

Comparison of Pharmacokinetic Interactions and Physiologically Based Pharmacokinetic Modeling of PCB 153 and PCB 126 in Nonpregnant Mice, Lactating Mice, and Suckling Pups

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Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants that can induce neurological defects in infants and children via placental and lactational transfer. To investigate the lactational transfer of PCBs and compare pharmacokinetic interactions among nonpregnant, lactating mice and suckling pups, quantitative time-course measurements of PCB accumulation in tissues were performed. On postnatal day 1, nonpregnant and lactating C57BL/6 mice were exposed to PCB 153 (2,2',4,4',5,5'-hexachlorobiphenyl, 20 mg/kg) alone or a mixture of PCB 153 (20 mg/kg) and PCB 126 (3,3',4,4',5-pentachlorobiphenyl, 0.2 mg/kg) by oral gavage. At 1, 3, 6, and 13 days after treatment, PCB 153 and PCB 126 were determined in nonpregnant and maternal tissues as well as in neonatal tissues by gas chromatography (GC). Coadministration of PCB 153 and PCB 126 increased PCB 153 retention in the liver and decreased PCB 153 accumulation in the fat of nonpregnant mice. Lactational transfer was confirmed to be an efficient elimination mechanism for the lactating mice but a major source of exposure in the pups. However, little or no significant pharmacokinetic interactions were observed in lactating mice and suckling pups. To describe pharmacokinetic interactions between PCB 153 and PCB 126, a physiologically based pharmacokinetic model for PCB 153 disposition was developed. The effects of PCB 126 on the fat content in liver and a diffusion permeation constant in fat were incorporated into the physiologically based pharmacokinetic (PBPK) model. This model successfully describes PCB 153 disposition altered by PCB 126 in nonpregnant mice.

Key Words: PCB; lactational transfer; pharmacokinetic interactions; mixture; PBPK modeling; fat content; diffusion permeation constant.

Polychlorinated biphenyls (PCBs) are ubiquitous environmental contaminants causing a variety of toxicities in humans

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and animals (ATSDR, 1999). They are detected in numerous and diverse biological compartments including human serums and have become quantitatively the most important organochlorine residues in the environment due to their lipophilic character and their slow metabolism (Carpenter, 1998; Muhlebach *et al.*, 1991). PCBs usually exist in a mixture form, and can comprise up to 209 different congeners (ATSDR, 1999). Although PCBs have been reported to induce a variety of toxic effects including immunologic, teratogenic, reproductive, carcinogenic, and neurological effects in both human and animal studies (Safe, 1989), the developing brain is particularly susceptible to PCB exposure (Schantz *et al.*, 1996). PCBs have been reported to induce neurobehavioral deficits in children born at contaminated sites (Carpenter, 1998). Many of the defects were related to the nervous system, including abnormalities on behavioral assessment and increased activity, greater incidence of behavioral problems, lower IQ, and impaired visual recognition (Schantz, 1996). These neurological defects are primarily due to the exposure of the fetus through placental and/or lactational transfer (Buck, 1996; Seegal, 1996).

A major molecular structural factor in the determination of toxic properties and potencies in PCBs is the presence of chlorine atom on the *ortho* positions, influencing propensity to adapt planar conformation (Safe, 1994). Some non-*ortho* or mono-*ortho* chlorine-substituted PCBs have coplanar structures and high affinity for the Ah receptor. Their toxic effects are mediated by the Ah receptor, and their toxic potencies are determined by the affinity for the Ah receptor. In contrast, the di to tetra *ortho*-chlorinated PCBs have nonplanar structures and no measurable affinity for the Ah receptor. Their mechanisms of action are not completely understood (Kodavanti and Tilson, 2000; van der Burght *et al.*, 1999). While both types of PCB congeners have been reported to cause neurodevelopmental deficits following *in utero* and/or lactational exposure (Hussain *et al.*, 2000; Saghir *et al.*, 2000), the mechanisms of action between planar and nonplanar PCBs differ significantly (Humphrey *et al.*, 2000) and interactive effects between 2 types of

PCBs are observed (van Birgelen *et al.*, 1996; van der Plas *et al.*, 1988).

The developing brain is different from the adult brain in both composition and function (Kalil *et al.*, 2000), and the vulnerability of the developing brain to toxicants is critically dependent on timing and duration of exposure. Exposure to environmental toxicants coincident with the ontogeny is more likely to cause toxicities if they interfere with developmental processes within the critical period (Rice and Barone, 2000). Therefore, the characterization of exact pharmacokinetic profiles of environmental toxicants during the critical period of development is important both for understanding mechanisms of developmental neurotoxicities and in facilitating interpretation of the results from animal studies.

PCB 153 (2,4,5,2',4',5',-hexachlorobiphenyl) and PCB 126 (3,4,5,3',4',-pentachlorobiphenyl) were selected as a prototype of PCB mixture to understand the potentially complex pharmacokinetic interactions associated with PCB mixtures. These congeners are the ones that appear most prevalently both in the environment and in human serum (Humphrey *et al.*, 2000). PCB 153 is a representative nonplanar congener that appears in the environment and mammalian tissues at the highest concentration (Muhlebach *et al.*, 1991). PCB 126 is the most toxic PCB congener with coplanar structure (Safe, 1994) and binds to the Ah receptor with highest affinity. Both congeners have been suggested to induce neurobehavioral deficits via gestational and lactational transfer (Hussain *et al.*, 2000). Pharmacokinetic interactions between coplanar PCBs or TCDD and nonplanar PCBs were previously reported in adult rat and mice (van Birgelen *et al.*, 1996; van der Plas *et al.*, 1988). However, no studies investigating pharmacokinetic interactions between these 2 congeners during the critical developmental period have been reported despite the potential importance of these interactions. In reality, PCBs are available to human beings as mixtures. Accordingly, the pharmacokinetic information on exposure to a single congener is not enough to describe pharmacokinetic profiles during the developmental period.

