Masking of Guar Gum-Induced Acceleration of Hexachlorobenzene Excretion by Its Rapid Excretion through Lactation in Adult Female Rats

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The effect of dietary fiber and polyunsaturated fatty acids on the excretion of an ingested stable, lipophilic environmental pollutant was investigated in nonpregnant and pregnant rats fed hexachlorobenzene (HCB) diet (10 μg/100 g of diet). Nonpregnant rats ingesting guar gum, with polyunsaturated fatty acid for 4 weeks after an HCB diet period for 2 weeks, had lower perirenal fat mass as well as total amount of HCB found in such fat compared with cellulose-ingesting groups ($P < 0.05$). A large proportion of HCB in dams disappeared from the dams during the 15-day lactation period and was transferred to their suckling pups through the milk in both dietary fiber groups. No significant difference in total amount of HCB in suckling pups was observed whether the dams ingested cellulose or guar gum diet during the lactation period. It was concluded that the excretion of HCB into milk was so rapid that the guar gum-induced acceleration of its excretion from the dams were masked.

Keywords: *Hexachlorobenzene; dietary fiber; lactation; suckling pups; milk*

INTRODUCTION

Many lipophilic chlorinated hydrocarbons, such as polychlorinated dibenzo-*p*-dioxins (PCDD), polychlorinated dibenzofurans (PCDF), polychlorinated biphenyls (PCB), and 1,1,1-trichloro-2,2-bis(*p*-chlorophenylethane) (DDT) are pollutants that have been found widely distributed in the global ecosystem (Miyata, 1983). Moreover, people are regularly exposed to these substances, especially through the consumption of fish, meat, and milk (Takayama et al., 1991; Paul, 1993). After absorption, these chemicals tend to accumulate in fat tissue and are retained for a long time without being metabolized (Yakusiji et al., 1984; Philips et al., 1989; Hashimoto et al., 1995).

We observed that the metabolism and excretion of a lipophilic and relatively metabolizable pentachlorobenzene (PECB) are markedly enhanced in rats fed a restricted diet containing fish oil or viscous dietary fiber (Ikegami et al., 1994; Umegaki et al., 1993, 1995). We concluded that such enhanced excretion of PECB was due to the reduction in fat tissue mass that resulted from this treatment. In a previous paper (Nakashima et al., 1997), we also showed that hexachlorobenzene (HCB) accumulated in dams during pregnancy was transferred to their suckling pups through milk in the early days after birth. In that study, we used HCB as an example of a stable lipophilic environmental pollutant. HCB is chemically stable and metabolized very slowly, through a well-known pathway. A previous study indicated the involvement of cytochrome P-450 3A in the microsomal oxidation of HCB to pentachlorophenol (PCP) and to tetrachlorobenzoquinone (TCBQ) (Van Ommen et al., 1989). PECB is also relatively rapidly metabolized and is oxidized to the same products as in the metabolism of HCB, that is, PCP and TCBQ (Besten et al., 1989; Umegaki and Ichikawa, 1989). Both compounds have been reported to be hepatotoxic and immunotoxic and to affect thyroid hormone homeostasis (Carlson and Tardiff, 1976; Vos et al., 1979; Besten et al., 1993).

Therefore, in the first experiment, we investigated whether ingestion of dietary fibers and polyunsaturated fatty acids would influence both fat tissue mass and the enhanced metabolism and excretion of HCB in nonpregnant rats. We observed previously that fish oil-fed rats had a smaller epididymal fat tissue mass compared with lard- or soybean oil-fed rats (Umegaki et al., 1995). Furthermore, in this study, we designed the fatty acid composition of the experimental diets using docosahexaenoic acid (DHA) and γ-linolenic acid, as shown in Table 2, and examined the influence of dietary fatt acid on the fat tissue mass and accumulation of HCB. In the second experiment, we examined the influence of dietary fiber on the metabolism and accumulation of HCB in dams as well as its transfer to suckling pups. In these experiments, nonpregnant and pregnant rats were treated with the minimum level of HCB (10 μg/100 g of diet, 31.5 nmol/100 g of diet) that would subsequently product detectable organ concentration of HCB. Therefore, it was expected that the biological effect of HCB on the nonpregnant rats, dams, and newborns would be very weak. In this paper, however, we will describe changes in the accumulation of HCB in nonpregnant rats and dams after their fat tissue

