipose and skin. 246-HCB adipose equivalents continued to rise through two-weeks post-dose and had increased to 50 times blood concentration at the end of the study.

Concentrations of equivalents in blood following dermal application of PCBs is shown in Table 1. MCB peak blood concentrations exceeded those of the other PCBs by an order of magnitude. Peak blood concentration levels were reached at progressively later times as the degree of chlorination increased, presumably reflecting the slower rates of dermal penetration and systemic absorption (Garner and Matthews, 1997, 1998). Radioactivity from the lower chlorinated biphenyls was removed rapidly from the blood, while blood equivalents from tetra- and hexachlorobiphenyl decreased very slowly, approximating a plateau.

Liver equivalents are shown in Table 2. Equivalents of each PCB reached peak levels quickly and declined at a relatively slow rate. 24-TCB liver equivalents remained higher than those of the other PCBs through 2 weeks post-dose.

All of the PCBs showed an affinity for skin (Table 3) and adipose (Table 4). Concentrations of MCB and 4-DCB equivalents in skin and adipose peaked in a profile similar to blood. 24-TCB remained elevated relative to the others and

declined only very slowly. Hexachlorobiphenyl concentrations in both of these tissues increased slowly through 2-weeks.

MCB and 4-DCB muscle concentrations of equivalents (Table 5) peaked quickly and declined proportionately with those of blood. 24-TCB muscle equivalents peaked more slowly but remained at levels roughly 5-10 times higher than the other PCBs. 246-HCB slowly increased to ca 8 ng-equivalents/g by 48h and plateaued through 2 weeks. Kidney equivalents of the PCBs did not reach elevated levels and showed no signs of accumulation (Table 6).

The excretion profile of absorbed radioactivity in feces and urine following dermal administration is shown in Figures 2 and 3, respectively. Nearly 60% of the absorbed radioactivity was recovered in the urine of MCB treated rats. The remainder of absorbed monochlorobiphenyl-derived radioactivity was recovered in the feces. MCB is known to be extensively and rapidly metabolized and excreted following other routes of administration (Matthews and Andersen, 1975; Hass et al 1977). 4-DCB was also nearly completely excreted within 2 weeks. Approximately 70 and 23% of absorbed radioactivity was recovered in the feces and urine following administration of 4-DCB. Urinary excretion of the tetra- and hexachlorobiphenyl was negligible and most absorbed radioactivity was excreted in the feces in two weeks (44 and 57% of absorbed dose, respectively).

Although the monochlorobiphenyl was extensively eliminated following both an IV and dermal administration, the profiles of elimination differed considerably (Figure 4). Recovery of MCB-derived radioactivity in urine was much greater following dermal administration. Since elimination of PCBs is generally considered to be determined primarily by rate of hepatic metabolism, the excretion profile data suggest that a difference in metabolism may exist between routes of administration. Excretion profile data from IV doses of the other PCBs did not show a significant difference from that of dermal doses (data not shown).

The profile of metabolites in excreta following equivalent IV and dermal doses of mono-, di-, tetra and hexachlorobiphenyls was investigated to determine if there was a route-dependent difference in metabolites formed. The profile of urinary metabolites following a single IV dose of the monochlorobiphenyl is shown in (Figure 5a). As has been previously reported a number of metabolites were observed (Parkinson and Safe 1981). Parent was not detected. The profile of radioactivity in the urine collected 12h after the dermal administration indicates a single major metabolite was found (Figure 5b). Though the scope of these studies did not include efforts to identify PCB-derived metabolites, the 4-CB derived metabolite did not coelute with 4'-hydroxyl-4 chlorobiphenyl, the principle metabolite formed hepatically following IV and PO exposure (Hass et al, 1977). Incubation of the urinary metabolite with b-glucuronidase/sulfatase (Sigma, St. Louis) produced a peak that was chromatographically distinct from 4'-hydroxyl-4 chlorobiphenyl. This marked difference suggested that, following

dermal administration, the liver (the principal organ of PCB metabolism) was not presented with the same substrates for metabolism as following an IV dose.

Comparison of urinary metabolite profiles following IV or dermal administration of the dichlorobiphenyl did not indicate the dramatic differences observed for MCB(Data not shown). However, a number of peaks present in the urine following an IV administration were absent following the dermal administration. Metabolite profiles of radioactivity in feces following a single dermal or IV dose of the tetrachlorobiphenyl and hexachlorobiphenyl showed little or no significant differences (data not shown). Structural identification of the metabolites formed from 4-chlorobiphenyl following dermal administration was not attempted at this point in our investigation.

Differences in metabolite profiles suggested extrahepatic metabolism of MCB, most likely in the dose site. To confirm that rat skin is capable of metabolizing PCBs, 1 cm^2 skin slices were incubated for 8 hours with several ^{14}C-labelled PCBs. These congeners were chosen to represent a spectrum of chlorination patterns including several known to be readily metabolized as well as congeners known to be more resistant to metabolism. The PCBs were applied at concentrations necessary to represent a 0.4 mg/kg dose applied to a 1 cm2 to a 250 g rat. Each of the PCBs was relatively well absorbed into the skin samples (Figure 6). HPLC analysis of radioactivity extracted from skin slices demonstrated that rat skin has significant metabolic activity for PCBs. The skin

completely converted 4-chlorobiphenyl to a single metabolite (Figure 7a). Though the scope of these studies did not include efforts to identify dermally formed, PCB-derived metabolites, the 4-CB derived metabolite did not coelute with 4'-hydroxyl-4 chlorobiphenyl, the principle metabolite formed hepatically following IV and PO exposure (Hass et al, 1977). The skin exhibited metabolic activity toward nearly all of the other PCBs, but not to the same extent as against 4- chlorobiphenyl (Figure 7b-d). The 24-TCB and 246-HCB, as expected, were not readily metabolized (data not shown. This pattern is expected given that hepatic metabolism activity varies with degree and position of chlorination.

DISSCUSSION

The metabolism and disposition of PCBs following IV or oral administration is known to be greatly influenced by the degree and position of chlorination of the PCB congener studied (Matthews and Anderson, 1975; Matthews and Tuey, 1980). PCBs of low chlorination rapidly distribute to the tissues and are quickly metabolized and eliminated. Most higher chlorinated PCBs are more slowly distributed among the tissues, metabolized, and eliminated. All PCBs tend to distribute first into highly perfused tissues such as liver and muscle and then accumulate in tissues with high lipid content and low perfusion such as adipose and skin. PCB distribution to tissues following dermal exposure followed similar patterns but was also influenced by varying degrees and rates of systemic absorption. The rate of PCB absorption from the skin is apparently much more complex than absorption from the GI tract and varies greatly among individual PCB congeners studied. The kinetics of PCB absorption involve a combination of sequential or parallel processes, including penetration across the stratum corneum, possibly metabolism in the epidermis and/or dermis, binding to proteins, and absorption into the systemic circulation (Garner and Matthews, 1997, 1998; Garner et al., 1998).

Peak tissue concentrations occurred at progressively later times, with the earliest maximum for the rapidly absorbed MCB and the latest times for the slowly absorbed 246-HCB. This lab has previously demonstrated that, for the four compounds studied in vivo, rate of absorption is MCB>>DCB>TCB>HCB

(Garner and Matthews, 1997; 1998) and the present data confirms that the peak tissue concentrations measured are a function of absorption rate. The maximum internal exposure to MCB was at 4h, with 37% of the dose present in all tissues but the tissues were essentially free of equivalents by 2 weeks post-dose. The slowly absorbed PCBs did not reach elevated concentrations in tissues until well after the unabsorbed dose had been removed from the site of application (Tables 1-5). 24-TCB tissue levels remained fairly constant after peak concentrations. 24-TCB internal exposure was greatest , with ca 85% of the total absorbed dose present in tissues at 72h post-administration. 246-HCB equivalents in tissues (predominately adipose and skin at later timepoints) continued to rise through 2 weeks post-dose. 24-TCB and 246-HCB equivalents concentrated to the largest degree in adipose tissue.

Excretion of absorbed radioactivity following dermal application was greatest for the lower chlorinated biphenyls, which were nearly completely excreted by 2 weeks post dose. The 24-TCB and 246-HCB were less extensively excreted (ca 40% and 60%, respectively) and, at two weeks, the excretion profiles had not reached plateau (again, reflecting slow systemic absorption). Elimination of PCBs is considered to be determined primarily by rate of hepatic metabolism, which is influenced by chlorine number and position (Matthews and Andersen, 1975; Matthews and Tuey, 1980). That is, PCBs with a high degree of chlorination and/or lacking vicinal hydrogens to facilitate arene oxide formation are slowly eliminated. Chlorination in the 4 position inhibits formation of arene

oxides at the sterically preferred position, between the 3 and 4 carbons. In the absence of metabolism to more hydrophillic molecules these very lipophillic compounds are very slowly eliminated. Elimination also affected tissue distribution. Those PCB congeners that are not metabolized are redistributed from the highly perfused tissues to the lipid rich tissues of adipose and skin.

Rat skin is known to posses several enzyme systems that mediate xenobiotic and endogenous substrate metabolism including cytochromes P450, epoxide hydrolase, glucuronyl transferase, sulfotransferase, and glutathione transferases (Mukhtar et al 1991, Bickers et al, 1981). The present work with skin samples incubated with PCBs demonstrated that rat skin has significant metabolic activity towards these compounds, particularly MCB. Dermally applied PCBs are also known to induce hepatic (Alvares and Kappas, 1977) and dermal (DeVito et al , 1993; Bickers et al, 1974; Finnen et al, 1984) P-450s in rodents and humans. Thus, transdermal metabolism of PCBs may influence both the absorption and disposition of xenobiotics and their metabolites. This conjecture is supported by the evidence of Kao and Hall (1987) who demonstrated that steroid absorption is mediated by metabolism. Kao et al (1985) also demonstrated that BaP and testosterone penetration was metabolism mediated in both rat and human skin. Conclusive evidence of PCB metabolism in skin was provided by the results of the skin culture experiments shown in Figure 7. This work demonstrates that those PCBs which possess structural characteristics that predispose them to rapid metabolism (MCB, 34-TCB, and 236-HCB) were the most extensively

metabolized in skin. The much more rapid absorption of MCB indicates that metabolism in skin may facilitate dermal absorption.

HPLC analysis of the urine of rats treated dermally with MCB showed a marked difference in metabolite profile between dermal and IV doses. The excretion profiles of the dermally treated animals, relative to those treated IV, showed a reduction in dose excreted via the feces with a concomitant increase in urinary excretion. This suggests that metabolite(s) formed during dermal absorption may be removed from the blood and excreted via the kidneys at a rate greater than they are absorbed by other tissues. Mehendale (1976) demonstrated in the isolated perfused rat liver that MCB metabolites formed in the liver after absorption of parent are very rapidly cleared via the bile and the rate of efflux of metabolites was minimal relative to biliary excretion. These experiments indicated that exogenously supplied MCB metabolites are absorbed by the liver and excreted via the bile at a slower rate than the parent compound . Thus, metabolite formed outside of the liver forms a separate pool from which excretion via the liver occurs at a slower rate, allowing urinary excretion to predominate. The formation of PCB metabolites during “first-pass” metabolism in the skin would allow for increased clearance of total equivalents from the system, since PCB metabolites are more rapidly excreted than parent. But greater exposure of the tissues to PCB metabolites, relative to other routes of exposure, is also possible. The metabolites formed dermally may also differ from those formed by other routes, potentially resulting in subtly different biological

responses. Dermal dose metabolite and excretion profiles of the more slowly absorbed and metabolized PCBs tend to more closely resemble those of equivalent IV doses, indicating that transdermal metabolism probably plays a smaller role in the fate of dermally absorbed PCBs with higher chlorination or chlorine substitution that limits rates of metabolism.

This report is the first to demonstrate the first-pass effect of skin metabolism and its effects on the disposition of PCBs. Dermal metabolism may strongly influence the bioavailability of PCBs encountered via dermal exposure. Further, the spectrum of parent and metabolites absorbed into the body following dermal exposure may vary significantly from that following other routes of exposure. Metabolic activity in the skin, a tissue generally considered a depot for PCBs (Matthews and Anderson, 1975), may mean that present risk assessment models for PCB exposure may underestimate exposure to PCB metabolites. The presence of PCB metabolites from first-pass metabolism may alter the disposition of other PCBs present systemically.

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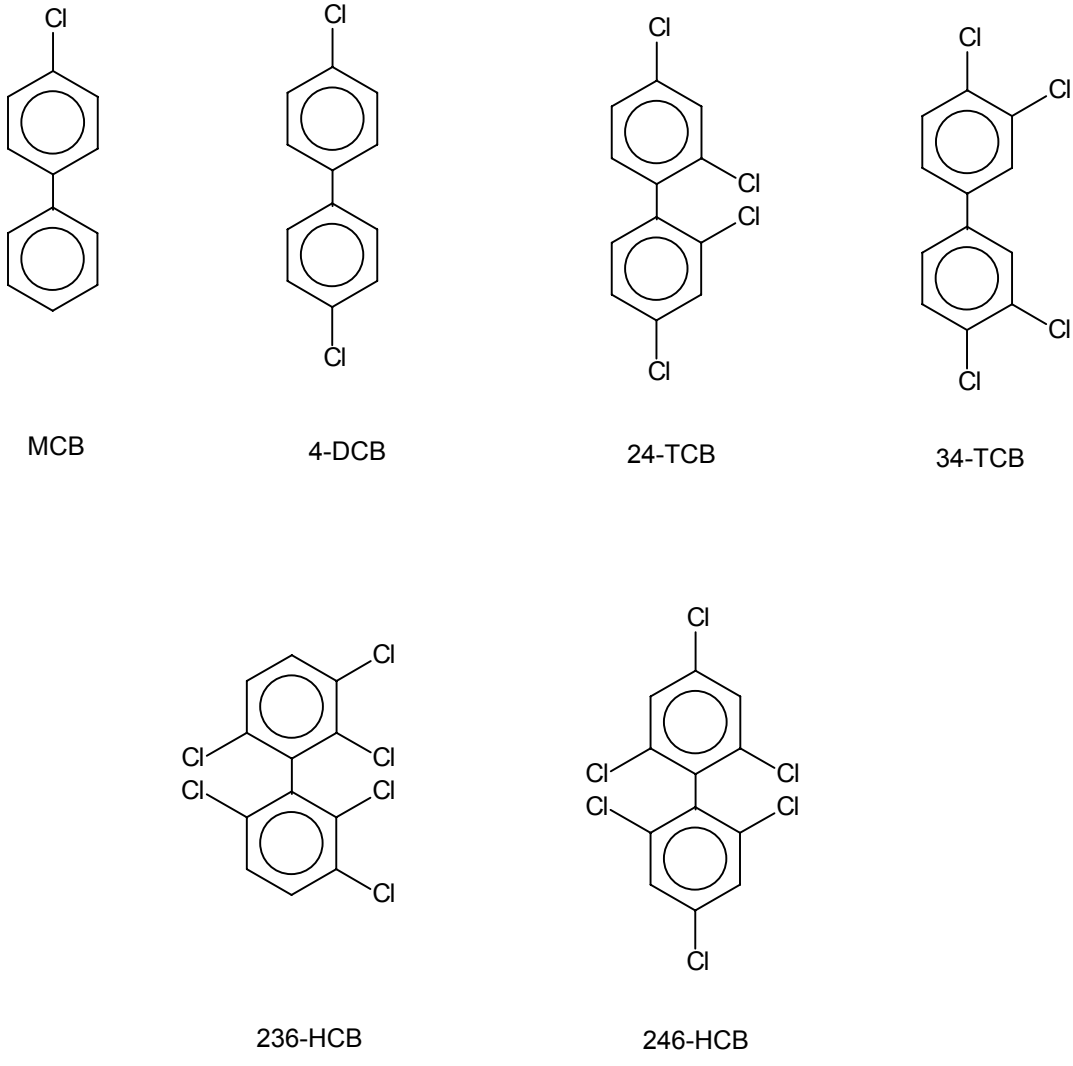

Figure 1. Structures of PCBs used in these experiments.

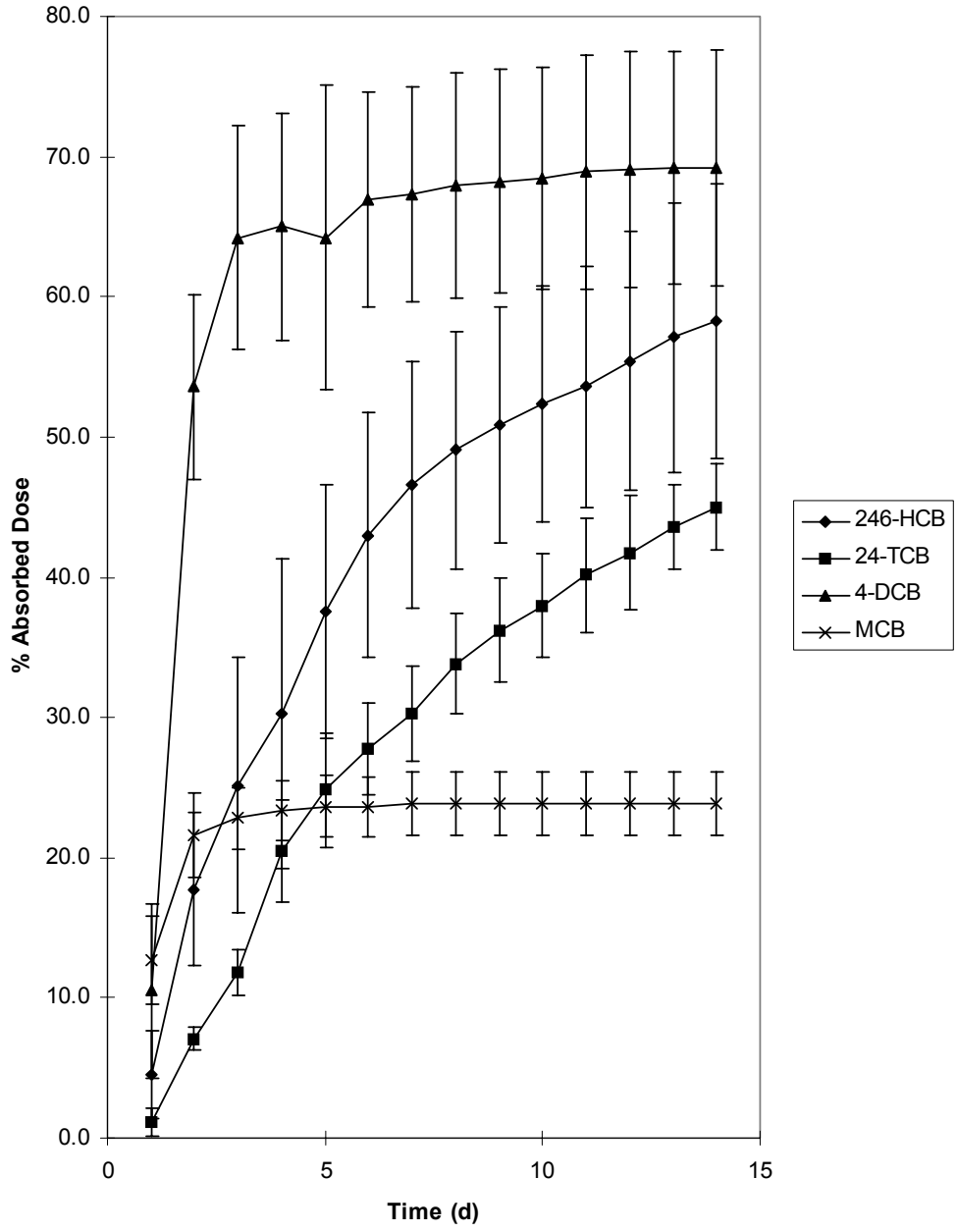

Figure 2. Percentage of total absorbed radioactivity recovered in the feces of F-344 rats following a single dermal administration of selected ^{14}C-labelled PCBs (0.4 mg/kg).

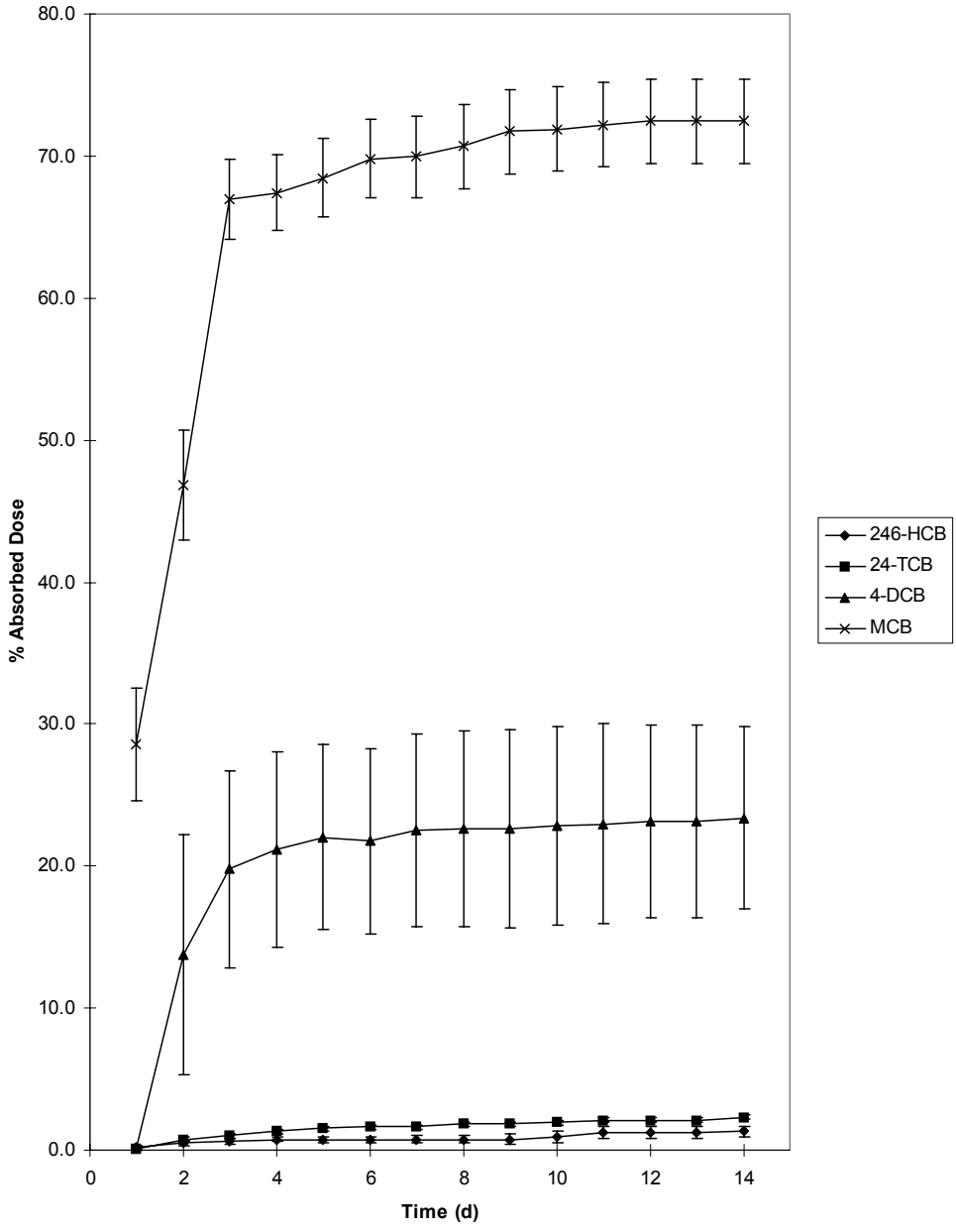

Figure 3. Percentage of total absorbed radioactivity recovered in the urine of F-344 rats following a single dermal administration of selected ^{14}C-labelled PCBs (0.4 mg/kg).

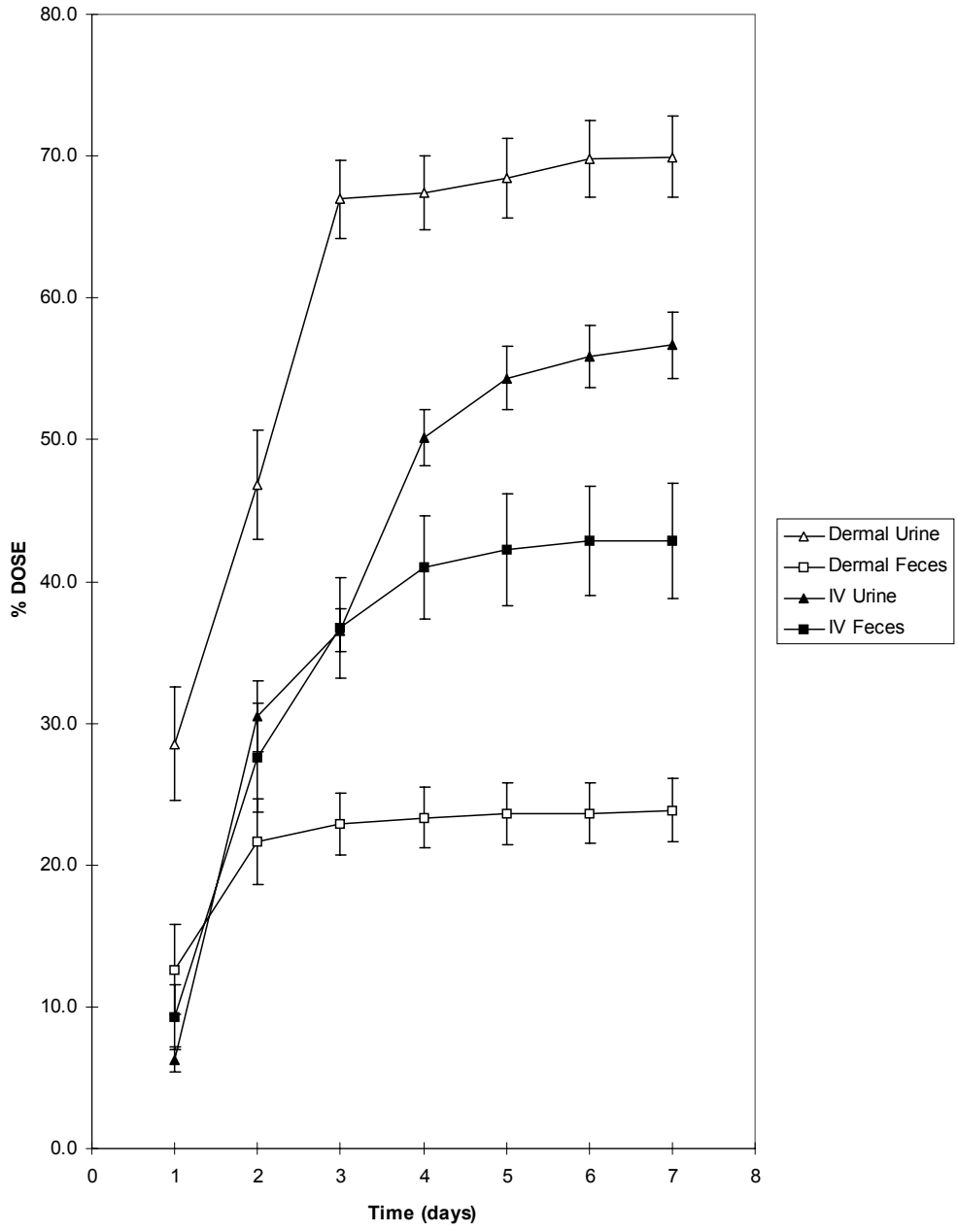

Figure 4. Percentage of total absorbed radioactivity recovered in the urine and feces of F-344 rats following either a single IV or dermal administration of ^{14}C-labelled MCB (0.4 mg/kg).