The objectives of our study were 3-fold. First, we wanted to investigate whether the coexposure to 2 different PCB congeners with distinct toxic mechanisms of action (PCB 153 and PCB 126) could change the pharmacokinetic profiles in mice. Second, we wanted to compare pharmacokinetic interactions among nonpregnant mice, lactating mice, and suckling pups because PCB congeners could be transferred to the fetus and suckling mice from their mothers (Orberg and Ingvast, 1977) and the extent of transfer was greater in lactation than through placenta (Vodicnik and Lech, 1980). Finally, physiologically based pharmacokinetic (PBPK) modeling, a simulation technique useful for species extrapolation and quantitative risk assessment (Dedrick and Bischoff, 1969), was used as a hypothesis-testing tool for deriving mechanistic insight for the observed pharmacokinetic data.

MATERIALS AND METHODS

Chemicals. PCB 153 and PCB 74 (2,4,4',5-tetrachlorobiphenyl) were purchased from Ultra Scientific (North Kingtown, RI). PCB 126 was obtained from Accustandard (New Haven, CT). The purities of all congeners used were over 98%, which was confirmed by both vendors. Pentane (HPLC grade), sea sand, and diethylether (> 99% purity) were purchased from VWR Scientific (Denver, CO). Sodium anhydrous sulfate and Florisil® (pesticide residue grade, 60–100 mesh) were purchased from Alltech Associates (Deerfield, IL).

Animals and treatment. C57BL/6 nonpregnant and pregnant female mice were purchased from Harlan Sprague Dawley Laboratory (Indianapolis, IN) and housed individually in cages at Painter Center, Colorado State University, which is fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). Eighteen nonpregnant mice and 24 pregnant mice were used in this study. The mice were maintained on a 12-h light/dark cycle at a constant temperature of 25°C and humidity of 55%. Diet (certified Teklad NIH-07 rodent diet) and tap water were provided *ad libitum*. The average number of pups from pregnant mice was 6. Because of the low fertility rate, we did not cull the litters and used all the pups.

On postnatal day (PND) 1, 12 lactating mice were exposed to PCB 153 alone (20 mg/kg bw) and the other 12 mice were exposed to a mixture of PCB 153 (20 mg/kg bw) and PCB 126 (0.2 mg/kg bw) through oral gavage. Corn oil was used as vehicle to dissolve both congeners. The volume of oral gavage was 5 ml/kg. The dosing time was between 0800 and 0900 h. At 1, 3, 6, and 13 days after oral gavage, 3 lactating mice and their pups in each group were anesthetized using isoflurane or CO₂ gas and then euthanized. The tissues of lactating mice (liver, fat, skin, and brain) and pups (liver, brain, and the remaining carcass excluding GI tract) were collected from each animal. Nonpregnant mice were treated the same way as lactating mice and their tissues were collected at 1, 6, and 13 days after treatment. At each time point, 3 mice per group were sacrificed. All samples were frozen with liquid nitrogen and stored at -70°C until analysis.

Extraction. Liver, fat, and brain samples were weighed (approximately 0.5 g/sample) and then transferred to stainless steel beakers. Sea sand and sodium sulfate anhydrous mixture (5 g/10 g) were added to the samples and then ground using a glass stirring rod until granular dry mass was obtained. PCB 74 (200 ng) was added to each of the ground samples as an internal standard. This congener has similar physicochemical characteristics but does not interfere with PCB 153 and PCB 126 in gas chromatography (GC) analyses. Twenty ml of pentane was added to each sample, which was then boiled on a hot plate with stirring. The boiling temperature was between 30°C and 40°C. The extracts were transferred to the filters and collected in glass tubes. For the extraction of the carcass from pups and skin tissues of adult mice, 10 ml of 50% sulfuric acid was added to each sample. After homogenization, 10 ml of pentane was added and vigorously shaken. When the layers separated, the upper layer was transferred to glass tubes. These processes were repeated 3 times. Collected extracts were concentrated to 2 ml with a nitrogen evaporator.

Clean up. Before clean up, the adsorbents (sodium sulfate anhydrous and Florisil®) were layered (the ratio of sodium sulfate to Florisil® is 1 to 2) and packed into a Florisil® glass column (Allen Scientific Glass, Denver, CO) and then the column was rinsed with 100 ml of pentane. The concentrated extract was transferred to the column. The column was eluted with 100 ml of pentane/diethylether mixture (6% diethylether in pentane). The elution rate was adjusted to 20 drops per 10 s. Finally, the collected solution was concentrated to 1.0 ml with a nitrogen evaporator. The recovery rate of extraction is about 60%.

Gas chromatographic analyses. A HP-5890 Series II Plus gas chromatograph (Hewlett Packard, Wilmington, DE) with ECD detector was used to determine PCB amount. The analysis condition was based on a previous method (Mills *et al.*, 1963). Briefly, DB-5 (crosslinked 5% phenylmethylsilicone, 30 m × 0.32 mm, Agilent Technologies, Palo Alto, CA) capillary column was used. The initial temperature was 60°C, programmed to 200°C at

30°C/min for 6 min, subsequently to 250°C at 3°C/min for 15 min. The flow of carrier gas, helium, was 5 ml/min. The make-up gas was pure nitrogen at a flow rate of 70 ml/min. The injector temperature was 225°C and the detector temperature was 320°C. The volume of injection was 2 μ l per sample.

The samples were quantitated using internal standard method as previously reported (Storr-Hansen, 1991). Standard solutions for PCB 153 and PCB 126 were prepared in the range from 5 ng/ml to 500 ng/ml. To each solution, PCB 74 (200 ng/ml) was added as an internal standard. Calibration curve was created and fitted using quadratic regression equation. The detection limit of GC was 5 ng/ml.

Statistics. Differences of tissue concentration between samples from various treatment groups and time points were tested for significance by two-way ANOVA, followed by Fisher's multiple comparison test. All analyses were performed with the statistical software, Minitab ($p < 0.01$; Windows version 12.0).

Construction of an interaction PBPK model. To explain pharmacokinetic interactions between PCB congeners, a PBPK model for the combination effects of PCB 153 and PCB 126 was developed. The previous PBPK model for PCBs (Lutz *et al.*, 1977) was composed of 5 lumped compartments and successfully described individual PCB disposition including PCB 153 using flow-limited transfer. However, this model could not explain the change of PCB 153 disposition by coexposure to PCB 126.

