Hexachlorobenzene and Pentachlorobenzene Accumulated during Pregnancy is Transferred to Pups at the Accumulation Ratio in Dams

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Hexachlorobenzene (HCB) and pentachlorobenzene (PECB) were used to clarify how the rate at which lipophilic environmental pollutants are metabolized will affect their transfer to pups. In this study, the ratio of PECB/HCB transference to fetuses and suckling pups was investigated in rats fed a diet containing HCB (35.1 nmol/100 g diet) and PECB (351 nmol/100 g diet). The amounts of HCB and PECB transferred to fetuses were 0.44% and 0.15% of the amounts consumed by their dams, respectively, and the PECB/HCB concentration ratio in fetuses was 3.4. In pregnant rats on the day before parturition, the PECB concentrations in organs and fat tissue were 3 to 4 times higher than those of HCB. After parturition, PECB rapidly disappeared from the body of nursing rats during the lactation period, especially when compared with HCB. On day 2 after birth, HCB and PECB concentrations in the stomach contents of suckling pups were highest, and the PECB concentration was 3.5 times higher than that of HCB. HCB in the stomach contents decreased gradually ($T_{1/2}$=5.7 d) and PECB decreased rapidly ($T_{1/2}$=2.8 d) during the 15 d after birth. These findings indicate that the PECB/HCB transferred to fetuses and suckling pups was the same ratio found in the blood of their dams.

Key words —— hexachlorobenzene, pentachlorobenzene, lactation, suckling pup, metabolic rate

INTRODUCTION

Many organochlorine environmental pollutants such as pesticides and herbicides have been found widely distributed in the global ecosystem.[1,2] Moreover, the presence of small amounts of polychlorinated dibenzo-*p*-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), polychlorinated biphenyls (PCBs), *pp'*-dichlorodiphenyl-trichloroethane (DDT), *pp'*-dichlorodiphenyltrichloroethylene (DDE), hexachlorobenzene (HCB) and dieldrin in normal human organs, tissues and breast milk has been confirmed for a considerable period of time.[3–7] In the body, fat tissue has an important role in storage of lipophilic pollutants,[8,9] and its tissue mass is very large, especially during pregnancy. However, the fat tissue that increases during pregnancy usually decreases during lactation.

We observed previously that HCB accumulated in dams was transferred to their fetuses through the placenta and to suckling pups through milk.[10] The amount transferred prenatally to the fetuses was very small, but a large amount of HCB ingested by dams was transferred to suckling pups in the early days after birth. In that study, we used HCB as a model compound of a stable lipophilic environmental pollutant. HCB is a chlorinated hydrocarbon and is retained in fat tissue and metabolized very slowly. A previous study with rat liver microsomes showed that cytochrome P-450 3A was involved in the oxidation from HCB both to pentachlorophenol (PCP) and tetrachlorobenzoquinone (TCBQ).[11] Pentachlorobenzene (PECB) is also a chlorinated hydrocarbon but is more rapidly metabolized than HCB, and is oxidized to give the same products as in the metabolism of HCB, such as PCP and TCBQ.[12,13] However, the half-lives of HCB and PECB are very different: 3 months for HCB and 5 days for PECB in adult

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rats.[14]

Although the transfer of these environmental pollutants from pregnant women to their infants and from lactating women to their breast fed babies is clearly a public health concern, detailed information on the mother-infant transfer of these chemicals is still sparse. Many reports have revealed that many of the pollutants found in human milk are metabolized very slowly[3-7] and easily stored in fat tissue. However, it is uncertain whether rapidly metabolized pollutants are transferred to breast milk. Therefore, the present study was performed to investigate the distribution of HCB, which is chemically stable and metabolized very slowly, and PECB, which is a relatively rapidly metabolized environmental pollutant, in rat dams, their fetuses and pups and their interaction in the transfer and distribution. The dams were continuously exposed to a small amount of HCB together with PECB, and the transfer of these pollutants to their fetuses and suckling pups was investigated. In this study pregnant rats were supplied a diet containing the minimum levels of HCB (35.1 nmol/100 g diet) and PECB (351 nmol/100 g diet) that would subsequently produce detectable organ concentrations of HCB and PECB. Therefore, it was expected that the biological effect of these chlorinated hydrocarbons on the dams and newborns would be very weak. We have determined whether the PECB that accumulated in dams during pregnancy was rapidly transferred to suckling pups immediately after birth as has been observed for HCB. We describe the distribution of these chlorinated hydrocarbons in the blood, organs and fat tissues of pregnant and nursing rats as well as transfer to fetuses through the placenta and suckling rats through the milk. Further, we discuss the ratio of PECB/HCB transference from dams to their fetuses and suckling pups.