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Table 1. Composition of the Experimental Diets[a]

ingredient	CEL-SO-HCB$^+$	CEL-SO-HCB$^-$	GG-SO-HCB$^-$	CEL-LA-HCB$^-$	GG-LA-HCB$^-$	CEL-DHA-HCB$^-$	GG-DHA-HCB$^-$
casein	20.0	20.0	20.0	20.0	20.0	20.0	20.0
DL-methionine	0.3	0.3	0.3	0.3	0.3	0.3	0.3
cornstarch	39.75	39.75	39.75	39.75	39.75	39.75	39.75
sucrose	10.0	10.0	10.0	10.0	10.0	10.0	10.0
dextrine	13.2	13.2	13.2	13.2	13.2	13.2	13.2
soybean oil	7.0	7.0	7.0	3.0	3.0	3.0	3.0
γ-linolenic acid				4.0	4.0		
DHA[b]						4.0	4.0
cellulose	5.0	5.0		5.0		5.0	
guar gum			5.0		5.0		5.0
AIN mineral mixture[c]	3.5	3.5	3.5	3.5	3.5	3.5	3.5
AIN vitamin mixture[d]	1.0	1.0	1.0	1.0	1.0	1.0	1.0
choline bitartrate	0.25	0.25	0.25	0.25	0.25	0.25	0.25
tert-butylhydroquinone	0.0014	0.0014	0.0014	0.0014	0.0014	0.0014	0.0014
hexachlorobenzene	10						

[a] Diets were prepared according to the recomendation of the American Institute of Nutrition (AIN-93G). Composition for all ingredients is given in grams per 100 g of diet except for hexachlorobenzene, which is given in micrograms per 100 g of diet. [b] Docosahexaenoic acid. [c] Mineral mixture was based on the AIN-93 G formulation. [d] Vitamin mixture was based on the AIN-93 G formulation.

Table 2. Fatty Acid Composition (Percent) of Dietary Fat[a]

fatty acid	CEL-SO-HCB$^-$/ GG-SO-HCB$^-$	CEL-LA-HCB$^-$/ GG-LA-HCB$^-$	CEL-DHA-HCB$^-$/ GG-DHA-HCB$^-$
$C_{14:0}$	tr[b]	tr	1.52
$C_{16:0}$	9.53	12.92	11.99
$C_{16:1}$	tr	tr	3.31
$C_{18:0}$	4.08	2.99	2.89
$C_{18:1}$	21.17	30.49	19.53
$C_{18:2}$	55.85	34.17	20.53
$C_{18:3}$	8.42	16.12	3.63
$C_{20:5}$	tr	tr	5.92
$C_{22:6}$	tr	tr	26.16
S:M:P[a]	1:1.6:4.7	1:1.9:3.2	1:1.4:3.3
$(n-6)/(n-3)$	6.6:1	2.1:1	0.6:1

[a] Ratio of saturated, monounsaturated, and polyunsaturated fatty acids. [b] Trace.

mass was reduced by ingestion of dietary fiber and fatty acids. Transfer of HCB to their suckling pups was also studied.

MATERIALS AND METHODS

Materials. HCB was purchased from Tokyo Kasei Kogyo (Tokyo, Japan) and recrystallized three times by methanol (purity 99%). γ-Linolenic acid and DHA were obtained from Idemitu Material Co. (Tokyo, Japan) and Nihon Suisan Co. (Tokyo, Japan), respectively. 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 diets are shown in Table 1. The diets were prepared according to the recommendation of the American Institute of Nutrition (AIN-93G diet) (American Institute of Nutrition, 1993). Cellulose and guar gum were used as dietary fiber. Soybean oil, γ-linolenic acid, and DHA were used as fat. The diet containing HCB was prepared by dissolving HCB (100 μg/L of ethanol) in soybean oil. To avoid oxidation of fatty acid, γ-linolenic acid or DHA was mixed with soybean oil daily, and the blended fats were added to the fat-free diet immediately before administration of the diet to rats. The fatty acid compositions of these experimental diets were designed to give high, medium, and low $(n-6)/(n-3)$ ratios as shown in Table 2. Rats were given free access to the diet and distilled water. Daily food intake was measured during the experimental period.

Animals. In study 1, female Sprague-Dawley rats (10 weeks of age) obtained from Japan Clea (Tokyo, Japan) were housed individually in stainless steel wire-bottomed cages in a room with a constant temperature of 23 $\pm$ 1 °C and a 12-h light/dark cycle. They were supplied with the diety containing HCB (Table 1, CEL-SO-HCB$^+$) for 2 weeks and then divided into seven groups of six rats each having similar mean body weights. Immediately, one group was anesthetized with ether and sacrificed by cardiac puncture. The other six groups were fed one of the HCB-free diets (Table 1). After 4 weeks of consuming these HCB-free diets, all rats were also sacrificed by cardiac puncture.

In study 2, 16 pregnant Sprague-Dawley rats were used. Sperm-positive rats (10 weeks old) were obtained from Japan Clea (Tokyo, Japan) on day 2 of pregnancy. They were divided into four groups and housed individually in plastic cages. Groups 1 and 4 were fed CEL-SO-HCB$^+$ and CEL-SO-HCB$^-$, respectively (Table 1), during pregnancy and lactation. Groups 2 and 3 were fed CEL-SO-HCB$^+$ only during pregnancy. Within 24 h of birth, litters were culled to 10 pups each, and groups 2 and 3 were fed CEL-SO-HCB$^-$ and GG-SO-HCB$^-$, respectively, during lactation. Two pups from each litter on days 2, 6, 10, and 15 after birth and all dams on day 15 after parturition were anesthetized and sacrificed by cardiac puncture.