In order to describe pharmacokinetic changes of PCB 153 upon coexposure to PCB 126, the PBPK model of Lutz *et al.* was modified as follows:

First, we incorporated time-dependent increase of partition coefficient in the liver. This was supported by several experimental observations. It has been shown that some coplanar PCBs (e.g., PCB 126) and 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) induce fat accumulation in the liver in a time-dependent manner (Koga *et al.*, 1990). Furthermore, PCB 126 could increase lipid content by 50% in murine liver 7 days after oral exposure (Nobuyuki *et al.*, 1990). In general, the partition coefficient in tissues depends on the *n*-octanol/water coefficient, K_{ow} , and profiles for water and lipid contents in each tissue (De Jongh *et al.*, 1997). Since PCB 153 is a lipophilic compound, its partition coefficient in tissues is highly proportional to lipid content in tissues. Therefore, the partition coefficient in the liver (PL) was modified to a time-dependent equation, $PL = 10 + \alpha \times \text{TIME}$ where α is the coefficient for time-dependent increase of partition coefficient and TIME is the minutes after PCB 153 exposure.

Second, the fat compartment was described as diffusion-limited, and a diffusion permeation constant (PAFC) from fat-blood to fat tissues was added. It has been suggested that PCB 153 circulates in the body in association with lipoproteins. To be absorbed in fat tissues, PCB 153 was suggested to be dissociated with lipoproteins by lipoprotein lipases (Noren *et al.*, 1999). Because TCDD and coplanar PCBs can inhibit lipoprotein lipase in the fat via Ah receptor activation (Olsen *et al.*, 1998), it is likely that a TCDD-like PCB congener such as PCB 126 can also inhibit lipoprotein lipase, thus decreasing the uptake of PCB 153 in the fat. Both *in vivo* and *in vitro* studies showed that lipoprotein lipase in adipocytes was inhibited as soon as 1 h after TCDD administration (Olsen *et al.*, 1998). Accordingly, diffusion permeation constant from fat-blood to fat tissues (PAFC) was assumed to be decreased by coexposure to PCB 126 and PCB 153.

Third, brain compartment was added as a major target organ because PCBs can induce neurotoxicities in both adult and young mice.

A schematic diagram for this PBPK model is presented in Figure 1. Other physiological and biochemical parameters were obtained from a report by Brown *et al.* (1997) and a previous article (Lutz *et al.*, 1977) and are shown in Table 1.

All PBPK model construction, simulation, and parameter estimations were performed using the Berkeley Madonna software package (version 8.01 for Windows, Kagi Shareware, Berkeley, CA).

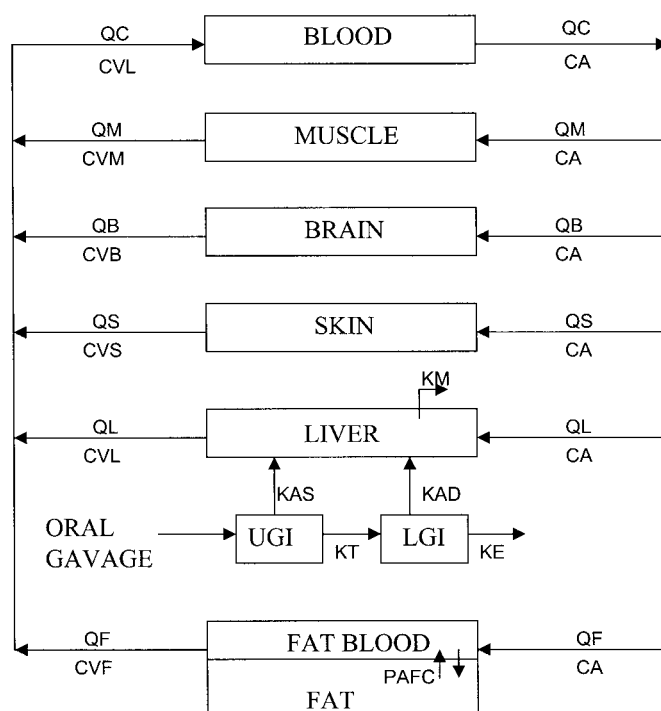


FIG. 1. Schematic diagram of the physiologically based pharmacokinetic model used to describe pharmacokinetic interaction on PCB 153 disposition in the nonpregnant mice. All compartments except fat are flow-limited. Fat tissue is described as a diffusion-limited compartment. The abbreviations are given in Table 1.

RESULTS

Effect of Coexposure to PCB 126 and PCB 153 on Body Weight Gain in Growing Pups

PCB 126 has been previously reported to suppress normal weight gain in growing rats (Van Birgelen *et al.*, 1994), so the body weights of nonpregnant mice, lactating mice, and suckling pups were monitored after treatment. At the dosing regimen applied, no differences in body weights were observed between the group exposed to PCB 153 alone and the group coexposed to PCB 153 and PCB 126 (data not shown).

Baseline Pharmacokinetic Interactions of Coexposure to PCB 126 and PCB 153: Distribution of PCB 153 in the Tissues of Nonpregnant Mice

Pharmacokinetic interactions indeed occurred in nonpregnant mice when PCB 153 and PCB 126 were given to them as a mixture. The most significant findings are: (1) time-dependent retention of PCB 153 in the liver when coexposed to PCB 126, and (2) a reduction in the rate of PCB 153 accumulation in the fat when coexposed to PCB 126. The details in relation to figures and tables are given below.

