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Effect of PCBs on the lactational transfer of methyl mercury in mice: PBPK modeling

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Abstract

MeHg and PCB exposure to lactating mice were analyzed and a physiologically-based pharmacokinetic (PBPK) model was developed to describe the lactational transfer of MeHg in mice. The influence of albumin on the lactational transfer of MeHg was incorporated into the PBPK model. Experimental results with lactating mice and their pups showed that co-exposure with PCB congeners increased the lactational transfer of MeHg to the pups, which was associated with the rise of albumin levels in maternal blood. Observed results were matched with PBPK model simulations conducted under the assumptions that (1) MeHg bound to plasma albumin is transferred to maternal milk, and (2) PCB congeners may increase the lactational transfer of MeHg by escalating albumin levels in maternal blood. Further refinement of PBPK model quantitatively described the pharmacokinetic changes of MeHg by co-exposure with PCBs in pup's tissues.

Keywords

MeHg; PCBs; PBPK; lactational transfer

1. Introduction

Mercury compounds are generally released into the environment as an inorganic form from both natural sources and anthropogenic activities (Gochfeld, 2003). Inorganic mercury can be converted into organic mercury, such as methyl mercury (MeHg) via microbial action in aquatic ecosystems and further accumulated in fish (Smith *et al.*, 1974). Pregnant and lactating women may have risk factors associated with neurodevelopmental effects in fetuses and babies by the exposure to MeHg if they regularly eat large amounts of contaminated fish (Mahaffey, 2000; Myers *et al.*, 2000; Ronchetti *et al.*, 2006). Polychlorinated biphenyls (PCBs) are chlorinated hydrocarbons composed of 209 different congeners. The congeners with co-planar configuration such as PCB 126 can bind to aryl hydrocarbon (Ah) receptors and initiate

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Conflict of Interest

Authors declare there is no conflict of interest with regards to the research described in the manuscript.

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biological actions similar to 2,3,7,8-tetrachlorodibenzo-dioxin (TCDD; Safe, 1994). PCB congeners with non-planar configuration such as PCB 153 do not bind to Ah receptors and their biological activities are different from TCDD. These congeners induce CYP2B activity by activating the constitutive androstane receptor (CAR) (Tilson and Kodavanti, 1997; Strathmann et al., 2006). Even though their toxic mechanism is different, both co-planar and non-planar PCBs increase toxicities in animals and humans. Environmental exposure to PCBs occurs via multiple routes, including oral ingestion of contaminated dairy products, meats, and fish, all of which contain mixtures of non-planar and co-planar congeners (Carpenter, 2006).

Both MeHg and PCBs are recognized as neurotoxicants (Rice, 1995). The developing brain is highly susceptible to both toxicants because of rapid growth and development (Boersma and Lanting, 2000; Nakai and Satoh, 2002). Epidemiological studies have indicated that the babies and children born at MeHg and/or PCB contaminated sites had shown reduced cognitive function and disrupted neurobehavioral characteristics even if their mothers did not show any neurotoxic symptoms (Buck, 1996; National Research Council, 2000). Animal studies commonly suggest that perinatal exposure to MeHg and PCBs might cause neurofunctional deficits (Bowers et al., 2004; Burbacher et al., 1990; Newland and Paletz, 2000).

Interactive effects between MeHg and PCBs have been reported previously. In an epidemiologic study, neurobehavioral deficits in children indicated a possible interaction between MeHg and PCBs when they are exposed to mercury and PCBs through perinatal exposure (De Guise *et al.*, 2001). Bemis and Seegal showed that co-exposure to MeHg and PCBs *in vitro* affects cytosolic calcium homeostasis in a non-additive manner and reduces dopamine content in rat's brain synergistically (Bemis and Seegal, 1999; Bemis and Seegal, 2000).

Pharmacokinetic interactions between PCB congeners have been investigated in both lactating and non-lactating mice (van Birgelen *et al.*, 1996; Lee et al., 2007). However, pharmacokinetic interactions between MeHg and PCB congeners during the lactational period have not been reported despite the potential importance. This study aimed to investigate the pharmacokinetic interactions between MeHg and PCB congeners (i.e., PCB 153 and PCB 126) during lactational period and to elucidate the possible mechanisms of the interactions using PBPK modeling. PCB 153 and PCB 126 were selected as a representative PCB mixture to present both coplanar and non planar PCB congeners. In this article, we report the effect of PCB congeners on the lactational transfer of MeHg and present a PBPK model describing lactational transfer of MeHg in mice following co-exposure with PCB congeners.