MATERIALS AND METHODS

Materials —— HCB and PECB were purchased from Tokyo Kasei Kogyo (Tokyo, Japan) and recrystallized three times from methanol (purity 99%). Other chemicals were purchased from Wako Pure Chemical Industries (Osaka, Japan). Diet components were purchased from Oriental Yeast (Tokyo, Japan).

Animals and Diet —— Eight pregnant rats (Sprague-Dawley strain) were used in this study. Sperm-positive rats (10 weeks old) were commercially obtained from Japan Clea (Tokyo, Japan) on day 5 of pregnancy. They were housed individually in plastic cages in a room kept at a constant temperature (23±1℃) and illuminated according to a 12-h light : dark cycle. Rats were fed an experimental diet with HCB (35.1 nmol/100 g diet) and PECB (351 nmol/100 g diet). The composition of the diet according to AIN-93[15] is shown in Table 1. The diet was prepared by dissolving HCB (3.5 mol/l ethanol) and PECB (35 mol/l ethanol) in soybean oil. Rats were given free access to diet and distilled water. Dams and suckling pups were weighed and daily food intake was measured at least five times weekly during the experimental period.

On the day before parturition, rats were divided into two groups. One group was anesthetized with ether, and blood taken with heparinized syringes by heart puncture. The organs, fat tissues, placenta and fetuses were removed from each rat and weighed. The other group was supplied the same diet without HCB and PECB during 15 d after parturition and on days 2, 5 and 11 after birth, two suckling pups from each of the litter of dams were similarly anesthetized and their blood collected. Organs and fat tissues were removed from each suckling pup and weighed. On day 15 after parturition, the remaining suckling pups and dams were similarly anesthetized and blood, organs and fat tissues were obtained. These collected samples were frozen immediately and stored at −20℃ until the analysis. All procedures were in accordance with the guidelines for the National Institute of Health and Nutrition.

Analytical Methods —— Blood (0.2—3 ml) was mixed with 1—5 ml of distilled water. Organs were homogenized in 4 volumes of water. HCB and PECB in the sample were extracted with *n*-hexane. To extract HCB and PECB, fat tissues were homogenized in 25 volumes of *n*-hexane. The *n*-hexane extracts were centrifuged at 600 *g* for 5 min. The *n*-hexane layer was concentrated, if necessary, and was cleaned by Florisil column chromatography (0.5 g of Florisil layered on 0.2 g of $NaSO_4$). The column was eluted with 5 ml of *n*-hexane. The eluate was evaporated and its volume was approximately adjusted with *n*-hexane. HCB and PECB were analyzed using a Shimadzu PARVUM QP-5000 gas chromatography/mass spectrometry (Shimadzu, Kyoto, Japan). A fused silica capillary column DB624 (0.25 mm × 30 m) (J & W Scientific Folsom, CA, U.S.A.) was used at a column temperature of 250℃ with 20 ml/min Helium as the carrier gas.

Statistical Analysis —— Data presented as individ-

ual group means±S.D. Statistical analysis was conducted by ANOVA. Differences in mean values between groups were tested by Student *t*-test (Table 2), Duncan's multiple-range test (Table 3 and Figs. 2—3) and the Kruskal–Wallis test for unequal variance in Table 4 and Fig. 1[16]. The Yukumus computer program (Yukumus, Tokyo) was used for statistical analysis of the data.

RESULTS

The mean body weight of pregnant rats on the day before parturition was unmistakably higher than that of nursing rats on day 15 after parturition ($p<0.05$) (Table 2). No significant differences in liver and brain weights were observed between the pregnant and nursing rats, but the kidney weight of the nursing rats was significantly higher than that of the pregnant rats ($p<0.05$). However, the perirenal fat tissue weight of the nursing rats was significantly lower than that of the pregnant rats ($p<0.05$).