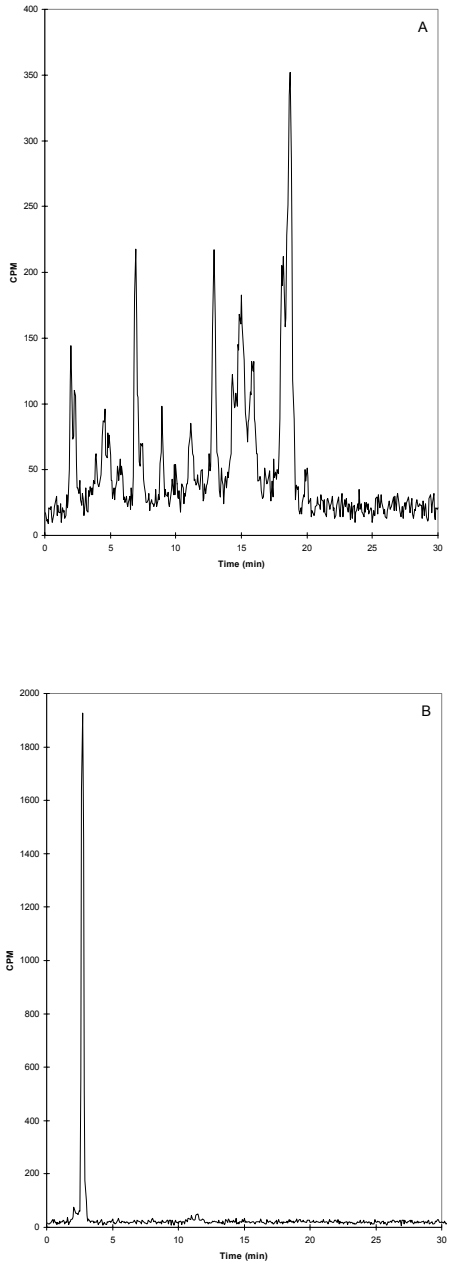

Figure 5. HPLC radiochromatograms of urine collected 12 hr following either a single IV (a) or dermal (b) administration of ^{14}C-labelled MCB (0.4 mg/kg).

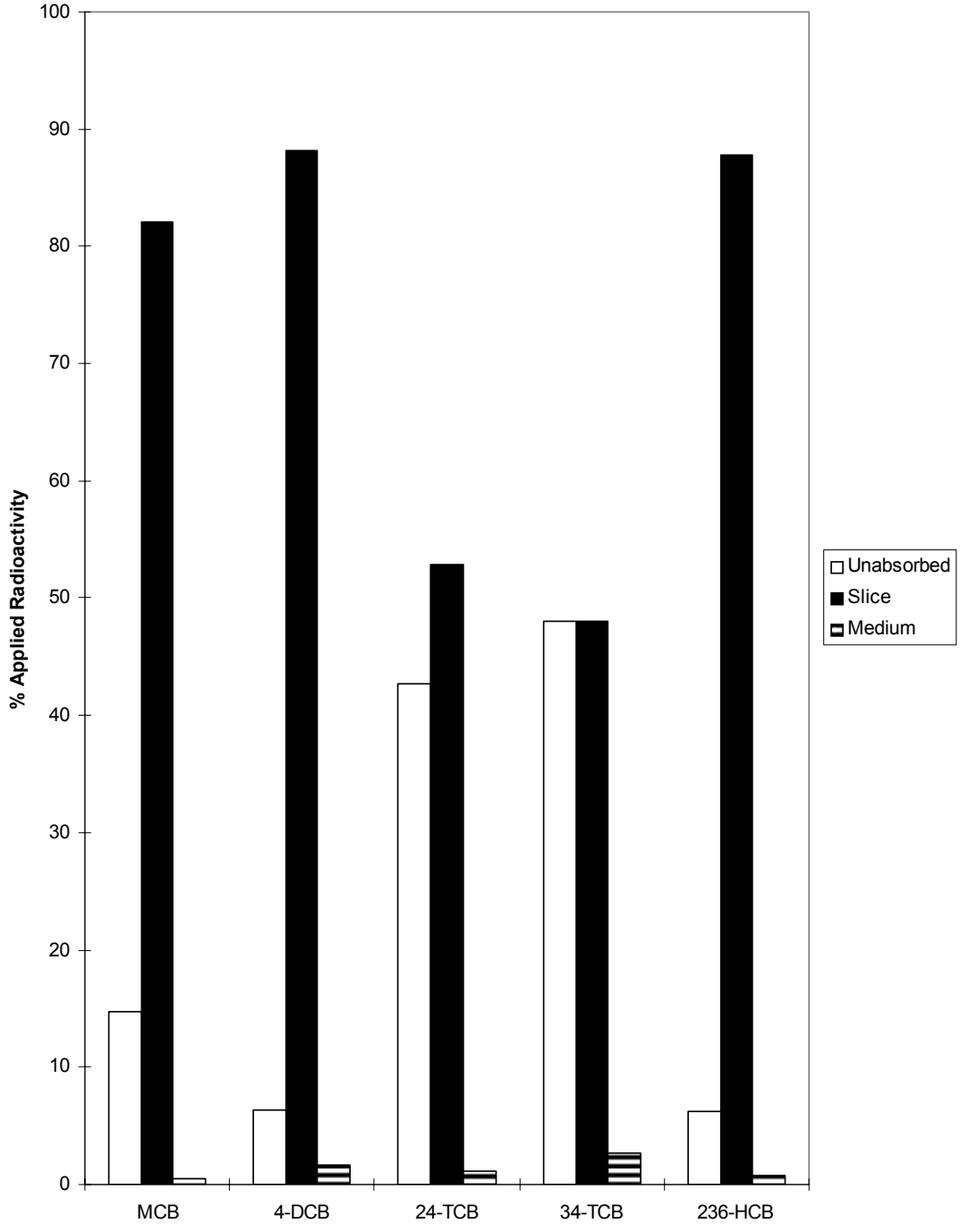

Figure 6. Percentage of applied radioactivity recovered following incubation selected ^{14}C-labelled PCBs with rat skin samples.

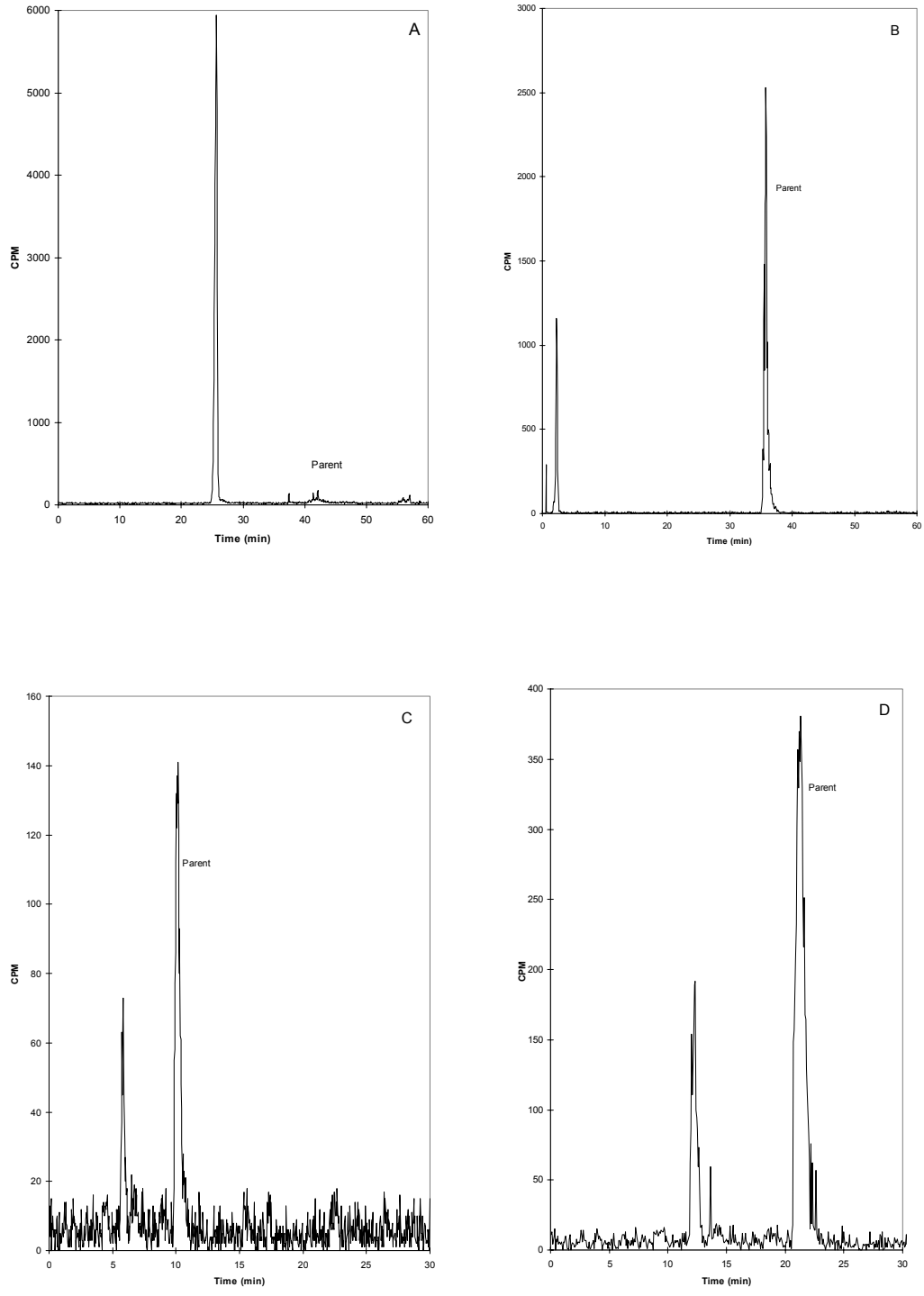

Figure 7. HPLC radiochromatograms of radioactivity extracted from skin slices incubated with ^{14}C-labelled (a) MCB, (b) 4-DCB, (c) 34-TCB, and (d) 236-HCB.

Table 1. Concentration and Distribution of PCB-Derived Radioactivity in Blood Following Dermal Administration of Selected PCBs (0.4 mg/kg) to F-344 Rats (N=3).

Time (h)	*MCB*	*4-DCB*	*24-TCB*	*246-HCB*
1	27 ± 8[a,b] (0.56 ± 0.17)	ND[c]	ND	ND
4	84 ± 13* (1.7 ± 0.3)	3.1 ± 0.9 (0.07 ± 0.02)	1.9 ± 0.8 (0.05 ± 0.02)	2.0 ± 0.9 (0.14 ± 0.07)
12	63 ± 16 (1.3 ± 0.3)	16.1 ± 5.6* (0.34 ± 0.12)	6.1 ± 2.4* (0.16 ± 0.06)	5.4 ± 1.9* (0.39 ± 0.14)
24	41 ± 12 (0.83 ± 0.25)	13.2 ± 6.2 (0.28 ± 0.13)	10± 3 (0.28 ± 0.09)	10 ± 1* (0.75 ± 0.04)
48	6.8 ± 0.9* (0.14 ± 0.02)	1.9 ± 1.5* (0.04 ± 0.03)	8.4 ± 2.3 (0.22 ± 0.06)	16 ± 4 (1.2 ± 0.3)
72	0.96 ± 0.52* (0.02 ± 0.01)	0.72 ± 1.18 (0.02 ± 0.03)	7.5 ± 2.3 (0.20 ± 0.06)	ND
96	0.72 ± 0.41 (0.01 ± 0.01)	<0.01	7.0 ± 3.2 (0.19 ± 0.08)	19 ± 1 (1.4 ± 0.09)
336	<0.01*	<0.01	3.0 ± 0.4 (0.08 ± 0.01)	4.4 ± 2.8* (0.32 ± 0.20)

[a] Mean ± S.D.
[b] ng Equivalents/g tissue (% absorbed dose).
[c] Not determined.
[d] Below detection limits.
* Significantly different ($p < 0.05$) from proceeding timepoint.

Table 2. Concentration and Distribution of PCB-Derived Radioactivity in Liver Following Dermal Administration of Selected PCBs (0.4 mg/kg) to F-344 Rats (N=3).

Time (h)	*MCB*	*4-DCB*	*24-TCB*	*246-HCB*
1	109 ± 21[a,b] (0.95 ± 0.18)			
4	232 ± 8* (2.01 ± 0.07)	15.6 ± 9.9 (0.014 ± 0.09)	7.95 ± 2.14 (0.09 ± 0.024)	4.26 ± 2.79 (0.13 ± 0.09)
12	149 ± 14* (1.29 ± 0.12)	61.5 ± 20.1* (0.56 ± 0.18)	70 ± 7.9* (0.79 ± 0.09)	16 ± 5* (0.50 ± 0.15)
24	91.3 ± 0.3* (0.79 ±	46.9 ± 10.2 (0.43 ± 0.09)	139 ± 19* (1.6 ± 0.2)	25.7 ± 7.3 (0.80 ± 0.23)
48	27.1 ± 5.1* (0.24 ± 0.04)	12.7 ± 0.6* (0.12 ± 0.05)	183 ± 5* (2.1 ± 0.1)	44.6 ± 13 (1.34 ± 0.40)
72	18 ± 2 (0.16 ±	5.88 ± 2.69 (0.05 ± 0.02)	101 ± 37* (1.1 ± 0.4)	ND[c]
96	13.7 ± 1.3* (0.012 ±	2.16 ± 0.17 (0.02 ± <0.01)	114 ± 29 (1.29 ± 0.32)	24.3 ± 0.9 (0.75 ± 0.03)
336	2.1 ± 0.2* (0.02 ±	0.44 ± 0.27* (<0.01)	62.1 ± 9.0* (0.70 ± 0.10)	11.2 ± 5.4* (0.35 ± 0.17)

[a] Mean ± S.D.
[b] ng Equivalents/g tissue (% absorbed dose).
[c] Not determined.
* Significantly different ($p < 0.05$) from proceeding timepoint.

Table 3. Concentration and Distribution of PCB-Derived Radioactivity in Skin Following Dermal Administration of Selected PCBs (0.4 mg/kg) to F-344 Rats (N=3).

Time (h)	*MCB*	*4-DCB*	*24-TCB*	*246-HCB*
1	35 ± 11[a,b] (1.4 ± 0.4)	ND[c]	ND	ND
4	437 ± 99* (16.7 ± 3.8)	8.8 ± 3.0 (0.35 ± 0.12)	7.6 ± 2.6 (0.38 ± 0.13)	0.51 ± 0.34 (0.07 ± 0.05)
12	173 ± 63* (6.6 ± 2.4)	41 ± 7* (1.6 ± 0.3)	42 ± 3* (2.1 ± 0.2)	6.6 ± 1.6* (0.90 ± 0.22)
24	66 ± 18 (2.5 ± 0.7)	36 ± 2 (1.4 ± 0.1)	145 ± 22* (7.2 ± 1.1)	14 ± 2* (2.0 ± 0.3)
48	46 ± 5 (1.8 ± 0.2)	16 ± 3* (0.64 ± 0.12)	209 ± 28* (10 ± 1)	20 ± 9 (2.7 ± 1.2)
72	12 ± 6* (0.47 ± 0.23)	7.6 ± 0.8* (0.30 ± 0.03)	209 ± 44 (10 ± 2)	ND
96	6.7 ± 7.3 (0.26 ± 0.28)	2.4 ± 1.2* (0.10 ± 0.05)	161 ± 49 (8.0 ± 2.4)	8.9 ± 7.7 (1.2 ± 1.0)
336	5.1 ± 5.8 (0.19 ± 0.22)	0.55 ± 0.40 (0.02 ± 0.02)	122 ± 22 (6.1 ± 1.1)	28 ± 10 (3.8 ± 1.3)

[a] Mean ± S.D.
[b] ng Equivalents/g tissue (% absorbed dose).
[c] Not determined.
* Significantly different ($p < 0.05$) from proceeding timepoint.

Table 4. Concentration and Distribution of PCB-Derived Radioactivity in Adipose Following Dermal Administration of Selected PCBs (0.4 mg/kg) to F-344 Rats (N=3).

Time (h)	*MCB*	*4-DCB*	*24-TCB*	*246-HCB*
1	115 ± 111 [a,b]			
	(3.2 ± 3.1)			
4	408 ± 85*	31.9 ± 14.2	2.69 ± 0.28	1.49 ± 1.24
	(12 ± 2)	(0.94 ± 0.42)	(0.10 ± 0.01)	(0.15 ± 0.12)
12	286 ± 39	106 ± 47	81.3 ± 75.2	7.84 ± 0.65*
	(8.0 ± 1.1)	(3.12± 1.4)	(3.0 ± 2.8)	(0.79 ± 0.07)
24	153 ± 18*	115 ± 28	245 ± 38*	22.5 ± 3.35*
	(4.3 ± 0.5)	(3.4 ± 0.8)	(9.0 ± 1.4)	(2.3 ± 0.4)
48	41.7 ± 9.8*	53.4 ± 47.6	726 ± 61*	50 ± 9*
	(1.2 ± 0.3)	(1.6 ± 1.4)	(26 ± 2)	(5.0 ± 0.9)
72	18.8 ± 1.7*	39.7 ± 6.9*	1630 ± 410*	ND[c]
	(0.53 ± 0.05)	(1.2 ± 0.2)	(60 ± 15)	
96	11 ± 2.9*	19.4 ± 4.9*	863 ± 72*	77.3 ± 3.42*
	(0.31 ± 0.08)	(0.57 ± 0.14)	(32 ± 3)	(7.8 ± 0.3)
336	<0.01*	1.24 ± 0.58*	901 ± 239	252 ± 69*
		(0.04 ± 0.02)	(33 ± 9)	(25.3 ± 6.9)

[a] Mean ± S.D.
[b] ng Equivalents/g tissue (% absorbed dose).
[c] Not determined.
* Significantly different (p< 0.05) from proceeding timepoint.

Table 5. Concentration and Distribution of PCB-Derived Radioactivity in Muscle Following Dermal Administration of Selected PCBs (0.4 mg/kg) to F-344 Rats (N=3).

Time (h)	*MCB*	*4-DCB*	*24-TCB*	*246-HCB*
1	8.86 ± 7.15[a,b] (1.0 ± 0.8)			
4	39.1 ± 15.5 (4.5 ± 1. 8)	2.54 ± 0.51 (0.31 ± 0.06)	2.24 ± 0.35 (0.34 ± 0.05)	0.07 ± 0.13 (0.03 ± 0.05)
12	27.8 ± 17.2 (3.2 ± 2.0)	3.09 ± 0.49 (0.37 ± 0.06)	14.4 ± 6.6 (2.2 ± 1.0)	1.75 ± 0.29 (0.72 ± 0.12)
24	9.25 ± 2.73 (1.1 ± 0.3)	6.24 ± 0.34 (0.75 ± 0.04)	33.2 ± 12.8 (5.0 ± 1.9)	3.28 ± 0.95 (1.4 ± 0.4)
48	2.56 ± 2.26 (0.29 ± 0.26)	2.37 ± 0.07 (0.29 ± 0.25)	42.7 ± 0.2 (6.4 ± 0.1)	7.49 ± 2.85 (3.1 ± 1.2)
72	1.94 ± 0.32 (0.22 ± 0.04)	0.59 ± 0.11 (0.07 ± 0.01)	78 ± 31 (11 ± 5)	ND[c]
96	0.71 ± 0.16 (0.08 ± 0.02)	0.42 ± 0.28 (0.05 ± 0.03)	55 ± 20 (8.3 ± 3.0)	6.95 ± 3.46 (2.9 ± 1.4)
336		0.09 ± 0.08 (0.01 ± 0.01)	16.9 ± 9.3 (2.5 ± 1.4)	10.29 ± 5.6 (4.2 ± 2.3)

[a] Mean ± S.D.
[b] ng Equivalents/g tissue (% absorbed dose).
[c] Not determined.

Table 6. Concentration and Distribution of PCB-Derived Radioactivity in Kidney Following Dermal Administration of Selected PCBs (0.4 mg/kg) to F-344 Rats (N=3).

Time (h)	*MCB*	*4-DCB*	*24-TCB*	*246-HCB*
1	149 ± 5.2[a,b] (0.15 ± 0.01)			
4	561.4 ± 82.6* (0.57± 0.08)	22.8 ± 16.0 (0.03 ± 0.02)	6.36 ± 1.9 (0.08 ± <0.01)	3.2 ± 1.3 (0.01 ± <0.01)
12	487± 40 (0.50 ± 0.04)	91 ± 23* (0.1 ± 0.03)	53.9 ± 8.3* (0.07 ± 0.01)	9.98 ± 5.85 (0.04 ± 0.02)
24	214 ± 18* (0.22 ± 0.02)	68.6 ± 9.52 (0.07 ± 0.01)	65.7 ± 10.5 (0.09 ± 0.01)	31.1 ± 2.51* (0.11 ± 0.01)
48	52.2 ± 9.2* (0.05 ± 0.01)	16.0 ± 6.4 * (0.02 ± 0.01)	118 ± 1* (0.16 ± <0.01)	71.5 ± 13.7* (0.26 ± 0.05)
72	28 ± 4.2* (0.03 ± <0.01)	9.63 ± 3.92 (0.01 ± <0.01)	130 ± 57 (0.17 ± 0.08)	ND[c]
96	18.9 ± 2.5* (0.02 ± <0.01)	5.42 ± 1.12 (0.01 ± <0.01)	69.0 ± 10 (0.09 ± 0.01)	31.6 ± 5.38* (0.12 ± 0.02)
336	1.61 ± 0.9* (<0.01)	1.32 ± 0.27* (<0.01)	39.1 ± 4.1* (0.05 ± 0.01)	15.6 ± 1.4* (0.06 ± <0.01)

[a] Mean ± S.D.
[b] ng Equivalents/g tissue (% absorbed dose).
[c] Not determined.
* Significantly different (p< 0.05) from proceeding timepoint.

Comparative Disposition of 3,3',4,4'-Tetrachlorobiphenyl (PCB 77) Following Dermal vs IV or Oral, Administration.

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ABSTRACT

A common route of human exposure to PCBs is via dermal contact. Thus, the disposition of 3,3',4,4'-tetrachlorobiphenyl (PCB 77) in rats was investigated following administration of a low dose (0.4 mg/kg) to skin for comparison with the fate of similar dose administered IV or PO. Absorption into skin was biphasic, with an initial rapid phase that slowed to a nearly negligible rate by 12h post-dose. PCB 77 metabolism in skin was demonstrated by the use of skin slices and evidence was provided to indicate metabolism may facilitate dermal absorption. However, only 31% of the dose penetrated into the skin in 48 hr. and it appears that PCB 77 administered dermally may inhibit its own metabolism. PCB 77 equivalents in all tissues peaked 4 days post dose. After 4 days elimination from most tissues was much slower than from blood, but blood levels were still higher than observed following iv or oral administration. Data presented herein suggest that the skin acts as a partial barrier to transdermal exposures of PCB 77; however, dermal absorption is significant and may be facilitated in part by biotransformation in the skin. These data further indicate that the half-life of PCB 77 absorbed dermally may be significantly longer than when it introduced into the body as a bolus by iv or po administration.

INTRODUCTION

Polychlorinated biphenyls (PCBs) were widely used in a variety of industrial and consumer products and over one million tons of PCBs were produced before their production was banned in the 1970s. Due to their chemical stability, these compounds are still ubiquitous environmental contaminants. (WHO, 1993). PCBs are virtually insoluble in water, but are readily adsorbed onto soil, dust, and surfaces in homes and in factories (WHO, 1993). Thus, the dermal route represents a major, if not the major, route of human exposure to PCBs. Consequently, development of data to characterize PCB penetration of skin is a critical part of any assessment of human health risks associated with exposure.

A number of authors have reported that PCBs are absorbed into the skin of animals (Vos and Beems, 1971; Wester et al, 1983; Fischer et al, 1989; Hughes, et al, 1992) and humans (Surber et al, 1990, Fischer et al, 1989). (Wester et al, 1993). There is evidence that dermal exposure may have been the major route of exposure to PCBs rather than inhalation in the workplace (Lees et al, 1987; Baker, et al, 1980). Dermal exposure of animals to high PCB levels has been reported to result in gross toxicities such as liver necrosis, renal tubular degeneration, and dermal hyperplasia (Vos and Beems, 1971). Exposure to much smaller amounts of PCB mixtures through the skin has been demonstrated to effect hepatic cytochrome P450s, thyroid function, and to alter thyroid hormone metabolism (Batomsky and Murthy, 1976; Batomsky and Wyse, 1975; Bickers et al, 1975).

PCB 77 (3,3',4,4'-tetrachlorobiphenyl) is a non-ortho-substituted coplanar PCB congener. Structure-toxicity studies suggest that the "coplanar" PCBs and their monoortho analogs contribute significantly to the toxicity of the PCB mixtures (Safe, 1994). PCB 77, considered one of the most toxic congeners, is thought to act as a strong aryl hydrocarbon receptor agonist, leading to adverse effects such as body weight loss, immunosuppression, thymic atrophy, hepatotoxicity, tumor promotion, and disturbances of steroid hormone action (Seo et al, 1995; Schmitz et al, 1995; Chu et al, 1995; Desauliners et al 1997; Morse et al 1993). These experiments were performed to characterize the dermal absorption by rats of PCB 77 following low level exposure to a dose (0.4 mg/kg) such as might be encountered by humans and to compare the fate of this dose with that of similar doses administered IV or PO.

MATERIALS AND METHODS

Animals

Adult male F-344 rats (175-275 g) were purchased from Taconic (Germantown, NY). Animals were furnished food (NIH 31 rat chow) and tap water ad libitum and maintained on a 12 hour light/dark cycle. Rats used in experiments were housed in glass metabolism cages that provided for the separate collection of urine and feces.

Xenobiotic

The U-[^{14}C]-3,3',4,4'-tetrachlorobiphenyl (PCB 77, Figure 1), (specific activity 27.3 mCi/mmol), was purchased from Sigma Chemical Co. (St. Louis, MO) and was determined to be at least 95% radiochemically pure by HPLC. Non-radiolabelled material was not used in these experiments.