2. Materials and Methods

2.1. Animals and Treatment

C57 BL/6 male and female mice were purchased from Harlan Sprague Dawley Laboratory (Indianapolis, IN) and housed at Painter Center in Colorado State University, which is fully accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC). The mice were maintained on a 12-hr 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*. Mating took place at Painter Center in Colorado State University according to the following breeding protocol. Briefly, two female mice were placed together with one male in a cage for 4 consecutive days. The day after mating was assigned as gestational day (GD) 0. The pregnancy rate was about 60%. The animals were weighed every three days during gestation. On postnatal day (PND) 0, 7, and 14, the offspring were counted and inspected for signs of overt toxicity. If the number of pups were lower than six, the dam and pups were not used in the study.

On PND 1, sixteen lactating mice (four mice per group) were exposed to MeHg alone (1 mg/kg bw), a mixture of PCB 153 (20 mg/kg bw) and PCB 126 (0.2 mg/kg bw), or a mixture of MeHg, PCB 153, and PCB 126 through oral gavage, respectively. Corn oil was used as a vehicle to dissolve the chemicals. The dosing time was between 8:00 and 9:00 a.m. It was confirmed that each dosing solution has the same concentration of MeHg and PCB congeners by atomic absorption spectrometry and gas chromatography, respectively. At 1, 3, 6, and 13 days after oral gavage, the lactating mice and their pups were anesthetized using isoflurane and then euthanized. The tissues from lactating mice (kidney, blood, and brain) and pups (brain, kidney, and the remaining carcass) were collected. All samples were frozen with liquid nitrogen and stored at -70°C until analysis.

2.2. Chemical analyses

The analyses of PCB congeners were based on the modified method by Lee et al. (2002). Briefly, tissue samples were digested by 60% of sulfuric acid and extracted by pentane. After cleaning up the extracts with Florisil[®], the concentrations of PCB 153 or PCB 126 were determined using a HP-5890 Series II Plus gas chromatography with an ECD detector (Hewlett Packard, Wilmington, DE). The analyses of mercury were based on the method by EPA (Smoley, 1992). Briefly, tissues samples were digested by the mixture of nitric acid and sulfuric acid and completely oxidized by potassium permanganate and potassium persulfate. The concentration of mercury was determined using a cold vapor atomic absorption spectrometry (Varian, Sugarland, TX).

2.3. Measurement of albumin in blood

Albumin determination was based on the bromocresol green (BCG) method reported previously (Tietz, 1970). Briefly, serum was collected by centrifugation from maternal blood. A 18 μL aliquot of each serum sample was mixed with 2.1 ml of BCG reagent provided by Amresco (Solon, OH). At pH=4.2, albumin bind with bromocresol green to produce a blue-green complex. The change in absorbance at 600 nm correlates with the concentration of albumin. The absorbance was measured at 600 nm.

2.4. PBPK Model Development

It has been reported: (1) MeHg and PCB congeners could affect the plasma levels of albumins (Matthews *et al.*, 1977; Yasutake *et al.*, 1989), (2) MeHg could bind to albumins extensively in plasma (Vodicnik and Lech, 1980; Sundberg *et al.*, 1998), and (3) plasma albumin could play a role in the lactational transfer of MeHg and PCB congeners (Spindler-Vomachka *et al.*, 1984; Sundberg *et al.*, 1998). To evaluate whether the effects of MeHg and/or PCB congeners on albumin levels in maternal blood could be related to the lactational transfer of these compounds, a PBPK model was developed describing lactational transfer of MeHg based on a previous model (Byczkowski and Lipscomb, 2001). The binding protein pool was incorporated into blood compartment. The physiological parameters (i.e., albumins) in this compartment were adopted from previous publications (Spindler-Vomachka *et al.*, 1984; McMullin *et al.*, 2003). Two different hypotheses were raised for the lactational transfer of MeHg. First hypothesis is unbound (or free) MeHg will be transferred from the mother to the pups by lactation. This hypothesis was based on the literature dealing with the lactational transfer of drugs and environmental contaminants (Corley *et al.*, 2003). Second hypothesis is that MeHg bound with albumins will be transferred from the mother to the pups by lactation. This hypothesis was based on the previous publications focusing on the lactational transfer of MeHg (Vodicnik and Lech, 1980; Sundberg *et al.*, 1998).