The mean body weight of pregnant rats on the day before parturition ($n=3$) and nursing rats on day 15 after parturition ($n=3$) fed the HCB and PECB-free diet during the experimental period (control group) was 352±31 g and 293±12 g, respectively. On day 2, 5, 11 and 15 after birth, the body weight of suckling pups fed by the control dams was 7.4±0.3 g, 12.6±1.1 g, 30.3±3.6 g and 46.3±3.2 g, respectively. Therefore, in both pregnant and nursing rats, no significant differences in body weight were observed between

Table 1. Composition of Diet[a]

Ingredient	g/100g diet
Casein	20.0
DL-Methionine	0.3
Cornstarch	39.75
Sucrose	10.0
Dextrine	13.2
Cellulose	5.0
Soybean oil	7.0
AIN Mineral mixture	3.5
AIN Vitamin mixture	1.0
Choline bitartrate	0.25
tert-Butylhydroquinone	0.0014
	nmol/100g diet
Hexachlorobenzene	35.1
Pentachlorobenzene	351

a) AIN-93 Purified Diets for Laboratory Rodents: *J. Nutr.*, **123**, 1939—1951 (1993).[15]

Table 2. Body, Organ and Fat Tissue Weights of Dams Fed a Diet Containing Hexachlorobenzene (HCB) and Pentachlorobenzene (PECB) at 31.5 and 315 nmol/100 g Diet, Respectively, before Parturition, and Weight of Their Fetuses[a]

Dams	Pregnant[d]	Nursing[e]
	g	
Body weight	360±24[b]	287±5.0[c]
Liver weight	13.5±0.9	12.3±0.6
Kidney weight	1.58±0.15[b]	1.94±0.16[c]
Brain weight	1.92±0.03	1.97±0.06
Perirenal fat weight	3.46±0.76[b]	1.61±1.06[c]
Placenta weight	0.45±0.05	
Fetus weight[f]	3.72±0.46	

a) Values represent means±S.D., $n=4$. *b*, *c*) Within a row, values not sharing a superscript letter are significantly different at $p<0.05$. *d*) On day 1 before parturition. *e*) On day 15 after parturition. *f*) Number of fetuses of 4 pregnant rats (16+10+14+14).

Table 3. Body, Organ and Fat Tissue Weights of Suckling Pups Nursed by Dams Fed a Diet Containing Hexachlorobenzene (HCB) and Pentachlorobenzene (PECB) at 31.5 and 315 nmol/100 g Diet, Respectively, before Parturition[a]

Days after birth	2	5	11	15
		g		
Body	7.8 ±1.1[b]	11.8 ±1.3[c]	29.1 ±3.5[d]	45.7 ±2.6[e]
Stomach contents	0.47±0.09[b]	0.47±0.15[b]	1.25±0.35[c]	1.19±0.17[c]
Liver	0.32±0.05[b]	0.43±0.05[c]	0.88±0.13[d]	1.50±0.10[e]
Kidney	0.09±0.01[b]	0.15±0.03[c]	0.32±0.03[d]	0.08±0.04[e]
Brain	0.26±0.03[b]	0.42±0.04[c]	0.97±0.07[d]	1.28±0.05[e]
Perirenal fat tissue	ND[f]	0.02±0.00[b]	0.08±0.02[c]	0.19±0.04[d]

a) Values represent means±S.D., $n=8$. *b*, *c*, *d*, *e*) Within a row, values not sharing a superscript letter are significantly different at $p<0.05$. *f*) Not determined.

control groups and groups fed the diet containing HCB and PECB, respectively (Table 2). Body, organ and fat tissue weights of suckling pups after birth are shown in Table 3. Normal growth was observed from suckling pups nursed by dams fed a diet containing HCB and PECB after birth.

HCB and PECB concentrations in the blood, organs, fat tissues, placenta and fetuses of the pregnant rats on the day before parturition and the nursing rats on day 15 after parturition are shown in Table 4. In both pregnant and nursing rats, both HCB and PECB concentrations were highest in perirenal fat tissue, followed by subcutaneous fat tissue. The concentrations of these chlorinated hydrocarbons were lowest in blood and followed by organs. In the pregnant rats, the concentrations of HCB and PECB in the perirenal fat tissue were 58 and 74 times higher than those in the blood, respectively. In nursing rats, the concentrations of HCB and PECB in the perirenal fat tissues were 91 and 141 times higher than those in the blood, respectively.