Preparation and Delivery of Dose Forms

Dermal dose formulations were prepared by dissolving sufficient radiolabelled PCB in acetone for delivery of 0.4 mg PCB/kg in a dose volume of 0.15 ml/kg. Approximately 24 hours prior to dosing, animals were anesthetized with ketamine:xylazine (60:9 mg/kg) and the fur on a 10 x 10 cm area was clipped from each animal's back. Dermal doses were administered onto a 1 cm^2 area on the backs of the animals using a 50 ml Hamilton HPLC syringe (Hamilton Co., Reno, NV) with a blunt-tipped needle and the solvent evaporated under a gentle stream of air. A stainless steel wire mesh shield (Shandon-Lipshaw Co., Pittsburgh, PA) was then glued over the dose site.

The applied dose was left on the dose site for up to 48 hours post-dose. Serial sacrifices were performed, with three animals per time-point at 1, 4, 8, 12, 24, 48, 72, 96, and 336 hr post-dosing. For animals held longer that 48 hr the dose site shield was removed and unabsorbed radioactivity was washed from the dose site with acetone. At sacrifice the dose site was excised and solubilized in 2N ethanolic NaOH.

Intravenous and oral dose formulations were prepared in Emulphor:ethanol:H_2O (1:1:8, v:v:v) for delivery of 0.4 mg/kg PCB/kg in a volume of 1 mL/kg. IV doses were injected into a lateral tail vein.

Analysis of Biological Samples

At sacrifice in the dermal studies (These same tissues were not assayed for oral and iv doses? And, if not, what tissues were taken for oral and iv?) blood, adipose tissue, muscle, skin (ears) and the entire kidney and liver were removed for assay of radiochemical content. Small tissue samples were oxidized to CO_2 with a Packard Oxidizer (Packard Instrument Co., Downer's Grove, IL). Livers were homogenized with a Polytron (Kinematica AG, Switzerland) and the homogenates oxidized. Oxidized samples were then assayed by liquid scintillation spectrometry (LSS). Urine and feces collected during the dermal, PO and IV studies were collected separately and total radioactivity determined. Aliquots of urine were added directly to Ultima Gold liquid scintillation solution

(source?) and assayed by LSS. Feces samples were homogenized, oxidized, and analyzed by LSS.

Profiles of radioactivity in excreta samples was determined by HPLC. Urine samples were filtered and injected directly onto an HPLC column. Feces samples were homogenized in an equal volume of H_2O and the homogenate extracted twice with 10 volumes of $CHCl_3$:methanol (8:2, v:v). The extract was filtered, evaporated, and reconstituted in acetone for analysis by HPLC. The HPLC system consisted of Waters model 510 pumps, a Rainin Microsorb MV C8 column with a IN/US b-RAM flow-through radioactivity detector (250mL lithium glass cell) and a gradient mobile phase flow of 2 mL/min. The gradient mobile phase conditions were: 100% H_20 initially, ramping linearly to 50% acetonitrile (MeCN) and holding for 10 minutes, then ramping to 100% MeCN over 5 minutes. Column recovery was quantitative.

Incubation of PCB 77 with Rat Skin Slices

Rats were anesthetized with ketamine and hair was removed from their backs with animal clippers. A cork ring (1 cm^2 annular space) was cemented to the skin and the skin and cork ring was excised with scissors and placed in 4 mL Waymouth's complete medium. The dose (15 uL acetone containing PCB) was then applied to the top of skin slices floating in the medium. Slices were

incubated for 8h at 37° C while atop a rotary shaker rotating at 90RPM. Slices were washed with acetone 5 times, minced, frozen in liquid nitrogen, and ground into a fine powder with a mortar and pestle. Skin powder was then extracted twice with 1 mL $CHCl_3$. The extract was filtered with a 0.45um filter and evaporated under a gentle stream of nitrogen, reconstituted in 1 mL acetone and the radioactivity profile determined by HPLC. The HPLC system consisted of Waters model 510 pumps, a Rainin Microsorb MV C8 column with a IN/US b-RAM flow-through radioactivity detector and a gradient mobile phase flow of 2 mL/min. The gradient mobile phase conditions were: 100% H20 initially, ramping linearly to 50% acetonitrile (MeCN) and holding for 10 minutes, then ramping to 100% MeCN over 5 minutes. Column recovery was quantitative.

RESULTS

PCB 77 is a highly lipophillic and would therefore be expected to be readily penetrate into the skin. However, dermal penetration of PCB 77 was incomplete and only 31% of the dose penetrated into the skin by 48 hr post-application (Figure 2). Penetration kinetics of radioactivity into the skin was biphasic, with an initial rapid absorption phase that slowed to a nearly negligible rate by 8h post-dose. Measured penetration data fit the curve

$$\%\text{Penetrated} = 31.2e^{-0.3454t} + 68.1e^{-0.00535t}$$

where t = time post application. The initial penetration half life was 2.4 hr but increased to 130 hr by ca 8hr post-dose.

A significant portion of the dose that penetrated the skin was still found in skin four days post-dose (Figure 3). The dose site skin served as an apparent reservoir for systemic absorption and beginning ca. 8hr after administration radioactivity in skin declined in a first order manner. The percent of the total dose that penetrated the skin and remained at time t was fitted to the curve:

$$\%\text{Penetrated Dose in Dose Site} = 9.63e^{-0.0139t}$$

where t = time post application. A half life of elimination from the dose site of 49 hr was calculated.

Earlier data from this laboratory suggest that the absorption of PCBs may be mediated either in whole or in part by transdermal metabolism (Garner and Matthews, 1997). The initial rapid disappearance of radioactivity suggested an active absorption process, perhaps mediated by metabolism. To test this hypothesis PCB 77 was incubated with whole rat skin samples. HPLC analysis of the radioactivity extracted from skin slices indicated the formation of a single more polar metabolite, demonstrating that the skin is metabolically active toward PCB 77 (Figure 4). Identification of this metabolite was outside the scope of this investigation and was therefore not pursued. PCB 77 has been demonstrated to inhibit its own metabolism (Gooch et al, 1989; Schlezinger et al., 1997; White et al. 1997a, 1997b). This data, coupled with the above HPLC data, suggested that PCB 77 may inhibit its own metabolism in the skin, thus accounting for the biphasic penetration curve as observed. That is, it appears that metabolism-mediated absorption of this PCB significantly predominated relative to its passive absorption. To test this hypothesis, the dose sites of rats were pretreated with 0.4 mg/kg doses of unlabelled PCB 77 4hr prior to application of equivalent doses of ^{14}C-PCB 77, and the penetration of radioactivity monitored for 4h hours. Pretreatment of the dose site with PCB 77 prior to a equivalent dose inhibited the penetration of the subsequent dose ca 75% (Table 1and Figure 5). The half-

life of penetration during the first 4 hr increased to 41 hr, a period nearly equivalent to the passive rate ($t_{1/2}$ = 49 h) of radioactivity efflux from the dose site measured beginning 8h after dose application.

Following dermal administration equivalents in all tissues peaked at approximately 4 days post-dose (Figure 6). This observation is consistent with the concept of the skin acting as a dose site reservoir and the slow elimination of PCB 77 that has been demonstrated previously. .As observed previously in iv dosed animals adipose and liver equivalents remained elevated relative to blood through 2 weeks post-dose. Elimination rates from liver and adipose were much lower than that of blood. Initial concentrations in all tissues were 10-100 times higher than blood, suggesting that PCB 77 rapidly partitions into the tissues following dermal administration.

Excretion of PCB 77 was determined following IV, PO or dermal doses of 0.4 mg/kg. Excretion of absorbed radioactivity following dermal administration was incomplete; only 22% of the absorbed dose was excreted by 1 week post-dose (Figure 7). Urinary excretion in all cases was negligible (< 1%) (data not shown). Excretion in feces following IV or PO was more rapid (Table 2) and accounted for 80-90% of administered dose in 7 days. Half lives of elimination were ca 3 days following IV or PO administration, but up to ca 18 days following dermal administration. Profiles of metabolites extracted from feces following dermal, IV, or PO administration were nearly identical (data not shown).

PCB 77 equivalents detected in blood following dermal administration were an order of magnitude greater than those following IV or PO administration. However, the rates of elimination of equivalents from the blood were similar for PO and dermal doses (Figure 8).

DISCUSSION

Dermal absorption of a number of structurally diverse PCBs has previously been reported to approximate first order (Fischer et al., 1989; Garner and Mathews, 1997, 1998). The rate of penetration into skin tends to decrease as the lipophilicity of the congeners studied increase. That is, the rate of penetration into the viable epidermis is probably limited more by penetration of the PCB into the relatively water-rich viable epidermis than by penetration through the lipid rich stratum corneum. PCB77 was initially rapidly absorbed into the skin of rats, but somewhat less rapidly than might have been predicted based on previous studies of dermal absorption of PCBs. The initial rate of PCB 77 absorption greatly exceeded that predicted from its K_{ow} (Garner and Mathews, 1998), much in the manner of 4-chlorobiphenyl, another PCB that undergoes extensive transdermal biotransformation (Garner and Mathews, 1997). If, as observed for 4-chlorobiphenyl, absorption of PCB 77 is facilitated by metabolism in skin, the P450 content of the epidermis would facilitate metabolism-mediated penetration (Noonan and Wester, 1989). This would be consistent with the observations of Jackson et al. (1993) who were unable to predict the extent of dermal penetration of PCB77 based solely on the properties of passive partition into the stratum corneum/epidermis of rats. They first suggested that transdermal metabolism of PCB 77 may facilitate its absorption.

Data presented herein further suggest that PCB 77 may also inhibit its own penetration into the skin, possibly by partial saturation or other inhibition of

its own metabolism. As rapid, metabolism-mediated absorption diminished, passive partitioning into the viable epidermis would then predominate. As demonstrated previously (Fischer et al., 1989; Garner and Mathews, 1997, 1998) passive penetration of a high K_{ow} PCB is extremely slow as the epidermis is a relatively polar substrate due to its high water and protein content. Thus, the slow phase of the dermal absorption of PCB 77 and its absorption following pretreatment of the dose site with unlabeled material was similar to that of poorly metabolized PCBs2,4,5,2',4',5'-hexachlorobiphenyl (Fischer et al., 1989) and 2,4,6,2',4',6'-hexachlorobiphenyl (Garner and Mathews, 1997).

The retention of PCB 77 equivalents in the dose site and their subsequent slow systemic release (Figure 3) may account for the elevated tissue and blood concentrations seen through 14 days post-dosing. This is consistent with our previous observation (Garner and Matthews 1998) that PCBs that are retained in the dermal dose site support higher tissue concentrations for an extended period of time than those which are not retained. This effect would be consistent with the significantly slower excretion of PCB 77 equivalents seen following dermal administration relative to IV or PO administration.

The complex distribution kinetics of PCB77 may be responsible for the unusual blood concentration and excretion profiles following a dermal exposure (Figures 7, 8). Contrary to other, less lipophillic compounds, PCBs have blood profiles

that are more greatly affected by distribution to tissues than by elimination (Matthews and Anderson, 1975). In addition, the rate of dose delivery as well as the amount delivered to the tissues determines the profile of disposition (Garner and Matthews, 1998). In the case of an IV dose of PCB 77, the central compartment received the entire dose instantaneously. Thus, highly perfused organs that have relatively low affinity for such lipophillic compounds, such as the liver would receive a large amount of material rapidly. Conversely, high affinity, low perfusion tissues such as skin or adipose receive most of an iv dose as a result of redistribution of material initially stored in other tissues. Mass action would drive metabolism and the net result would be more rapid elimination of PCB 77 from the central compartment and from the animal. The “first-pass” effect of the liver would also contribute to the lower PCB 77 blood concentrations following PO administration. The rate of systemic absorption is very slow following dermal administration; therefore, the rate of transport to the liver is considerably less, resulting in lower rate of metabolism and excretion. The very much slower systemic absorption following dermal administration would thus result in greater distribution to the less perfused, lipid rich tissues of adipose and skin. Indeed, peak adipose tissue concentrations of PCB 77 following dermal administration are ca 100 times greater than those following either IV or PO administration (data not shown). Due to the retention of PCB 77 at the dose site and lipid rich tissues, efflux from these reservoirs would dominate total systemic equivalents for several days after the dose was removed. This efflux would thus

account for the elevated blood concentrations observed following dermal administration relative to those observed following iv or po administration.

In summary, data presented herein suggests that the skin acts as a partial barrier to transdermal exposures of PCB 77; however, dermal absorption is significant and may be facilitated in part by biotransformation in the skin. These data further indicate that the half-life of PCB 77 absorbed dermally may be significantly longer than when it introduced into the body as a bolus by iv or po administration.

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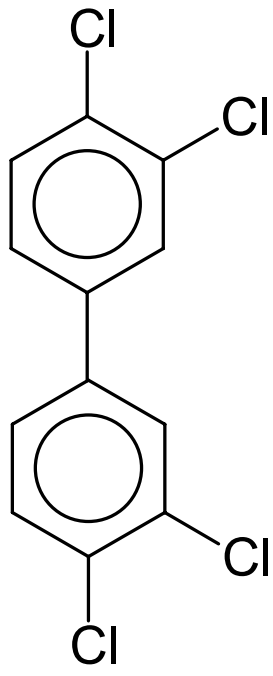

Figure 1. Structure of 3,3',4,4'-Tetrachlorobiphenyl (PCB 77)

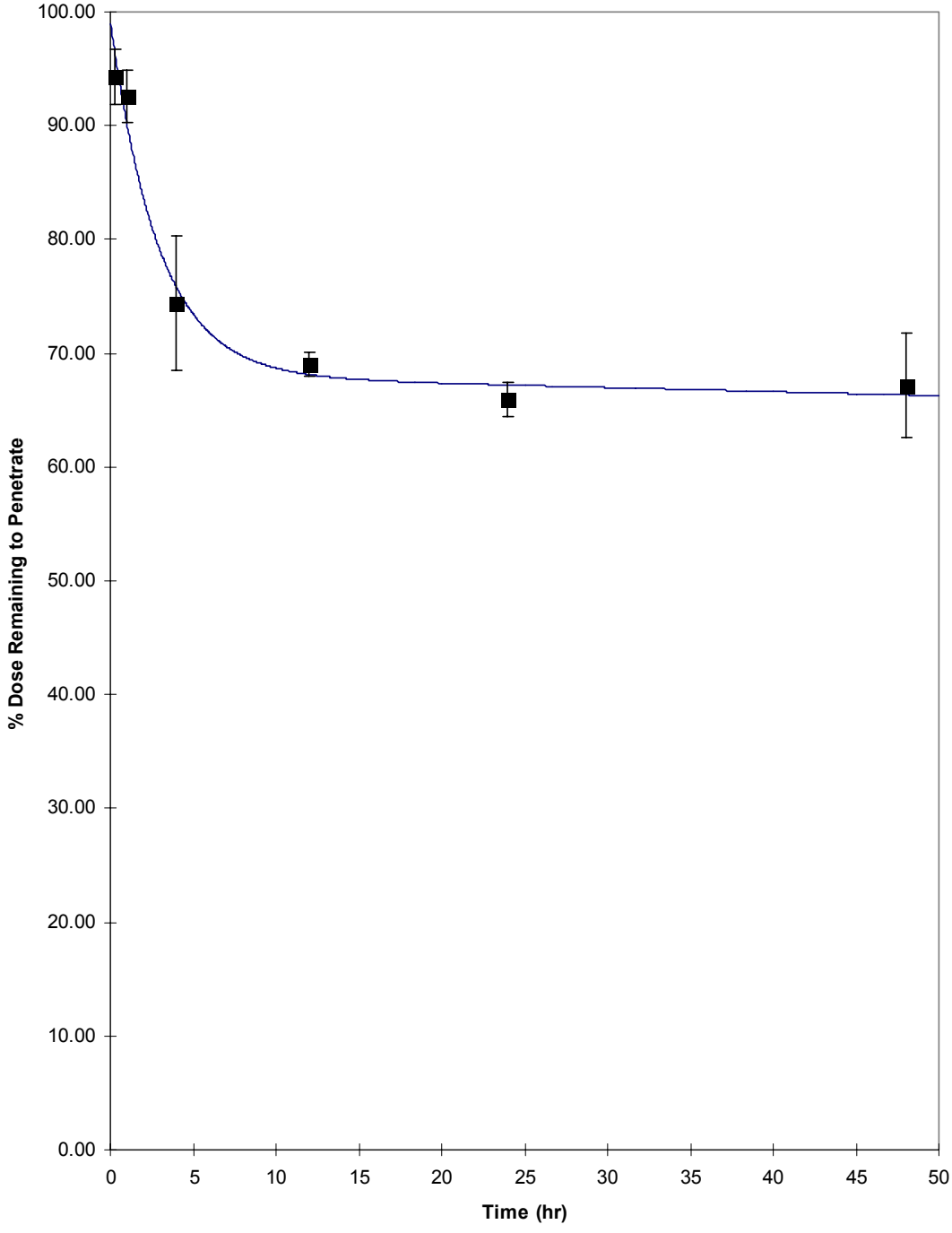

Figure 2. Dermal penetration of a single application of PCB77 (0.4 mg/kg) to F-344 rats.

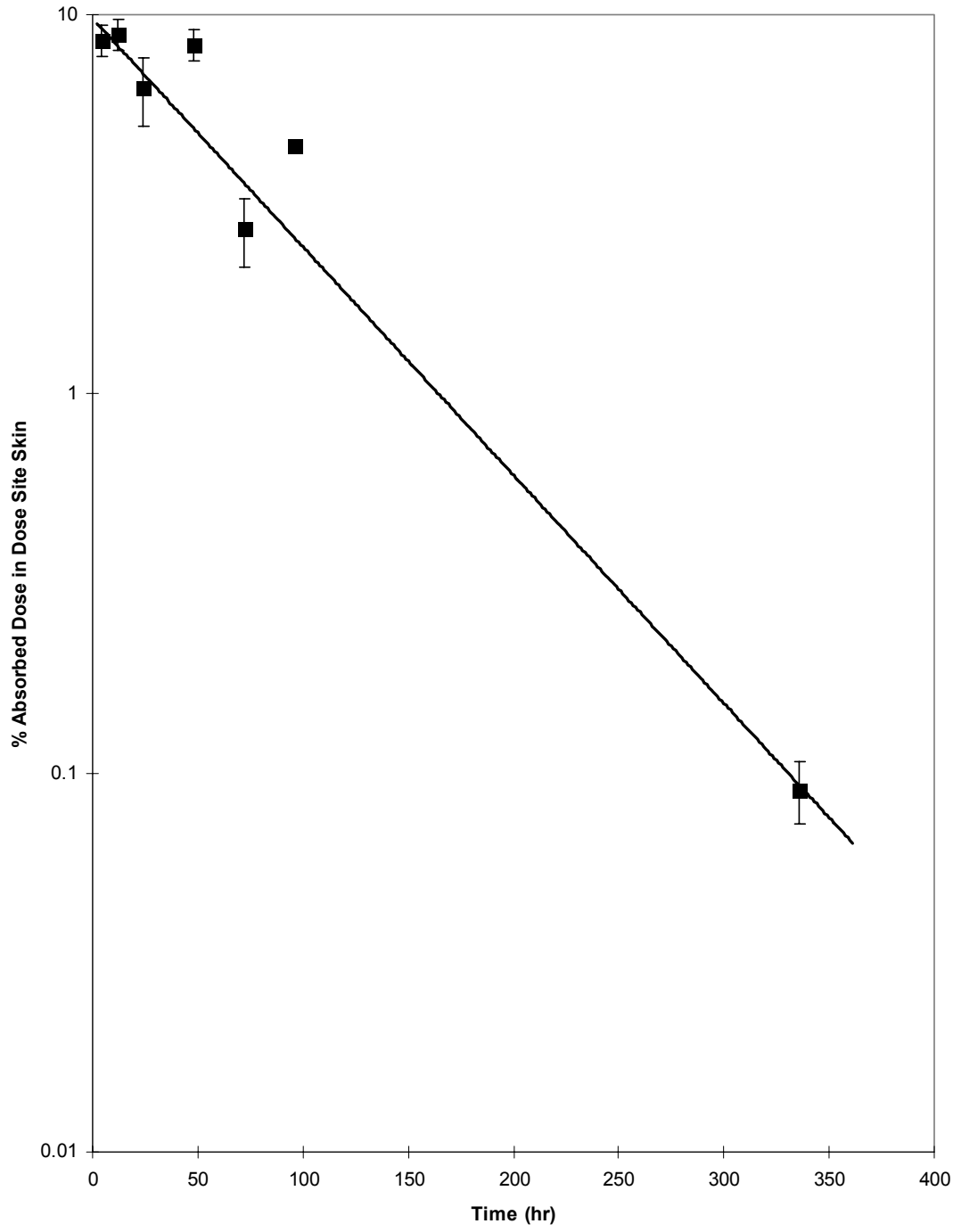

Figure 3. Time course of radioactivity present in the dose site skin following a single application of PCB77 (0.4 mg/kg) to F-344 rats.

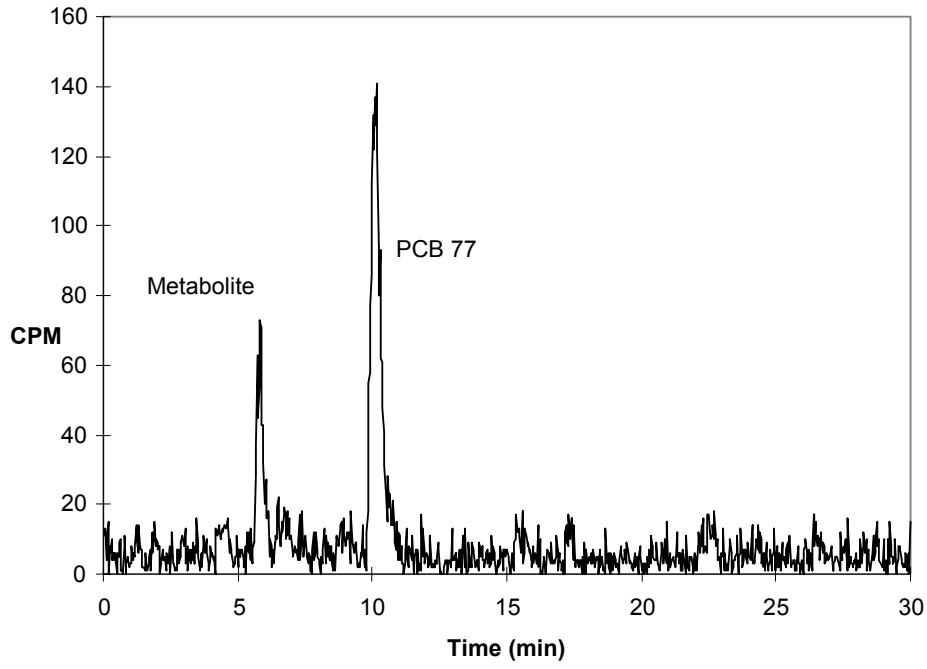

Figure 4. HPLC Radiochromatogram of Skin Extract Following Incubation of [^{14}C]-PCB77 with Rat Skin Slices for 8h.

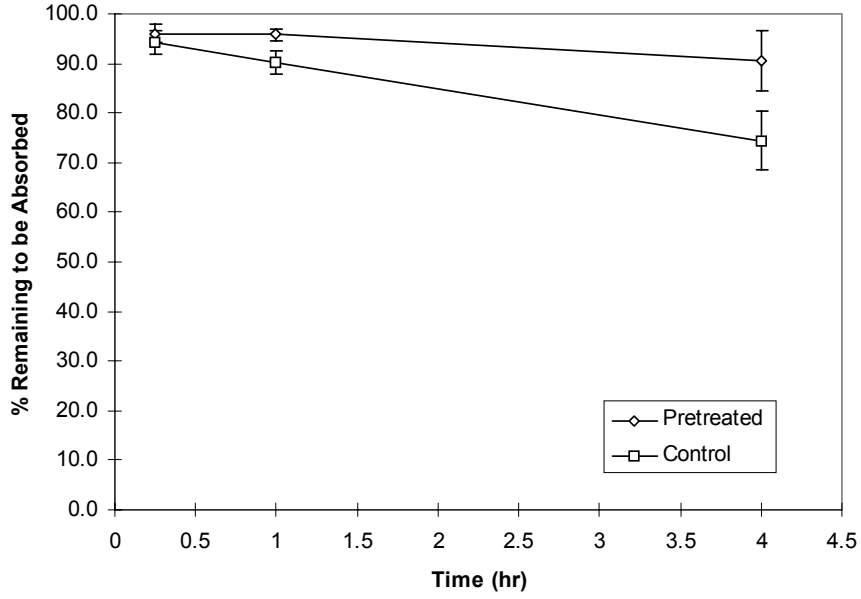

Figure 5. Penetration of PCB 77 into Dose Site Skin Following 4h Pretreatment of Dose Site with Identical Dose.

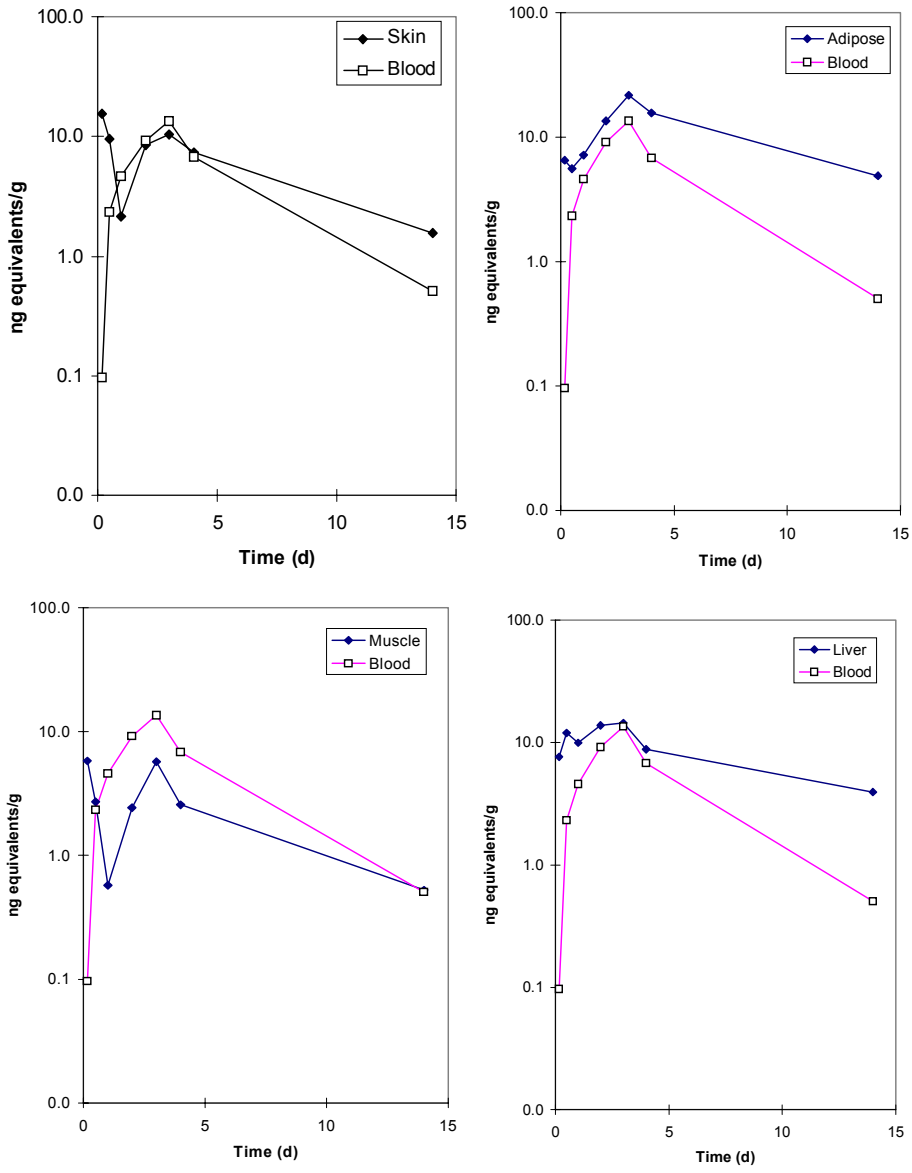

Figure 6. PCB 77 Equivalents in Selected Tissues Following Dermal Administration (0.4 mg/kg)

(Error bars excluded for clarity.)