2.4.1. Model structure—The model structure was presented in Figure 1, which was constructed based on a previous model (Byczkowski and Lipscomb, 2001). The blood

compartment was separated into the serum compartment and the binding protein compartment (i.e., albumin). Chemical transfer from mother to the pups was described based on two different hypotheses: First, chemicals bound with the transport protein, albumin, could be transferred from mother to the pups. In this scenario, chemicals in the binding protein compartment could be moved to the mammary gland, and subsequently transferred to the pups. Second, unbound chemicals could be transferred from mother to the pups. In this scenario, chemicals in serum compartment could be moved to the mammary gland, and subsequently transferred to the pups. In the following section, we described mathematical expressions of chemical transfer in the defined compartments.

2.4.2. Mathematical expression of the model—The mathematical expressions of the model were formulated as follows. Equation (1) represents a tissue mass balance equation for a flow-limited transport process.

$$\frac{dA_i}{dt} = Q_i \times (C_D - C_{Vi}) \quad (1)$$

, where Q_i is blood flow to i th tissue; A_i is amount in i th tissue. C_B is arterial blood concentration; C_{Vi} is venous blood concentration leaving i th tissue. $C_{Vi} = C_i/P_i$, where P_i is tissue/blood partition coefficient, C_i is concentration in i th tissue. $C_i = A_i/V_i$; where V_i is the volume of i th tissue. Equation (2)–(3) are the tissue mass balance equations for a diffusion-limited transport process.

$$\frac{dA_{iB}}{dt} = Q_i \times (C_B - C_{Vi}) + P_{Ai} \times \left(\frac{C_i}{P_i} - C_{Vi} \right) \quad (2)$$

$$\frac{dA_i}{dt} = P_{Ai} \times \left(C_{Vi} - \frac{C_i}{P_i} \right) \quad (3)$$

, where Q_i is blood flow to i th tissue; A_{iB} is amount in the tissue blood of i th tissue. P_{Ai} is the diffusion permeation constant of i th tissue. For the tissue compartments eliminating compounds from the body, an additional equation describing the elimination of compounds is added.

$$\frac{dA_i}{dt} = Q_i \times (C_B - C_{Vi}) - K_{Ei} \times C_{Vi} \quad (4)$$

, where K_{Ei} is the elimination constant of compound in i th tissue.

Binding of the compounds with binding proteins (albumins for MeHg) and the disposition in the body are described as follows.

$$\frac{dA_{UT}}{dt} = K_{FT} - K_{ET} \times C_{UT} - K_{BT} \times C_p \times C_{UT} \quad (5)$$

$$\frac{dA_{BT}}{dt} = K_{BT} \times C_p \times C_{UT} - K_e \times C_{BT} \quad (6)$$

, where A_{UT} is the amount of binding proteins unbound with compounds; K_{FT} is the formation constant of binding proteins; K_{ET} is the degradation constant of binding proteins unbound with compounds; K_{BT} is the association constant of binding proteins with compounds; A_{BT} is the amount of binding proteins bound with compounds; C_p is the plasma concentration of the compound; K_e is the elimination constant of binding proteins bound with compounds. The equations for mammary tissue/milk are described based on two different hypotheses presented in previous section. In equations (7) and (8), we described that chemicals are transferred to the pups without binding to transport proteins.

$$\frac{dA_{MA}}{dt} = Q_{MA} \times (C_B - C_{VMA}) - N \times \frac{dA_{SUCK}}{dt} \quad (7)$$

, where A_{MA} is the amount of compounds in mammary tissue/milk; C_{VMA} is venous blood concentration leaving mammary tissue/milk; N is the number of pups. The second term (dA_{SUCK}/dt) is identical to pup litter suckling rate.