HCB concentration in blood of control nursing rats was 0.32±0.12 nmol/l, but PECB in blood was not detected. HCB and PECB in blood of suckling pups fed by control nursing rats was not detected.

On day 15 after parturition, nursing rats fed the diet containing HCB and PECB only during pregnancy had significantly lower concentrations of HCB and PECB in the blood, organs and fat tissues compared with the pregnant rats ($p<0.01$). On day 15 after parturition, HCB concentrations in the liver, kidney, brain and perirenal fat tissue of nursing rats were approximately 1/5, 1/

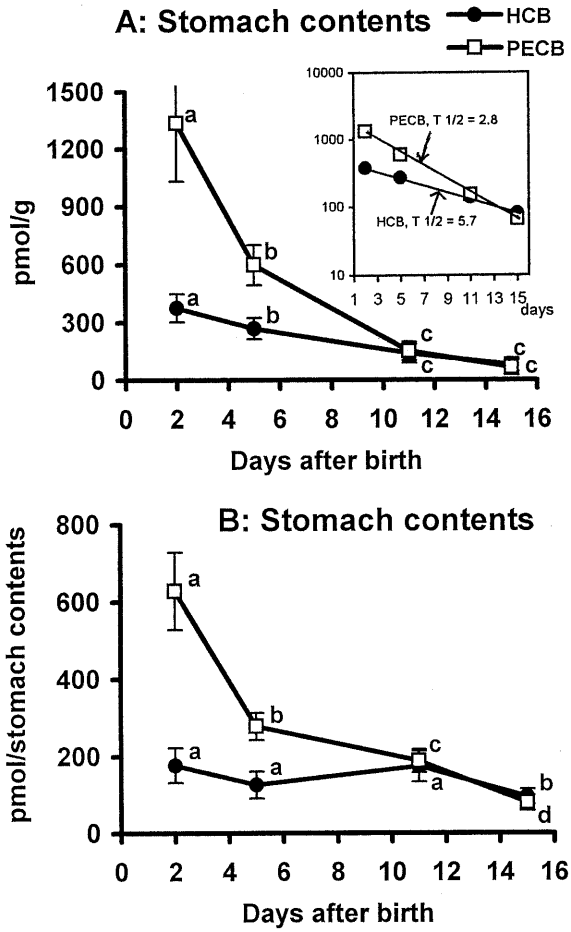

Fig. 1. Hexachlorobenzene (HCB) and Pentachlorobenzene (PECB) Levels in the Stomach Contents of Suckling Pups Nursed by Dams Fed a Diet Containing HCB and PECB at 31.5 and 351 nmol/100 g Diet, Respectively, before Parturition

A: Concentration of HCB and PECB in the stomach contents. B: Total amount of HCB and PECB in the stomach contents. On day 2, 5, 11 and 15 after birth, two suckling pups from each litter of four dams were killed. Values are mean±S.D. of 8 suckling rats. a, b, c, d: Values not sharing a superscript letter are significantly different from day 2 after birth at $p<0.05$.

Table 4. Hexachlorobenzene (HCB) and Pentachlorobenzene (PECB) Concentrations in Blood, Organ and Fat Tissue of Dams Fed a Diet Containing HCB and PECB at 31.5 and 315 nmol/100 g Diet, Respectively, before Parturition[a)]

	HCB		PECB	
Dams	Pregnant[b)]	Nursing[c)]	Pregnant[b)]	Nursing[c)]
Blood, nmol/l	21.1±5.0	3.29±1.08*	54.9±6.2	1.89±1.36*
Liver, pmol/g	25.7±6.2	5.15±2.36*	75.5±15.7	4.47±2.19*
Kidney, pmol/g	35.4±5.4	5.55±1.81*	126.2±23.3	6.08±1.77*
Brain, pmol/g	17.9±1.1	3.22±1.08*	86.2±12.7	4.95±1.03*
Perirenal fat tissue, pmol/g	1234±118	300±122*	4095±570	266±123*
Subcutaneous fat tissue, pmol/g	479±108	12.2±5.9*	1752±398	26.9±8.6*
Placenta, pmol/g	20.4±2.7		81.6±11.6	
Fetus, pmol/g	10.8±2.3		36.7±5.5	

a) Values represent means±S.D., $n=4$. *Significantly different from pregnant rats, $p<0.01$. *b)* On day 1 before parturition. *c)* On day 15 after parturition.