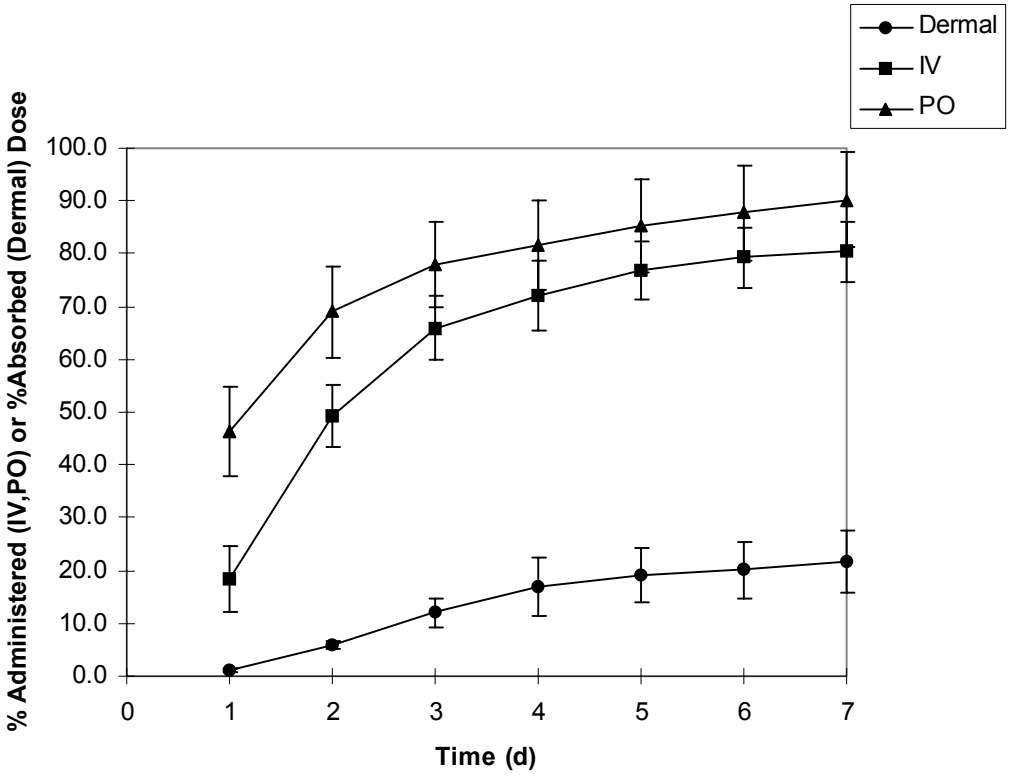

Figure 7. Excretion of Radioactivity Following IV, PO, and Dermal Administration (0.4 mg/kg) to Rats.

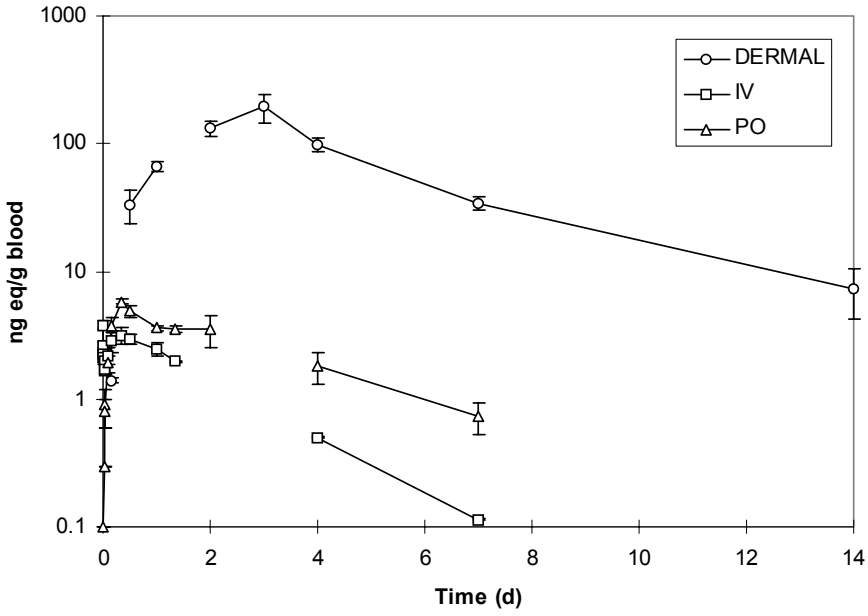

Figure 8. Concentration of PCB 77-Derived Equivalents in Blood Following IV, PO, and Dermal Administration (0.4 mg/kg) to Rats

Table 1. *Rate Constants and Half-lives of Penetration of PCB 77 into Dose Site Skin Following 4h Pretreatment of Dose Site with Identical Dose.*

	K_{abs} (h^{-1})	$t_{1/2}$ (h)
Pretreated	*0.017*	*40.7*
Control	*0.064*	*10.8*

Table 2. *Rate Constants and Half-lives of Excretion of Radioactivity Following IV, PO, and Dermal Administration of PCB 77 (0.4 mg/kg) to Rats.*

Dose Route	K_{elim} (d^{-1})	$t_{1/2}$ (d)
Dermal	*0.04*	*17.5*
IV	*0.23*	*3.0*
PO	*0.26*	*2.6*

PART 2: THE CATECHOL METABOLITES OF POLYCHLORINATED BIPHENYLS: EFFECTS ON METABOLISM AND FUNCTION OF ESTROGEN.

PCB metabolites and mechanisms of PCB-mediated toxicity
C. Edwin Garner

PCB Toxicity:

The mechanisms of PCB toxicity are poorly understood, but coplanar PCBs with dioxin-like actions have been extensively studied (Goldstein and Safe 1989; Leece et al. 1985; Safe 1984, 1990, 1994; Safe et al. 1985a). A few highly potent, dioxin-like PCB congeners bind to the Ah receptor, which regulates the synthesis of a variety of proteins, including cytochrome P450 1A1/1A2. This receptor was identified in the cytosol of mouse liver cells (Poland et al. 1976) and, subsequently, in extrahepatic tissues of laboratory animals, mammalian cell cultures, human organs and cell cultures, and also nonmammalian species. Not all the congeners showed the same affinity for the Ah receptor; affinity was found to be determined by the chlorine substitution pattern (Bandiera et al. 1982). Those congeners capable of assuming coplanar conformation and therefore structurally similar to 2,3,7,8-TCDD (unsubstituted or monosubstituted in positions 2 and 2') bind with the highest affinity. Initial binding to the Ah receptor is followed by an activation and transcription step and subsequent accumulation of occupied nuclear receptor complexes. These complexes interact with a specific DNA sequence in the CYP1A1 or CYP1A2 genes (which regulate the expression of cytochrome P-450IA1 and IA2 isozymes), leading to enhancement of gene expression.

The PCB congeners that exhibit Ah receptor-mediated responses constitute only a small fraction of the total number of possible congeners that are routinely identified in environmental samples, and may be present only as trace components in commercial PCB mixtures (Jones 1988; McFarland and Clarke 1989). Therefore, it is reasonable to assume that bulk of congeners that act by other mechanisms also contribute in a large part to the toxicity of PCB mixtures. PCB congeners also induce P-450 activities that are not mediated through the Ah receptor (Denomme et al. 1983; Scheutz et al. 1986; Hansen, 1998) primarily in the liver. In addition to inducing 3-methylcholanthrene- and phenobarbital-inducible isozymes of P-450, PCB congeners can induce other P-450 isozymes including CYP3A (Scheutz et al. 1986) and CYP4A1 (Huang and Gibson 1992; Koga et al. 1994), as well as other drug metabolizing enzymes such as epoxide hydrolase, glutathione S-transferase, and glucuronyl transferase (Goldstein and Safe 1989; Parkinson et al. 1983; Safe 1990). A number of PCB toxicities may therefore be mediated by changes in endogenous metabolism and/or through PCB metabolites.

PCBs and estrogenicity.

PCB mixtures are known to have a net estrogenic effect (Bitman and Cecil 1970; Jansen et al. 1993). Estrogenic effects exhibited by PCB congeners are not mediated through the Ah receptor. Metabolism of PCBs leads to the formation of

hydroxylated metabolites or their conjugates, which are more readily excreted (Figure 1). Results from studies in rodents found hydroxylated coplanar PCB metabolites to be less toxic than the parent compounds and reported that they did not induce Ah receptor-mediated responses (Koga et al. 1990; Shimada 1987; Yoshimura et al. 1987). However, phenolic PCB metabolites bind to the estrogen receptor and elicit estrogenic responses (Korach et al. 1988; Jansen et al. 1993). Some nonplanar PCBs are weakly estrogenic in immature rodents (Jansen et al. 1993; Li et al. 1994). Hydroxylated metabolites of lower chlorinated nonplanar PCBs bind to the estrogen receptor *in vitro* and are uterotrophic *in vivo* (Korach et al. 1988). Recent studies have reported retention of hydroxylated PCB metabolites in tissues of mink and mice (Klasson-Wehler et al. 1993), in rat plasma and in human blood (Bergman et al. 1994; Klasson-Wehler et al. 1997). Bergman et al. (1994) showed that there is selective retention of hydroxylated PCB metabolites in blood in humans, seals, and rats. Administration of Aroclor 1242, 2,2',5,5'-tetrachlorobiphenyl, or 2,2',4,4',5,5'-hexachlorobiphenyl increased uterine weight in immature rats (Jansen et al. 1993; Li et al. 1994). This effect was not observed after administration of the dioxin-like congener 3,3',4,4'-tetrachlorobiphenyl (Jansen et al. 1993). Jansen et al. (1993) suggested that some PCB congeners may increase gonadotrophin-releasing hormone, may produce effects beyond the receptor for gonadotrophin-releasing hormone, or may affect production and release of luteinizing hormone from the pituitary by mechanisms unrelated to estrogenic action. Li et al. (1994) suggested that induced biotransformation of 2,2',4,4',5,5'-hexachlorobiphenyl

may have contributed to its estrogenicity since induction of pentoxyresorufin O-dependentylase (PROD, P-4502B1 activity) peaked in the same dose range at which marked estrogenicity of the congener was observed. The capacity of PCB metabolites to bind to the estrogen receptor and to promote growth in estrogen sensitive tissues suggests that PCBs may have effects on reproduction and development. Additionally, estrogen sensitive transformed cells may clonally expand due to elevated "estrogen equivalents" in target tissues, contributing to the promotional effect of PCBs.

PCBs and carcinogenicity.

Commercial PCB mixtures appear to be complete carcinogens possessing both initiating and promoting activities (Kimbrough et al. 1975; Norback and Weltman 1985; Kimura, et al., 1976; Schaeffer et al. 1984; Mayes, et al, 1997; Silkworth et al., 1997), but the specific role of the individual congeners within the Aroclor mixtures has not been determined. Studies with PCB congeners suggest that congeners resembling phenobarbital (PB) as inducers (i.e., di-ortho substituted) promote the formation of preneoplastic focal lesions in rodents (Buchmann et al. 1991; Laib et al. 1991). The mechanism of promotion is not known, but results from non-coplanar PCB studies have suggested that inhibition of intercellular communication may be one indicator of tumor-promoting activity (Hemming et al. 1992). However, this may not be a principal mechanism since many other structurally diverse congeners also show promoting activity (Hemming et al.

1993; Silberhorn et al. 1990).

The combination of PCB- mediated alterations of metabolic capacity and the resulting metabolites of PCBs may play a key role in genotoxic and tumorigenic effects of PCBs. The metabolism-mediated formation of arene oxides can lead to the formation of both detoxification products, which are excreted, and potentially toxic adducts that bind covalently to macromolecules such as RNA, DNA, or proteins. For example, incubation of 4-chlorobiphenyl with Aroclor 1248-induced rat liver microsomes resulted in the formation of protein, DNA, and RNA adducts with 4-chlorobiphenyl equivalents (Wyndham and Safe 1978). Binding of PCB congeners was also found to be related to the rate of metabolism, such that 2,2',3,3',6,6'-hexachlorobiphenyl exhibited more binding to protein, DNA, and RNA than the poorly metabolized 2,2',4,4',5,5'-hexachlorobiphenyl (Morales and Matthews 1979).

Recently, PCB catechol metabolites have been identified as toxicologically significant genotoxic compounds. PCB catechols can be further oxidized to semiquinones and quinones (McLean et al., 1996a; Amaro et al, 1997). PCB-derived quinones react with both nitrogen and sulfur nucleophiles of the cell and may explain, in part, the toxic effects of individual PCBs and PCB formulations (McLean et al., 1996a; Amaro et al, 1997). Free radicals and oxidative DNA damage are produced during oxidation of lower chlorinated biphenyls to catechols and quinones (McLean et al., 1996a; 1996b). PCB catechols also

redox cycle, producing superoxide radicals that may be genotoxic (McClean, et al. 1997). Additionally, PCB catechols may directly adduct DNA through quinone intermediates (Oakley et al., 1996a; Amaro et al, 1997).

There is limited information regarding genotoxic effects in humans after exposure to PCBs. One study described a slight increase in sister chromatid exchanges in lymphocytes after exposure to PCBs following a fire in an electric station (Melino et al. 1992). An additional study reported an increased percentage in chromosomal aberrations in lymphocytes from subjects involved in the manufacturing of PCBs (Kalina et al. 1991). Evidence of PCB-mediated genotoxicity in animals is mixed. Oral administration of Aroclors 1242 or 1254 to rats did not induce chromosomal damage in bone marrow cells or spermatogonial cells (Garthoff et al. 1977; Green et al. 1975a, 1975b). Similar results were reported for Aroclor 1254 after intraperitoneal administration to mice (Bruce and Heddle 1979). However, evidence of transient DNA damage was reported in rats administered Aroclor 1254 (Robbiano and Pino 1981), but the damage was quickly repaired by enzymatic systems.

PCBs did not exhibit mutagenic activity when tested in *Salmonella typhimurium* with or without activation systems. *In vitro* testing in Chinese hamster cells resulted in negative mutagenic responses in (Hattula 1985). However, Aroclor 1254 induced chromosomal damage in human lymphocytes (Sargent et al. 1989). Aroclor 1254 induced unscheduled DNA synthesis in cultured rat liver

cells (Althaus et al. 1982). The weak *in vitro* and *in vivo* evidence of genotoxicity suggest that the PCB mixtures tested do not pose a genotoxic threat to humans. It should be noted, however, that it is well established that addition of liver microsomal preparations from PCB-treated animals to *in vitro* genotoxicity assays potentiates the genotoxic activity of numerous carcinogens by activation to reactive intermediates and proximate carcinogens (Hayes 1987). Additionally, PCB congeners themselves are generally poor microsomal substrates (Schnellmann, et al, 1986; C. E. Garner, unpublished data; H. B. Matthews, personal communication) so the lack of PCB-mediated in vitro mutagenicity/genotoxicity following “activation” may be artifactual.

The results of the oral studies with rats exposed to PCB mixtures (Kimbrough et al. 1975; Norback and Weltman 1985; Schaeffer et al. 1984) provide sufficient evidence to conclude that PCBs are carcinogenic in animals and potentially carcinogenic in humans. The induction of benign hepatomas in mice orally exposed to Aroclor 1254 (Kimbrough and Linder 1974) provides supporting evidence of tumorigenicity. PCBs as a group have been classified as probable human carcinogens by IARC (1987) and by EPA (IRIS 1995). NTP (1989) has concluded that PCBs are reasonably anticipated to be carcinogenic in humans based on sufficient evidence of carcinogenicity in animals. Again, there is insufficient information about which constituents of the PCB mixtures are carcinogenic.

The mechanisms by which PCBs promote carcinogenesis and act as complete carcinogens have not been elucidated, and structure-activity relationships of PCB congeners are unclear (Buchmann et al. 1991; Hayes 1987; Laib et al. 1991; Luebeck et al. 1991; Safe 1989; Sargent et al. 1992). Recently, workers have tentatively suggested that PCB-mediated tumorigenicity may be attributable to the effects of PCBs on estrogen metabolism (Mayes, et al, 1997; Silkworth et al., 1997; Segura-Aguilar, 1997). Additionally, other workers have suggested that PCB catechol metabolites are both indirectly and directly genotoxic (Oakley et al., 1996a; Amaro et al, 1997; McLean et al., 1996a; 1996b) and therefore contribute to the carcinogenicity of PCBs. In the following chapters, data will be introduced that supports the role of both PCB catechol metabolites and estrogen metabolism in PCB-mediated carcinogenesis. Additionally, further work will be forwarded to suggest that PCB catechol metabolites may serve to promote the growth of estrogen receptor sensitive transformed cells.

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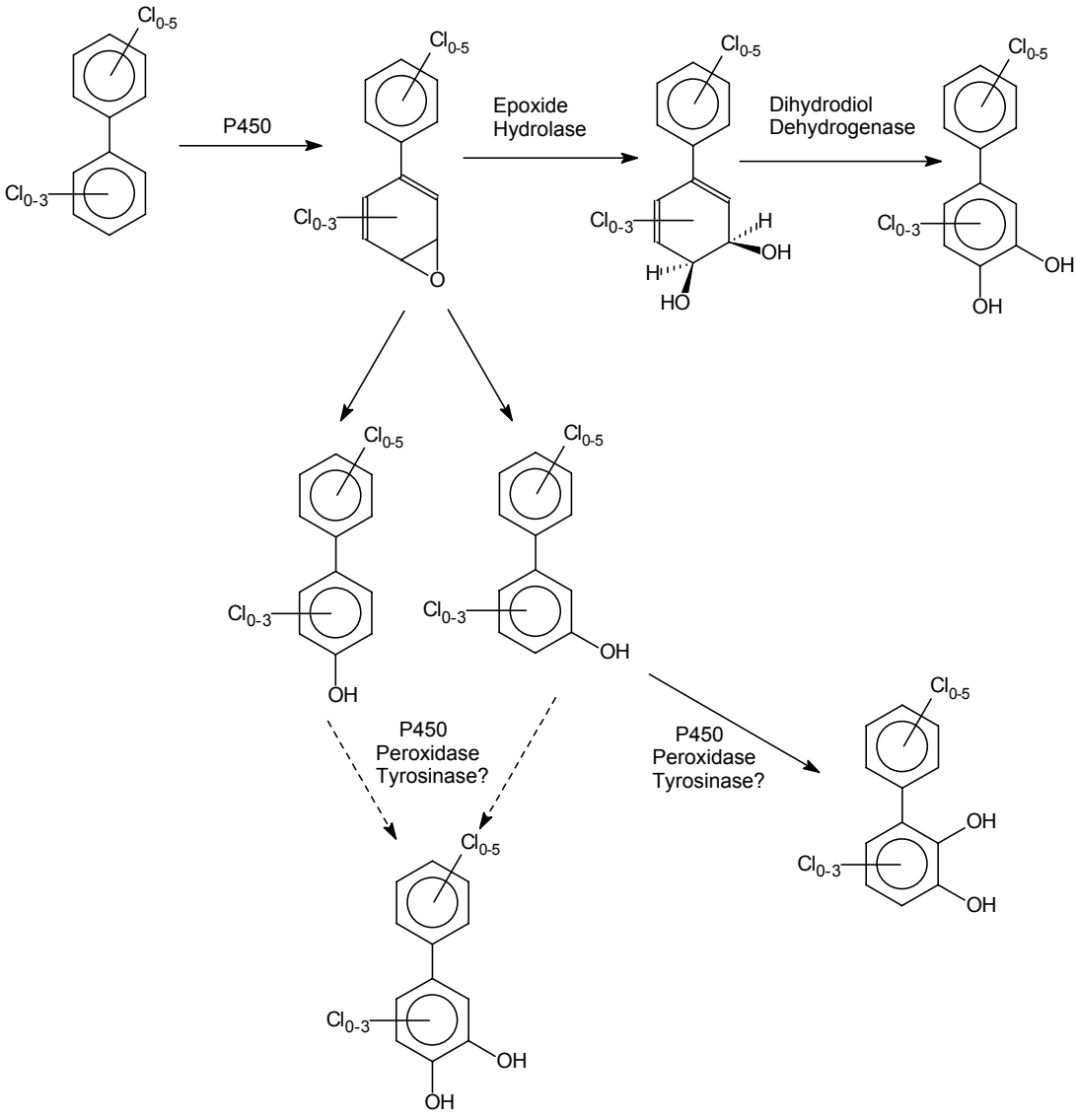
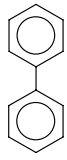
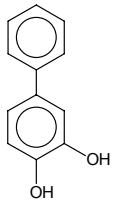
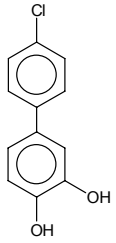
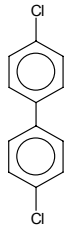
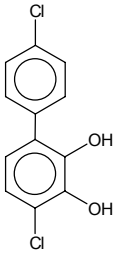
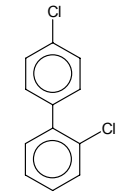
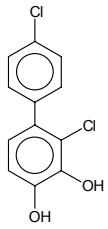
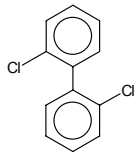
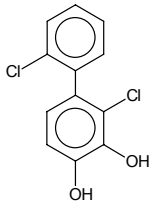
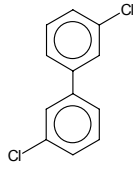
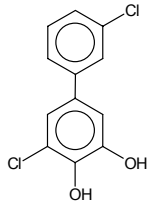
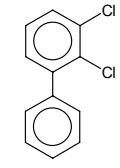
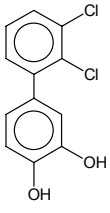
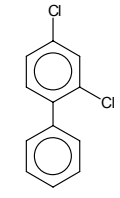
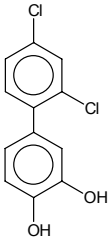
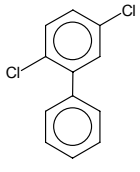
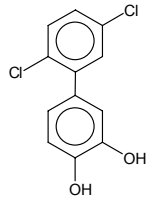
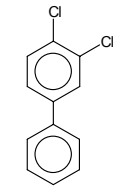
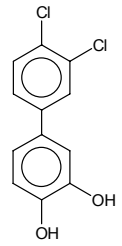
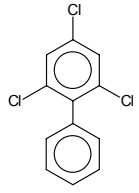
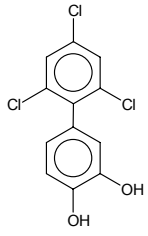
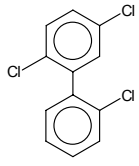
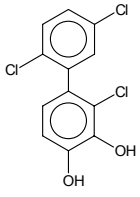
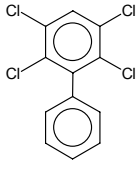
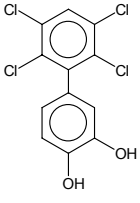
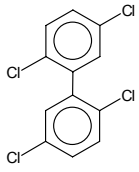
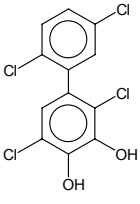


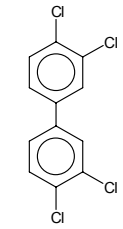
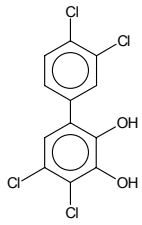
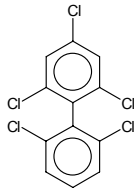
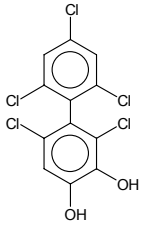
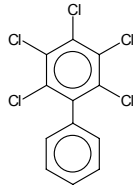
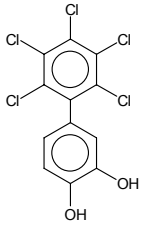
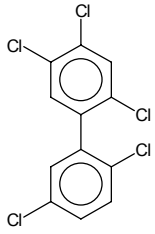
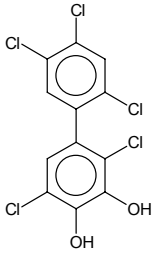
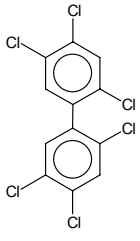
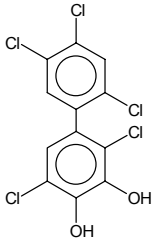
Figure 1. Pathways of PCB metabolism.

Table 1. PCBs known to form catechol metabolites in experimental animals.

Parent Molecule	Catechol Metabolite	Species	Reference
		Rat	Raig and Ammond (1972)
		Rat Rabbit Goat Pig Frog	Safe et al. (1975a) Safe et al. (1975b) Safe et al. (1975c) Safe et al. (1975d) Safe et al. (1976)
		Rat	Goto et al. (1975)

		Monkey	Greb et al. (1975)
		Rat	Goto et al. (1975)
		Rat	Goto et al. (1975)
		Rat	Goto et al. (1975) Goto et al. (1974)
		Monkey	Greb et al. (1975)
		Rat	Tulp et al. (1977)

		Rat	Goto et al.(1974b)
		Rat	Goto et al. (1975) Goto et al. (1974b)
		Monkey	Greb et al. (1975)
		Rat	Goto et al. (1975) Goto et al. (1974b)
		Rat Mouse Guinea Pig	Mio and Sumino (1985)

		Rat	Koga et al. (1989)
		Rat	Goto et al. (1975)
		Rat	Goto et al. (1975) Goto et al. (1974b)
		Rat	Chen et al. (1976)
		Rabbit	Sundstrom and Wachmiester (1975) Hutzinger et al. (1974)

Catechol Metabolites of Polychlorinated Biphenyls (PCBs) Inhibit the Catechol-O-methyltransferase-Mediated Metabolism of Catechol Estrogens.