Figure 2A shows the change of PCB 153 distribution in the liver between the group exposed to PCB 153 alone and the

TABLE 1
PBPK Model Parameters

Parameter	Abbreviation	Value	Parameter estimation
Physiological Parameters			
Body weight (kg)	BW	0.025	Literature ^a
Fraction of brain	BF	0.020	Literature ^a
Fraction of blood	BLF	0.049	Literature ^a
Fraction of fat	FF	0.056	Literature ^a
Fraction of skin	SF	0.208	Literature ^a
Fraction of muscle	MF	See legend ^b	Literature ^a
Fraction of GI tract	GF	0.042	Literature ^a
Fraction of liver	LF	0.080	Literature ^a
Cardiac output (l/h)	QC	$16.5 \times (BW)^{0.75}$	Literature ^a
Blood flow of brain	QBF	0.033	Literature ^a
Blood flow of fat	QFF	$0.07 \times (BW)^{0.75}$	Literature ^a
Blood flow of skin	QSF	0.083	Literature ^a
Blood flow of muscle	QMF	See legend ^c	Literature ^a
Blood flow of GI tract	QGF	0.141	Literature ^a
Blood flow of liver	QLF	0.159	Literature ^a
Partition coefficients			
Brain	PB	2.5	Fitted ^d
Fat	PF	300	Literature ^e
Skin	PS	35	Literature ^e
Muscle	PM	5	Literature ^e
Liver	PL	10	Literature ^e
Biochemical parameters			
Diffusion permeation			
constant in fat	PAFC	10	Fitted ^d
Absorption coefficient in upper GI tract	KAS	0.415	Fitted ^d
Absorption coefficient in lower GI tract	KAD	0.077	Fitted ^d
Elimination coefficient in upper GI tract	KT	10	Fitted ^d
Elimination coefficient in lower GI tract	KE	0.073	Fitted ^d
Metabolic constant (l/h)	KM	0.006	Literature ^e

^aBrown *et al.*, 1997.

^b $1 - (BF + BLF + FF + SF + GF + LF)$.

^c $1 - (QBF + QFF + QSF + QLF + QGF)$.

^dOptimized by fitting model output to experimental data.

^eLutz *et al.*, 1977.

group coexposed to PCB 153 and PCB 126. At 1 day after treatment, there were no differences in the concentration of PCB 153 between the 2 groups. However, the concentration of PCB 153 in the liver of animals exposed to both congeners was more than 2 times higher than that in the liver of mice exposed to PCB 153 alone at 13 days after treatment.

Similar results were shown in the change of PCB 153 distribution in the brain between the group exposed to PCB 153 alone and the group coexposed to PCB 153 and PCB 126 (Fig. 2B). At 13 days posttreatment, the concentration of PCB 153 in the group coexposed to PCB 153 and PCB 126 was higher than that in the group exposed to PCB 153 alone, but this difference was not statistically significant.

Coexposure of PCB 153 and PCB 126 changed the distri-

bution pattern of PCB 153 in the fat. As shown in Figure 2C, the group exposed to PCB 153 alone showed that the concentration of PCB 153 in fat increased rapidly until reaching plateau (6 days after treatment). On the contrary, the concentration of PCB 153 in the group coexposed to PCB 153 and PCB 126 increased slowly during that time, suggesting a reduction of the rate of uptake.

Skin is the major deposit for PCBs (Vodicnik and Lech, 1980). Figure 2D shows the change of PCB 153 distribution in skin between the group exposed to PCB 153 alone and the group coexposed to PCB 153 and PCB 126. There were no significant differences in PCB 153 concentration between the group exposed to PCB 153 alone and the group coexposed to PCB 153 and PCB 126.

Postnatal Pharmacokinetics: Comparison of PCB 153 Distribution on PND 2 and 14 among Nonpregnant, Maternal, and Pup Tissues

The pharmacokinetic interactions described above for nonpregnant mice after coexposure to PCB 153 and PCB 126 did not occur in lactating mice and suckling pups. However, there was strong evidence to confirm earlier studies that lactation is an efficient mechanism to transfer of PCB 153 to the pups. Consequently, the lactating mice had the lowest PCB 153 concentrations in the liver and brain among 3 groups of mice and the pups had the highest concentrations in the liver in early lactation period.

PCB 153 has been reported to be accumulated mostly in fat and skin tissues and transferred readily from lactating mice to their offspring (Vodicnik and Lech, 1980). We investigated differences in lactational transfer between the group exposed to PCB 153 alone and in combination with PCB 126. Also, we attempted to compare pharmacokinetic interactions among nonpregnant mice, lactating mice, and their pups. The tissue concentrations of PCB 153 in mice of different stages were compared at PND 2 and 14 after oral gavage at PND 1. Figure 3 shows the differences of PCB 153 concentration in the liver among nonpregnant mice, lactating mice, and their pups. Interestingly, the concentrations of PCB 153 in neonatal livers were much higher than nonpregnant and lactating mice on PND 2. However, PCB 153 in neonatal livers was rapidly decreased; thus, there were no longer any differences in PCB 153 concentrations of livers between nonpregnant mice and suckling pups by PND 14. It seems that rapid growth of neonatal liver could be a confounding factor for this result. However, constant concentration of PCB 153 in neonatal carcass was observed during the 2 weeks after birth, despite the rapid increase of body weight. It suggests that such rapid growth of tissue and body weight may not be a significant confounding factor. Coexposure to PCB 126 increased the retention of PCB 153 in the liver of nonpregnant mice but not in the liver of lactating mice and their pups. Figure 4 shows the concentrations of PCB 153 in the brains of nonpregnant mice,