Sample Collection and Analysis. All rats were sacrificed by cardiac puncture, and blood was collected with heparinized syringes. Liver, kidney, and perirenal fat (abdominal portion) were removed from each rat, weighed, and then stored at -20 °C until needed for analysis.

Blood (0.2−3 mL) was mixed with 1−5 mL of distilled water. Organs were homogenized in 4 volumes of water. HCB in the sample was extracted with *n*-hexane. To extract HCB, 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 Na_2SO_4). The column was eluted with 5 mL of *n*-hexane. The eluate was evaporated and its volume was appropriately adjusted with *n*-hexane. HCB was analyzed using a Hitachi 663-30 gas chromatograph with an electron capture detector (Hitachi, Tokyo, Japan). The column (3 mm $\times$ 3 m) packed with 5% OV-210 coated on Gas Chrom Q was used at 250 °C temperature, with 50 mL/min N_2 as the carrier gas.

Statistical Analysis. Data are presented as individual group means $\pm$ SEM. Statistical analysis was conducted by one-way ANOVA in study 2. Differences in mean values between groups were tested by Duncan's multiple-range test and the Kruskal−Wallis test for unequal variance in Table 6 (Yonezawa et al., 1988). Differences were considered significant at $P < 0.05$. The Yukumus computer program (Yukumus, Tokyo, Japan) was used for statistical analysis of the data.

RESULTS AND DISCUSSION

Study 1. Dietary fibers and fatty acids did not influence body weight and relative weight of liver and kidney of nonpregnant rats but did significantly affect

Table 3. Effect of Dietary Fibers and Fatty Acids on Body Weight and Relative Weights of Organs and Fat Tissue of Nonpregnant Rats Fed the Diet without HCB for 4 Weeks after Being Fed the Diet Containing HCB at 10 μg/100 g of Diet for 2 Weeks[a]

dietary group	body wt (g)	rel wt (g/100 g of body wt) liver	kidney	perirenal fat tissue
After Administration of HCB for 2 Weeks				
CEL-SO-HCB$^+$	242 ± 14	4.08 ± 0.65	0.65 ± 0.03	1.12 ± 0.14^{bc}
After Dietary Treatment for 4 Weeks				
CEL-SO-HCB$^-$	270 ± 20	3.43 ± 0.42	0.61 ± 0.05	1.35 ± 0.22^{ab}
GG-SO-HCB$^-$	257 ± 14	3.61 ± 0.13	0.61 ± 0.03	1.01 ± 0.16^{c}
CEL-LA-HCB$^-$	270 ± 20	3.71 ± 0.26	0.60 ± 0.03	1.34 ± 0.09^{a}
GG-LA-HCB$^-$	255 ± 11	3.70 ± 0.31	0.72 ± 0.18	0.72 ± 0.18^{d}
CEL-DHA-HCB$^-$	258 ± 8	3.51 ± 0.11	0.61 ± 0.03	0.98 ± 0.16^{c}
GG-DHA-HCB$^-$	260 ± 12	3.64 ± 0.14	0.64 ± 0.04	0.89 ± 0.10^{cd}

[a] Values are expressed as mean ± SD or six rats per group. Values in the same column with different superscript letters are significantly different at $P < 0.05$.

relative weight of perirenal fat tissue (Table 3). Rats fed soybean oil or γ-linolenic acid in combination with guar gum had significantly lower perirenal fat tissue weight compared with those fed the diet containing cellulose. Among the groups fed cellulose diets, perirenal fat tissue weight was lower in the DHA-ingesting rats than in those ingesting soybean oil or γ-linolenic acid. Among the groups fed guar gum diets, however, perirenal fat tissue weight was lower in γ-linolenic acid-ingesting rats than in those ingesting soybean oil.

In the present experiment, we used a small amount of HCB as a chemically stable lipophilic chemical to examine the influence of dietary fibers and fatty acids on the metabolism and accumulation of HCB in female adult rats. No significant alterations in the amount of HCB ingested during the initial 2 weeks was observed among these seven dietary groups: the group of rats sacrificed immediately after the 2-week CEL-SO-HCB$^+$ diet period (23.0 ± 1.6 μg); the CEL-SO-HCB$^-$ diet group (22.3 ± 1.8 μg); the GG-SO-HCB$^-$ diet group (23.5 ± 2.5 μg); the CEL-LA-HCB$^-$ diet group (24.6 ± 3.5 μg); the GG-LA-HCB$^-$ diet group (24.2 ± 3.6 μg); the CEL-DHA-HCB$^-$ diet group (23.5 ± 2.5 μg); and the GG-DHA-HCB$^-$ diet group (24.0 ± 2.8 μg).