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ABSTRACT

The catechol metabolites of estradiol are potent signalling molecules and are hypothesized to be central to estrogen-linked carcinogenesis. Methylation by catechol-O-methyltransferase (COMT) is the principal means of catechol estrogen (CE) deactivation in the liver and other tissues. The present studies were conducted to determine the effects of PCBs and catechol PCB metabolites on the COMT-mediated catabolism of 4- and 2-hydroxyestradiol (4-OHE_2 and 2-OHE_2, respectively) in vitro and in vivo. Liver homogenates of female SD rats treated with Araclor 1254 for 21 days (5 mg/kg/day) showed a 30% and 40% reduction of COMT activity towards 2-OHE_2 and 4-OHE_2, respectively. Incubation of [^{3}H]-b-estradiol with these same liver homogenates, followed by HPLC analysis demonstrated an elevation of CEs and a nearly complete reduction in levels of methylated catechol estrogens. In classical enzyme kinetics studies, the catechol PCBs were demonstrated to be good substrates for COMT, with K_ms approximately equivalent to those of the CEs. Catechol PCBs were also mixed -type inhibitors of the O-methylation of CEs. The data suggest that PCBs significantly alter the metabolism of catechol estrogens in vivo and that this effect may be mediated by catechol PCB metabolites. Further, methyltransferase inhibition by PCB catechols may contribute to PCB-mediated endocrine effects and liver carcinogenesis.

INTRODUCTION

Polychlorinated biphenyls (PCBs) were widely used in a variety of industrial and consumer products for several decades before their production was banned in the 1970s. As a result of their extensive use and their chemical stability, PCBs are still ubiquitous environmental contaminants that are frequently found as complex mixtures of isomers in air, water, soil, dust, and on surfaces in homes and in factories (WHO, 1993). Initial concern regarding PCBs focused on their accumulation in the tissues of fish, birds, terrestrial and marine mammals, and humans (Wasserman et al., 1979). However, concern has recently expanded to consider the action of PCBs and their metabolites as hormonal mimics (LeBlanc, 1995; Hileman, 1994). Hormonal effects mediated by PCBs and their metabolites frequently result from changes in the activities of enzymes responsible for the metabolism and clearance of naturally occurring hormones (Segura-Aguilar et al., 1997). Recently commercial PCB mixtures have been demonstrated to induce liver and mammary tumors in female rats (Mayes, et al, 1997; Silkworth et al., 1997) and this effect has tentatively been attributed to the effects of PCBs on estrogen metabolism.

Catechol metabolites of estradiol (catechol estrogens, CEs) are potent signalling molecules (Ball and Knuppen, 1980) and are hypothesized to be central to estrogen-linked carcinogenesis (Yager and Liehr, 1996; Cavalieri and Rogan, 1998; Stack et al, 1998; Weisz et al, 1998; Liehr, 1998; Cavalieri et al, 1997;

Stack et al, 1996; Li and Li, 1984, Seraj et al. 1996). Catechol estrogen metabolites are also capable of metabolic redox cycling between quinone and hydroquinone forms, resulting in free radical generation (Figure 1). Several types of direct and indirect free radical-mediated DNA damage are induced by estrogens in vitro and in vivo. Among these are: DNA single strand breaks, 8-hydroxylation of guanine bases, and DNA adduct formation by malondialdehyde, a decomposition product of free radical-induced lipid peroxides (Liehr, 1997; Han and Liehr, 1994, 1995; Liehr et al, 1986). In addition, catechol estrogens may also induce genotoxicity by direct formation of DNA base adducts (Cavalieri and Rogan, 1998; Cavalieri et al, 1997). Methylation by catechol -O-methyltransferase (COMT) is the principal means of catechol estrogen (CE) deactivation in the liver and other tissues (Yager and Liehr, 1996; Ashburn et al, 1993; Ball and Knuppen, 1980).

PCB catechols are metabolites of PCBs formed by further oxidation of phenolic PCB metabolites or by hydrolysis of the epoxide intermediates that are thought to be involved in the formation of most hydroxy metabolites (Figure 2). Catechol metabolites are formed from a significant number of asymmetrical and symmetrical PCBs (Goto et al, 1975). Formation of catechols from heavily chlorinated biphenyls requires dechlorination and stepwise hydroxylation. These metabolites have been shown to form in rats, mice, rabbits, monkeys and amphibians (Garner et al., in press) PCB catechols can be further oxidized to semiquinones and quinones (McLean et al., 1996a; Amaro et al, 1997). PCB-derived quinones react with both nitrogen and sulfur nucleophiles of the cell and

may explain, in part, the toxic effects of individual PCBs and PCB formulations (McLean et al., 1996a; Amaro et al, 1997). Free radicals and oxidative DNA damage are produced during oxidation of lower chlorinated biphenyls to catechols and quinones (McLean et al., 1996a; 1996b). PCB catechols also redox cycle, producing superoxide radicals that may contribute to genotoxicity (McClean, et al. 1997). Additionally, PCB catechols may directly adduct DNA through quinone intermediates (Oakley et al., 1996a; Amaro et al, 1997). The PCB catechols are potential substrates of the ubiquitous enzyme catechol-O-methyltransferase (COMT), which inactivates potentially reactive catechol metabolites of a number of molecules, including those of the hormone estradiol (Ashburn et al, 1993; Ball and Knuppen, 1980). Indeed, these PCB metabolites have been found to be O-methylated, but the mechanism by which methylated metabolites are formed is presently not known.

Exogenous catechols, such as the flavinoid quercetin, have been demonstrated to inhibit COMT (Zhu and Liehr, 1996) and therefore to potentiate estrogen-mediated carcinogenesis in susceptible tissues (Zhu and Liehr, 1994). We hypothesize that PCB catechol metabolites may, in a similar manner, contribute to PCB-mediated carcinogenesis through inhibition of catechol O-methyl transferase. Lowered clearance of CEs and of PCB-catechols would, as the catechols redox cycle, lead to elevated oxidative stress and subsequently, genotoxicity. The present studies were conducted to determine the effects of PCBs and model catechol PCB metabolites on the COMT-mediated catabolism

of 4- and 2-hydroxyestradiol (4-OHE_2 and 2-OHE_2, respectively) both in vitro and in vivo.

Materials and Methods

Reagents

All reagents used in the synthesis of the PCB catechols were purchased from Aldrich Chemical Company, (Milwaukee, WI). The 3,4-biphenyldiol was purchased from Ultra Scientific Co., (Kingstown, RI). Dithiothreitol (DTT), purified porcine hepatic COMT, 17-b-estradiol, epinephrine, 4-hydroxy-estradiol, 2-hydroxy-estradiol, and S-adenosyl-L-methionine (SAM) were purchased from Sigma Chemical Co. (St. Louis). Araclor 1254 was a gift from Monsanto Chemical Co. (St. Louis). Unlabelled 2-methoxy-3-hydroxy-estradiol, 3-methoxy-2-hydroxy-estradiol, 4-methoxy-3-hydroxy-estradiol, 3-methoxy-4-hydroxy-estradiol, estrone, and estriol were purchased from Steraloids (Wilton, NH).

The [^{3}H-methyl]-S-adenosyl-L-methionine (14.9 Ci/mmol) and [6,7-^{3}H]-17-b-estradiol (40 Ci/mmol) used in these studies were purchased from New England Nuclear (Boston).

Synthetic methods.

The 2,5-dichloro-3',4'-biphenyldiol, 2,5-dichloro-2',3'-biphenyldiol, and 2,4,6-trichloro-3',4'-biphenyldiol (Figure 3) were synthesized in these laboratories as described previously (Garner et al, 1998), which is a variation of the Cadogan biphenyl coupling reaction. Briefly, dimethoxyanilines were coupled with chlorinated benzenes to yield the chlorinated dimethoxybiphenyls. The catechols were then produced by demethylation with boron tribromide. Structural identification was determined by NMR and GC/MS. All test compounds were determined to be greater than 95% pure by TLC and gas chromatography.

Animal studies

Female Sprague-Dawley rats (150-175 g at initiation of study) were housed in polycarbonate cages and supplied food and water *ad libitum*. Four rats were administered Araclor 1254 (5 mg/kg/day) dissolved in corn oil for 21 days. Four control animals received equivalent volumes (1.0 mL/kg) of the corn oil vehicle.

Preparation of S9.

Rats were sacrificed by decapitation, exsanguinated, and their livers removed. All tissue samples were kept at 0-4 °C. The livers were immediately placed in

ice cold 0.15M KCl containing 2.5mM HEPES (pH 7.4) and rinsed three times. Each liver was minced with scissors and then homogenized with a Potter-Elvjhem glass homogenizer. Homogenates were then centrifuged at 10,000g for 30 minutes. Protein content of each supernatant was determined by the method of Lowry using bovine serum albumin (BSA) as a standard. The supernatants were diluted to a protein concentration of 1 mg/mL, transferred to Nunc Cryotubes in 300 mL aliquots and snap frozen in liquid nitrogen before storage at -70 °C.

Incubation of ^{3}H-estradiol with S9.

Hepatic S9 from Aroclor treated and control rats was incubated with [6,7-^{3}H]-17-b-estradiol to determine the effect of PCB treatment on the hepatic production of catechol estrogens and their methylated metabolites. Briefly, to each incubation solution (total volume 800 mL 0.1M phosphate, pH 7.4) was added: 4 nmol [6,7-^{3}H]-17-b-estradiol (25 mCi), 1 mmol NADPH, 0.03 mmol SAM, 15 mmol $MgCl_2$, and 0.4 mmol DTT. Control S9 samples were also incubated with ^{3}H-estradiol in the presence of 100 mmol pyrogallol, a potent COMT inhibitor. Incubations were initiated by addition of 100 mL S9 (100 mg protein). After 30 minutes at 37 °C, the reactions were stopped by addition of 1500 mL cold MeOH. The samples were vortexed and then centrifuged at 1500 g for 15 min. The protein pellets were washed as above with an additional volume of MeOH and the

extracts combined. The MeOH extracts were filtered (0.45 mm) and reduced to dryness in vacuo. Samples were reconstituted in 200 mL MeOH immediately prior to analysis by HPLC. Each sample was injected onto an HPLC system consisting of a Waters C18 column (9x150mm) and a gradient mobile phase with a flow rate of 1.5 mL/min. The gradient initial conditions were 20% MeOH in H_2O, ramping linearly to 70% MeOH over 30 minutes. Column eluent was monitored with a Applied Biosystems variable wavelength flow-through detector set at 210 nm. Radiochemical detection was performed with a b-RAM flow-through radioactivity detector (IN/US Systems, Tampa, Fl) equipped with a 1 mL liquid:liquid cell using Ultima-Flow liquid scintillate (Packard Instruments, Meriden, CT) and a column effluent:pump flow ratio of 1:4. Peak identification was determined by coelution with unlabeled authentic standards.

Incubation of [^{3}H-methyl]-S-adenosyl-L-methionine and unlabelled estradiol with S9.

Hepatic S9 from Aroclor treated and control rats was incubated with [^{3}H-methyl]-S-adenosyl-L-methionine and *unlabelled* 17-b-estradiol to determine the effect of PCB treatment on the hepatic production of methylated metabolites of catechol estrogens. Briefly, to each incubation solution (total volume 800 mL 0.1M phosphate, pH 7.4) was added: 4 nmol 17-b-estradiol, 1 mmol NADPH, 0.03 mmol SAM, 25 mCi [^{3}H-methyl]-S-adenosyl-L-methionine, 15 mmol $MgCl_2$, and 0.4 mmol DTT. Incubations were initiated by addition of 100 mL S9 (100

mg protein). After 30 minutes at 37 °C, the reactions were stopped by addition of 1500 mL cold MeOH. The samples were vortexed and then centrifuged at 1500 g for 15 min. The protein pellets were washed as above with an additional volume of MeOH and the extracts combined. The MeOH extracts were filtered (0.45 mm) and reduced to dryness *in vacuo*. Samples were reconstituted in 200 mL MeOH immediately prior to analysis by HPLC. Each sample was injected onto an HPLC system consisting of a Waters C18 column (9x150mm) and a gradient mobile phase with a flow rate of 1.5 mL/min. The gradient initial conditions were 20% MeOH in H_2O, ramping linearly to 70% MeOH over 30 minutes. Column eluent was monitored with a Applied Biosystems variable wavelength flow-through detector set at 210 nm. Radiochemical detection was performed with a b-RAM flow-through radioactivity detector (IN/US Systems, Tampa, Fl) equipped with a 1 mL liquid:liquid cell using Ultima-Flow liquid scintillate (Packard Instruments, Meriden, CT) and a column effluent:pump flow ratio of 1:4. Peak identification was determined by coelution with unlabeled authentic standards.

Determination of COMT activities

COMT assays were performed in 13x100mm glass test tubes. Briefly, the incubation mixture consisted of the following reagents with final concentrations in 300 mL: 0.07M Tris-HCl, pH 8.0; 0.03 M $MgCl_2$; 2 mCi [^{3}H-methyl]-S-adenosyl-L-methionine; 3.3 mM SAM; 0.7 mM DTT; substrate (delivered in DMSO) 0-120

mM. Incubations were initiated by addition of purified enzyme or hepatic S9.. The tubes were placed in a shaking water bath at 37 °C. Blanks were tubes with no substrate. Reactions were stopped at 20 minutes by addition of 0.5 mL of 0.5 M borate buffer, pH 10. The radiolabelled O-methylated products were extracted into 4 mL hexane. Aliquots were counted by liquid scintillation spectrophotometry (LSS).

COMT Inhibition Assays

COMT assays were performed with 2-OHE_2 or 4-OHE_2 at 0-120 mM in the presence of selected PCB catechols at concentrations 0, 1.0, and 10 mM. Incubations were initiated by addition of purified enzyme. Reactions were stopped at 20 minutes by addition of 0.5 mL of 0.5 M borate buffer, pH 10. The radiolabelled O-methylated products were then extracted into 4 mL hexane. Aliquots were counted by LSS. Unlabelled O-methylated catechol estrogens (10 mg each) were then added to each sample and the hexane was then removed in vacuo. Samples were reconstituted in 250 mL MeOH immediately prior to analysis by HPLC. Each sample (150 mL injection volume) was injected onto an HPLC system consisting of a Waters C18 column (9x150mm) and a gradient mobile phase with a flow rate of 1.5 mL/min. The gradient initial conditions were 20% MeOH in H_2O, ramping linearly to 70%MeOH over 15 minutes. Column eluent was monitored with a Applied Biosystems variable wavelength flow-through detector set at 210 nm. The UV absorbing peaks corresponding to the

methylated products of 2-OHE_2 or 4-OHE_2 were collected as they eluted from the column and the radiochemical content of these fractions were then determined by LSS.

Enzyme Kinetics

Kinetic analysis was performed using Winnonlin nonlinear regression software (Scientific Consulting Inc., Cary, NC).

RESULTS

Araclor treatment significantly reduced hepatic COMT activities in vitro:

Hepatic COMT activity was determined in S9 of female SD rats treated with Araclor 1254 (5 mg/kg/day) for 21 days. At saturating conditions, there was a 36% and 28% reduction ($p < 0.05$) in the O-methylation of 4-hydroxyestradiol and 2-hydroxyestradiol, respectively (Table 1).

Effects of Araclor treatment on in vitro catechol estrogen metabolism:

HPLC radiochemical profiles were determined following incubation of treated and control S9 with ^{3}H-estradiol. Profiles of 3H-estradiol metabolites in the control incubates included significant amounts of O-methylated catechol estrogens and no detectable free estradiol 2-, and 4-catechols (Figure 4A.) Estradiol metabolite profiles from the experimental incubates were significantly different (Figure 4B). There was a complete absence of methylated 2- and 4-hydroxy estradiol and significantly larger 4-hydroxyestradiol peak relative to 2-hydroxyestradiol. The reduction in catechol estrogen O-methylation following Araclor treatment seen in the above experiments was repeated when we incubated S9 with *unlabelled*

estradiol and 3H-methyl S-adenosinyl-L-methionine (data not shown). When COMT was inhibited by pyrogallol in control S9 and the 3H-estradiol metabolite profile determined, the ratio of 4-OHE_2 to 2-OHE_2 was ca 1 (Table 2). Araclor treated rats also were shown to produce more 4 hydroxyestradiol than 2-hydroxyestradiol, with a 4-OHE_2/2-OHE_2 ratio of ca 4 (Table 2). The increased ratio of 4- to 2-hyroxyestradiol did not reflect an increased production of the free catechols, since the Araclor treated rats produced less of both 4-hydroxyestradiol and 2-hydroxyestradiol than control animals (Table 2). Instead the altered ratio reflected a greater inhibition of 2- hydroxyestradiol production relative to the production of 4-hydroxy estradiol.

Incubation of PCB catechols with COMT.

The biphenyl catechols were effective substrates of purified COMT with Km's similar to that of the catechol estrogens (Table 3).

Position of hydroxyl groups on the PCB catechols was critical for COMT methylation. The 3,4 catechols were readily methylated (Figure 5) but the single PCB 2,3-catechol tested here was a poor COMT substrate (data not shown). Within the set of PCB catechols tested, activity of COMT toward PCB 3,4-catechols generally increased with increasing degree of chlorination (Figure 5). Activity of COMT toward the trichloro PCB catechol was nearly equivalent to that of 2-OHE_2 and greater than that of the 4-OHE_2 and epinephrine (Figure 5). Affinity for COMT, expressed as 1/Km, was greatest for 2-hydroxyestradiol and

lowest for 4-hydroxyestradiol and 246-TCB (Table 3). No pattern was apparent for between affinities and velocities of the catechol PCBs. Intrinsic clearance, generally expressed as Vmax/Km, was greatest for the 2-hydroxyestradiol and lowest for 4-hydroxy estradiol. PCB catechol Cl_{intr} was low relative to the endogenous substrate 2-hydroxyestradiol (Table 3).

Inhibition of COMT by PCB catechols.

Since the PCB catechols were substrates of COMT, we performed experiments with model compounds to determine if these catechols were inhibitors of COMT and to determine the nature of the inhibition. The biphenyl catechols were demonstrated to be potent inhibitors of the methylation of both 2- and 4-hydroxyestradiol by purified COMT (Figure 6, 7). Inhibition was concentration dependent (Table 4, 5. and Figures 6, 7). Chlorination was necessary for maximum inhibition but increasing chlorination did not result in increased potency in this small series of compounds. COMT inhibition potency may be a function of, among other things, both chlorine position and degree of chlorination.

Kinetic analysis revealed that PCB catechols inhibited COMT mediated O-methylation of 2- and 4-hydroxyestadiol in a mixed-type mechanism. In all cases Vmax was significantly reduced for both substrates in a concentration dependent manner. The effects on Km varied, with no readily apparent pattern to these

effects. As inhibitor concentration increased the intersection point shifted toward the x axis in a concentration dependent manner, suggesting that the inhibition became progressively more noncompetitive in nature. For these mixed type inhibitors, K_i's in the range of 0.3-0.5 mM were estimated.

Regardless of the mechanism, intrinsic clearance of 2- and 4-hydroxyestradiol was reduced significantly by PCB catechols (Tables 4 and 5). Intrinsic clearance of 2- hydroxyestradiol was more sensitive to change than that of 4-hydroxyestradiol. In other words, equal concentrations of inhibitor produced a greater reduction in 2-hydroxyestradiol clearance than that of 4-hydroxyestradiol. In both cases though, Cl_{int} was reduced between 80 and 98% by the model compounds reported here. Clearance of 2-OHE_2 by COMT was reduced up to 98% by the most potent PCB catechol, 2,5-DC-3',4'-BPD (Table 4).

DISCUSSION

To determine the effect of PCB treatment on estradiol metabolism, PCBs were administered to female rats at a dose previously demonstrated to induce liver tumors (Mayes, et al, 1997). The amount of free catechol estrogens produced following incubation of estradiol with subcellular fractions from PCB-treated rats and the COMT activity for methylation of CEs was measured. Data obtained demonstrate that PCB treatment resulted in an increase in free CEs, which are normally cleared by COMT and a concurrent decrease in the capacity of hepatic catechol clearance by COMT. The two effects, when coupled, may account for a mechanism that contributes to PCB-mediated tumorigenesis. Such a mechanism has been demonstrated previously in the hamster kidney model, wherein the catechol flavinoid quercetin potentiated CE mediated tumorigenesis (Zhu and Liehr, 1994; 1996).

In vitro, PCB catechols are a substrate of COMT, with kinetic properties similar to those measured with endogenous substrates. Affinities (1/Km) for PCB-catechols were similar to those of the CEs and the catecholamine, epinephrine. PCB-catechols also significantly inhibited the O-methylation of 2-OHE_2 and 4-OHE_2. Generally, the Vmax for O-methylation of 2-OHE_2 and 4-OHE_2 was reduced by PCB catechols in a concentration dependent manner. The Km of 2-hydroxyestradiol increased in most of cases with the exception of the higher concentration of the 2,4,6-TCB, where there was no significant change. This suggested that inhibition of 2-OHE_2 O-methylation was similar to a linear mixed-

type mechanism. The system is considered to be a mixture of partial competitive and pure noncompetitive inhibition (Seegal, 1975). The Vmax of 4-OHE_2 also decreased with inhibitor concentration. However, the Km for 4-OHE_2 O-methylation did not change or increased as inhibitor concentration increased. The data points in this assay showed considerable scatter, making elucidation of the exact inhibition mechanism problematic. Nevertheless, this difference in kinetic behavior suggests that PCB catechols may inhibit methylation of 4-OHE_2 by a partial noncompetitive mechanism. The shift in curve intersection point that was observed in all cases indicates that the inhibition mechanism is complex and may vary with inhibitor concentration.

The extended pi orbital system of the biphenyl catechols may contribute to their mechanism and potency of inhibition of COMT. After a catechol is in the active site of COMT, one hydroxyl proton is removed to form a phenolate ion. The phenolate then attacks the S-methyl group of SAM in an SN_2-like manner (Vidgren et al., 1994). The efficacy of a substrate in nucleophillic attack on the methyl group would be a function of the electron density on the phenolate. With a PCB catechol, in the event of 4-hydroxy proton ionization, the negative charge would be delocalized, with the electron density effectively transferred to the distal ring (Fig. 7). The resulting intermediate would be a poor nucleophile relative to a non-delocalized phenolate, yielding a non-productive or dead-end EI complex, thus contributing to the noncompetitive nature of the inhibition. The 3-phenolate could not delocalize the charge to the same extent. It would, in a manner similar

to the endogenous substrates, be productive, contributing to the partial competitive inhibition of COMT. In vitro, with each PCB catechol we were able to detect two O-methylated products by HPLC (data not shown), though the ratios heavily favored a single product. A single product would be expected if 4-enolate rearrangement yielded a complete dead-end complex. In the case of quercetin there is also both a competitive and non-competitive inhibition of COMT (Zhu and Liehr, 1994; 1996). Zhu and Liehr (1994) were able to detect a 3-methyl ether of quercetin, but not the 4-methoxy product. The electron density of quercetin 4-phenolate would be more diffuse than the 3-phenolate due to conjugation, resulting in a less reactive substrate occupying the active site. The CEs and catecholamines, which have no opportunity to delocalize the electron density beyond the single aromatic ring, have productive 3- and 4-enolate intermediates and COMT produces two products, though not necessarily to equal extents (Ball and Knuppen, 1976).

The above suggests two possible mechanisms that may work in conjunction or independently for PCB-mediated carcinogenesis. PCB catechols may reduce their own clearance via COMT. Free PCB catechols may be a significant source of genotoxicity if they redox cycle after conversion to the semiquinone/quinone. These putative redox-active metabolic intermediates may be genotoxic by creating reactive oxygen intermediates and/or the quinone may adduct

DNA/RNA. Secondly, CE clearance may be reduced following PCB dosing, and may mediate genotoxicity in a similar manner.

A large portion of free PCB catechols are probably cleared as the glutathione conjugates (Amaro, 1997). Treatment of experimental animals with individual PCBs and mixtures has been demonstrated to reduce glutathione (Hori et al, 1997) and to increase activity of glutathione-S-transferase (Parki et al., 1977; Kamohara et al., 1984; Miranda et al., 1992). Therefore, after long term exposure to PCBs, glutathione reserves may be depleted. Regardless of the state of tissue glutathione levels, both the free and O-methylated catechol estrogens can themselves be conjugated with GST. Once conjugated, though, the glutathionyl-catechol estrogens cannot be deactivated through methylation (Ball and Knuppen, 1980). Although the conjugation of quinones with glutathione is associated with the process of detoxification, the reaction frequently facilitates quinone-induced toxicity. Thiol conjugates of quinones retain the ability to redox cycle and generate reactive oxygen species themselves (Monks et al, 1992; Jeong et al 1996; Butterworth et al, 1998). In fact, GSH conjugates are more readily reduced to the semiquinones (O'Brien, 1991). In the case that COMT mediated catechol clearance is reduced, free catechol equivalents may be shunted through GST pathways and therefore increase total redox cycling equivalents. The possibility is high that these two mechanisms may work in conjunction and further work needs to be done to elucidate their relative contributions.

Disruption of 2-OHE_2 O-methylation following PCB treatment raises an interesting third possible mechanism for PCB carcinogenicity. Recent reports have hypothesized that catechol estrogen methyl ether products of COMT may protect against estrogen-induced cancers in susceptible tissues (Zhu and Conney, 1998; Seegers et al, 1997;). Further, 2-methoxyestradiol (2-$MeOHE_2$) suppresses tumor growth in vivo and inhibits angiogenesis activity in vitro (Attalla et al., 1996; 1998). In vivo, 2-methoxyestradiol arrests cells in mitosis by interfering with the dynamics of the mitotic spindle, causing a prominent G2/M arrest and micronuclei formation followed by apoptosis (Seegers et al., 1997). In cultured cells 2-methoxyestradiol induces large increases in the protein p53, causing the cells to undergo apoptosis at the G1-S checkpoint of the cell cycle (Mukhopadhyay and Roth, 1997). If the synthesis of 2-$MeOHE_2$ is inhibited and if 2-$MeOHE_2$ contributes to the regulation of p53 synthesis, then cells that have genomic damage (regardless of the source), may be less able to respond to genomic damage by cell cycle arrest/apoptosis via p53. This combination would result in cells which are "genomically fragile", susceptible to low levels of genomic damage due to their reduced ability to enter into cell cycle arrest. Exposure to PCB, and thus PCB catechols, may result not only in increased levels of reactive CEs, but also a decreased ability to repair genetic damage.

In summary, these experiments demonstrate that, following treatment of rats with PCB, the COMT-mediated metabolism of CEs was reduced. Secondly, that PCB

catechols are substrates and potent inhibitors of COMT The above information taken together supports the hypothesis that the catechol metabolites of PCBs may contribute to PCB-mediated carcinogenesis partly through inhibition of catechol O-methyl transferase. Chronic exposures to PCB may enhance metabolic redox cycling of both PCB catechols and catechol estrogens and this, in turn, may contribute to PCB-induced tumorigenesis.

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Table 1. Catechol O-methyltransferase mediated methylation of 4-hydroxyestradiol and 2-hydroxyestradiol in S9 prepared from the livers of SD female rats (n=4) following oral administration of Aroclor 1254 (10 mg/kg/day) for 21 days.

Substrate		COMT Activity nmol/min/mg protein	Percent Change
2-hydroxyestradiol 10 μM	Control	0.051 ± 0.002	-28
	Treated*	0.037 ± 0.009	
4-hydroxyestradiol 10 μM	Control	0.072 ± 0.008	-36
	Treated*	0.046 ± 0.011	

* = Significantly different $p < 0.05$.