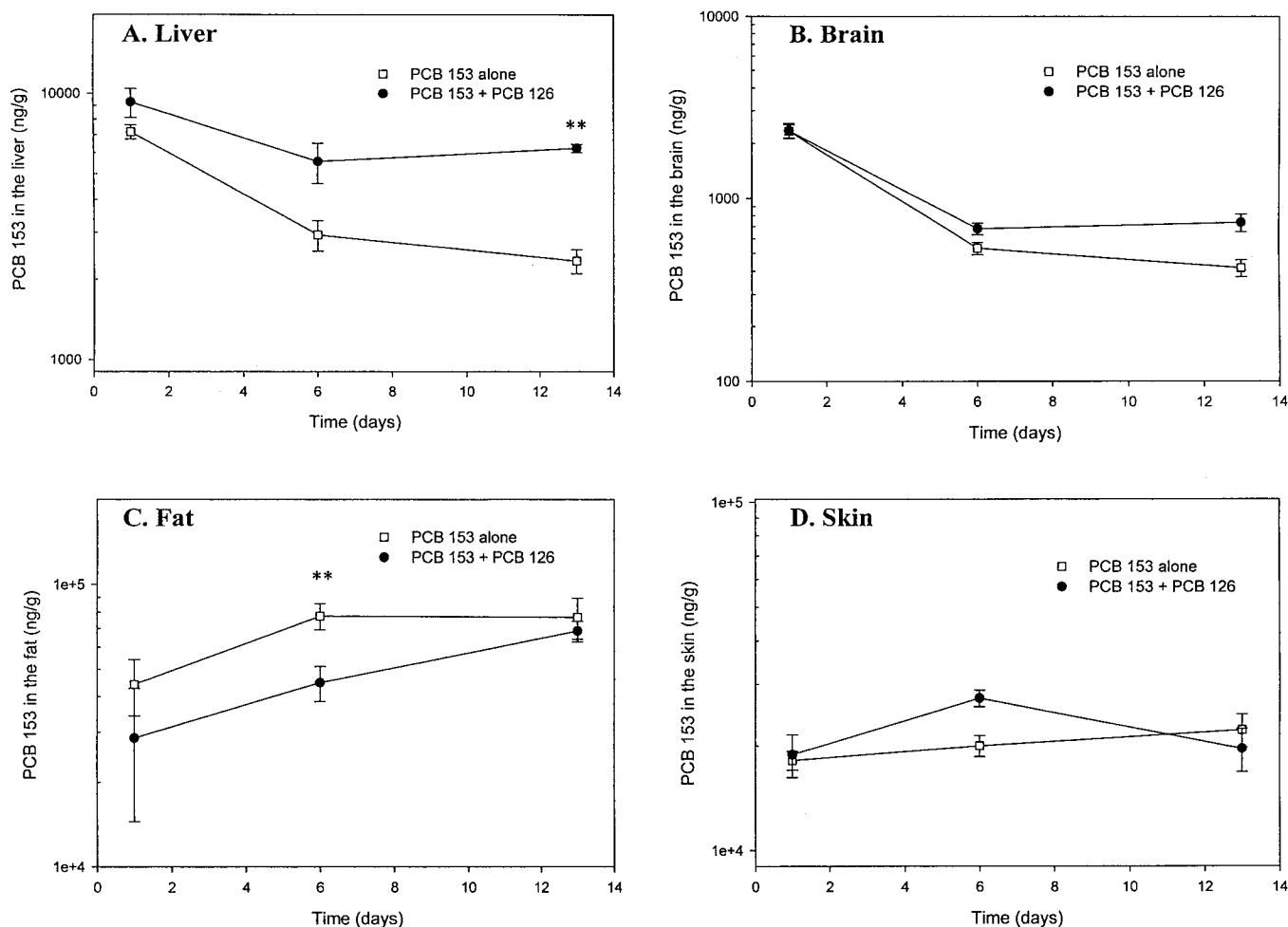


FIG. 2. The concentration of PCB 153 in the tissues of nonpregnant mice as a function of time. The nonpregnant C57BL/6 female mice were exposed to PCB 153 (20 mg/kg bw) or PCB 153 (20 mg/kg bw) + PCB 126 (0.2 mg/kg bw) through oral gavage. One, 6, and 13 days after oral gavage, the nonpregnant mice were sacrificed by CO₂ and their tissues were collected. Values are expressed as ng PCB 153/g tissue. Data points represent mean \pm SEM. **Significantly different from the corresponding group exposed to PCB 153 alone ($p < 0.01$).

lactating mice, and suckling pups. At PND 2, the concentrations of PCB 153 were higher in the brains of nonpregnant mice than those of lactating mice and suckling pups. By PND 14, there was no difference between nonpregnant mice and suckling pups. In contrast, the concentrations of PCB 153 in the brain of lactating mice were significantly lower than those brains of suckling pups and nonpregnant mice at PND 14. Coexposure to PCB 126 did not change the concentration of PCB 153 in the brains of lactating mice and pups. Figure 5 shows the difference of PCB 153 concentration in fat tissues between nonpregnant and lactating mice. In nonpregnant mice, PCB 153 concentration increased at PND 14 from at PND 2, whereas, PCB 153 did not change or slightly decreased at PND 14 in lactating mice. Coexposure of PCB 126 did not change the disposition of PCB 153 in fat tissues at PND 14. In both groups, PCB 153 tissue concentration was higher in nonpregnant mice than that in lactating mice at PND 14.

Time-Course Distribution of PCB 153 in Suckling Pups with or without Coexposure to PCB 126

Figure 6A shows the change of PCB 153 distribution in pup liver between the groups exposed to PCB 153 alone and the group coexposed to PCB 153 and PCB 126. In both groups, the concentration of PCB 153 in neonatal liver decreased over time. There were no significant differences in PCB 153 concentration at each time point between 2 groups. This result was different from that of nonpregnant mice, which showed that coexposure of PCB 126 and PCB 153 increased the retention of PCB 153 in the liver (Fig. 2A).

There were no significant differences in PCB 153 distribution in pup brain between the 2 groups (Fig. 6B). The concentration of PCB 153 in the brain of both groups has decreased after treatment.