In all groups, the HCB concentration was highest in the fat tissue and lowest in the blood, and the total amount of HCB was highest in the fat tissue and lowest in the kidney (Table 4). Because of its lipophilic properties, HCB accumulated predominantly in fat tissue. The concentration and total amount of HCB in liver, kidney, and perirenal fat tissue were lower in rats sacrificed after the 4-week HCB-free diet period than in rats sacrificed immediately after the 2-week HCB diet period. In the blood, however, the HCB concentration measured after the HCB-free diet period was higher than or similar to that measured immediately after the HCB diet period. The livers of the group fed the GG-DHA-HCB$^-$ diet were lower in both concentration and total amount of HCB than those of other groups. The HCB concentration in blood and the concentration and total amount of HCB in kidney were significantly higher in the GG-LA-HCB$^-$ and GG-DHA-HCB$^-$ diet groups than in the other groups. The total amount of HCB in perirenal fat tissue was significantly lower in the rats fed guar gum than in those fed cellulose regardless of the difference in the concomitantly given lipids. From the results, we suspected that the ingestion of guar gum, and especially in combination with γ-linolenic acid or DHA, somehow accelerated the excretion of HCB, thereby markedly reducing the total amount of HCB retained in the body.

We have already reported from a study with male weaned rats that guar gum- or fish oil-fed rats had smaller perirenal and epididymal fat tissues, and that the ingestion of the guar gum or fish oil results in significantly lower accumulation of PECB in perirenal, epididymal, and subcutaneous fat tissue, and that the reduction in fat tissue mass could enhance the metabolism and excretion of PECB (Umegaki and Ichikawa, 1990; Umegaki et al., 1993; Ikegami et al., 1994). Similar findings were reported by Birnbaum (1983) with hexachlorobiphenyl (HCBP), by Geyer et al. (1990) with TCDD 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD), and by Dole et al. (1962) and Mitjavla et al. (1981) with DDT. When the fat mass was significantly decreased by starvation, restricted feeding, fish oil feeding, and viscous indigestible polysaccharides feeding, the body's capacity to store lipophilic chemicals is reduced and the mobilization of the chemical accumulated there into blood is increased. As a result, the concentrations of such chemicals in the blood and liver increase and the metabolism of the chemicals by the hepatic drug-metabolizing enzyme is accelerated. In the present study, we observed similar fat-reducing and metabolism-accelerating effects of dietary fiber and fatty acids on female adult rats.

As shown in Tables 3 and 4, although no significant difference in the HCB concentration in perirenal fat tissue was observed among the six groups, except the CE-SO-HCB$^-$ diet group, the perirenal fat tissue weight of the guar gum- or DHA-fed rats was lower than that

Table 4. Effect of Dietary Fibers and Fatty Acids on HCB Levels in Blood, Liver, and Perirenal Fat of Nonpregnant Rats Fed the Diet without HCB for 4 Weeks after Being Fed the Diet Containing HCB at 10 μg/100 g of Diet for 2 Weeks[a]

	concentration				total amount		
dietary group	blood (μg/L)	liver (ng/g)	kidney (ng/g)	perirenal fat tissue (μg/g)	liver (ng/liver)	kidney (ng/kidney)	perirenal fat tissue (μg/perirenal fat tissue)
After Administration of HCB for 2 Weeks							
CEL-SO-HCB$^+$	1.79 ± 0.43^{a}	6.52 ± 1.46^{a}	4.45 ± 0.60^{a}	0.79 ± 0.10^{a}	64.96 ± 12.76^{a}	7.05 ± 0.5^{a}	2.32 ± 0.41^{a}
After Dietary Treatment for 4 Weeks							
CEL-SO-HCB$^-$	1.61 ± 0.34^{a}	4.56 ± 0.97^{b}	2.65 ± 0.55^{c}	0.47 ± 0.05^{c}	40.33 ± 9.05^{b}	4.26 ± 0.93^{c}	1.85 ± 0.37^{b}
GG-SO-HCB$^-$	1.88 ± 0.12^{a}	3.58 ± 0.74^{bc}	3.62 ± 0.32^{b}	0.55 ± 0.10^{b}	44.49 ± 7.39^{b}	5.18 ± 0.57^{bc}	1.28 ± 0.13^{c}
CEL-LA-HCB$^-$	1.59 ± 0.15^{a}	4.32 ± 0.90^{b}	3.01 ± 0.40^{bc}	0.54 ± 0.03^{b}	43.49 ± 6.47^{b}	4.65 ± 1.06^{bc}	1.88 ± 0.31^{b}
GG-LA-HCB$^-$	2.55 ± 0.56^{b}	4.05 ± 0.50^{b}	4.81 ± 0.65^{a}	0.58 ± 0.04^{b}	38.08 ± 4.19^{b}	7.28 ± 1.12^{a}	1.07 ± 0.18^{c}
CEL-DHA-HCB$^-$	1.84 ± 0.28^{a}	4.40 ± 0.73^{b}	3.40 ± 0.31^{b}	0.56 ± 0.08^{b}	39.50 ± 5.84^{b}	5.18 ± 0.23^{b}	1.42 ± 0.13^{b}
GG-DHA-HCB$^-$	2.21 ± 0132^{b}	3.18 ± 0.77^{c}	3.96 ± 0.66^{a}	0.48 ± 0.10^{bc}	29.34 ± 4.24^{c}	6.42 ± 1.27^{a}	1.12 ± 0.18^{c}