$$\frac{dA_{SUCK}}{dt} = K_{MILK} \times C_{MA} \quad (8)$$

, where K_{MILK} is the milk transfer rate and assumed to be the milk production rate. In equations (9) and (10), we described that chemicals are transferred to the pups bound with transport proteins.

$$\frac{dA_{BT}}{dt} = K_{BT} \times C_p \times C_{UT} - K_e \times C_{BT} - N \times \frac{dA_{SUCK}}{dt} \quad (9)$$

$$\frac{dA_{SUCK}}{dt} = K_{MC} \times C_{TT} \times C_{BT} \quad (10)$$

, where K_{MC} is chemical transfer rate from milk to pups and assumed to be dependent on milk production rate; C_{TT} is concentration of total transport proteins in plasma. The equation describing body weight growth of lactating pup (BWP) was adapted from previous literature (Corley *et al.*, 2003):

$$BWP = BWPI + ADULT \times \left(\frac{e^{AGE-1}}{e^{HALF} + e^{AGE-1}} \right) \quad (11)$$

, where $BWPI$ is the initial body weight of a pup; $HALF$ is the one month after birth in mice; $ADULT$ is the normal weight of an adult mouse; AGE is the month after birth in mice.

2.4.3. Parameter values—Physiological constants such as blood flow and tissue volume were taken directly from a published literature (Brown *et al.*, 1997). Other parameters including physicochemical parameters, metabolic constants, and changes during lactational period followed the values or mathematical expressions in previous literatures (Byczkowski and Lipscomb, 2001; Lee *et al.*, 2007). All parameters mentioned above were presented in Table 1.

2.4.4. Simulation software—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).

2.5. Statistical analysis

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. Time and treatment groups were considered as factors. All analyses were performed with the statistical software, Minitab ($p < 0.05$; Windows version 12.0).

3. Results

3.1. Tissue dosimetry of MeHg and albumin levels

Figure 2 shows the tissue levels of mercury in pups and the levels of albumin in maternal blood. In Fig. 2(A), the group exposed to MeHg only showed that the concentration in pup's brain increased gradually from PND 2 through PND 14. The group exposed to MeHg + PCB congeners showed that the concentration of mercury in pup's brain attained maximal level at PND 7. At PND 7, the tissue concentration was higher in the group exposed to MeHg + PCB congeners than the group exposed to MeHg only at a statistically significant level ($p < 0.05$). In Fig. 2(B), the concentration of mercury in pup's carcass was higher in the group exposed to MeHg + PCB congeners than the group exposed to MeHg only. The concentration of mercury in pup's carcass was statistically different at every time point ($p < 0.05$). In Fig. 2(C), the concentration of mercury in pup's kidney was statistically higher in the group exposed to MeHg + PCB congeners than in the group exposed to MeHg only at PND 7 and PND 14 ($p < 0.05$). Overall results indicate that co-exposure with PCB congeners to the lactating mice increases the lactational transfer of MeHg to the pups.

The albumin levels in maternal blood were compared between the group exposed to MeHg only and the group exposed to MeHg + PCB congeners (Fig. 2(D)). There was a statistically significant difference of albumin levels at PND 2 between the group exposed to MeHg only and the group exposed to MeHg + PCB congeners ($p < 0.05$). These results indicate that co-exposure with PCB congeners increased the levels of albumin in maternal blood and the levels of albumin in maternal blood may be associated with the lactational transfer of MeHg in mice.

In Table 2, the time-course concentration of MeHg in maternal tissues was presented. Overall, the mean concentration of MeHg in maternal tissues was higher in the group exposed to MeHg + PCB congeners than the group exposed to MeHg only. *Tissue dosimetry of PCB congeners.* Fig. 3 showed the level of PCB congeners in pup's tissues. In Fig. 3(A), the group exposed to PCB congeners only showed that the concentration of PCB 153 in pup's brain reached the maximum level at PND 2 and decreased after that. The group exposed to MeHg + PCB congeners showed that the concentration of PCB 153 reached the maximum level at PND 4 and decreased after that. The difference of PCB 153 was not statistically significant between two groups. In Fig. 3(B), the levels of PCB 153 in pup's carcass did not show any statistically significant differences between two groups. In Fig. 3(C), the levels of PCB 153 in pup's liver gradually decreased with time. The levels of PCB 153 in the group exposed to MeHg + PCB congeners were statistically significant from those in the group exposed to PCB congeners only at PND 4 ($p < 0.05$). Fig. 3(D) showed a time-course concentration of PCB 126 in pup's liver. The levels of PCB 126 gradually increased with time and reached maximal levels at PND 14 in both groups. These levels were not statistically different between two groups. PCB 126 was not detected in other tissues, which was consistent with previous findings (Lee *et al.*, 2002). These results indicate that the lactational transfer of PCB congeners from mother to the pups were not different between the group exposed to PCB congeners only and the group exposed

