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High-Fat Diet Enhances the Accumulation of Hexachlorobenzene (HCB) by Pregnant Rats during Continuous Exposure to HCB

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To investigate the influence of a high-fat diet on HCB distribution and accumulation, pregnant rats in study 1 were fed a high-fat or control diet containing HCB, and, in study 2, pregnant rats were given a single HCB dose by intragastric gavage and HCB-free high-fat or control diet. In study 1, the high-fat diet group had higher HCB concentrations in fat tissues and liver than did the controls. In study 2, although the total amounts of HCB in the fat tissue and liver were greater in the high-fat diet group than in the controls, no significant differences in HCB concentration were observed between the two groups. The high-fat diet group also showed more fecal excretion of HCB. Therefore, HCB accumulation in rats fed a high-fat diet was enhanced more by continuous exposure to HCB than by administration of a single dose.

KEYWORDS: Hexachlorobenzene; high-fat diet; pregnant rat; continuous exposure; fecal excretion

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), *p*,*p*′-dichlorodiphenyltrichloroethane (DDT), *p*,*p*′-dichlorodiphenyltrichloroethylene (DDE), hexachlorobenzene (HCB), and dieldrin in normal human organs, tissues, and breast milk has been confirmed for a considerable period of time (*3*−*5*).

In previous papers, we reported that fat tissues have an important role in the storage of lipophilic organochlorine environmental pollutants in the body, and the tissue mass increases during pregnancy and decreases during lactation (*6*−*9*). We also observed that large amounts of HCB, a stable and lipophilic organochlorine chemical, have accumulated in the fat tissues of the pregnant rats during pregnancy with the increase of fat tissue mass. However, after parturition, a large proportion of the HCB accumulated in dams disappeared during the lactation period and was transferred to their pups through the milk in the early days after birth. Therefore, it is important to reduce the amount of HCB accumulated in the fat tissue during pregnancy.

Dale et al. (*10*), Mitjavila et al. (*11*), and Wyss et al. (*12*) have reported that the amount of highly lipophilic chemicals in fat tissue decreased with food restriction or starvation and that their mobilization from fat tissues, metabolism, and excretion were accelerated at the same time. We have also observed that the metabolism and excretion of pentachlorobenzene (PECB), a relatively metabolizable, lipophilic organochlorine chemical, were increased in young rats by restricted feeding, a fish oil diet, and a viscous indigestible polysaccharide diet (*6*, *7*, *10*−*14*). The enhanced metabolism and excretion of PECB were due to the small mass of fat tissue that resulted from those treatments. These results suggest that diet or food components that alter the fat tissue mass might affect the distribution, metabolism, and accumulation of the chemicals.

Because dietary fat influences fat tissue mass, it was considered that a high-fat diet might influence the mobilization, metabolism, excretion, and accumulation of these lipophilic chemicals. Moreover, because of the lipophilic nature of HCB, it was expected that its absorption and accumulation in the fat tissues of pregnant rats would be influenced by a high-fat diet. In study 1, we compared the distribution and amount of HCB accumulated in the pregnant rats fed the control or a high-fat diet containing a small amount of HCB (continuous exposure) during pregnancy. In this study, cellulose was added to the high-fat diet to maintain a constant metabolizable energy concentration. However, we have reported previously in a study using young rats that the dietary fibers in diet decreased both the fat tissue mass and the absorption of lipophilic organochlorine chemicals (*6*, *12*, *14*). Therefore, it was considered that the large amount of cellulose in a high-fat diet might influence both the fat tissue mass and the absorption of HCB. In study 2, we determined the amount of HCB excreted in the feces of the

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Table 1. Composition of the Experimental Diets[a]

ingredient	control diet[b]	high-fat diet[c]
casein	20.0	20.0
L-cystine	0.3	0.3
cornstarch	39.7486	
dextrin	13.2	
sucrose	10.0	
glucose	15.0	
soybean oil	7.0	28.3
cellulose	5.0	31.6486
AIN mineral mixture[d]	3.5	3.5
AIN vitamin mixture[e]	1.0	1.0
choline bitartrate	0.25	0.25
tert-butylhydroquinone	0.0014	0.0014

[a] Composition for all ingredients is given in grams per 100 g of diet. [b] Diets were prepared according to the recommendation of the American Institute of Nutrition (AIN-93G). [c] Carbohydrates in the high-fat diet were replaced by soybean oil and glucose with minimal but adequate levels. Cellulose was added to the diet to maintain a constant metabolizable energy concentration. [d,e] Mineral and vitamin mixtures were based on the AIN-93 formulation.

pregnant and nonpregnant rats fed the control or a high-fat diet without HCB but given a single dose of HCB by intragastric gavage. Furthermore, we compared the amount and concentration of HCB accumulated in pregnant rats of study 1 (continuously exposed to a small amount of HCB) with that in pregnant rats of study 2 (a single dose of HCB).