Fat tissues in neonatal mice were not significant enough to

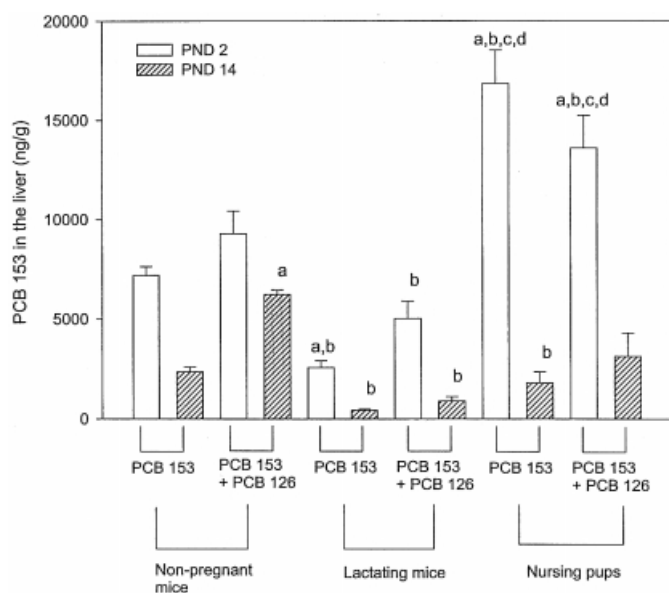


FIG. 3. Comparison of concentrations of PCB 153 in the liver of non-pregnant mice, lactating mice, and suckling pups. On PND 1, the nonpregnant and lactating mice were exposed to PCB 153 (20 mg/kg bw) or PCB 153 (20 mg/kg bw) + PCB 126 (0.2 mg/kg bw) via oral gavage. On PND 2 and PND 14, the nonpregnant mice, lactating mice, and suckling pups were sacrificed and their livers were collected. Bars represent mean and SEM. Statistic differences were evaluated with Tukey's multiple comparison after 2-way ANOVA (a, statistically different from nonpregnant mice exposed to PCB 153 only at same date; b, statistically different from nonpregnant mice exposed to PCB 153 + PCB 126 at same date; c, statistically different from lactating mice exposed to PCB 153 only at same date; d, statistically different from lactating mice exposed to PCB 153 + PCB 126 at same date; $p < 0.01$).

be reliably collected and analyzed. Therefore, PCB 153 was measured in pup carcass without liver, brain, and gastrointestinal (GI) tract. As shown in Figure 6C, the concentration of PCB 153 in the carcass increased until 3 days after treatment and then decreased slightly in the group exposed to PCB 153 alone, whereas it remained at almost the same level in the group coexposed to PCB 153 and PCB 126. There were no significant differences in PCB concentration at each time point between the 2 groups.

Mechanistic Consideration for Pharmacokinetic Interactions between PCB Congeners and PBPK Modeling

The preceding results suggested that coexposure to PCB 126 increased the retention of PCB 153 in the liver and decreased the absorption of PCB 153 in the fat of nonpregnant mice, but not in those of lactating mice and nursing pups. As indicated earlier, the mechanistic basis for coexposure to PCB 126-induced pharmacokinetic interactions was hypothesized to be: (1) time-dependent fat accumulation in the liver, and (2) the decrease of PCB 153 uptake in the fat due to the lack of dissociation of PCB 153:lipoprotein complex through the inhibition of lipoprotein lipase. In order to address these mechanistic considerations and quantitatively analyze the effects of

PCB 126 on tissue distribution of PCB 153, an interaction PBPK model for PCB 153 was developed.

Through parameter optimization of 2 coefficients (α , PAFC), the change of pharmacokinetic profiles of PCB 153 was simulated successfully (Fig.7). The result of parameter optimization is shown in Table 2. Thus, a PBPK model incorporating fat accumulation in the liver and inhibition of lipoprotein lipase activity in the fat adequately described the change of PCB 153 disposition induced by PCB 126.

DISCUSSION

Our most significant results can be summarized as follows: First, coexposure to PCB 153 and PCB 126 increased the retention of PCB 153 in the liver and decreased the rate of PCB 153 accumulation in the fat of nonpregnant mice. Second, the incorporation of a time-dependent increase of partition coefficient in the liver and the decrease of diffusion permeation constant in the fat into a PBPK model could adequately account for pharmacokinetic changes of PCB 153 in nonpregnant mice. Third, coexposure to PCB 126 and PCB 153 may not affect the extent of lactational transfer of PCB 153. While lactational transfer is an efficient elimination mechanism for

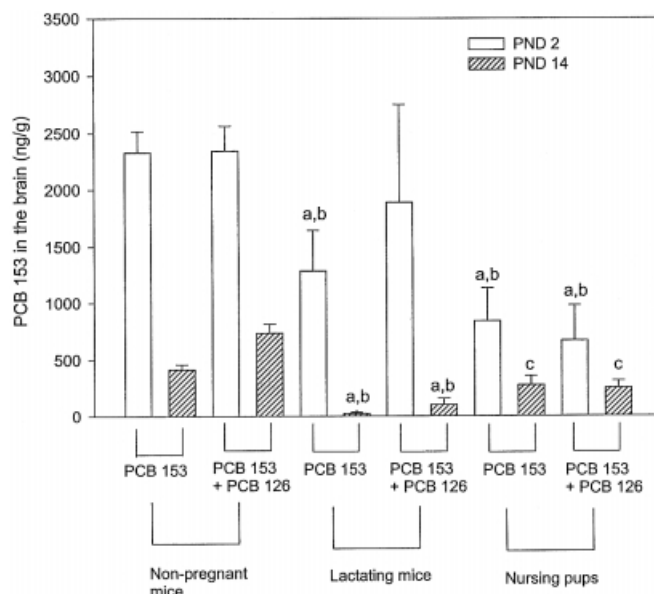


FIG. 4. Comparison of concentrations of PCB 153 in the brain of non-pregnant mice, lactating mice, and suckling pups. The nonpregnant and lactating mice were exposed to PCB 153 (20 mg/kg bw) or PCB 153 (20 mg/kg bw) + PCB 126 (0.2 mg/kg bw) via oral gavage on post natal day 1 (PND 1). On PND 2 and PND 14, nonpregnant, lactating mice and suckling pups were sacrificed and their brains were collected. Bars represent mean and SEM. Statistic differences were evaluated with Tukey's multiple comparison after 2-way ANOVA (a, statistically different from nonpregnant mice exposed to PCB 153 only at same date; b, statistically different from nonpregnant mice exposed to PCB 153 + PCB 126 at same date; c, statistically different from lactating mice exposed to PCB 153 only at same date; d, statistically different from lactating mice exposed to PCB 153 + PCB 126 at same date; $p < 0.01$).