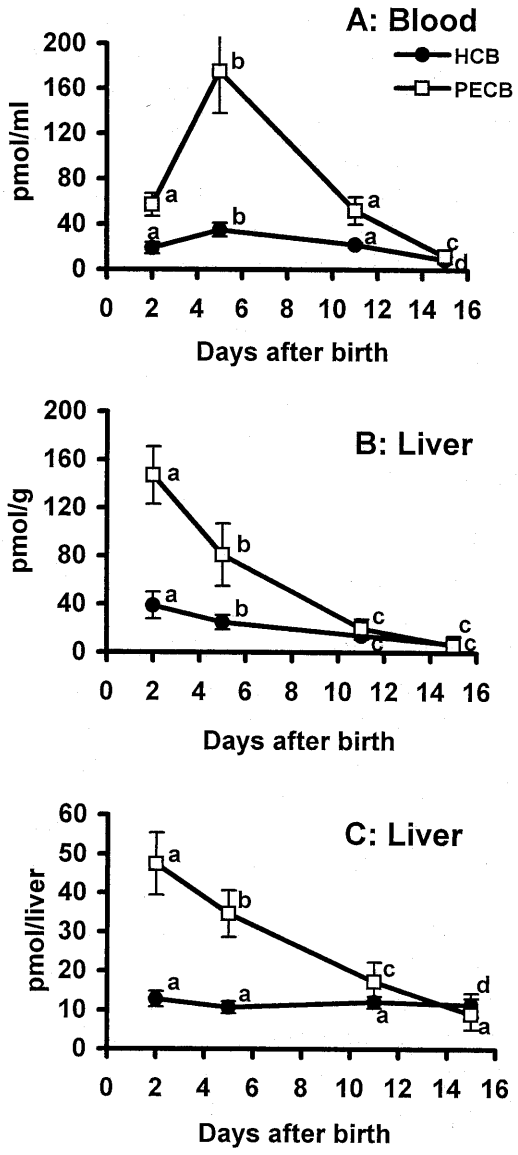

Fig. 2. Levels of Hexachlorobenzene (HCB) and Pentachlorobenzene (PECB) in the Blood and Liver of Suckling Pups Nursed by Dams Fed a Diet Containing HCB and PECB at 31.5 and 351 nmol/100 g Diet, Respectively, before Parturition

A: Concentration of HCB and PECB in the blood. B: Concentration of HCB and PECB in the liver. C: Total amount of HCB and PECB in the liver. On day 2, 5, 11 and 15 after birth, two suckling pups from each litter of four dams were killed. Values are mean ±S.D. of 8 suckling rats. a, b, c, d: Values not sharing a superscript letter are significantly different from day 2 after birth at $p<0.05$.