MATERIALS AND METHODS

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

Diets. The compositions of the control and high-fat diets are shown in **Table 1**. The control diet composition was based on the AIN-93G diet (*15*). Basically, the decision regarding the glucose content of the high-fat diet was based on previous work done by Lanoue and Koski (*16*). Carbohydrates in the high-fat diet with minimal but adequate levels of carbohydrate (15%) were substituted for soybean oil and glucose. Cellulose was added to the high-fat diet to maintain a constant metabolizable energy concentration. Both diets supplied 3.95 kcal/g of diet. In study 1, pregnant rats were treated with the minimum level of HCB (35.1 nmol/100 g of diet, 10 μg/100 g of diet) that would subsequently produce a detectable organ concentration of HCB. The diets containing HCB were prepared by dissolving HCB (3.51 mol/L of ethanol) in soybean oil.

Animals. Sperm-positive pregnant rats of the Sprague−Dawley strain (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 °C) and illuminated according to a 12-h light/dark cycle. Rats were given free access to food and distilled water. Rats were weighed, and daily food intake was measured at least four times weekly during the experimental period.

S**tudy 1.** Eight pregnant rats were used in this study. They were divided into two groups (control and high-fat diet groups) of four rats each. Control and high-fat diet groups were fed the control and high-fat diet containing HCB, respectively, during pregnancy. On the day before parturition, all rats were anesthetized with ether and killed by cardiac puncture. For the analysis of HCB, blood samples were obtained using heparinized syringes, and organs, tissues, placentas, and fetuses were dissected and weighed.

Study 2. Twelve pregnant rats were used in this study. The pregnant rats were divided into two groups (control and high-fat diet groups), respectively, of six rats each. The control and high-fat diet groups of pregnant rats were fed the control and high-fat diet without HCB, respectively, for 15 days. Five days before being sacrificed, all rats were given by intragastric gavage a single dose of HCB (351 nmol, 100 μg) dissolved in 0.5 mL of soybean oil. Feces were collected for 5 days after the administration of HCB. On the day before parturition, all rats were anesthetized with ether and killed by cardiac puncture. Using heparinized syringes, blood samples were obtained, and organs, fat tissues, placentas, and fetuses were dissected, weighed, and stored at −20 °C. All procedures were in accordance with the National Institute of Health and Nutrition guidelines for the care and use of laboratory animals.

Table 2. Body and Organ Weights of Pregnant Rats Fed the Control or High-Fat Diets Containing HCB at 35.1 nmol/100 g of Diet during Pregnancy[a]

	control diet	high-fat diet
body wt (g)	361 ± 22	356 ± 20
liver (g)	13.56 ± 0.53	14.18 ± 0.77
kidney (g)	1.55 ± 0.08	1.51 ± 0.09
perirenal fat tissue (g)	3.42 ± 0.60a	4.67 ± 0.66b
placenta (g)	0.50 ± 0.07	0.48 ± 0.08
fetus (g)	3.96 ± 0.22	3.75 ± 0.21

[a] All values represent mean ± SD, $n = 4$. Number of fetuses: control (13 + 12 + 16 + 10), high-fat (17 + 11 + 12 + 12). Within a row, values not sharing a letter are significantly different at $P < 0.05$.

Analytical Methods. Blood (3 mL) was mixed with 5 mL of distilled water. Organs were homogenized with 4 volumes of water. Feces were dried, weighed, and pulverized. HCB in the samples was extracted with *n*-hexane. To extract HCB, fat tissues were homogenized with 25 volumes of *n*-hexane. The HCB in *n*-hexane was cleaned by Florisil column chromatography (0.5 g of Florisil layered on 0.2 g of $NaSO_4$). HCB was analyzed using a Shimadzu PARVUM QP-5000 gas chromatograph−mass spectrometer (Shimadzu, Kyoto, Japan) as described previously (*7*).