[a] Values are expressed as mean ± SD or six rats per group. Values in the same column with different superscript letters are significantly different at $P < 0.05$.

of the cellulose- or soybean oil-fed rats, respectively. Therefore, the total amount of HCB in the perirenal fat tissue was lower in the rats fed with guar gum or DHA than in rats fed cellulose or DHA, respectively (Table 3). We then studied the relationship between the relative weight (grams/100 g of body weight) and the total amount of HCB in the perirenal fat tissue as well as the relation of total amount of HCB in the fat tissue with the concentration in blood and the total amount of kidney and liver based on the data from rats fed the HCB-free diet for the last 4 weeks. As shown in Figure 1, a marked positive correlation was noted between the relative weight and the total amount of HCB in perirenal fat tissue ($r = 0.816$). However, the total amount of HCB in this tissue showed a negative correlation with the blood concentration ($r = -0.719$) as well as with the total amount in the kidney ($r = -0.557$). A low correlation was observed in the total amount of HCB in the perirenal fat tissue and that in the liver ($r = 0.309$).

These observations suggest that increased mobilization of HCB accumulated in fat tissues into blood enhanced the metabolism of HCB in the liver and the excretion via the kidney. HCB incorporated into the liver is considered to be rapidly metabolized by the hepatic drug-metabolizing enzymes transported into the blood stream and excreted through the kidney or bile.

Study 2. In study 2, we studied whether guar gum, which accelerated the metabolism and excretion of HCB accumulated in female rats, affects the transfer of HCB stored in dams to suckling pups through the milk. On the day before parturition, the pregnant rats fed HCB (CEL-SO-HCB$^+$) during pregnancy weighed 373 ± 14.4 g ($n = 12$) and those not fed HCB (CEL-SO-HCB$^-$) during the same period weighed 382 ± 10.6 g ($n = 4$). Food intakes from the second day of pregnancy until the day before parturition were 337 ± 18 g in the dams fed HCB and 329 ± 15 g in those not given HCB. Thus, exposure to a small amount of HCB during pregnancy did not significantly affect body weight and food intake.

Food intake of dams given guar gum during the lactation period was significantly lower than those of dams in the other three groups given cellulose instead of guar gum (Table 5). Body weights of dams were not significantly different among these four groups, but the weights of suckling pups on day 15 after birth were significantly lower in those nursed by the guar gum-ingesting dams than in those nursed by the cellulose-ingesting dams. Dams did not show significant group differences in the weights of the liver, kidney, and perirenal fat. However, the suckling pups nursed by the guar gum-ingesting dams had significantly lower weights of the liver and perirenal fat tissue than those nursed by the cellulose-ingesting dams.

In dams, the HCB concentration was highest in the perirenal fat tissue and lowest in the blood, and the total amount of HCB in the perirenal fat tissue was larger than in the liver (Table 4). On day 15 after parturition, both groups of dams exposed to HCB only during pregnancy (CEL-SO-HCB$^-$) and GG-SO-HCB$^+$) showed significantly lower values than those exposed to HCB throughout the experiments in terms of the concentration and/or total amount of HCB in blood, liver, and perirenal fat tissue. Because HCB had rapidly disappeared from the body of the nursing rats during the 15 days of lactation, there were no significant differences in the concentrations and total amount of HCB in the