*Table 2. Percent of total radioactivity as 4- or 2-hydroxyestradiol following incubation of 3H-Estradiol with S9 from rats treated with Aroclor 1254 or vehicle controls**

	Control	**Araclor**	**% Change**
4-OHE_2	5.17 ± 0.57	3.62 ± 0.87	-30.1
2-OHE_2	5.58 ± 0.22	0.82 ± 0.20	-85.4
4OH/2OH Ratio	0.93	4.43	

*Control incubations included 100 mM Pyrogallol

Table 3. Kinetic parameters: Catechol-O-Methyltransferase mediated methylation of selected polychlorinated biphenyl catechols and catechol hormones.

Substrate	*Vmax (nmol/min/mg prot.)*	*Km (mM)*	*1/Km*	*Cl_{intr}*
3,4-BPD	0.09 ± 0.00	0.53 ± 0.04	1.9	0.16
2,5-DC-2',3'-BPD	<0.01 ± <0.01	0.35 ± 0.14	2.9	<0.01
2,5-DC-3'4'-BPD	0.60 ± 0.12	5.87 ± 1.51	0.2	0.10
2,4-DCBPD	0.25 ± 0.02	0.32 ± 0.06	3.1	0.80
2,4,6-TC-3'4'-BPD	3.94 ± 0.48	2.43 ± 0.42	0.4	1.62
2-OH-E2	3.61 ± 0.53	0.39 ± 0.10	2.6	9.27
4-OH-E2	1.05 ± 0.16	2.25 ± 0.46	0.4	0.47
Epinephrine	0.91 ± 0.13	1.46 ± 0.41	0.7	0.62

Table 4. Kinetic parameters: Inhibition of Catechol-O-Methyltransferase mediated methylation of 2-Hydroxyestradiol by selected polychlorinated biphenyl catechols

	Vmax (nmol/min/mg prot.)	*Km (mM)*	*Cl_{intr}*	*% Cl_{intr} Change*
No Inhibitor	3.61 ± 0.53	0.39 ± 0.10	9.27	-------
BPB 1 mM	1.52 ± 0.47	2.25 ± 1.39	0.68	-92.7
BPB 10 mM	0.85 ± 0.10	1.70 ± 0.42	0.50	-94.6
2,5-DC-BPD, 1 mM	0.60 ± 0.06	1.16 ± 0.17	0.52	-94.4
2,5-DC-BPD, 10 mM	0.15 ± 0.03	1.44 ± 0.46	0.11	-98.8
2,4,6-TC-BPD, 1 mM	0.76 ± 0.07	0.82 ± 0.12	0.92	-90.0
2,4,6-TC-BPD, 10 mM	0.18 ± 0.04	0.31 ± 0.13	0.58	-93.7

Table 5. Kinetic parameters: Inhibition of Catechol-O-Methyltransferase mediated methylation of 2-Hydroxyestradiol by selected polychlorinated biphenyl catechols

	Vmax (nmol/min/mg prot.)	*Km (mM)*	*Cl_{intr}*	*% Cl_{intr} Change*
No Inhibitor	1.05 ± 0.16	2.25 ± 0.46	0.47	-------
2,5-DC-BPD, 1 mM	0.20 ± 0.04	2.06 ± 0.77	0.10	-79.1
2,5-DC-BPD, 10 mM	0.10 ± 0.03	2.03 ± 1.36	0.05	-89.7
2,4,6-TC-BPD, 1 mM	0.19 ± 0.01	3.20 ± 0.17	0.04	-90.9
2,4,6-TC-BPD, 10 mM	0.14 ± 0.02	3.75 ± 0.98	0.05	-89.4

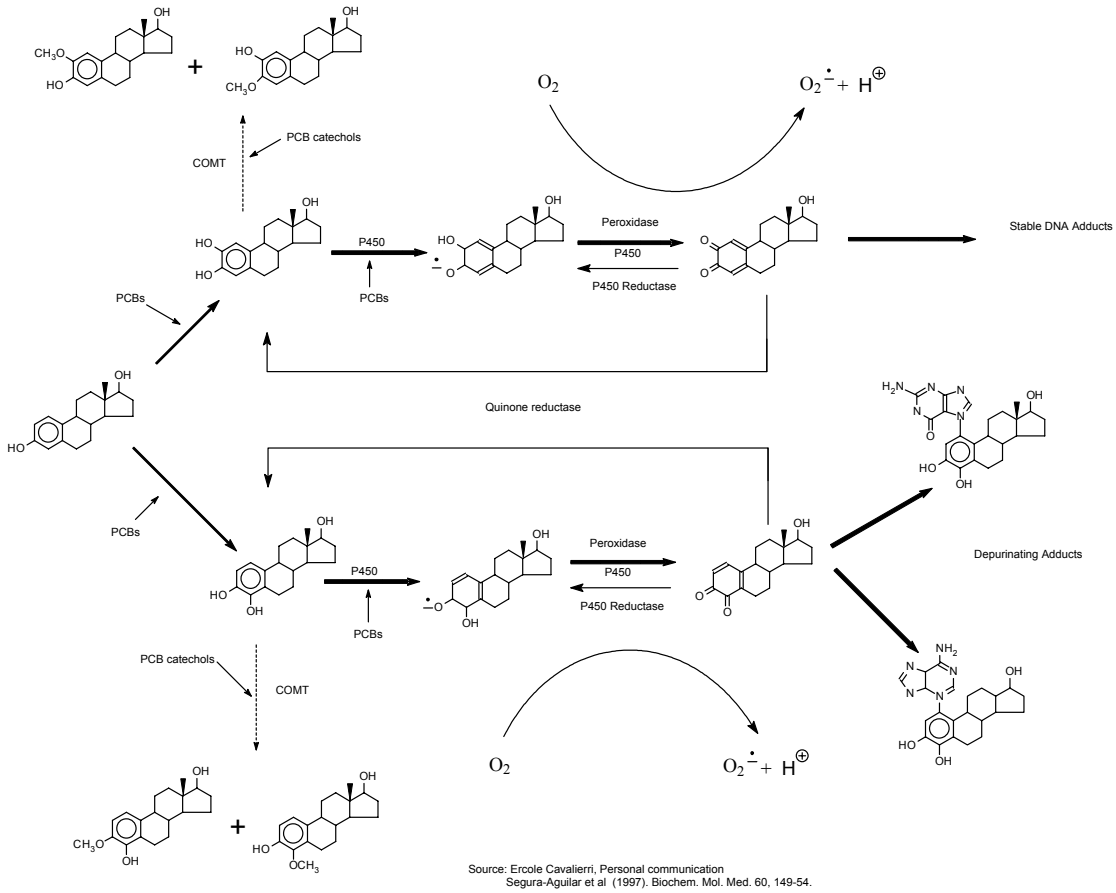


Figure 1. Potential effects of PCBs and PCB metabolites on estradiol metabolism and clearance of catechol estrogens.

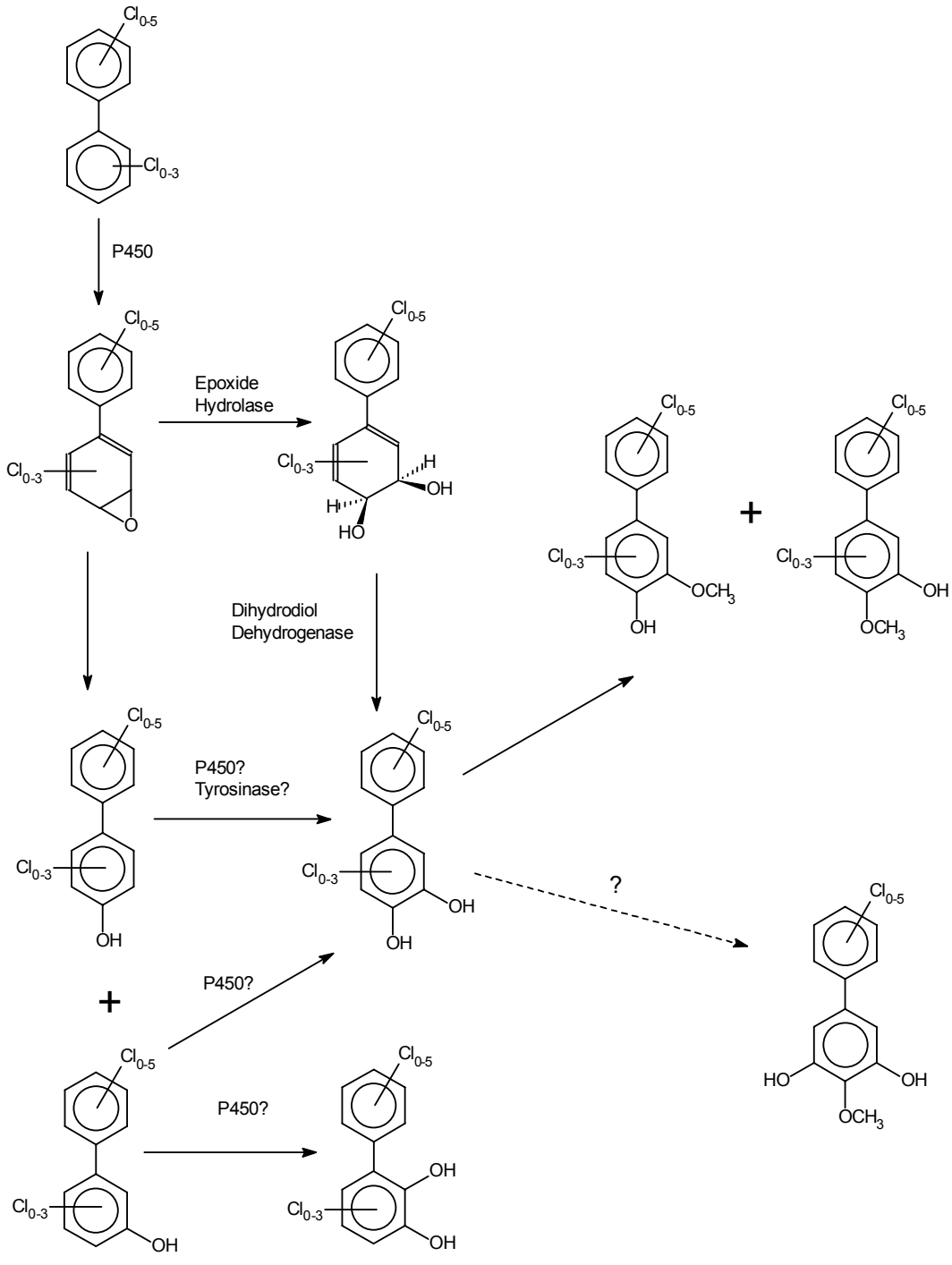


Figure 2. Pathways of PCB metabolism.

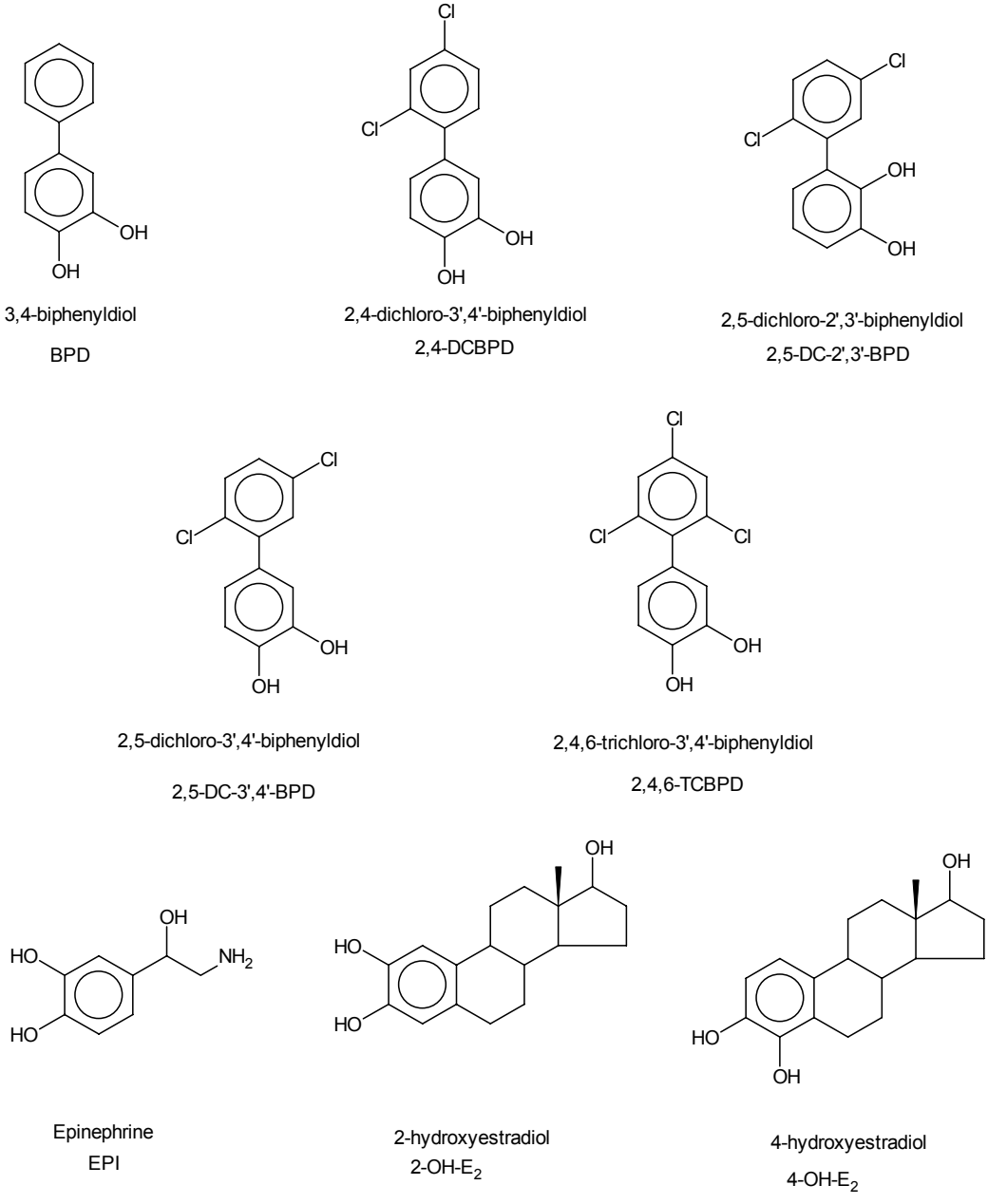
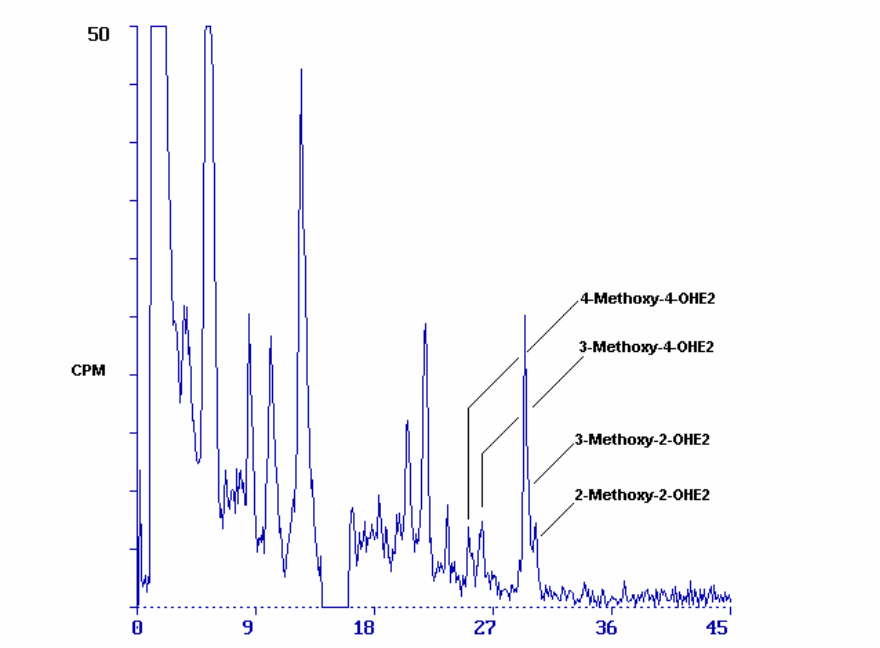


Figure 3. Model catechols used in the present studies.

A



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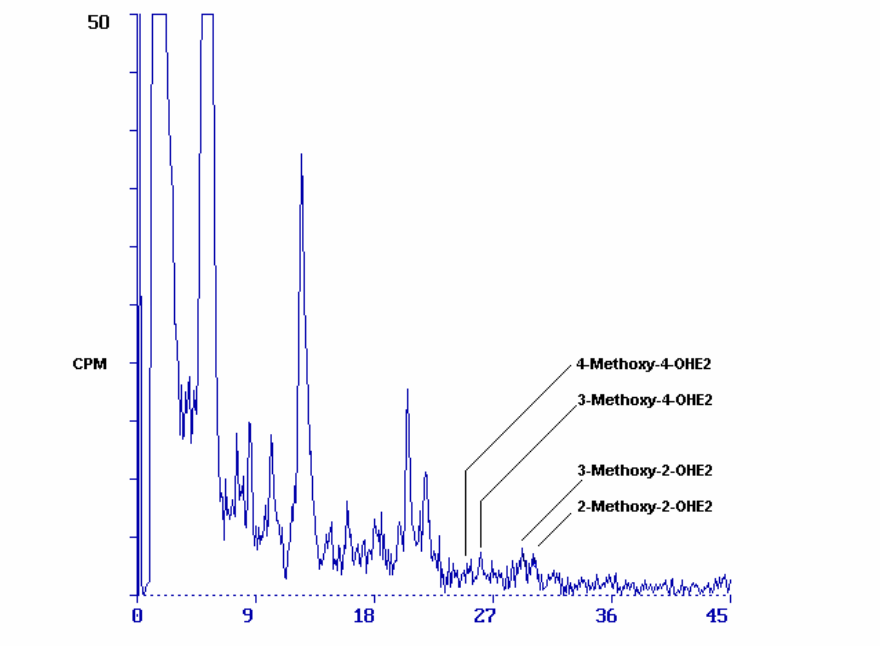

Figure 4. HPLC radiochromatograms of the products of incubation of 3H-estradiol with hepatic S9 from rats treated with: A. Vehicle only or B. Aroclor 1254 (5 mg/kg/day) for 21 days.

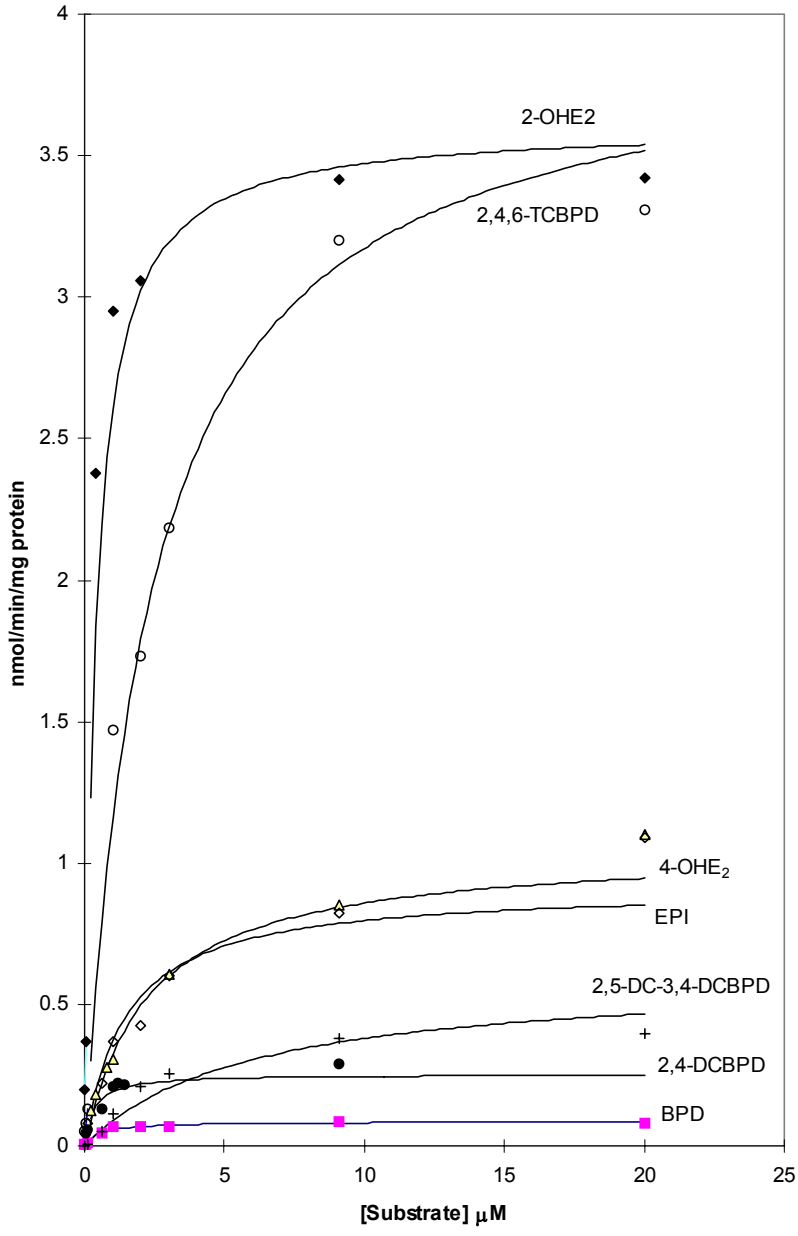

Figure 5. O-Methylation of selected PCB catechols and endogenous catechol hormones by COMT in vitro.

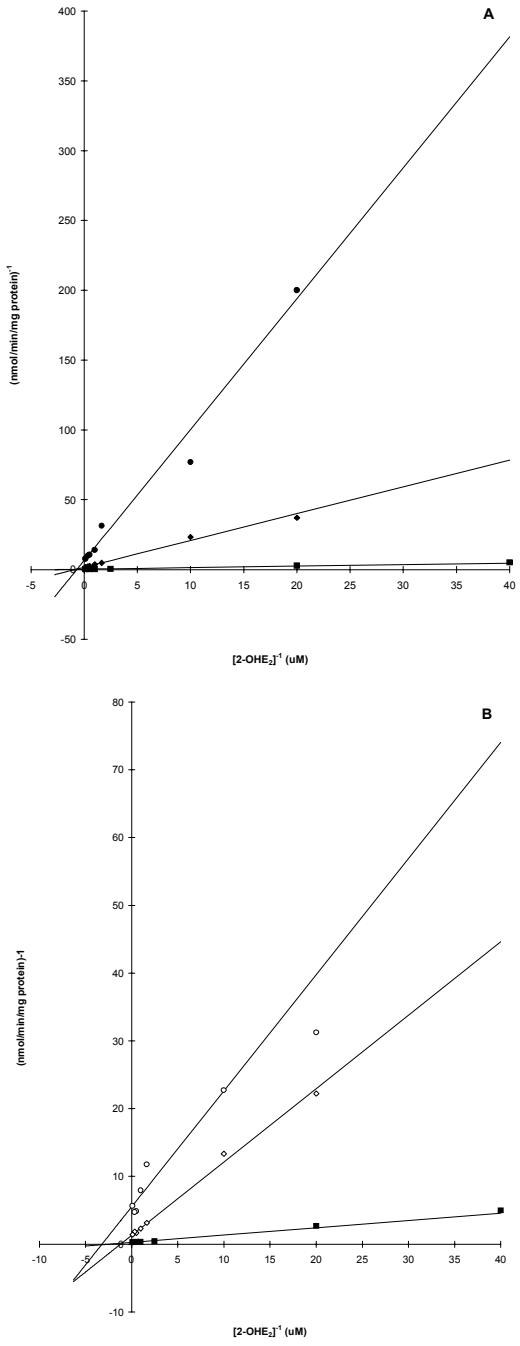

Figure 6. Concentration dependent inhibition of the COMT mediated O-methylation of 2-OHE_2 by A. 2,5-DCBPD and B. 2,4,6-TCBPD.

Key: No inhibitor; 2,5-DCBPD, 1 uM; 2,5-DCBPD, 10 uM; 2,4,6-TCBPD 1 uM; 2,4,6-TCBPD, 10 uM.

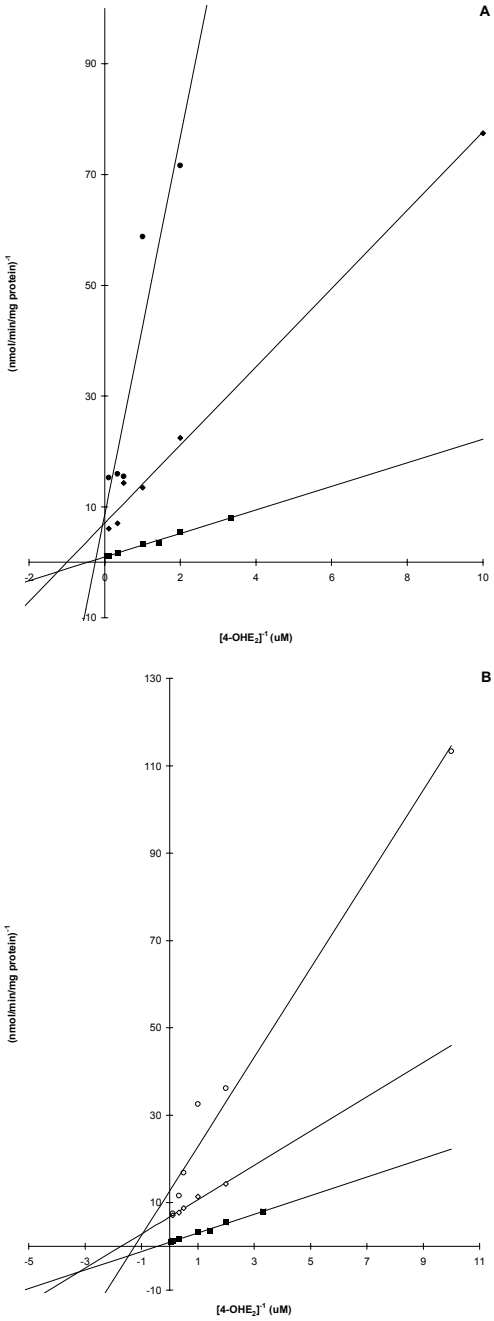

Figure 6. Concentration dependent inhibition of the COMT mediated O-methylation of 4-OHE2 by A. 2,5-DCBPD and B. 2,4,6-TCBPD.

Key: No inhibitor; 2,5-DCBPD, 1 uM; 2,5-DCBPD, 10 uM; 2,4,6-TCBPD 1 uM; 2,4,6-TCBPD, 10 uM.

In Vitro Estrogenicity of the Catechol Metabolites of Selected Polychlorinated Biphenyls.