Hepatic lipids were extracted according to the methods of Folch et al. (*17*). Triacylglyceride concentration was measured using a triglyceride test kit (Wako Pure Chemical Industries).

Statistical Analysis. Data are presented as the mean ± SD of each group. Statistical analysis was conducted by ANOVA. Differences in mean values among groups were tested by Duncan's multiple-range test. Student's *t* test was used for all pairwise comparisons. Differences were considered to be significant at $P < 0.05$. The Yukmus computer program (Yukmus, Tokyo, Japan) was used for statistical analysis of the data.

RESULTS

Study 1. Body and organ weights on the day before parturition of pregnant rats fed the control and high-fat diets containing HCB were shown in **Table 2**. The high-fat diet did not influence the body and organ weights of pregnant rats or the fetus weight. However, the perirenal fat tissue weight of pregnant rats fed the high-fat diet was higher than that of rats fed the control diet.

Food and HCB intakes from day 5 of pregnancy to the day before parturition were 281 ± 10 g and 99.6 ± 4.8 nmol, respectively, in the pregnant rats fed the control diet containing HCB and 274 ± 16 g and 96.2 ± 5.7 nmol, respectively, in those fed the high-fat diet containing HCB. Therefore, there were no significant differences in food and HCB intakes between the pregnant rats fed the control and high-fat diets.

On the day before parturition, the HCB concentration was highest in the fat tissues of the pregnant rats fed the diet containing HCB (**Table 3**). It was considered that a large amount of HCB had accumulated in the fat tissues of the pregnant rats during pregnancy. The HCB concentration in the perirenal and subcutaneous fat tissues and liver and the total amounts of HCB in the perirenal fat tissue and liver of the pregnant rats fed the high-fat diet containing HCB were significantly higher than

Table 3. HCB Levels in the Blood, Organs, and Fat Tissues of Pregnant Rats Fed the Control or High-Fat Diets Containing HCB at 35.1 nmol/100 g of Diet during Pregnancy[a]

	control diet	high-fat diet
blood (nmol/L)	12.36 ± 1.29	12.86 ± 2.01
liver		
concentration (pmol/g)	32.38 ± 2.52a	39.28 ± 3.23b
total amount (pmol/organ)	429.3 ± 25.4a	556.8 ± 23.9b
kidney		
concentration (pmol/g)	33.87 ± 4.23	31.95 ± 4.79
total amount (pmol/organ)	52.52 ± 5.34	48.26 ± 5.08
perirenal fat tissue		
concentration (nmol/g)	1.01 ± 0.13a	1.44 ± 0.12b
total amount (nmol/tissue)	3.44 ± 0.55a	6.65 ± 0.86b
subcutaneous fat tissue (nmol/g)	0.35 ± 0.04a	0.49 ± 0.05b
placenta		
concentration (pmol/g)	18.73 ± 3.07	24.88 ± 3.35
total amount (pmol/organ)	9.39 ± 2.86	11.93 ± 3.11
fetus		
concentration (pmol/g)	2.55 ± 0.32a	3.54 ± 0.26b
total amount (pmol/fetus)	10.10 ± 0.82a	13.28 ± 0.97b

[a] All values represent mean ± SD, $n = 4$. Within a row, values not sharing a letter are significantly different at $P < 0.05$. On day 1 before parturition. Number of fetuses: control (13 + 12 + 16 + 10); high-fat (17 + 11 + 12 + 12).

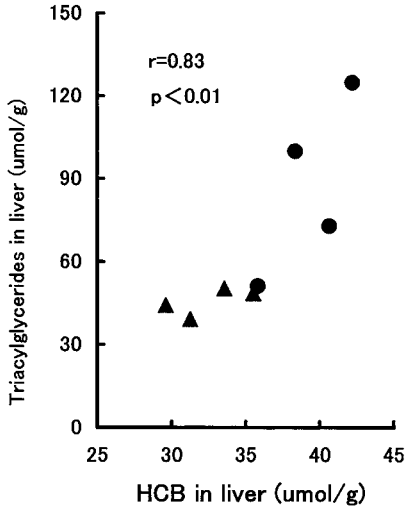

Figure 1. Correlation between the concentrations of hexachlorobenzene (HCB) and triacylglycerides in the liver of pregnant rats fed the control (▲) and high-fat diets containing HCB (●). Each data point represents one rat.

those of the pregnant rats fed the control diet ($P < 0.05$). However, no significant differences in the HCB concentration in blood, kidney, and placenta or the total amount of HCB in kidney and placenta were observed between the pregnant rats fed the control diet and those fed the high-fat diet.