to MeHg + PCB congeners. In Table 3, time-course concentrations of PCB congeners in maternal tissues were presented. Overall, tissue concentrations of PCB congeners were not different between two groups.

3.2. PBPK modeling and simulation: relationship between lactational transfer of MeHg and albumin levels in maternal blood

PBPK simulation results for the lactational transfer of MeHg with diverse albumin levels in maternal blood were presented in Fig. 4. The simulation output, based on the assumption that free MeHg without binding to albumin can be transferred to pups, implies that the lactational transfer of MeHg could be reversely proportional to the albumin levels in maternal blood (Fig. 4 (A)). The simulation output, based on the assumption that MeHg bound with albumin can be transferred to pups, implies that the lactational transfer of MeHg could be directly proportional to albumin levels in maternal blood (Fig. 4 (B)). Both results indicate that the lactational transfer of MeHg may be dependent upon the levels of albumin in maternal blood.

3.3. PBPK modeling: refinement and validation

Fig. 5 represented the refined simulation results of mercury disposition in pup's tissues. The final model assumed that MeHg bound with albumin is transferred to the pups based on above findings. The parameters reflecting the physiology and metabolism of growing mice were optimized to describe experimental results. The final parameters were presented in Table 1. Adjusting the formation constant of albumin in maternal blood can describe pharmacokinetic changes of mercury in pups by co-exposure with PCB congeners. Increase of albumin formation was associated with increase of mercury in pups by co-exposure with PCB congeners.

4. Discussion

4.1. Lactational transfer and tissue disposition of MeHg and PCB congeners

The present study demonstrated that co-exposure of MeHg and PCB congeners to the lactating mice could elicit pharmacokinetic interactions for the lactational transfer from the mother to the pups. Experimental findings indicate that co-exposure of MeHg and PCB congeners increase the lactational transfer of MeHg, not PCB congeners. Albumin levels in maternal blood seem to be associated with increased lactational transfer of MeHg. Lactational transfer of MeHg is directly proportional to albumin levels in maternal blood. PBPK modeling indicates that MeHg bound with albumin is transferred to pups and co-exposure with PCB congeners could increase lactational transfer of MeHg by inducing the levels of albumins in maternal blood.

Previously, we presented a PBPK model to describe the pharmacokinetics of PCB153 in lactating mice and their pups. The lactational PBPK model described the mass transfer of PCB153 into developing organism during lactation and further helped to understand the kinetic change of PCB153 with or without PCB126 during lactational stage (Lee *et al.*, 2007). Current study demonstrated the pharmacokinetic interactions between MeHg and PCB congeners on the lactational transfer as well. It has been known that MeHg could be transferred from the mother to the babies during perinatal period (Pitkin *et al.*, 1976). Many studies have focused on MeHg transfer during gestational period, showing that MeHg could be easily transferred from the mother to the fetus during gestational period (Risher *et al.*, 2002). Some animal studies suggested that the amount of lactational transfer of MeHg may be lower than that of gestational transfer (Nordenhall *et al.*, 1995). However, it is still important to know the pharmacokinetic profiles on the lactational transfer and tissue disposition of MeHg because MeHg can induce neurotoxicities from early stage of development and important brain development occurs during postnatal development. The developing brain is more sensitive to environmental