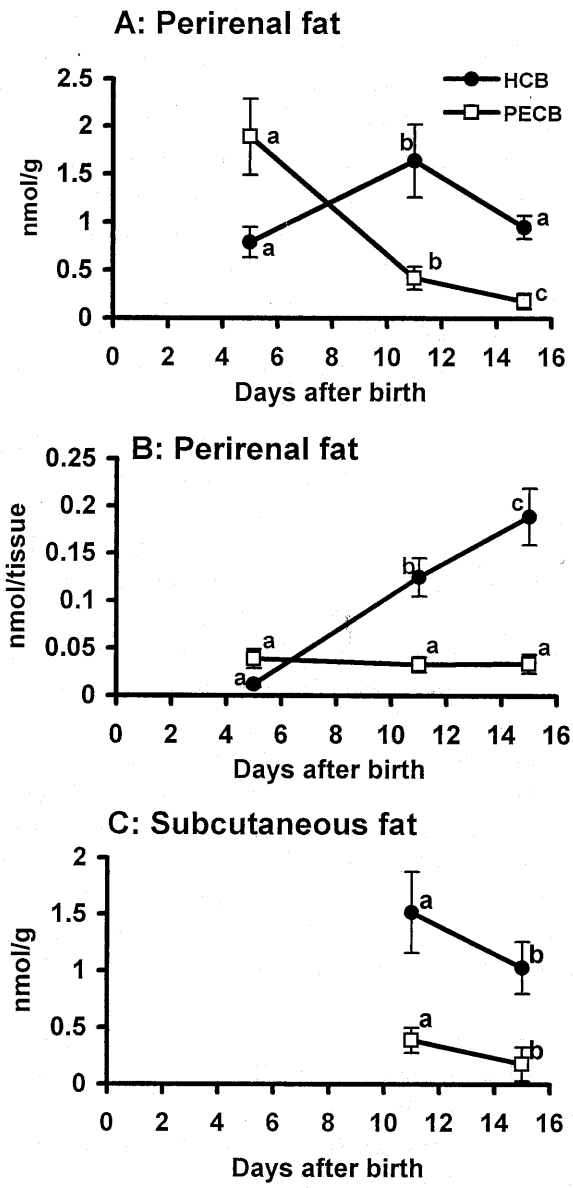

Fig. 3. Levels of Hexachlorobenzene (HCB) and Pentachlorobenzene (PECB) in the Fat Tissues of Suckling Pups Nursed by Dams Fed a Diet Containing HCB and PECB at 31.5 and 351 nmol/100 g Diet, Respectively, before Parturition

A: Concentration of HCB and PECB in the perirenal fat tissue. B: Total amount of HCB and PECB in the perirenal fat tissue. C: Concentration of HCB and PECB in the subcutaneous fat tissue. On day 5, 11 and 15 after birth, two suckling pups from each litter of four dams were killed. Values are mean±S.D. of 8 suckling rats. a, b, c: Values not sharing a superscript letter are significantly different from day 2 after birth at $p<0.05$.

6, 1/6 and 1/4 of those in pregnant rats, respectively. On the other hand, PECB concentrations in the liver, kidney, brain and perirenal fat tissue of nursing rats were approximately 1/17, 1/21, 1/17 and 1/15 of those in pregnant rats, respectively. Therefore, PECB had rapidly disappeared from the body of the nursing rats during the 15 d of lactation compared with HCB.

The HCB and PECB concentrations in the fetuses were lower than those in the blood of their dams and placenta, respectively (Table 4) ($p<0.05$). Although PECB concentration in the placenta was significantly higher than that in the blood of dams ($p<0.05$), no significant difference in HCB concentration was observed between the

placenta and the blood of dams. The possible transfer of HCB and PECB from dams to fetuses was estimated when the pregnant rats were fed the diet containing HCB and PECB from day 5 of pregnancy to the day before parturition. The average body weight and litter size of the fetuses were 3.75 g and 13.5 respectively. Since the HCB and PECB concentrations in the fetuses were 10.8±2.3 pmol/g and 36.7±5.5 pmol/g, respectively, the estimated amounts of HCB and PECB transferred from a dam to litter were about 542 pmol (40 pmol per fetus) and 1843 pmol (137 pmol per fetus), respectively. Based on the food intake (392±25 g) of the pregnant rats from day 5 of pregnancy to the day before parturition, the diet intake of HCB and PECB was 123 nmol and 1235 nmol, respectively. Therefore, the amounts of HCB and PECB transferred from dams to their fetuses were estimated to be about 0.44% and 0.15%, of the respective amounts consumed by the dams.

In order to study the transfer to their suckling pups of both HCB and PECB accumulated in dams during pregnancy through milk, the concentrations of HCB and PECB in the stomach contents, blood, liver, and perirenal and subcutaneous fat tissues of suckling pups were determined on days 2, 5, 11 and 15 after birth (Figs. 1—3). On day 2, the PECB concentration and total amount in the stomach contents were approximately 4 times higher than that of HCB (Fig. 1). However, although the HCB concentration in stomach contents decreased gradually, the PECB concentration decreased rapidly during the postnatal period. The total amounts of PECB and HCB in the stomach contents also showed the same tendency as the concentrations. On days 11 and 15 after birth, no significant differences were observed between the concentrations of HCB and PECB in the stomach contents. Therefore, the half-lives of HCB and PECB in the stomach contents of suckling pups after birth were estimated to be 5.6 d and 2.9 d, respectively.