Triacylglyceride concentration in the liver of the pregnant rats fed the high-fat diet containing HCB was significantly higher than that of rats fed the control diet. Relationships between the concentrations of HCB and triacylglycerides in liver were examined. As shown in **Figure 1**, a marked positive correlation was noted between the concentrations of HCB and triacylglyceride.

HCB was also detected in the fetuses of both the control and high-fat diet groups. However, the HCB concentration in fetuses of both groups was significantly lower than those found in the blood and placenta of their respective dams ($P < 0.05$). With the ingestion of HCB from day 5 of pregnancy to the day before parturition, the amounts of HCB transferred from dams to fetuses were estimated to be about 0.13 and 0.18% of the amounts consumed by the dams fed the control and high-fat diets, respectively. Therefore, the HCB concentration and the total amount of HCB (the amount of HCB transferred from a dam to her litter) in fetuses in the high-fat diet group were significantly higher than those in the control group ($P < 0.05$).

Table 4. Body and Organ Weights of Pregnant Rats Fed the Control or High-Fat Diet without HCB but Given a Single Dose of 100 μg of HCB 5 Days before Sacrifice[a]

	control diet	high-fat diet
body wt (g)	354 ± 19	366 ± 24
liver (g)	13.15 ± 0.49	14.51 ± 0.97
kidney (g)	1.50 ± 0.07	1.63 ± 0.09
perirenal fat tissue (g)	3.27 ± 0.53a	4.58 ± 0.56b
placenta (g)	0.49 ± 0.06	0.47 ± 0.10
fetus (g)	3.66 ± 0.21	3.83 ± 0.29

[a] All values represent mean ± SD, $n = 6$. Number of fetuses: control diet group (17 + 13 + 13 + 10 + 12 + 9); high-fat diet group (16 + 13 + 14 + 12 + 10 + 12). Within a row, values not sharing a letter are significantly different at $P < 0.05$.

Table 5. HCB Levels in Blood, Organs, and Fat Tissues of Pregnant Rats Fed the Control or High-Fat Diet without HCB but Given a Single Dose of 100 μg of HCB 5 Days before Sacrifice[a]

	control diet	high-fat diet
blood (nmol/L)	24.56 ± 2.34	23.90 ± 2.20
liver		
concentration (pmol/g)	122.0 ± 27.4a	164.2 ± 23.7b
total amount (pmol/organ)	1604 ± 238a	2383 ± 305b
kidney		
concentration (pmol/g)	76.35 ± 11.23a	94.66 ± 8.80b
total amount (pmol/organ)	116.2 ± 14.3a	152.3 ± 12.5b
perirenal fat tissue		
concentration (nmol/g)	5.65 ± 0.40	5.95 ± 0.31
total amount (nmol/tissue)	18.6 ± 1.9a	27.3 ± 1.5b
subcutaneous fat tissue (nmol/g)	3.24 ± 0.27	3.23 ± 0.35
placenta		
concentration (pmol/g)	37.46 ± 4.14	43.82 ± 5.30
total amount (pmol/organ)	16.35 ± 1.93	20.63 ± 2.54
fetus		
concentration (pmol/g)	9.58 ± 1.92	11.34 ± 2.52
total amount (pmol/fetus)	35.04 ± 6.63	43.63 ± 8.73

[a] All values represent mean ± SD, $n = 6$. On day 1 before parturition. HCB in placenta and fetus, $n = 6 \times 3$. Within a row, values not sharing a letter are significantly different at $P < 0.05$. Number of fetuses: control diet group (17 + 13 + 13 + 10 + 12 + 9); high-fat diet group (16 + 13 + 14 + 12 + 10 + 12).

Study 2. The high-fat diet did not influence the body weight and the weights of the liver, kidney, placenta, and fetuses of pregnant rats (**Table 4**). However, the perirenal fat tissue weight of pregnant rats fed the high-fat diet was significantly higher than that of rats fed the control diet ($P < 0.05$).