contaminants than adult brain because of rapid growth during short time period (Eriksson, 1997). Therefore, even slight changes of toxicants in the developing brain can elicit morphological damages and functional deficits. In addition to PCB congeners which we selected, other environmental toxicants coexist with MeHg especially in the water (Ritter *et al.*, 2002). Since most risk assessments are based on the single chemical studies, the research on pharmacokinetic interactions will ultimately contribute to risk assessments for environmental contaminants. Numerous studies have reported that MeHg and PCBs interfere with biological process critical for CNS development. For example, MeHg and PCBs have been shown to disrupt cytosolic Ca^{2+} homeostasis, a critical component for proper cellular and neuronal functions. Co-exposure of MeHg and PCBs affected cytosolic Ca^{2+} homeostasis in a non-additive manner (Bemis and Seegal, 2000). These results may indicate that co-exposure of MeHg and PCBs affects normal brain development in a non-additive manner. Pharmacokinetic interactions between MeHg and PCBs may be the first step to induce non-additive toxic effects. Epidemiological studies on developmental neurotoxicity of MeHg have shown a discrepancy with regard to neuropsychological defects. Despite high exposure to MeHg, the defects were seen in children from the Faeroe Islands, but not in children from the Seychelles (Davidson *et al.*, 2006; Grandjean *et al.*, 2001). It is postulated that the children in the Faeroe Islands were exposed to PCBs as well as to MeHg, so the observed differences in children are due to co-exposure of MeHg and PCBs. Our study may imply that the developmental neurotoxicity is partly due to the increased lactational transfer of MeHg by pharmacokinetic interactions between MeHg and PCBs.

4.2. Importance of binding proteins for pharmacokinetic interactions between MeHg and PCB congeners

The transport of chemicals into milk seems to follow the same pathways as that of nutrients. However, the real mechanisms have not been thoroughly investigated. In general, passive diffusion across the membrane is believed to be the major transport mechanisms of xenobiotics (Corley *et al.*, 2003). In addition, carrier proteins may be involved in the transfer of toxic chemicals into the milk (Oskarsson *et al.*, 1998). Some studies showed that MeHg or PCB congeners could affect the levels of albumin or lipoproteins in blood (Matthews *et al.*, 1977; Yasutake *et al.*, 1989). It is well known that MeHg could bind to albumin with high affinity and PCB congeners could bind to lipoproteins (Matthews *et al.*, 1977; Yasutake *et al.*, 1989). The lactational transfer of MeHg or PCB congeners with plasma proteins has been suggested previously (Spindler-Vomachka *et al.*, 1984; Sundberg *et al.*, 1999). Lactational transfer of MeHg or PCB congeners may be changed if MeHg or PCB congeners could affect the levels of binding proteins (i.e., albumin or lipoproteins) in maternal blood. In this study, we successfully demonstrated that co-exposure of MeHg and PCB congeners did not change the level of lipoproteins but changed the level of albumin. Subsequent PBPK modeling approach indicated that the lactational transfer of MeHg increased with increasing albumin levels in maternal blood following co-exposure with PCB congeners. The route by which albumin is transferred from maternal blood to milk is still unclear. However, it is postulated that the transfer results from leakage through the mammary epithelium via a paracellular route (Geursen and Grigor, 1987). In general, the passage of molecules through mammary glands is impeded by a gasket-like structure called tight junctions. Tight junctions become leaky allowing nutritional components to pass into the milk, which is a paracellular transport. Milk itself is produced and stored in alveolar units in mammary glands. There is no evidence that either MeHg or PCB congeners damage the mammary epithelium in mice. It is unlikely that co-exposure of MeHg and PCB congeners affects the paracellular transport. Albumin is synthesized in the liver, secreted to the plasma, and transferred to the milk. PCB congeners have increased the expression and secretion of albumin in the hepatocytes (Borlak *et al.*, 2002), which may be the possible mechanism on the increase of albumin levels in maternal plasma following co-exposure with PCB congeners. Increase of albumin levels in maternal

plasma will lead to increasing transfer of albumin to milk. Subsequently, the transfer of MeHg bound with albumin will be increased.

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Fig. 1. Schematic diagrams of the PBPK model describing the lactational transfer of MeHg with or without PCB congeners. The model structure was constructed by based on a published model (Byczkowski and Lipscomb, 2001).

Fig. 2.

The tissue concentrations of mercury in the pup and the levels of albumin in maternal blood. Each data point represents mean $\pm$ SEM. *means the statistically significant difference from the corresponding group ($p < 0.05$). The unit of mercury concentration is ng/g wet tissue. (A) The time-course concentration of mercury in pup's brain. (B) The time-course concentration of mercury in pup's carcass. (C) The time-course concentration of mercury in pup's kidney. (D) The time-course concentration of albumin in maternal blood.