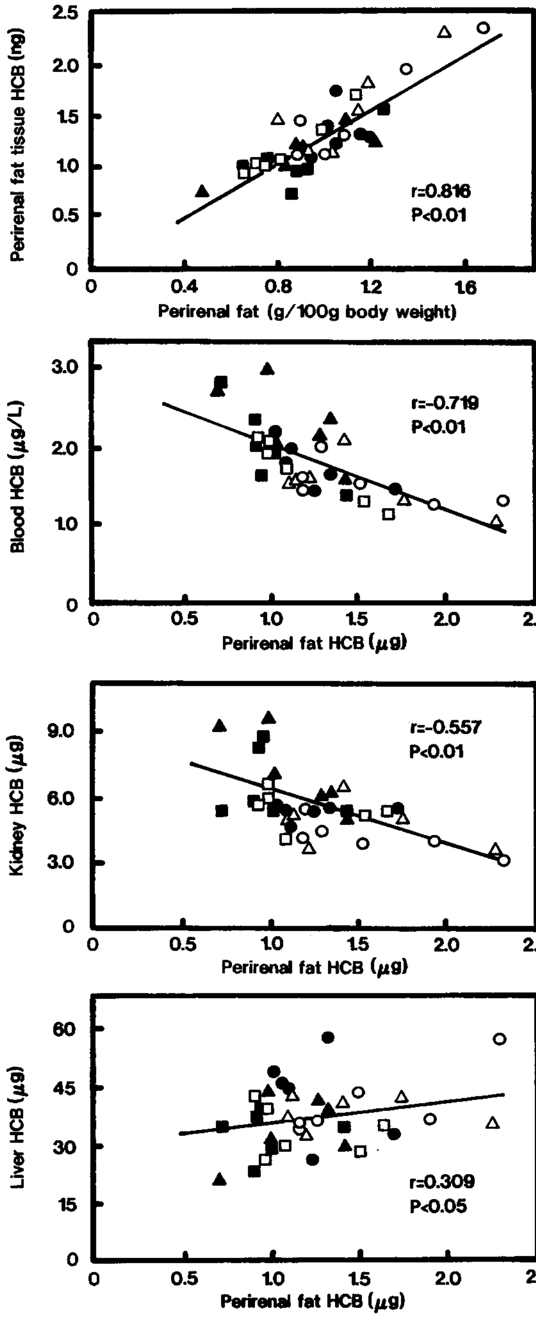

Figure 1. Correlation between the relative weight (g/100 g of body weight) and total amount of HCB in the perirenal fat tissue as well as the correlation of HCB in the fat with that in the blood, kidney, or liver in the rats fed the HCB-free diet for the last 4 weeks. Each data point represents one rat (○, rats fed the diet CEL-SO-HCB$^-$; ●, rats fed the diet GG-SO-HCB$^-$; △, rats fed the diet CEL-LA-HCB$^-$; ▲, rats fed the diet GG-LA-HCB$^-$; □, rats fed the diet CEL-DHA-HCB$^-$; ■, rats fed the diet GG-DHA-HCB$^-$).

blood, liver, and perirenal fat tissue between these two groups. Furthermore, no significant differences were found in the levels of HCB when these two groups of dams given HCB only during pregnancy were compared with the group of dams given HCB-free diet throughout the experiment. Therefore, we conclude that a large proportion of the HCB that accumulated in dams during pregnancy was transferred to their suckling pups through milk.

To study the effect of dietary fiber on the transfer of HCB accumulated during pregnancy to their suckling

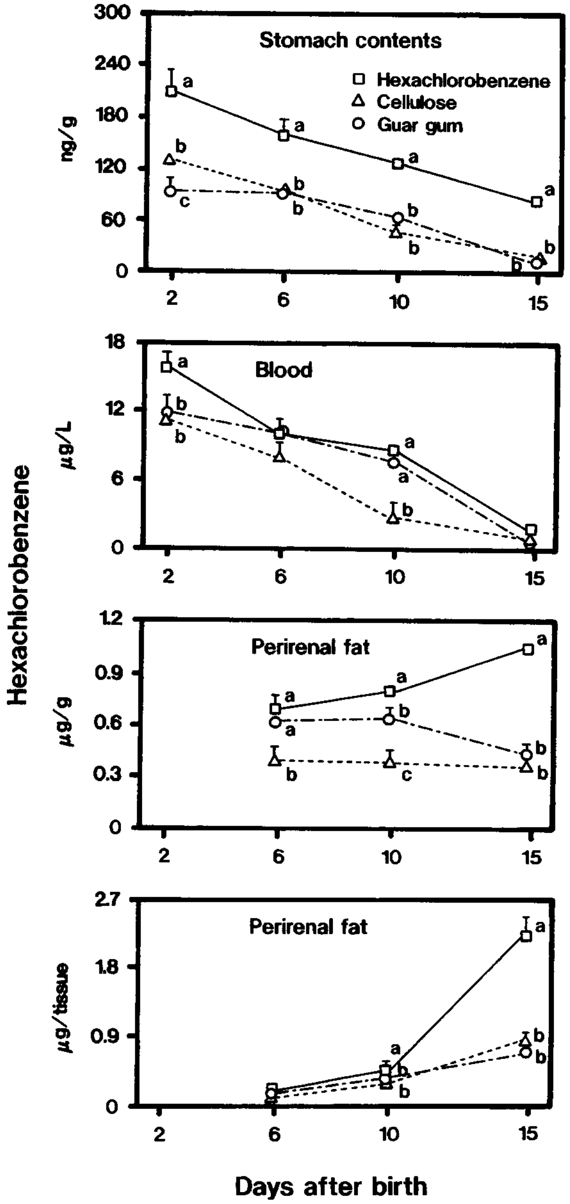

Figure 2. Concentration of HCB in the stomach contents, blood, liver, and subcutaneous fat tissue of suckling pups nursed by the dams fed the HCB diet (CEL-SO-HCB$^+$) during pregnancy and having ingested cellulose diet with (CEL-SO-HCB$^+$) or without HCB (CEL-SO-HCB$^-$) or guar gum diet without HCB (GG-SO-HCB$^-$) for 15 days after parturition. Values are expressed as mean ± SD, $n = 8$. Values in the same day with different superscript letters are significantly different at $P < 0.05$.

pups through the milk, suckling pups nursed by the cellulose- or guar gum-ingesting dams were examined on days 2, 6, 10, and 15 after birth in terms of HCB concentration in their stomach contents, blood, and perirenal fat tissue (Figure 2). The HCB concentration in the stomach contents decreased gradually during the 15-day lactation period but was consistently higher in the suckling pups nursed by the dams exposed to HCB throughout the experiment than in those nursed by the dams exposed to HCB only during pregnancy. The HCB concentration in the stomach contents of suckling pups was not significantly different whether the dams ingested guar gum or cellulose during the lactation period; however, on day 2 after birth, the value was exceptionally lower in those nursed by the guar gum-ingesting dams than in those nursed by the cellulose-ingesting dams.