No significant difference in the HCB concentration in blood was observed between the groups fed the control and high-fat diets (**Table 5**). The concentration and total amount of HCB in the liver and kidney of pregnant rats fed the high-fat diet were higher than that of pregnant rats fed the control diet ($P < 0.05$). In the perirenal fat tissue, although the total amount of HCB of pregnant rats fed the high-fat diet was higher than that of rats fed the control diet ($P < 0.05$), no significant difference in HCB concentration was observed between the rats fed the control and high-fat diets. No significant difference in subcutaneous fat tissue HCB concentration was observed between the rats fed the control and high-fat diets.

No significant difference in the concentration and total amount of HCB in placenta and fetus was observed between the pregnant rats fed the control and high-fat diets. The HCB concentration

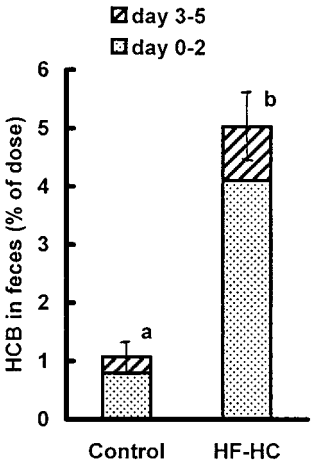

Figure 2. Fecal excretion of hexachlorobenzene (HCB) by pregnant rats fed the control and high-fat diets without HCB during 5 days after a single dose of 100 μg of HCB. Values are means ± SD. Values not sharing a letter are significantly different ($P < 0.05$).

in fetuses of both groups was significantly lower than those found in the blood and the placenta of their respective dams ($P < 0.05$). In total, the amounts of HCB transferred from dams to their fetuses were estimated to be about 0.12 and 0.15% of the dose given to the dams fed the control and high-fat diets, respectively.

The cumulative fecal excretion of HCB for 5 days after the administration of HCB is shown in **Figure 2**. A large part of HCB was excreted in the feces during 2 days after administration. The HCB excretion of pregnant rats fed the high-fat diet was higher than that of corresponding rats fed the control diet ($P < 0.05$). However, the amount of HCB excreted from the pregnant rats fed the control and high-fat diets was <5% of the dose administered.

DISCUSSION

In studies 1 and 2, we used HCB as an example of a stable lipophilic environmental pollutant. In previous papers (*8*, *9*, *18*, *19*), we have already reported that the HCB accumulated in dams during pregnancy was transferred to their suckling pups through milk in the early days after birth. In these previous papers and in study 1, pregnant rats were treated with the minimum level of HCB (35.1 nmol/100 g of diet; HCB intakes of the control and high-fat diet groups were 99.6 ± 4.8 and 96.2 ± 5.7 nmol, respectively) that would subsequently produce a detectable organ concentration of HCB. Therefore, it was expected that the biological effect of HCB on the dams would be very weak. HCB has been reported to be hepatotoxic and immunotoxic and to affect thyroid hormone homeostatis (*20*−*23*). Although the present study did not confirm such negative effects, it is important to address the question of whether prenatal or postnatal exposure to HCB produces long-term harmful effects.

To determine the fecal excretion of HCB, pregnant rats in study 2 received a single dose of 100 μg (351 nmol) of HCB by intragastric gavage. The amount was ~3.5−3.7 times higher than the HCB intake of the pregnant rats in study 1. However, in the present study there is no evidence that the small amount of HCB harmed the pregnant rats. Moreover, the amount of HCB of the pregnant rats fed the high-fat diet that contains large amounts of cellulose and soybean oil was higher than that of pregnant rats fed the control diet (**Figure 2**).

In studies 1 and 2, we observed that the total amount of HCB accumulated in the fat tissues and liver of the pregnant rats fed the high-fat diet was higher than that of the rats fed the control diet (**Tables 3** and **5**). In a previous paper, we have already noted a marked positive correlation between the relative fat tissue weight and the distribution of trichloroethylene in perirenal and epididymal fat tissue (*24*). In this study, because of the increase of these fat tissue weights of the pregnant rats fed the high-fat diet (**Table 5**), it was considered that large amounts of HCB had accumulated in these fat tissues of the pregnant rats. Ikegami et al. (*6*) and Umegaki et al. (*7*) had already reported that a small fat tissue mass, compared with a large mass, could enhance the metabolism and excretion of PECB. Similar observations were made by Birnbaum (*25*) using hexachlorobiphenyl and by Decad et al. (*26*) using 2,3,7,8-tetrachlorobenzofuran.