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ABSTRACT

In Vitro Estrogenicity of the Catechol Metabolites of Selected Polychlorinated Biphenyls. C. Edwin Garner, Wendy N. Jefferson, L. T. Burka, H. B. Matthews, and Retha R. Newbold (1999) *Toxicol. Appl. Pharmacol* **154***,* 188-197*.*

A considerable body of work has demonstrated that phenolic PCB metabolites, structural analogues to estradiol, bind to the soluble estrogen receptor (ER) and that hydroxy PCB-ER complexes will translocate into the nucleus and bind to ER response elements in cultured cells. Although catechol estrogens exhibit weak estrogenic activity, the catechol PCB metabolites which are structurally similar to these ER agonists have gone untested for potential estrogenicity. In the present work we have assessed the estrogenicity of this second group of PCB metabolites, the catechols. The test compounds used in the present study were chosen to elucidate the effects of chlorine and catechol position on in vitro estrogenicity. Cultured HeLa cells, transfected with the estrogen reporter gene ERET81CAT and mouse ER cDNA, were incubated with PCB catechols. The cells were harvested at 28 hours post-transfection and assayed for chloramphenicol acetyl transferase (CAT) activity.

The responses elicited by the PCB catechols tested fell within the range of effect measured for the catechol estrogens and phenolic PCBs, and were within the range previously reported for other “environmental estrogens” such as nonylphenol and o,p’-DDT. Maximal measured responses were achieved at concentrations approximately 2-3 orders of magnitude higher than that of 17-b-estradiol, indicating that PCB catechols have estrogenic activity *in vitro*. The extent of chlorination and position of the catechol (3,4 vs 2,3 substitution) were

important in determining estrogenicity in the compounds tested. The 2,3-catechol showed no detectable activity in this system, while activity of the 3,4-catechols increased with the degree of chlorination. The observed estrogenicity of PCB catechols suggests that further oxidative metabolism of estrogenic PCB phenolic metabolites would not necessarily result in lowering the total estrogenic burden of a PCB exposed organism. The present results imply that if estrogenic activity is assigned to an individual phenol, the potential contribution of its catechol metabolites to the total estrogenic burden should also be taken into consideration.

INTRODUCTION

Polychlorinated biphenyls (PCBs) were widely used in a variety of industrial and consumer products for several decades before their production was banned in the 1970s. As a result of their extensive use and their chemical stability, PCBs are still ubiquitous environmental contaminants that are frequently found as complex mixtures of isomers in air, water, soil, dust, and on surfaces in homes and in factories (WHO, 1993). Initial concern regarding PCBs focused on their accumulation in the tissues of fish, birds, terrestrial and marine mammals, and humans (Wasserman et al., 1979). However, concern has recently expanded to consider the action of PCBs and their metabolites as "environmental estrogens" or hormonal mimics that alter the reproduction and development of higher animals (Fox, 1992; Fuller and Hobson, 1986; Harding and Addison, 1986; LeBlanc, 1995). Such effects have been demonstrated in fish (Hogan and Brauhn, 1975; Mac and Edsall, 1991), birds (Hileman, 1994), and mink (Heaton et al., 1995).

The reproductive/hormonal effects mediated by PCBs and their metabolites have been attributed to disruption of the natural biological role of estrogen. This disruption may result from the induction of enzymes responsible for the metabolism and clearance of naturally occurring hormones (i.e. estrogen) or PCB metabolites binding to estrogen receptors (Alvares and Kappas, 1977; Sundström et al., 1976, Parkinson and Safe, 1981; De Vito et al 1993; Soontornchat et al., 1994; Bradlow et al., 1995; Segura-Aguilar et al., 1997).

The parent PCBs themselves are not effective estrogen receptor agonists (Korach et al., 1987). A considerable body of work has demonstrated that phenolic PCB metabolites bind to the soluble estrogen receptor (ER) (Korach et al., 1988; Jansen et al., 1993; Ramamoorthy, et al., 1997, Connor et al., 1997) and that PCB phenol -ER complexes will translocate into the nucleus and bind to ER response elements in cultured cells (Korach et al., 1997). The ER binding effects of phenolic PCB metabolites have been demonstrated *in vivo* to elicit a uterotrophic response in laboratory animals (Jansen et al., 1993; Korach et al., 1988; Ramamoorthy, et al., 1997, Connor et al., 1997; Fielden et al , 1997).

The present work addresses the estrogenicity of a second group of PCB metabolites, the catechols. PCB catechols are formed by numerous species by further oxidation of phenolic PCBs or by hydrolysis of epoxide intermediates that are thought to be involved in the formation of most hydroxy metabolites (Figure 1). Catechol PCBs have been demonstrated to form from a number of PCBs in several species (Table 1).

MATERIALS AND METHODS

Reagents

All reagents used in the synthesis of the catechols and phenols were purchased from Aldrich Chemical Company, (Milwaukee, WI). The 3,4-biphenyldiol was purchased from Ultra Scientific Co., (Kingstown, RI). Diethylstilbestrol, 17-b-estradiol, 4-hydroxy-estradiol, and 2-hydroxy-estradiol were purchased from

Sigma Chemical Co. (St. Louis). The antiestrogenic compound ICI 182,780 was a gift from Dr. Alan Wakeling, Zeneca Pharmaceuticals (Alderly Park, Cheshire, UK)

Synthetic methods.

The 2,5-dichloro-3',4'-biphenyldiol, 2,5-dichloro-4'-biphenylol, 2,5-dichloro-2',3'-biphenyldiol, 2,4,6-trichloro-4'-biphenylol and 2,4,6-trichloro-3',4'-biphenyldiol (Figure 1) were synthesized in these laboratories using the method of Mullin et al (1981) which is a variation of the Cadogan biphenyl coupling reaction. Briefly, mono- or dimethoxyanilines were coupled with chlorinated benzenes to yield the chlorinated mono- or dimethoxybiphenyls. The phenol or catechols were then produced by demethylation with boron tribromide. Structural identification was determined by NMR and GC/MS. All test compounds were determined to be greater than 95% pure by TLC and gas chromatography.

Estrogenicity assay

In vitro estrogenicity of the catechol PCB metabolites was determined using a transcriptional activation assay as previously reported in detail (Miglioaccio et al., 1992; Shelby et al., 1996). Briefly, HeLa cells, which do not constituatively express the estrogen receptor a or b (Shelby et al., 1996, R. Newbold, unpublished results), were grown in DMEM/F12 medium (1:1) without phenol red, supplemented with 5% fetal bovine serum and penicillin-streptomycin (Sigma). On the day of transfection, cells were cotransfected by electroporation

with vectors containing the estrogen reporter a gene ERET81CAT and mouse ER cDNA in media containing insulin/transferrin/selenium (ITS) (Sigma, St. Louis) in the absence of serum. The vectors were the gift of John Couse of the National Institute of Environmental Health Sciences, RTP,NC**.** The ERE sequence was 5'TCGACCAGGTCAGCGTGACCGGAGCTGAAAGAAAAGATTGACCCCGGATCC3' (Miglioaccio et al. 1992). Test material was dissolved in DMSO or ethanol for delivery into cell culture solutions. Triplicate incubations of cells with various concentrations of test compound were harvested at 28 hours post-transfection and assayed for chloramphenicol acetyl transferase (CAT) protein using a CAT ELISA kit (Boehringer Mannheim, Indianapolis, IN) according to manufacturers instructions. 17β-Estradiol at a concentration of 10 nM was used as the positive control for all experiments. Experiments were repeated a minimum of three times.. To assure that inter-assay comparisons were valid, cells from each assay were examined for signs of cytotoxicity (cellular swelling, blebbing, etc.) by light microscopy. Data from assays wherein substrate concentration was high enough to elicit cytotoxicity were not included. In separate control experiments, the potent antiestrogen ICI 182,780 was added at a concentration of 1μM one hour prior to the addition of each test compound, including estradiol and the catechol estrogens, to demonstrate that measured activity was mediated through binding to the estrogen receptor. These were repeated at least three times and statistical significance was calculated using Student's t-test.

RESULTS

Each of the catechol biphenyls induced CAT activity (Figure 3, Table 2). No signs of cytoxicity were visible at PCB catechol and phenol concentrations up to and including 10 mM (50 mM for BPD), but at concentrations higher than this maximum, 100% of the cells were killed. That is, only cellular debris was visible. Elucidating the mechanism of toxicity was outside of the scope of these investigations. Estradiol and the catechol estrogens were not cytotoxic at the concentrations used in these studies. The cytotoxicity of the PCB metabolites precluded the development of a classical dose-response curve. The concentrations which elicited maximal responses were approximately 3 orders of magnitude lower than that of 17-b-estradiol, indicating that PCB catechols are weak estrogens *in vitro*. However, estrogenicity of the chlorinated 3,4-catechols were within the same range as the catechol estrogens (Figure 3). The responses elicited by the PCB catechols tested here were similar to the phenolic PCBs (Figures 3,4) and approximately within the range seen from other "environmental estrogens" such as nonylphenol, o,p'-DDT when tested in this system (Shelby et al., 1996). This activity was completely blocked in all cases by the antiestrogen ICI-182,780 (Figure 4). The ability of ICI to completely block the activity of these compounds suggests that these compounds bind to the receptor and their estrogenic activity is mediated through the estrogen receptor.

Though the set of PCB catechols tested in the system was small, the data suggest that both chlorine substitution and catechol position have an effect on *in vitro* estrogenicity. Position of hydroxylation (3,4 vs 2,3 substitution) was important in determining estrogenicity of the catechols tested. The 3,4 substituted dichlorobiphenylcatechol, (3,4-DCBPD) was much more effective in activating the ER response elements than 2,3-catechol of the same PCB, 2,3-DCBPD, which showed almost no activity in this system**.** The three 3,4-catechols tested in this system were all more active than the 2,3-catechol. Of the 3,4-catechols tested, activity increased as the degree of chlorination increased. The trichloro biphenylcatechol (3,4-TCBPD) elicited activity at concentrations an order of magnitude lower than those at which dichlorobiphenyldcatechol (3,4-TCBPD) or biphenyldiol (BPD) became active.

DISCUSSION

This study is the first to demonstrate the *in vitro* estrogenicity of catechol metabolites of PCBs. Effects of the PCB catechols tested here were within the level of effect seen with other "environmental estrogens" such as nonylphenol and o,p'-DDT when tested in this system (Shelby et al., 1996). The estrogenic activity of the chlorinated biphenyl catechols also were within the effect range of the catechol estrogens, which are known to posses *in vivo* estrogenic properties (Ball and Knuppen, 1980). This finding is significant because a number of PCBs have been reported to be metabolized to catechols in a number of species

(Table 1 and references within). In some cases catechols may be major metabolites, constituting up to 80% of the total metabolite profile (Greb et al., 1975; Goto et al., 1974). The estrogenicity of PCB catechols also implies that further oxidative metabolism of estrogenic PCB phenolic metabolites would not necessarily result in lowering the total estrogenic burden of a PCB-exposed organism.

Several authors have demonstrated that PCBs with low chlorine content have uterotrophic and reproductive effects *in vivo* and that this activity can be linked to the ease of conversion of these compounds to hydroxylated compounds that could have estrogenic activities or alter estrogen metabolism (Jansen et al., 1993; Matta et al., 1997; Torok, 1975; 1978; Orberg, 1978; Desaulineiers, 1997, Li and Hansen, 1995; Gellert, 1978). Indeed, some authors have demonstrated that uterotrophic effects result from administration of hydroxylated PCB metabolites (Korach et al., 1988; Jansen et al., 1993; Ramamoorthy et al., 1997, Connor et al., 1997; Fielden et al., 1997). The liver readily converts administered phenolic PCB metabolites to the catechols when unsubstituted carbons are adjacent to the phenol (Safe et al., 1975a; Amaro et al., 1996). In the studies of Jansen et al (1993), 2,4,6-trichloro-4'biphenylol administered IP had a greater uterotrophic response *in vivo* than 3,5,3',5'-tetrachloro-4,4'- biphenyldiol at every concentration tested . The former compound possesses structural characteristics that would allow for easy conversion to the catechol *in vivo*

(Mathews and Anderson, 1975; Safe et al., 1975b; Sundström et al., 1976) while the latter cannot be further metabolized.

The present results imply that if estrogenic activity is assigned to an individual phenol, the contribution of its catechol metabolites to the total estrogenic burden should also be taken into consideration. Recently, Das et al (1997) demonstrated that mouse uterine cells possess a separate, non-ER mediated signalling pathway responsive to catechol estrogens. These findings, coupled with the structural similarities of PCB catechols with estrogen catechols, suggest that PCB catechols may also mimic the effects of catechol estrogens in the uterus. Taken together, the estrogenic effects of phenolic PCBs may involve a complex combination of events, involving interactions of both phenols and catechols with the ER; and, potentially, the catechols with other non-ER mediated pathways.

This group is presently conducting experiments to determine the *in vivo* estrogenic potential of PCB catechols and to compare their potencies with PCB and phenolic analogues.

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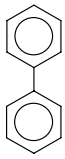
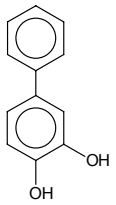
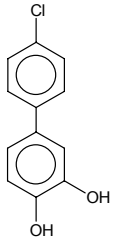
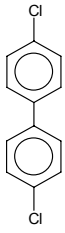
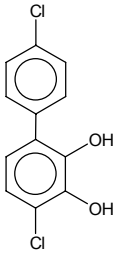
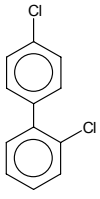
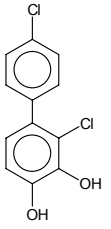
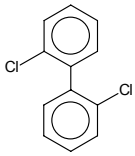
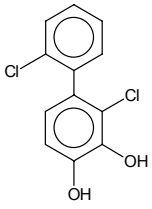
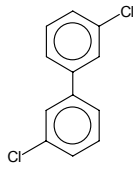
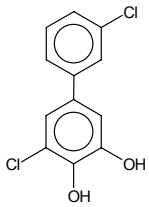
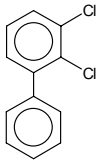
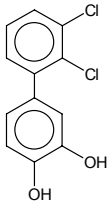
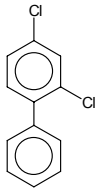
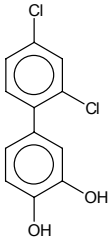
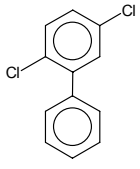
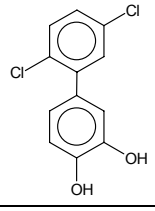
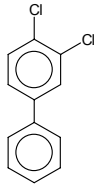
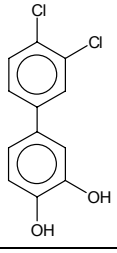
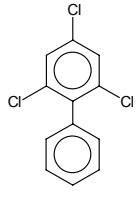
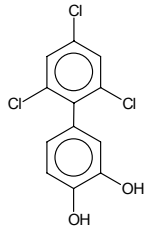
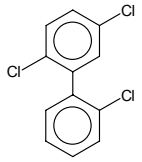
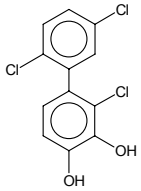
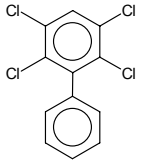
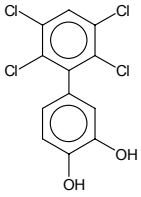
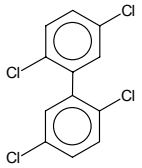
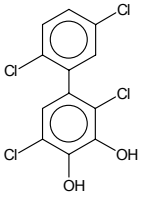
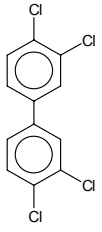
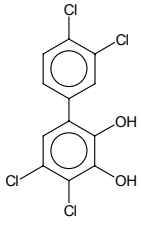
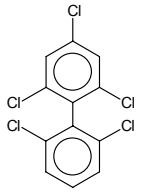
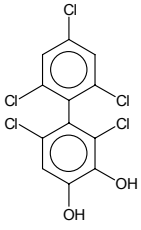
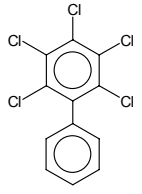
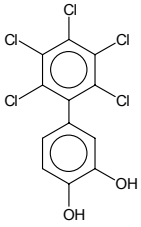
Table 1. PCBs known to form catechol metabolites in experimental animals.

Parent Molecule	Catechol Metabolite	Species	Reference
 biphenyl		Rat	Raig and Ammond (1972)
 4-chlorobiphenyl		Rat Rabbit Goat Pig Frog	Safe et al. (1975a) Safe et al. (1975b) Safe et al. (1975c) Safe et al. (1975d) Safe et al. (1976)
 4,4'-dichlorobiphenyl		Rat	Goto et al. (1975)
 4,2'-dichlorobiphenyl		Monkey	Greb et al. (1975)
 2,2'-dichlorobiphenyl		Rat	Goto et al. (1975)

 3,3'-dichlorobiphenyl		Rat	Goto et al. (1975)
 2,3-dichlorobiphenyl		Rat	Goto et al. (1975) Goto et al. (1974)
 2,4-dichlorobiphenyl		Monkey	Greb et al. (1975)
 2,5-dichlorobiphenyl		Rat	Tulp et al. (1977)
 3,4-dichlorobiphenyl		Rat	Goto et al.(1974b)
 2,4,6-trichlorobiphenyl		Rat	Goto et al. (1975) Goto et al. (1974b)

 2,5,2'-trichlorobiphenyl		Monkey	Greb et al. (1975)
 2,3,5,6-tetrachlorobiphenyl		Rat	Goto et al. (1975) Goto et al. (1974b)
 2,5,2',5'-tetrachlorobiphenyl		Rat Mouse Guinea Pig	Mio and Sumino (1985)
 3,4,3',4'-tetrachlorobiphenyl		Rat	Koga et al. (1989)
 2,4,6,2',6'-pentachlorobiphenyl		Rat	Goto et al. (1975)
 2,3,4,5,6-pentachlorobiphenyl		Rat	Goto et al. (1975) Goto et al. (1974b)

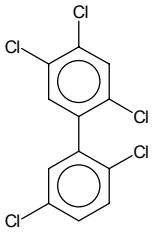
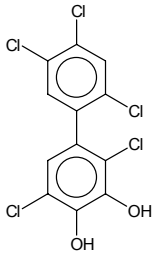
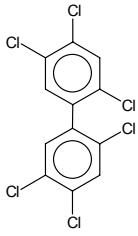
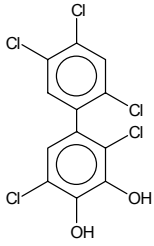
 2,4,5,2',5'-pentachlorobiphenyl		Rat	Chen et al. (1976)
 2,4,5,2',4',5'-hexachlorobiphenyl		Rabbit	Sundstrom and Wachmiester (1975) Hutzinger et al. (1974)

Table 2. Transcriptional Activation of Estrogen Receptor in HeLa Cells.

	Compound	Concentration at Maximum Measured Response (nM)	Percent of Maximum Estradiol Response*
Negative Control	Control		0.40 ± 0.21
Positive Control	Estradiol	10	100.00
Catechol Estrogens	2-OH-Estradiol	10,000	29.42 ± 1.83
	4-OH-Estradiol	1,000	38.78 ± 0.85
PCB Phenols	MH-DCB	10,000	20.31 ± 0.52
	MH-TCB	10,000	44.20 ± 2.56
PCB Catechols	BPD	50,000	41.60 ± 1.82
	3,4-DCBPD	10,000	18.62 ± 1.71
	2,3-DCBPD	10,000	5.62 ± 0.71
	TC-BPD	10,000	35.70 ± 5.76

*Responses were normalized to maximum response to b-estradiol measured at 10 nM. Data represent mean ± SD of a minimum of three separate experiments.

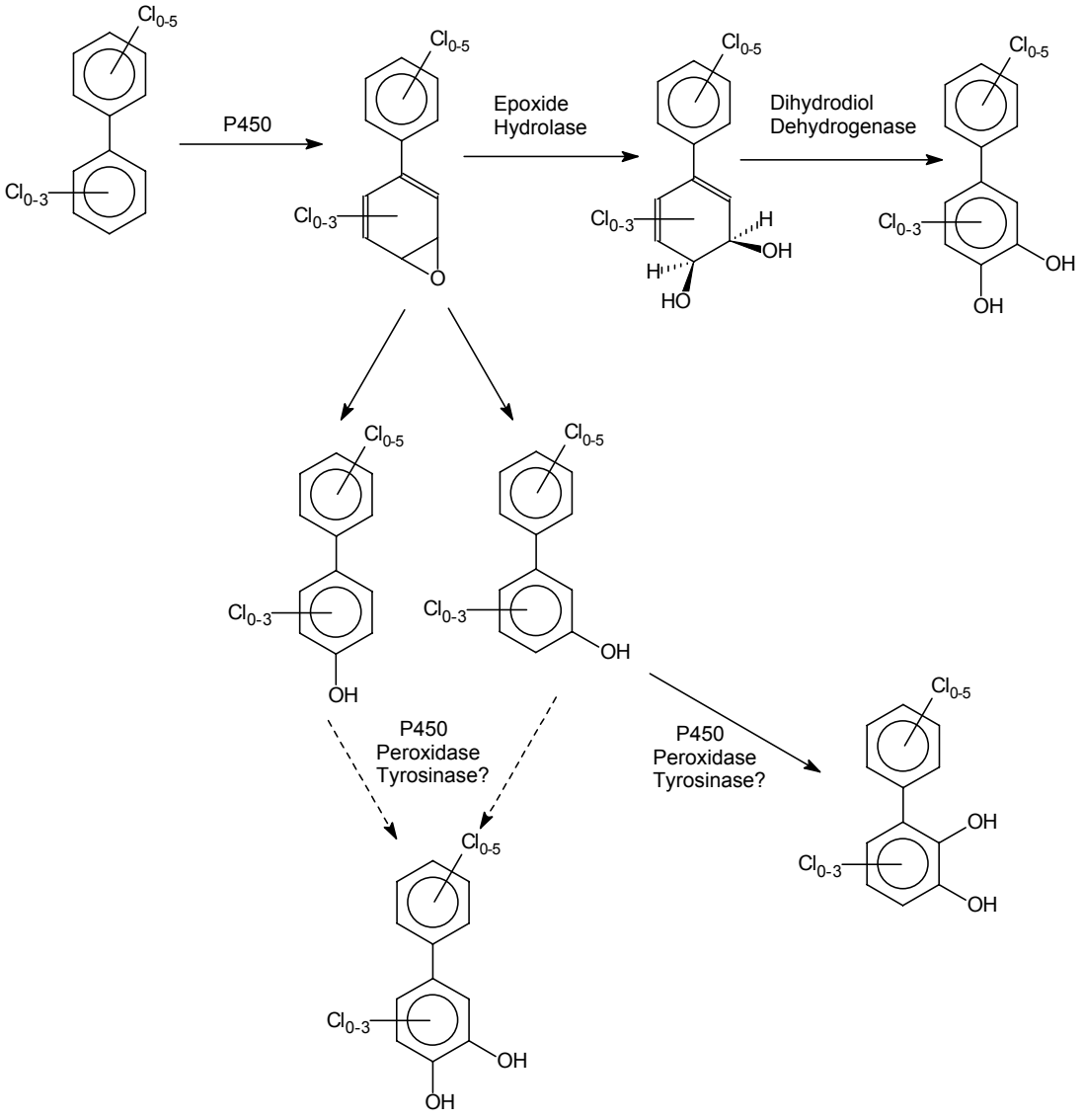


Figure 1. Metabolic formation of catechol metabolites of PCBs.

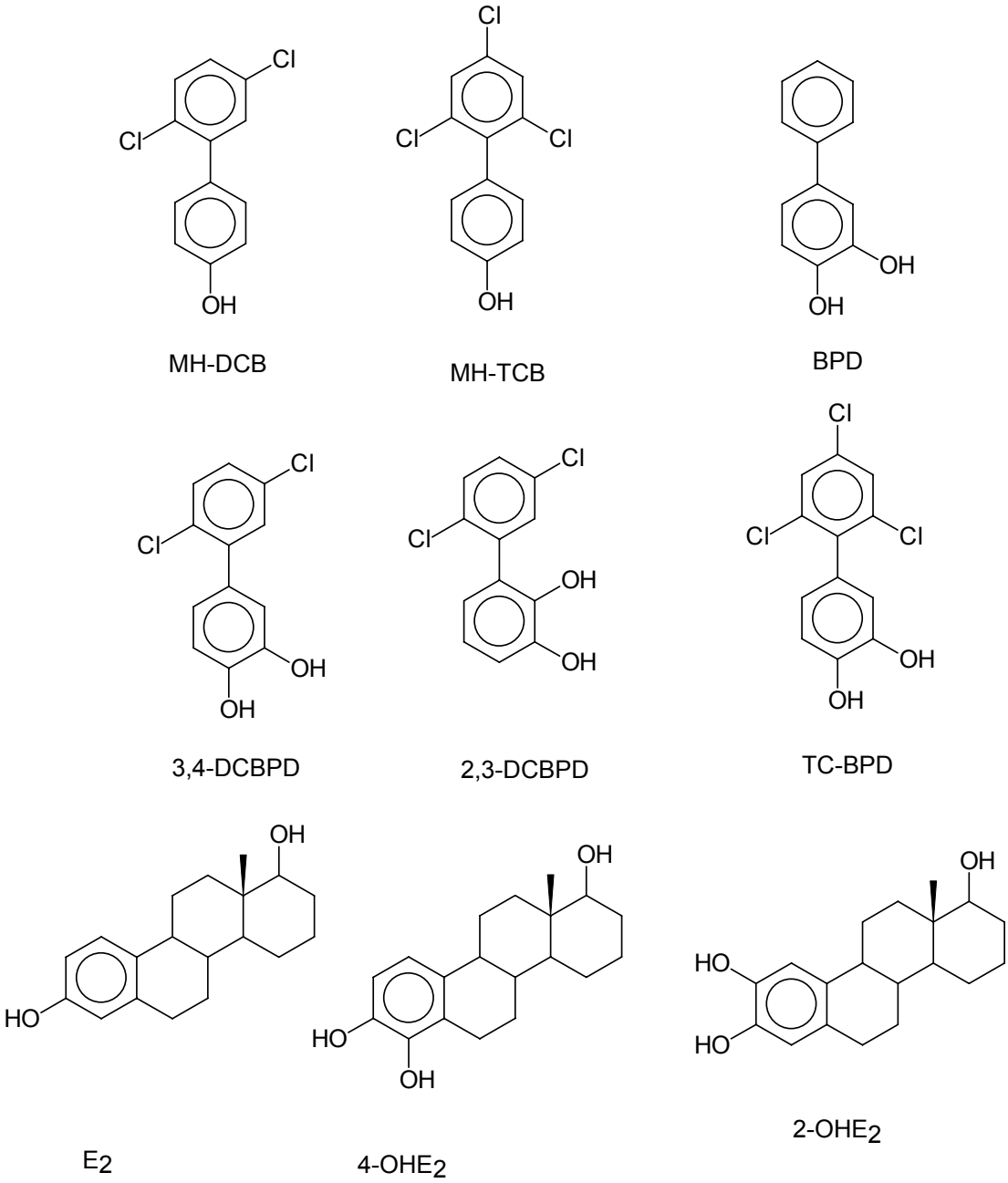


Figure 2. Structures of PCB catechol and phenolic metabolites used in these experiments.