Fig. 3.

The tissue concentrations of PCB congeners in the pup. Each data point represents mean $\pm$ SEM. *means the statistically significant difference from the corresponding group ($p < 0.05$). The unit of PCB concentration is ng/g wet tissue. (A) The time-course concentration of PCB 153 in pup's brain. (B) The time-course concentration of PCB 153 in pup's carcass. (C) The time-course concentration of PCB 153 in pup's liver. (D) The time-course concentration of PCB 126 in pup's liver.

Fig. 4.

PBPK simulation results for the lactational transfer of MeHg against albumin levels in maternal blood. At each figure we presented the simulation results for first two hypotheses respectively. (A) The simulation results for the lactational transfer of MeHg to the pups based on the assumption that free MeHg without binding to albumin can be transferred to pups. Albumin levels varied from 50% to 100% of normal levels in mice. (B) The simulation results for the lactational transfer of MeHg to the pups based on the assumption that MeHg bound with albumin can be transferred to pups. Albumin levels varied from 50% to 100% of normal levels in mice.

Fig. 5. PBPK simulation of MeHg in the pup's tissues comparing the group exposed to MeHg only with the group exposed to MeHg + PCB congeners. The only difference of the simulations between the group exposed to MeHg only and the group exposed to MeHg + PCB congeners is the levels of albumin in maternal blood.

Table 1**PBPK model parameters**

Parameter	Abbreviation	Value	Parameter estimation
Physiological Parameters			
Maternal Parameters			
Maternal Body weight (kg)	BW	0.025	Literature ^a
Fraction of brain	BF	0.020	Literature ^a
Fraction of plasma	BLF	0.022	Literature ^a
Fraction of RBC	RBCF	0.027	Literature ^a
Fraction of fat	FF	0.056	Literature ^a
Fraction of hair	HF	0.002	Literature ^a
Fraction of rapidly perfused	RF	0.100	Literature ^a
Fraction of slowly perfused	SSF	See legend ^b	Literature ^a
Fraction of gut	GF	0.021	Literature ^a
Fraction of gut lumen	IF	0.021	Literature ^a
Fraction of liver	LF	0.080	Literature ^a
Fraction of kidney	KF	0.0017	Literature ^a
Fraction of mammary gland	MAF	0.044	Literature ^a
Cardiac output (l/hr)	QC	16.5*BW**0.75	Literature ^a
Blood flow of brain	QBF	0.033	Literature ^a
Blood flow of fat	QFF	0.07	Literature ^a
Blood flow of kidney	QKF	0.091	Literature ^a
Blood flow of GI tract	QGF	0.141	Literature ^a
Blood flow of liver	QLF	0.159	Literature ^a
Blood flow of mammary gland	QMAF	0.1*QRF	Literature ^a
Blood flow of rapidly perfused	QRF	0.183	Literature ^a
Blood flow of slowly perfused	QSSF	See legend ^c	Literature ^a
Pup's parameters			
Pup's Body weight (kg)	BWP	See Text	Literature ^a
Fraction of blood	BLPF	0.049	Literature ^a
Fraction of brain	BPF	0.020	Literature ^a
Fraction of kidney	KPF	0.0017	Literature ^a
Fraction of liver	LPF	0.080	Literature ^a
Fraction of gut	GPF	0.021	Literature ^a
Fraction of gut lumen	IPF	0.021	Literature ^a
Fraction of body	CARF	See legend ^d	Literature ^a
Cardiac output (l/hr)	QCP	18*BWP**0.75	Literature ^a