On the other hand, when the fat tissue mass is small, a large amount of HCB that did not accumulate in fat tissues is mobilized into the blood and liver. However, a positive correlation was observed between the concentrations of HCB and triacylglycerides in liver (**Figure 1**). The triacylglyceride concentration in the liver of the pregnant rats fed the high-fat diet was higher than that of rats fed the control diet. It has been also reported that the concentration of triacylglycerides in the liver correlated with an increase in the amount of residues of 1,1′-(2,2,2-trichloroethylidene)bis(4-chlorobenzene) (DDT) and dieldrin in the liver (*27*). These findings indicate that the triacylglyceride concentration in the liver determined the concentration of lipophilic chemicals in the organ. This may be related to the high solubility of the lipophilic chemicals in the organ. Further study will be needed to clarify the toxicological meaning of the presence of high concentrations of both lipophilic chemicals and triacylglycerides in the liver.

Ando et al. (*5*) reported that HCB was detected in all preparations of human placenta, maternal blood, and cord blood from a group of general Japanese subjects and fetuses that were exposed to the lipophilic chlorinated compounds passing through the placental barrier. In the present study 0.12−0.18% of HCB ingested by dams was detected in the fetuses of both the control and high-fat diet groups, although the HCB concentration in fetuses of both groups was lower than those found in the blood and placentas of their dams (**Tables 3** and **5**). Abbott et al. (*28*) and Quinby et al. (*29*) reported that chlorinated compounds were detected in the fat tissue of stillborn infants. The amount of HCB transferred to the fetus was much smaller than that to the suckling pups through milk (*8*, *18*, *19*), suggesting that fetuses were also exposed to lipophilic chlorinated compound passing through the placental barrier. Furthermore, the amount of HCB accumulated in fetuses of pregnant rats fed the high-fat diet seemed to be higher than that in fetuses of pregnant rats fed the control diet.

In previous papers, we observed that dietary fiber, especially viscous indigestible polysaccharides, decreased the accumulation of lipophilic organochlorine chemicals in the body by reducing the absorption of these compounds and the fat tissue mass (*6*, *24*). We fed pregnant rats a high-fat diet to which was added a large amount of cellulose to maintain a constant metabolizable energy concentration in this study. We obtained the result that the amount of HCB accumulated in fat tissues of the high-fat diet group was higher than that of the control diet group (**Tables 3** and **5**). Because dietary fiber and lipids such as mineral oil (*31*) influence the lipophilic chlorinated compound absorption, we examined the fecal excretion of HCB in pregnant rats fed the control and high-fat diets. The amount of HCB excreted in the feces of rats fed the high-fat diet was 5−6 times higher than that of rats fed the control diet (**Figure 2**). Thus, the high-

fat diet containing a large amount of cellulose decreased the absorption of HCB. HCB absorptions by the groups fed the control and high-fat diets were about 99 and 95%, respectively.

As shown in **Figure 2**, we observed greater fecal excretion of HCB in the pregnant rats fed the high-fat diet, but the fecal excretion of HCB for 5 days after the administration of HCB was only 5% of the administered amount. We had no data concerning the metabolism of HCB by intestinal microorganisms. However, Rozman et al. (*31*) reported that HCB is very stable, having a half-life of >3 months in rats. Therefore, we considered that >95% of the administered HCB crossed the gastrointestinal wall unchanged and was transported via the blood stream to be accumulated in fat tissue due to its lipophilic property.

The HCB concentration in the fat tissues and liver of pregnant rats fed the high-fat diet containing HCB was higher than that of the control rats (**Table 3**). On the other hand, no significant differences in the HCB concentrations were observed between the rats fed an HCB-free high-fat or control diet and instead given HCB in one dose 5 days before being sacrificed (**Table 5**). Therefore, in rats fed the high-fat diet, it was considered that a low-level continuous exposure to HCB led to the accumulation of a larger amount of HCB than did a single exposure. The following explanation was considered: when a large amount of triacylglycerides was accumulated in tissues such as fat tissues and the liver, the tissue's capacity to store HCB was increased and the mobilization of the accumulated lipophilic chemicals into the blood was reduced. As a result, the accumulation of HCB in tissues that contained the large amount of triacylglycerides was increased.