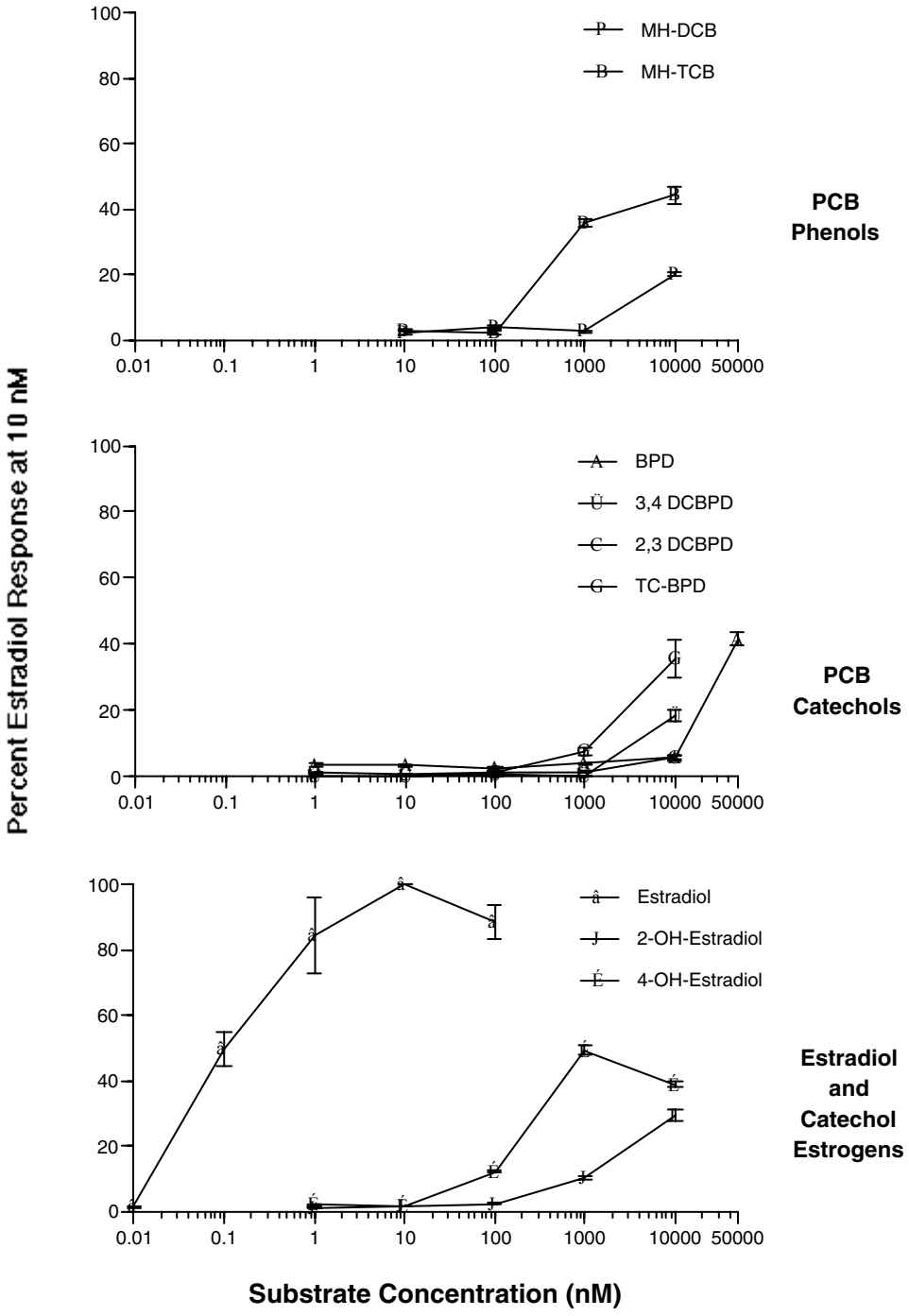


Figure 3. Estrogenic activation of CAT reporter gene in HeLa cells transfected with vectors containing the mouse estrogen receptor (mER) and ERET81CAT reporter.

Responses were normalized to maximum response to b-estradiol measured at 10 nM. Data represent mean ± SD of a minimum of three separate experiments.

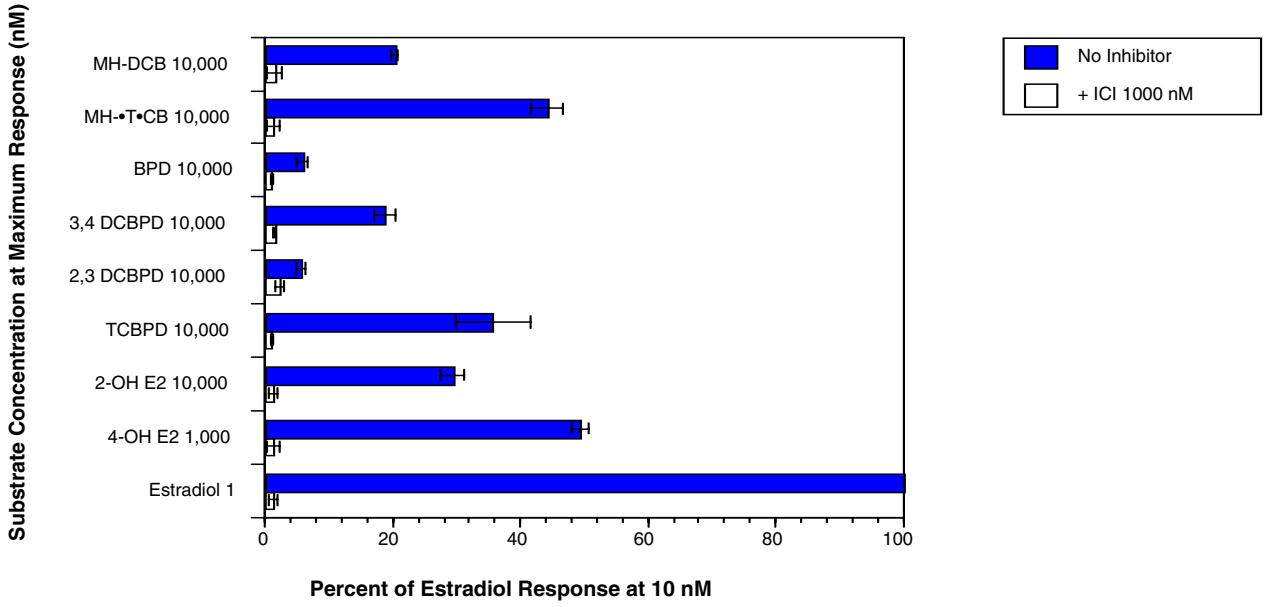


Figure 4. Effect of the antiestrogen ICI 182,780 on maximal activation of CAT reporter gene in mER containing HeLa cells .

Responses were normalized to maximum response to b-estradiol measured at 10 nM. Data represent mean ± SD of a minimum of three separate experiments. Differences in responses were statistically significant at $p < 0.05$.

GENERAL SUMMARY

C. Edwin Garner

The studies described in Parts 1 and 2 were part of a two prong investigation into the toxicokinetics and the biochemical mechanisms of polychlorinated biphenyl mediated toxicities. Part 1 investigated the absorption, disposition and excretion of PCBs following dermal administration. Our goals were to elucidate rate and mechanism of absorption in model PCBs and to then extrapolate those findings to model the absorption of complex mixtures of PCB congeners. Additional goals were to determine the mechanism of dermal absorption and to determine if chlorine position and content affected the disposition and excretion of absorbed radioactivity. In the course of these investigations, evidence of transdermal metabolism was discovered. This raised the possibility that following dermal exposure to PCBs, the organism may possibly be “dosed” with metabolites in addition to parent, and that the exposed organism may “see” more metabolites than during oral exposure. In other words, transdermal metabolism may be a means of delivering hydroxylated PCB metabolites directly to the systemic circulation. Part 2 investigated the interaction of one class of these possible metabolites, the catechols, with enzymes and receptors associated with estrogen metabolism and function.

Our first finding was that the rate and therefore the extent of dermal penetration of PCBs varied greatly over the range of PCB chlorination. Penetration through the stratum corneum into the viable epidermis was a first-order process and the

rate constants appeared to be a function of K_{ow}. Within a group of structurally related molecules, K_{ow} is a measure more of a compound's tendency to partition out of water (hydrophobicity) than its absolute affinity for lipid (lipophilicity) (Leo et al., 1971). Therefore, the rate of penetration into the viable epidermis is probably limited more by penetration of the PCB into the relatively water-rich viable epidermis than by penetration through the lipid rich stratum corneum. The relationship between dermal penetration rates and Kow developed here (Chapter 2, Equation 2) was used to predict the extent of dermal penetration of Aroclor 1254, a once commonly used commercial mixture of PCBs. Penetration rates of the PCB components of Aroclor 1254 (WHO, 1993) were estimated from log K_{ow} (Rappaport and Eisenreich, 1984). According to our rat model, 24 hours following application of Aroclor 1254 to the skin, approximately 35% of the mass of PCBs would penetrate the stratum corneum into the viable epidermis. Wester et al. (1983) measured ca 50% absorption of a 54% chlorine PCB mixture 24h following a single application to clipped guinea pig skin. The model underpredicted absorption, but this model was developed for the rat and has not been generalized for species differences and does not take into account accelerated penetration due to transdermal metabolism. Additionally, the PCB mixture used by Wester was not characterized and therefore may have contained components that were more rapidly absorbed. The model was also used to predict the absorption of 2,4,5,2'4',5'-hexachlorbiphenyl 72 and 120 hours post application and these predictions were compared to that measured by Fischer et al (1989). We predicted ca 25 and 36% of the applied dose would be

absorbed at 72 and 120 hours, respectively. Fischer measured 26 and 45% absorbed at those times. The predictions were considerably more accurate when measuring the penetration of a poorly metabolizable PCB. The model's utility may be improved considerably if transdermal rates of metabolism could be predicted.

After modeling the passive penetration of Aroclor 1254 we were able to examine the congener specific contribution to body burden. Greater than 85% of the components with 4 or less chlorines, ca 60% of pentachlorobiphenyls, and only 18% of hexachlorobiphenyls are predicted to penetrate the skin in 48 hr. Penetration of PCBs with 7 or more chlorines is predicted to be less than 1%. Sixty percent of the total absorbed PCBs will be pentachlorobiphenyls and less than 5% will be hexachlorobiphenyls. The high mass contribution (62%) of the pentachlorobiphenyls in the penetrated PCBs is a function of their large mass proportion in Aroclor 1254 (ca 50%). The physical act of dermal penetration is therefore predicted to enrich the profile of systemically absorbed PCBs with lower chlorinated species. The more rapidly absorbed PCBs are also more rapidly hepatically cleared, resulting in lower potential contribution to total parent body burden. However, these lower chlorinated biphenyls are also more likely to be metabolized transdermally, therefore body burden of the hydroxyPCBs may be significantly different from those following inhalation or oral exposure. The MCB urine HPLCs demonstrated that the system is exposed to a very different set of metabolites following dermal administration when a PCB is extensively

dermally metabolized. Penta- and hexachlorobiphenyls, which are the predominant species environmentally (WHO, 1993) are predicted to be slowly released from the epidermis/dermis reservoir, and because of their slow clearance, account for most of the unmetabolized PCB body burden resulting from a hypothetical exposure.

MCB and PCB 77 (initially) both penetrated into the viable epidermis at rates faster than their Kow would predict. Skin slice experiments demonstrated that metabolism at the stratum corneum /epidermal interface contributed to the penetration of both of these compounds. The slice experiments also indicated that if a PCB possesses vicinal, unsubstituted carbons, it will be readily metabolized by the skin. The skin possesses cytochrome P450 activities in the epidermis that are approximately 80% of hepatic activities (Noonan and Wester, 1989). This data supports the suggestions of Noonan and Wester (1989) and Jackson et al. (1993) that penetration of highly lipophillic compounds into viable epidermis and subsequently into the systemic circulation may be rate limited by metabolism. The work of Holland et al (1984) and Kao et al (1985) further suggest that the absorption of lipophillic compounds such as benzo(a)pyrene is increased following induction of metabolic enzymes

The dermal experiments also demonstrated that the epidermis served as a reservoir for penetrated dose, especially with the poorly metabolized tetra and hexachlorobiphenyls. MCB and DCB dose site equivalents peaked very quickly

in the viable epidermis and were very rapidly removed to the systemic circulation, again possibly via metabolism. The retention of the highly chlorinated PCBs may be a function of partitioning into adipose, sebaceous glands, and/or adsorption to epithelial proteins. Albumin is the major binding constituent in skin and other tissues (Menczel et al. 1985) and PCBs adsorb tightly to such proteins (Matthews, et al., 1984). Bidmon et al. (1990) has reported that the hair follicles and sebaceous glands of rat skin serve as a reservoir for the hormone estradiol following penetration. Regardless of the mechanism, the viable epidermis/dermis served as a slow release reservoir for systemic absorption of the higher chlorinated PCBs. In the case of HCB ca 15% of the penetrated dose was still present in the dose site skin 12 days after the dose was removed from the dose site. Those PCBs that retained in the dose site also had elevated tissue concentrations that peaked at times much later than unabsorbed dose was removed. These same compounds were also excreted much more slowly than those that were not retained in the dose site.

The rate of systemic absorption of PCBs was not characteristic of a simple first order process but is kinetically complex (Figure 1a.). This suggests that systemic absorption of PCBs involves a combination of sequential processes, including penetration across the stratum corneum, metabolism in the epidermis and/or dermis, affinity for lipophillic sites on proteins and/or partition into sebum, and finally absorption into the systemic circulation. The skin favored the rapid absorption of less chlorinated PCBs which, following absorption, are quickly

metabolized in the liver and, presumably, in skin to more polar compounds that are quickly eliminated. The relatively rapid metabolism and elimination of these compounds would result in lower body burdens of parent. Higher chlorinated PCBs do not penetrate into the skin as rapidly but are retained in the site of exposure and are very slowly absorbed systemically. The combination of slow systemic absorption with slow elimination from the tissues results in higher body burdens of the higher chlorinated PCBs.

It follows from the above that systemic exposure to PCBs following dermal exposure is dependent on more than just the amount of PCB to which the skin is exposed. Absorption following oral exposure is a simpler process since essentially oral absorption is complete for PCBs (Figure 1b.) (Albro and Fishbein, 1970, Mathews and Andersen, 1975; Garner and Matthews, unpublished results). Skin P-450s involved in the metabolism are inducible (Holland et al (1984); Kao et al (1985); DeVito et al , 1997) so dermal PCB exposure of an organism following exposure to a P-450 inducer would result in more complete absorption of PCB equivalents. Elevated skin metabolism would result in elevated metabolite exposure, but these compounds are for the most part, eliminated quickly relative to parent PCB (Matthews and Andersen, 1975). Predictions of the effect on PCB systemic absorption that would result from altered transdermal metabolism would be difficult to make accurately, but this work sheds some light on the possibilities. In the case of PCB 77, initial penetration exceeded that predicted from our dermal model, most likely as the

result of transdermal metabolism. When metabolism was (presumably) self-inhibited, the penetration rate fell to a rate that suggested passive processes. The dose site then became a reservoir that supported elevated tissue concentrations for up to 14 days post-application. Slow release to the system also resulted in extremely slow elimination relative to oral or IV exposure. The example of PCB 77 suggests that multiple dermal exposures of PCBs in the same region of skin may make significant changes in kinetic behavior of the subsequent exposures.

That a dermally exposed organism may absorb PCB metabolites directly into the systemic circulation led us to investigate the potential toxicological properties of PCB metabolites. The toxicological significance of PCB metabolites is continuing to be investigated. Slowly it is becoming apparent that the PCB congeners that exhibit Ah receptor-mediated responses constitute only a small fraction of the total number of possible congeners that are routinely identified in environmental samples, and may be present only as trace components in commercial PCB mixtures (Jones 1988; McFarland and Clarke 1989; Hansen, 1998). Therefore, it is reasonable to assume that bulk of congeners that act by other mechanisms also contribute in a large part to the toxicity of PCB mixtures (Hansen, 1998). Direct dosing of monohydroxylated, lower chlorinated PCB metabolites to yield high concentrations of PCB catechols has been demonstrated in several species by a number of authors Safe and Ruzo, 1975; Safe et al, 1975; Jones et al, 1979). We therefore were interested in exploring

the interaction of the catechol metabolites of PCBs with two toxic endpoints of PCB toxicity: carcinogenesis and endocrine disruption.

Recently commercial PCB mixtures have been demonstrated to induce liver and mammary tumors in female rats (Mayes, et al, 1997; Silkworth et al., 1997) and this effect has tentatively been attributed to the effects of PCBs on the metabolism of estrogen to its catechol metabolites. The catechol estrogens (CEs) are potent signalling molecules (Ball and Knuppen, 1980) and are hypothesized to be central to estrogen-linked carcinogenesis (Yager and Liehr, 1996; Cavalieri and Rogan, 1998; Stack et al, 1998; Weisz et al, 1998; Liehr, 1998; Cavalieri et al, 1997; Stack et al, 1996; Li and Li, 1984, Seraj et al. 1996). Catechol estrogen metabolites are also capable of metabolic redox cycling between quinone and hydroquinone forms, resulting in free radical generation. Several types of direct and indirect free radical-mediated DNA damage are induced by estrogens in vitro and in vivo. Among these are: DNA single strand breaks, 8-hydroxylation of guanine bases, and DNA adduct formation by malondialdehyde, a decomposition product of free radical-induced lipid peroxides (Liehr, 1997; Han and Liehr, 1994, 1995; Liehr et al, 1986). In addition, catechol estrogens may also induce genotoxicity by direct formation of DNA base adducts (Cavalieri and Rogan, 1998; Cavalieri et al, 1997). Methylation by catechol -O-methyltransferase (COMT) is the principal means of catechol estrogen (CE) deactivation in the liver and other tissues (Yager and Liehr, 1996; Ashburn et al, 1993; Ball and Knuppen, 1980).

To determine the effect of PCB treatment on estradiol metabolism, PCBs were administered to female rats at a dose previously demonstrated to induce liver tumors (Mayes, et al, 1997). The amount of free catechol estrogens produced following incubation of estradiol with subcellular fractions from PCB-treated rats and the COMT activity for methylation of CEs was measured. Data obtained demonstrate that PCB treatment resulted in an increase in free CEs, which are normally cleared by COMT and a concurrent decrease in the capacity of hepatic catechol clearance by COMT. Model catechol PCBs were demonstrated to be good substrates for COMT, with K_ms approximately equivalent to those of the CEs. Catechol PCBs were also mixed -type inhibitors of the O-methylation of CEs. The data suggest that PCBs significantly alter the metabolism of catechol estrogens in vivo and that this effect may be mediated by catechol PCB metabolites. The two effects, when coupled, may account for a mechanism that contributes to PCB-mediated tumorigenesis.

The interaction of catechol PCB metabolites with both the synthesis and clearance of catechol estrogens has significant toxicological ramifications in addition to cancer and this effect may be the central mechanism that links a number of disparate PCB toxicities.

The PCBs are known to:

1) Disrupt estrogen metabolism and estrus cyclicity (Orberg and Kihlstrom 1973; Crisp et al, 1998)

2) Decrease implantation and cause increased spontaneous abortion (Seiler et al,1994; Torok, 1975, 1978; Orberg and Kihlstrom 1973).
3) Decrease dopamine levels, synthesis, and uptake in the brain (Chishti et al, 1996; Choksi, et al., 1997; Seegal et al., 1996),
4) Disrupt pituitary/hypothalamal communication (Byrne et al, 1987; Thomas, 1990; Gould et al, 1997; Quabius et al , 1997).
5) Exert neurotoxic effects in the brain during development, leading to behavior and intelligence effects (Tilson and Kodvanti, 1997; Seegal, et al.1996).

The mechanisms of the toxicities listed above are not known. There is extensive evidence, though, in the literature that suggests a common element: catechol estrogens and COMT. The catechol estrogens or COMT are central to the regulation of each of the processes listed above. Disruption of endogenous CE metabolism by PCB catechols would offer plausible mechanisms to be investigated further.

As neuroendocrine molecules, CEs modulate estrous cyclicity and estradiol production by controlling the production and uptake of norepinephrine (NE) in the neurons that connect the hypothalamus and pituitary gland in the brain (Ghraf and Heimke, 1983). CEs inhibit the synthesis of NE by inhibiting the activity of the enzyme tyrosine hydroxylase (TH). The catechol estrogens inhibit this enzyme by competing with its pterin cofactor for binding sites on the enzyme.

O-methylation of the catechol prevents this competetion (Foreman and Porter, 1980). TH is the critical step in dopamine and norepinephrine synthesis and a reduction in the levels of these neurotransmitters in the pituitary reduces estrogen production by the ovaries and follicle stimulating hormone (FSH) and leutinizing hormone (LH) production by the pituitary (Franks, 1983). PCB catechols could disrupt this process in two ways. First, the PCB catechols could increase pituitary/hypothalamal CEs by inhibiting their COMT-mediated clearance. Second, the PCB catechols, which are structurally similar to the CEs, may act in the same manner as the CEs, eliciting a similar effect. Disruption of pituitary LH and FSH biosynthesis would have effect on cyclicity and hormonal surges. In the female animal, the balance and timing of surges of these hormones determines whether the uterus and the egg do their respective work with sufficient timing to produce a implanted, fertilized egg.

CEs also work in the uterus lining to control the implantation of the ovum in the uterine epithelium. Uterine wall 4- and 2-OHE2 ratios favor 2-OHE2 during periods where the uterine lining is resistant to implantation (Mondschein et al 1985; Dey et al, 1986; Chakraborty et al, 1989; Paria et al, 1990). During the peri-implantation period, COMT gene expression is significantly elevated in areas in which the egg can implant. 2-OHE2 is the preferred COMT substrate and so 2-OHE2 Is cleared at a greater rate. Therefore the CE's are selectively removed by COMT to favor 4-OHE2 in the regions that the egg may implant (Chakraborty, et al, 1990a; 1990b; Inoue and Creveling, 1995). During this period, if the egg

implants elsewhere or misses, the COMT levels drop back to normal. Elevated CE during implantation results in a resorption or the egg detaches and fails to implant. If PCB catechols disrupt COMT activities and therefore alter CE ratios or if they mimick CE interaction with critical proteins, the implantation period may be negatively affected. The abortifacient drug RU-486 is thought to act in a similar mechanism (Inoue and Creveling, 1995).

If PCB catechols inhibit COMT and therefore disrupt catechol estrogen metabolism, then it may be that PCBs exposure might result in a lowered fertility rate, either through failed implantation or disrupted cyclicity. PCBs do indeed have such effects but there is no solid mechanistic explanation. Rats, mice, pigs, and monkeys all have a decreased implantation rate and an increased resorption rate following exposure to PCBs. Their litter sizes are also reduced and they undergo increased spontaneous abortions. These same animal models also show modifications in hormone surge timing during the estrous cycle. hypothalamal/pituitary dopamine and norepinephrine neurotransmitter levels drop in rats and mice treated with PCBs and he activity of tyrosine hydroxylase is inhibited significantly by lower chlorinated PCBs. So it may be that the effects of PCBs on CEs or catechol PCB metabolites themselves may influence hypothalamal/pituitary neurotransmitter signals that ultimately affect the timing of hormonal signals that result in reproductive failure. These two potential effects, one direct, the other indirect, when combined, would make it difficult for a PCB exposed organism to reproduce.

Developmental exposure to PCBs results in persistent neurobehavioral alterations in humans monkeys and nonprimates; similar neurobehavioral effects are observed across species. The most common finding in animal studies was that developmental exposure to PCBs results in behavioral hyperactivity and alterations in higher cognitive processes or learning. In humans, developmental delays and impaired cognitive function have also been reported. (Tilson et al, 1990; Rogan and Gladen, 1992; Jacobson, et al. 1985; Jacobsen et al 1990) The mechanism of PCB-induced neurotoxicity is not fully understood but it is known that lower chlorinated PCBs can cause frank neuronal death (Carpenter et al., 1997). The catecholamine neurotransmitters are known to redox cycle, producing oxidative stress and neuronal death (Noble et al 1994; Miller et al. 1996; Burke et al, 1998). It is also known that catecholamine O-methylation is the principal means for reducing the risk of such neurotoxicity (Miller et al, 1996). Inhibition of COMT by PCB catechols would therefore increase the risk of such neurotransmitter-mediated toxicities.

In summary, this thesis makes two general conclusions. First, that there are significant route-specific effects to systemic absorption of PCBs following dermal exposure. Further evidence forwarded suggests that PCB metabolite exposure following such exposures may be elevated because of “first-pass” effects at the site of exposure. The second half of this document gives further evidence that

PCB metabolites may mediate PCB toxicities by mimicking endogenous metabolites or by disrupting endogenous metabolic pathways.

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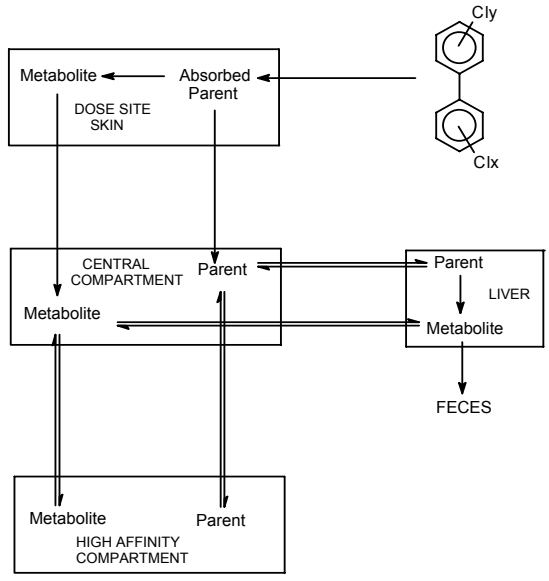
a. DERMAL MODEL



b. ORAL MODEL

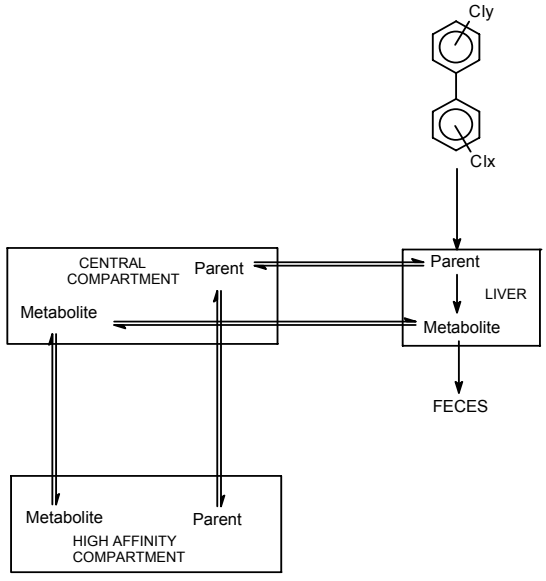


Figure 1. Models of PCB absorbance, disposition, and elimination.