Parameter	Abbreviation	Value	Parameter estimation
Blood flow of brain	QBPF	0.033	Literature ^a
Blood flow of kidney	QKPF	0.091	Literature ^a
Blood flow of liver	QLPF	0.159	Literature ^a
Blood flow of GI tract	QGPF	0.141	Literature ^a
Blood flow of body	QCARF	See legend ^e	Literature ^a
Partition Coefficients of MeHg			
Brain	PB	3.0	Literature ^f
Slowly perfused	PS	2.0	Literature ^f
Fat	PF	0.15	Literature ^f
Liver	PL	3.0	Literature ^f
Gut	PG	1.0	Literature ^f
Kidney	PK	5.0	Literature ^f
Rapidly perfused	PR	1.0	Literature ^f
Mammary gland	PM	0.2	Literature ^f
Biochemical Constants of MeHg (l/hr) ^g			
Transfer to RBC	KRBC	1.5	Literature ^f
Urinary elimination	KU	0.00006	Literature ^f
Transfer to hair	KH	0.000007	Literature ^f
Hairy elimination	KL	0.0001	Literature ^f
Interconversion between brain and brain blood	KBR	0.01	Literature ^f
Demethylation in brain	KBRI	0.000012	Literature ^f
Excretion of inorganic Hg from brain	KBRIL	0.001	Literature ^f
Demethylation in liver	KC	0.00001	Literature ^f
Interconversion between gut and gut lumen	KR	0.002	Literature ^f
Transfer from liver to gut lumen	KB	0.0001	Literature ^f
Excretion from gut lumen	KFC	0.0002	Literature ^f
Demethylation in gut lumen	KD	0.0001	Literature ^f
Excretion of inorganic Hg from gut lumen	KFI	0.0002	Literature ^f
Albumin formation (–PCBs)	KFT	7.0 (0 ≤ T ≤ 96) 14.56 (T ≥ 96)	Literature ^h
Albumin formation (+PCBs)	KFT	14.56	Literature ^h
Albumin degradation	KET	0.008	Literature ^h
Binding of albumin with MeHg	KBT	0.0166	Fitted
Degradation of MeHg bound with albumin	Ke	0.001	Literature ^h
Transfer of MeHg from mother to pups	KMILK	0.00233/N ⁱ	Literature ^f

Parameter	Abbreviation	Value	Parameter estimation
Transfer of MeHg bound with albumin from mother to pups	KMC	2.2×10^{-9}	Fitted

^aBrown *et al.*, 1997

^b $1 - (\text{BF} + \text{BLF} + \text{RBCF} + \text{FF} + \text{HF} + \text{RF} + \text{GF} + \text{IF} + \text{LF} + \text{KF} + \text{MAF})$

^c $1 - (\text{QBF} + \text{QKF} + \text{QFF} + \text{QGF} + \text{QLF} + \text{QMAF} + \text{QRF})$

^d $1 - (\text{BLPF} + \text{BPF} + \text{KPF} + \text{LPF} + \text{GPF} + \text{IPF})$

^e $1 - (\text{QBPF} + \text{QKPF} + \text{QLPF} + \text{QGPF})$

^fByczowski and Lipscomb, 2001

^gAll parameters should be allometrically scaled to body weight.

^hMcMullin *et al.*, 2003

ⁱN is the number of pups.

Table 2

Summary of MeHg concentrations in the maternal tissues (ng/g wet tissue).

	Time	Lactating dams (MeHg only)	Lactating dams (MeHg + PCB congeners)
Kidney	Day 1	882±389	1354±700
	Day 3	1292±344	2096±516
	Day 6	711±90	1271±595
	Day 13	205±54	955±189
Blood	Day 1	177±3	418±35
	Day 3	143±16	358±160
	Day 6	115±40	376±180
	Day 13	39±2	117±30
Brain	Day 1	102±40	199±18
	Day 3	217±100	253±74
	Day 6	149±13	327±239
	Day 13	89±7	217±10

Data represents mean±SEM.

Table 3

Summary of PCB 153 levels in maternal tissues (ng/g wet tissue).

	Time	Lactating dams (PCB congeners)	Lactating dams (MeHg + PCB congeners)
Liver	Day 1	5065	4256
	Day 3	3425±648	4461±453
	Day 6	1375±255	2426±155
	Day 13	1525±617	1279±898
Fat	Day 1	43030	35468
	Day 3	38908±4340	45727±2322
	Day 6	45110±2355	37913±1455
	Day 13	20688±5858	23296±6386
Brain	Day 1	ND	ND
	Day 3	819±25	645±38
	Day 6	ND	ND
	Day 13	389±197	124±58

ND not determined

Data represents mean±SEM.