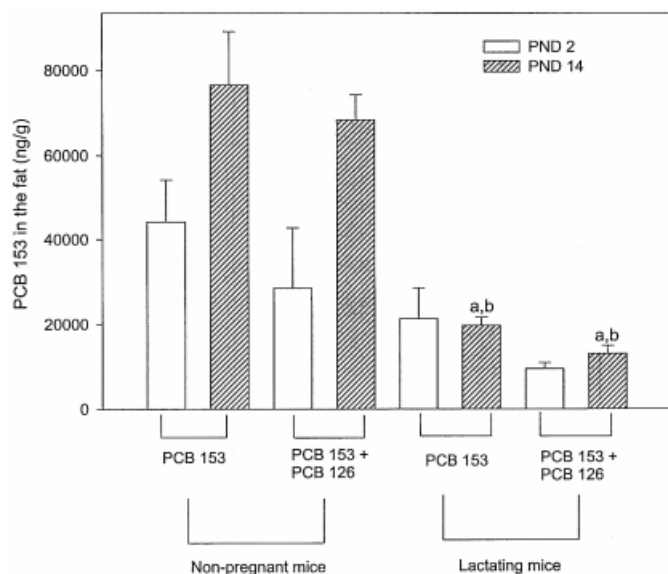


FIG. 5. Comparison of concentrations of PCB 153 in the fat of nonpregnant mice and lactating mice. The nonpregnant and lactating mice were exposed to PCB 153 (20 mg/kg bw) or PCB 153 (20 mg/kg bw) + PCB 126 (0.2 mg/kg bw) via oral gavage on PND 1. On PND 2 and PND 14, the nonpregnant and lactating mice were sacrificed and their fat tissues were collected. Bars represent mean and SEM. Statistic differences were evaluated with Tukey's multiple comparison after 2-way ANOVA (a, statistically different from nonpregnant mice exposed to PCB 153 only at same date; b, statistically different from nonpregnant mice exposed to PCB 153 + PCB 126 at same date, $p < 0.01$).

the lactating mice, it is a major source of exposure in the pups. Fourth, interactive effects of PCB 126 on the disposition of PCB 153 are different among nonpregnant mice, lactating mice, and their pups.

The present study demonstrated that coexposure of C57BL/6 female mice to a coplanar PCB congener (PCB 126) together with a nonplanar congener (PCB 153) affects the redistribution of nonplanar congener. In general, PCB 126 is assumed to exert its biological or toxicological actions through an Ah receptor-mediated mechanism (Safe, 1984). TCDD, which is also assumed to bind and transduce biological action via interactions with the Ah receptor, was previously shown to increase the retention of PCB 153 in B6C3F1 mice (van Birgelen *et al.*, 1996). Therefore, the effect of PCB 126 on the increase of PCB 153 retention may be due to the action through Ah receptor mediated process. TCDD is also able to induce fat accumulation in the liver (van Birgelen *et al.*, 1996). Accordingly, the increase of fat content in the liver may increase PCB 153 retention due to the highly lipophilic properties of PCB 153, which confers the highest affinity for fat among PCB congeners. Recently it was also reported that PCB 126 could reduce essential fatty acid and thus resulted in the increase of lipid content in the liver (Matsusue *et al.*, 1999). These studies suggest that TCDD and dioxin-like PCB congeners can increase the retention of nonplanar lipophilic congeners in the liver through the increase of lipid content in liver. In our

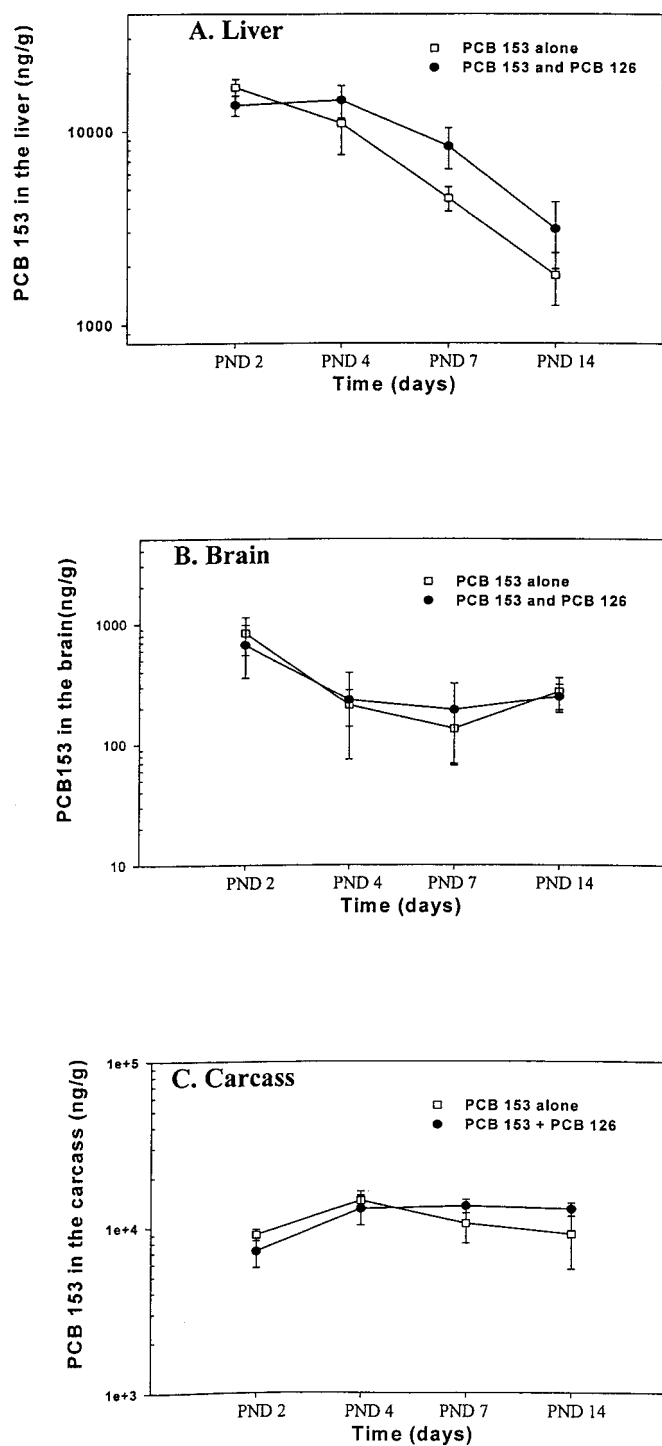


FIG. 6. The concentration of PCB 153 in the tissues of suckling pups as a function of time. The lactating mice were exposed to PCB 153 (20 mg/kg bw) or PCB 153 (20 mg/kg bw) and PCB 126 (0.2 mg/kg bw) through oral gavage on PND 1. At each time, the suckling pups were sacrificed by CO₂ and their tissues were collected. Values are expressed as the amount of PCB 153 per 1 g of tissue. Data points represent mean \pm SEM. PND, postnatal day. (A) Liver, (B) brain, (C) carcass.