The blood HCB in the suckling pups decreased gradually after birth in all groups. The suckling pups whose dams were given HCB throughout the experiment showed higher blood HCB concentration than other suckling pups on day 2 after birth. The suckling pups nursed by the dams ingesting HCB-free diet during lactation showed lower values than the other suckling pups on day 10 after birth.

As expected, the HCB concentration in perirenal fat tissue of the suckling pups was lower on day 6 after birth in those nursed by the dams ingesting the HCB-free cellulose diet than that in those nursed by the other dams. This value on day 10 after birth was highest in the suckling pups nursed by the dams exposed to HCB throughout the experiment and lowest in those nursed by the dams ingesting the HCB-free cellulose diet during lactation. On day 15 after birth, this value in the suckling pups nursed by the dams exposed to HCB throughout the experiment was higher than in the other suckling pups. The HCB concentration in the perirenal fat tissue of suckling pups was consistently higher than the corresponding values for the dams (Table 6).

The total amount of HCB in the perirenal fat tissue of suckling pups increased rapidly in those nursed by dams exposed to HCB throughout the experiment, but in the other suckling pups increase in this value was gradual. As a result, the value for the former suckling pups came to be significantly higher than that for the other suckling pups on days 10 and 15 after birth, although there had been no significant difference among them on day 6.

Intake of HCB was 297.3 ± 11.8 μg in the dams fed the HCB during pregnancy and lactation and 115.5 ± 5.3 μg in the dams fed the HCB only during pregnancy. Therefore, HCB intake of dams during pregnancy and lactation was ~12 times higher than those (22.3−24.6 μg) of nonpregnant rats given HCB for 2 weeks. However, the concentration (0.57 ± 0.04 μg) and the total amount (0.92 ± 0.30 μg) of HCB in the perirenal fat tissue of the dams (Table 6) were lower than those of nonpregnant rats (Table 4; concentration = 0.79 ± 0.10 μg; total amount = 2.32 ± 0.41 μg), but in the blood and liver of the former dams, the HCB levels were higher than those of the nonpregnant rats. We have previously shown that the fat tissue weight of dams was significantly decreased by lactation (Nakashima et al., 1997). This fat tissue reduction during lactation accelerated the mobilization of HCB into blood and decreased both the concentration and the total amount of HCB in perirenal fat tissue, so that the concentrations of HCB in the blood and organs increased. HCB increased in the mammary glands and milk as well. Thus, a greater portion of HCB that had been accumulated in the dams during pregnancy was considered to be transferred to suckling pups in the early days of lactation.

As shown in study 2, in the dams exposed to HCB during pregnancy but not lactation, the HCB concentration in the blood, liver, and perirenal fat tissues after 15 days of lactation was remarkably lower (Table 6) than the value seen in nonpregnant rats fed the diet without HCB for 4 weeks in study 1 (a 40% decrease in the HCB concentration in the perirenal fat tissue was observed after 4 weeks of HCB withdrawal) (Table 4). HCB disappeared so rapidly from the body of the dams

Table 5. Effect of Dietary Fibers on Body and Organ Weights of Dams Fed the Diet Containing HCB at 10 μg/100 g of Diet and That of Their Suckling Pups[a]

	dietary treatment, pregnant period/nursing period			
	CEL-SO-HCB$^+$/ CEL-SO-HCB$^+$	CEL-SO-HCB$^+$/ CEL-SO-HCB$^-$	CEL-SO-HCB$^+$/ GG-SO-HCB$^-$	CEL-SO-HCB$^-$/ CEL-SO-HCB$^-$
Dams (on Day 15 after Parturition, $n = 4$)				
food intake (g)	509 ± 70^a	485 ± 14^a	389 ± 15^b	451 ± 10^a
body wt (g)	302 ± 14	286 ± 12	287 ± 14	303 ± 19
Relative Wt (g/100 g of Body Wt)				
liver	4.33 ± 0.26	4.06 ± 0.17	4.32 ± 0.21	4.03 ± 0.40
Kidney	0.65 ± 0.03	0.64 ± 0.01	0.62 ± 0.01	0.65 ± 0.03
perirenal fat	0.51 ± 0.12	0.66 ± 0.16	0.43 ± 0.10	0.65 ± 0.15
Pups (on Day 15 after Birth, $n = 8$)				
body wt (g)	46.1 ± 1.4^a	48.0 ± 1.9^a	38.8 ± 0.8^b	46.6 ± 1.4^a
stomach content (g)	1.44 ± 0.21	1.65 ± 0.22	1.11 ± 0.06	1.45 ± 0.24
liver wt (g)	1.48 ± 0.04^a	1.59 ± 0.09^a	1.22 ± 0.02^b	1.54 ± 0.08^a
brain wt (g)	1.34 ± 0.06	1.29 ± 0.03	1.23 ± 0.01	1.24 ± 0.01
perirenal fat wt (g)	0.22 ± 0.02^a	0.24 ± 0.02^a	0.16 ± 0.01^b	0.26 ± 0.02^a

[a] Values are expressed as mean ± SD or six rats per group. Values in the same column with different superscript letters are significantly different at $P < 0.05$.