On day 2 after birth, PECB concentration in the blood of suckling rats was 3 times higher than that of HCB, and whilst the PECB concentration increased rapidly, the HCB concentration increased gradually for 5 d after birth (Fig. 2). On day 5 after birth, the PECB concentration in blood was approximately 5 times higher than that of HCB. While the PECB concentration decreased rapidly, the HCB concentration decreased gradually. On day 15 after birth, no significant differences were observed between the HCB and PECB concentration in the blood.

On day 2 after birth, PECB concentration and the total amount of PECB in the liver was 4 times higher than that of HCB (Fig. 2). However, while the PECB concentration and the total amount of PECB decreased rapidly, the HCB concentration and the total amount of HCB decreased gradually during the postnatal days. On days 11 and 15 after birth, no significant difference was observed between the HCB and the PECB concentration in the liver. On day 15 after birth, no significant difference was observed between the total amounts of HCB and PECB in the liver.

On day 5 after birth, PECB concentration in perirenal fat tissue was 2 times higher than that of HCB (Fig. 3). However, while the PECB concentration decreased rapidly after day 5, the HCB concentration was increased on day 11 after birth and decreased on day 15 after birth. Thus HCB concentrations were 4 times higher than those of PECB on days 11 and 15 after birth ($p < 0.05$). Although the total amount of HCB in perirenal fat tissue increased rapidly after birth, that of PECB did not alter. Therefore, on days 11 and 15 after birth, the total amount of HCB in perirenal fat tissue was 5 times higher than that of PECB. In subcutaneous fat tissue, HCB concentration on days 11 and 15 after birth was respectively 4 and 5 times higher than that of PECB.

DISCUSSION

In a previous study, we had already observed that prenatal transfer of HCB to fetuses through the placenta was very small, but a large amount of HCB was transferred from dams to their suckling pups through milk in the early days after birth.[10] In this study, we investigated whether the same phenomenon could be observed with respect to PECB, which is a rapidly metabolized lipophilic chlorinated compound. Because of its lipophilic nature, PECB accumulates in the fat tissue of pregnant rats and was expected to appear in the mother's milk during lactation. In this study, pregnant rats were supplied with a diet containing both PECB and HCB. The concentration of PECB in the diet was 10 times higher than that of HCB because of the short half-life of PECB.

However, PECB concentrations in organs and fat tissues of pregnant rats were only 3 to 4 times higher than those of HCB (Table 4). Studies using young rats (7 weeks old)[9,17] have shown that the amounts of HCB and PECB excreted in the feces within 5 d were, respectively, 20% and 3% of the administrated dose. From these results, although the rats were continuously fed a diet containing small amounts of HCB and PECB during pregnancy, about 80% of the administered HCB and 97% of the PECB was retained in the body. Both HCB and PECB have been shown to metabolize to PCP, but HCB is metabolized very slowly while PECB is metabolized quite rapidly.[18,19] Umegaki and Ikegami[17] have shown that about 50% of PECB and 0.1% of HCB were metabolized to PCP during 5 d after a single dose administration of either PECB (20 mg/rat) or HCB (105 mg/kg body weight) by intragastric gavage to rats. Therefore, the ratio of PECB/HCB in pregnant rats on the day before parturition (Table 4) was suitable and consistent with those of similar experiments conducted by Umegaki *et al.* and Umegaki & Ikegami.[9,17]

Chlorinated hydrocarbons have been detected in the placenta, maternal blood and cord blood in humans,[20] and in the fat tissue of stillborn infants.[21,22] In a previous paper, 0.39% of the HCB ingested by dams was estimated to be transferred to fetuses.[10] The amount of HCB transferred from dams to fetuses in this study was estimated to be 0.44% of their total intake (Table 4). However, as the average numbers and the whole weight of fetuses in this study was larger than those of the previous study, the total amount of HCB transferred from dams to fetuses was larger in this study than the previous study. Therefore, there was no significant difference between the concentration of HCB in fetuses in this study (10.8 ± 2.3 pmol/g) and that in the previous study (10.13 ± 0.86 pmol/g). In addition, eventhough the PECB concentrations in the organs and fat tissues of the pregnant rats were 3 to 4 times higher than those of HCB (Table 4), PECB did not clearly affect the transfer of HCB to fetuses. Moreover, the concentration of PECB in fetuses was 3.4 times higher than that of HCB (Table 4). These findings suggest that these chlorinated hydrocarbons might be transferred from dams to their fetuses at the same PECB/HCB ratio that is found in the dams, passing through the placental barrier.