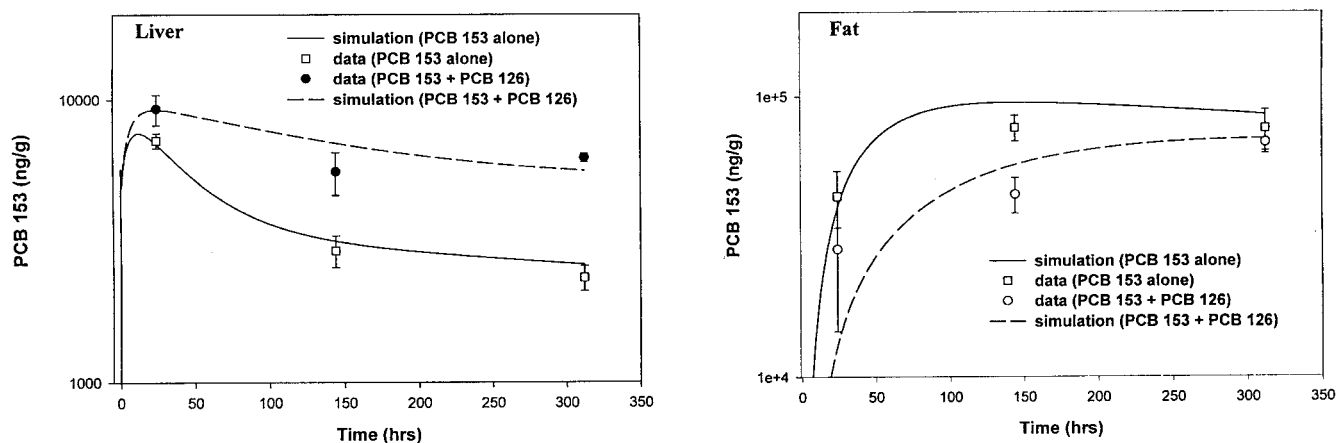


FIG. 7. Model simulations (solid and dashed line) of PCB 153 in the tissues of nonpregnant mice. Modifications of partition coefficient in the liver and diffusion permeation constant in the fat successfully simulated the change of PCB 153 disposition induced by coexposure of PCB 126 + PCB 153.

studies, we adopted this mechanism because it is consistent with our experimental results. Contrarily to our findings, some previous studies showed different results (De Jongh *et al.*, 1993). They showed that coexposure of coplanar PCB with nonplanar PCB increased the retention of coplanar PCB in the liver. It seems that the difference is due to the dose of PCBs. The dose of nonplanar PCB they used was 100 mg/kg, which is 5 times higher than the dose we used.

Since PCBs have high lipophilicity and low metabolism, the adipose tissue is the major storage site of most PCB congeners except dioxin-like congeners (Muhlebach *et al.*, 1991). However, our results showed that there was a decreased uptake of PCB 153 into the fat of nonpregnant mice coexposed to PCB 153 and PCB 126. One possible mechanism is the decrease of lipoprotein lipase activity in adipose tissue by TCDD or coplanar PCBs. In support of this possibility, Olsen *et al.* (1998) showed that TCDD and coplanar PCBs induced a statistically significant time- and dose-dependent decrease in lipoprotein lipase activity in preadipocyte cell line and suggested that different PCB and dioxin congeners could reduce lipoprotein lipase activity through the Ah receptor activation. Lipoprotein lipase on the membrane of adipocyte hydrolyzes lipoproteins into free fatty acids and proteins. PCB 153 could bind to lipoproteins in the liver and be transported to other tissues via

lipoproteins (Noren *et al.*, 1999). In fat tissues, PCB 153 is dissociated from lipoproteins by lipoprotein lipase and released into the fat (Gallenberg and Vodcnik, 1987). Therefore, the inhibition of lipoprotein lipase activity by coplanar PCB in the fat could result in the decrease of PCB 153 uptake by adipose tissues.

Through the incorporation of effects of lipid accumulation in the liver and inhibition of lipoprotein lipase in the fat, the observed pharmacokinetic changes of PCB 153 in nonpregnant mice could be successfully simulated using PBPK modeling. It should be noted that the interaction PBPK model in this article is still preliminary in nature. The time-dependent change of partition coefficient for the liver compartment provided an adequate fit to the data collected during the experimental period of 14 days. However, if the experimental period is very long, the changes of partition coefficient in the liver may become unrealistically large. Of course, such long-term accumulation of fat must be verified with experimental results if subsequent PBPK model refinement is to be attempted. Further, as the effects of PCB 126 may be related to receptor binding (i.e., Ah receptor), the concentration of PCB 126 may become an important factor regarding the fat accumulation in the liver in multiple dose level studies. Refinement of this interaction PBPK model is in progress in our laboratory.

PBPK models have been increasingly useful mechanistic tools to describe and estimate the disposition of chemicals in biological systems (Dedrick and Bischoff, 1969). The purpose for the construction of the PBPK model in this study is to quantitatively account for observed interactive effects between PCB 153 and PCB 126 at different stages of development. As in any modeling work in toxicology, the ultimate goal is to predict target tissue dosimetry following the establishment of a validated model. In this regard, the PBPK model for lactational transfer of PCB 153 is now being developed in our laboratory to simulate pharmacokinetic differences between lactating mice and nonpregnant mice.

TABLE 2
Modified PBPK Parameters to Fit Pharmacokinetic Changes of PCB 153 Affected by PCB 126

Parameter	Abbreviation	Before modification	After modification
Partition coefficient in liver	PL	10	$10 + 0.045 \times \text{TIME}$
Diffusion permeation constant in fat	PAFC	10	0.318

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