Many lipophilic organochlorine chemicals are widely distributed in the global ecosystem, and most people are exposed to these chemicals, especially through the consumption of fish, meat, and milk. After a prolonged exposure to these chemicals, absorbed chemicals accumulate for a long time without being metabolized in the tissues that contain triacylglycerides. Although the biological effect of HCB on the dams was very weak in the present study, further study is needed to address the question of whether prenatal exposures to HCB produce long-term harmful effects. This study demonstrated that the amount of HCB accumulated in dams fed the high-fat diet containing HCB during pregnancy was higher than that of dams fed the control diet. Although prenatal transfer of HCB to rat fetuses was very small, further study will be required to determine the risk factors associated with the transfer and accumulation of these chemicals in fetuses.

LITERATURE CITED

(1) Miyata, H. Real situation and problem of environmental, human and food exposure to dioxin related compounds. *J. Food Hyg. Soc. Jpn.* **1993**, *34*, 1−11 (in Japanese).

(2) Paul, M., Ed. *Polyhaloid Biphenyl, Occupational and Environmental Reproductive Hazards for Authors and Editors*; Williams and Wilkins: Baltimore, MD, 1993.

(3) Hashimoto, S.; Yamamoto, T.; Yasuhara, A.; Morita, M. PCDD, PCDF, planar and other PCB levels in human milk in Japan. *Chemosphere* **1995**, *31*, 4067−4075.

(4) Ando, M.; Transfer of 2,4,5,2′,4′,5′-hexachlorobiphenyl and 2,2-bis(*p*-chlorophenyl), 1,1,1-trichloroethane (*p,p*′-DDT) from maternal to newborn and suckling rats. *Arch. Toxicol*. **1978**, *41*, 179−186.

(5) Ando, M.; Hirano, S.; Itoh, I. Transfer of hexachlorobenzene (HCB) from mother to new-born baby through placenta and milk. *Arch. Toxicol*. **1985**, *56*, 195−200.

(6) Ikegami, S.; Umegaki, K.; Kawashima, Y.; Ichikawa, T. Viscous indigestible polysaccharides reduce accumulation of pentachlorobenzene in rats. *J. Nutr*. **1994**, *124*, 754−760.

(7) Umegaki, K.; Ikegami, S.; Ichikawa, T. Fish oil enhanced pentachlorobenzene metabolism and reduces its accumulation in rats. *J. Nutr.* **1995**, *125*, 147−153.

(8) Nakashima, Y.; Ohsawa, S.; Umegaki, K.; Ikegami, S. Hexachlorobenzene accumulated by dams during pregnancy is transferred to suckling rats during early lactation. *J. Nutr*. **1997**, *127*, 648−654.

(9) Nakashima, Y.; Ikegami, S. Hexachlorobenzene and pentachlorobenzene accumulated during pregnancy is transferred to pups at the accumulation ratio in dams. *J. Health Sci.* **2000**, *46*, 89−97.

(10) Dale, E.; Gains, T. B.; Hayes, W. J., Jr. Storage and excretion of DDT in starved rats. *Toxicol. Appl. Pharmacol*. **1962**, *4*, 89−106.

(11) Mitjavila, S.; Carrera, G.; Fernandetz, Y. Evaluation of the toxic risk of accumulated DDT in the rats: During fat mobilization. *Arch. Environ. Contam. Toxicol*. **1981**, *10*, 471−481.

(12) Wyss, P. A.; Muhlebach, S.; Bickel, M. H. Pharmacokinetics of 2,2′,4,4′,5,5′-hexachlorobiphenyl (6-CB) in rats with decreasing adipose tissue mass. *Drug Metab. Dispos*. **1982**, *10*, 657−661.

(13) Umegaki, K.; Ikegami, S.; Ichikawa, T. Effect of restricted feeding on the absorption, metabolism and accumulation of pentachlorobenzene in rats. *J. Nutr. Sci. Vitaminol*. **1993**, *39*, 11−21.

(14) Umegaki, K.; Ikegami, S.; Itoh, T.; Ichikawa, T. Effects of food restriction on distribution, accumulation and excretion of pentachlorobenzene and hexachlorobenzene in rats. *J. Food Hyg. Soc. Jpn*. **1993**, *34*, 404−410.