On day 2 after birth, PECB concentrations in the stomach contents, blood and liver of suckling pups were also 3 to 4 times higher than those of HCB shown in their dams (Figs. 1, 2 and Table 4). Therefore, the ratio of PECB/HCB in pregnant rats was the same as that in their 2-day-old suckling pups. It was considered that the ratio of PECB/HCB in the stomach contents of suckling pups was consistent with that in their mothers. Since the dams were fed a diet free of HCB and PECB after parturition, it was natural that PECB in the organs and fat tissues of the dams was rapidly metabolized and disappeared, but HCB was metabolized slowly (Table 4). After birth PECB concentration in the stomach contents of suckling pups decreased rapidly and that of HCB decreased gradually (Fig. 1). As the half-lives of HCB and PECB in adult rats were reported to be 3 months and 5 d, respectively,[17,23] PECB was considered to metabolize and excrete 18 times faster than HCB. On the other hand, as shown in this study, the disappearance of HCB and PECB in nursing rats after parturition was faster than that in non-pregnant rats, respectively. From these results it was considered that a large proportion of HCB that accumulated during pregnancy was transferred from dams to suckling pups through the milk without being metabolized in nursing rats. However, as PECB is metabolized 18 times faster than HCB, PECB accumulated during pregnancy was transferred from dams to suckling pups in lower proportions than HCB.

After birth, the amount of PECB transferred from dams to their suckling pups through the milk was larger than that of HCB (Fig. 1) in this study. However, although the amount of HCB in perirenal fat tissue of suckling pups increased after birth, as shown in our previous paper,[23] no significant increase in that of PECB was observed. The results indicated that the rate of metabolism was rapid for PECB, and very slow for HCB in suckling pups after birth.

Small amounts of organochlorine environmental pollutants including HCB, PCDDs, PCDFs, PCBs and DDT have been found in human milk from the general population.[6,22,24,25] Because of their lipophilic properties, these compounds accumulate in human fat tissue and are excreted in human milk during lactation. It has already been reported that the concentration of these pollutants in human milk decreased during the transition stage from colostrum to mature

human milk, and that the levels in human milk decreased from the first to the second child.[5] These results were confirmed by several studies in rats and monkeys treated with HCB, PCBs, PCDDs or PCDFs.[20,26-28] Furthermore, from our own studies, it was ascertained that large amount of pollutants were transferred to suckling pups through milk in the early days after birth and the accumulation disappeared rapidly from the mother's body.[10,23]

On the other hand, the HCB and PECB concentrations in the stomach contents of suckling pups were highest on day 2 after birth (Fig. 1). HCB and PECB in the blood increased during the initial 7 days after birth and then decreased (Fig. 2). While HCB concentrations in the liver and perirenal fat tissues were slowly decreased from day 2 and day 11 after birth, respectively, PECB concentration decreased rapidly after birth. Thus, the decrease of HCB and PECB concentrations in suckling pups was faster than the half-lives of these pollutants in adult rats, as reported by Rozman *et al.*[14] and our studies using non-pregnant female rats and young rats.[13,17] These results are interpreted as follows: 1) HCB and PECB were rapidly transferred from dams to their suckling pups; 2) rapidly metabolized and excreted; 3) diluted as a result of the rapid growth of suckling pups.

As we are usually exposed to small amounts of many kinds of chlorinated environmental pollutants such as PCDDs, PCDFs, PCBs, HCB, DDT and dieldrin in our foods and the environment, human breast milk also contains small amounts of these chlorinated environmental pollutants. Therefore, since many kinds of chlorinated hydrocarbons are transferred to suckling infants through milk, it may be conjectured that these chlorinated hydrocarbons influence each other. From this study, we concluded that several pollutants accumulated in dams were independently transferred to fetuses through the placenta and to suckling pups through milk.

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