(15) American Institute of Nutrition. AIN-93 purified diet for laboratory rodents; final reports of the American Institute of Nutrition ad hoc writing committee on the reformation of the AIN-73A rodent diet. *J. Nutr*. **1993**, *123*, 1939−1951.

(16) Lanoue, L.; Koski, K. G. Glucose-restricted diets alter milk composition and mammary gland development in lactating rat dams. *J. Nutr*. **1994**, *124*, 94−102.

(17) Folch, J.; Lees, M.; Sloane Stanley, G. H. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem*. **1957**, *226*, 497−509.

(18) Nakashima, Y.; Ohsawa, S.; Umegaki, K.; Ikegami, S. Masking of guar gum-induced accumulation of hexachlorobenzene excretion by its rapid excretion through lactation in adult female. *J. Agric. Food Chem.* **1998**, *46*, 2241−2247.

(19) Nakashima, Y.; Ohsawa. S.; Ikegami, S. High-fat diet enhances accumulation of hexachlorobenzene in rat dams and delays its transfer from rat dams to suckling pups through milk. *J. Agric. Food Chem.* **1999**, *47*, 1587−1592.

(20) Besten, D. C.; Bennik, M. H. J.; Bruggeman, I.; Schielen, P.; Kuper, F.; Brouwer, A.; Koeman, J. H.; Vos, J. G.; Van Bladeren, P. J. The role of oxidative metabolism in hexachlorobenzene-induced porphyria and thyroid hormone homeostasis: a comparison with pentachlorobenzene in a 13-week feeding study. *Toxicol. Appl. Pharmacol*. **1993**, *119*, 181−194.

(21) Carlson, G. P.; Tardiff, R. G. Effect of chlorinated benzenes on the metabolism of foreign organic compounds. *Toxicol. Appl. Pharmacol.* **1976**, *36*, 383−394.

(22) Courtnay, K. D.; Copeland, M. F.; Robbins, A. The effect of pentachloronitrobenzene, hexachlorobenzene and related compounds on fetal development. *Toxicol. Appl. Pharmacol*. **1976**, *35*, 239−394.

(23) Rush, G. F.; Smith, J. H.; Maita, K.; Bleavins, M.; Aurelich, R. J.; Ringer, R. K.; Hook, J. P. Prenatal hexachlorobenzene toxicity in the milk. *Environ. Res*. **1983**, *31*, 116−124.

(24) Nakashima, Y.; Ikegami, S. Guar gum induces trichloroethylene (TCE) accumulatation in the body by reducing TCE absorption and fat tissue mass. *J. Agric. Food Chem.* **2001**, *49*, 3499−3505.

(25) Birnbaum, I. S. Distribution and excretion of 2,3,6,2′,3′,6′- and 2,4,5,2′,4′,5′-hexachlorobiphenyl in senescent rats. *Toxicol. Appl. Pharmacol*. **1983**, *70*, 262−272.

(26) Decad, G. M.; Birnbaum, L. S.; Matthews, H. B. Distribution and excretion of 2,3,7,8-tetrachlorobenzofrane in C57BL/6J and DBA/2J mice. *Toxicol. Appl. Pharmaco*l. **1981**, *59*, 564−573.
(27) Ando, M. Studies of the effect of dietary protein and fat content upon DDT metabolism in rat liver. *J. Toxicol. Environ. Health* **1982**, *10*, 11−22.
(28) Abbott, D. C.; Goulding, R.; Tatton, J. O. G. Organochlorine pesticide residues in human fat in Great Britain. *Br. Med. J.* **1968**, 111, 146−154.
(29) Quinby, G. H.; Armstrong, J. F.; Durham, W. F. DDT in human milk. *Nature* **1965**, *207*, 726−728.
(30) Rozman, K.; Rozman, T.; Grain, H. Enhanced fecal elimination of stored hexachlorobenzene from rats and rhesus monkeys by hexadecane or mineral oil. *Toxicology* **1981**, *22*, 33−44.
(31) Rozman, K.; Rozman, T.; Grain, H. Stimulation of nonbiliary intestinal excretion of hexachlorobenzene in rhesus monkeys by mineral oil. *Toxicol. Appl. Pharmacol.* **1983**, *70*, 255−261.

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