Table 6. Effect of Dietary Fibers on HCB Levels in Blood, Liver, and Perirenal Fat of Dams Fed the Diet Containing HCB at 10 μg/100 g of Diet[a]

	dietary treatment, pregnant period/nursing period			
	CEL-SO-HCB$^+$/ CEL-SO-HCB$^+$	CEL-SO-HCB$^+$/ CEL-SO-HCB$^-$	CEL-SO-HCB$^+$/ GG-SO-HCB$^-$	CEL-SO-HCB$^-$/ CEL-SO-HCB$^-$
blood (μg/L)	6.2 ± 0.9^a	0.11 ± 0.08^b	0.09 ± 0.02^b	0.09 ± 0.04^b
liver (ng/g)	15.5 ± 3.6^a	1.05 ± 0.60^b	0.15 ± 0.27^b	0.13 ± 0.06^b
liver (ng/tissue)	198.8 ± 30.5^a	12.40 ± 7.11^b	1.69 ± 0.58^b	1.29 ± 0.33^b
perirenal fat tissue (ng/g)	569.9 ± 41.6^a	8.60 ± 1.68^b	11.08 ± 0.48^b	8.13 ± 0.48^b
perirenal fat tissue (ng/tissue)	920.8 ± 303.1^a	16.13 ± 4.92^b	12.03 ± 1.54^b	11.27 ± 2.32b

[a] Values are expressed as mean ± SD or four rats per group. Values in the same column with different superscript letters are significantly different at $P < 0.05$.

during lactation that the HCB concentration in blood, liver, and perirenal fat tissue after 15 days of lactation was not significantly different between the dams given HCM only during pregnancy and those fed an HCB-free diet throughout the experiment. We observed a similar result in our previous study, in which a greater portion of HCB that accumulated in dams during pregnancy was postnatally transferred to their suckling pups through milk immediately after birth (Nakashima et al., 1997). This rapid elimination of HCB from dams during lactation seemed to mask the difference, which would otherwise have been seen, in the inhibitory effects of guar gum and cellulose on the HCB accumulation in the dam's liver as well as in the dam's perirenal fat tissue.

A low level of HCB was detected even in the dams fed the HCB-free diet. We have suggested a reason for this in our previous paper (Nakashima et al., 1997). As a small amount of HCB could be detected in the presently used HCB-free diet as well as in commonly used pellet diets, we concluded that the small amount detected in the dams fed the HCB-free diet was probably due to HCB which had contaminated the basal diets.

In suckling pups on day 15 after birth, body weight was smaller in the guar gum-ingesting lactating dam group than in the corresponding cellulose-ingesting groups, but no significant difference in birth weight was observed among these groups. That is, weight gain was smaller in the guar gum-ingesting dam group than in the corresponding cellulose-ingesting group. Regarding the perirenal fat tissue of those born to and nursed by the dams given HCB only during pregnancy, the total tissue mass was smaller in the guar gum-ingesting dam group than in the corresponding cellulose-ingesting group, but the tissue HCB concentration was higher in the former. Consequently, the total amount of HCB in the perirenal fat mass of the suckling pups showed no significant difference between the two groups (Figure 1). The HCB concentration in the stomach contains and blood of suckling pups was highest on day 2 after birth and decreased rapidly through 15 days after birth in all groups, whereas the total amount of HCB in the perirenal fat tissue of suckling pups increased after birth. Therefore, the HCB that transferred from dams to suckling pups through milk accumulated in fat tissue.

Chronic administration of HCB to animals affects thyroid hormone homeostasis, disturbs porphyrin metabolism, produces hepatic, renal, and adrenal lesions, and induces lipid peroxidation (Carlson and Tardiff, 1976; Courtney et al., 1976; Rozman et al., 1997; Vos et al., 1979; Besten et al., 1993). To investigate the elimination of HCB in normal animals while avoiding harmful biological effects of HCB, we used a small dose that would nevertheless produce detectable concentrations in organs. Actually, in the present study, there was no evidence of its harmful effects on nonpregnant rats, pregnant and lactating rats, or their suckling pups. Although the present study did not confirm such negative effect, it is important to address the question whether prenatal and/or postnatal exposure to HCB produces long-term harmful effects; because infants are in a period of rapid growth and development, their susceptibility to toxic substances might be high. The harmful effects of HCB and the mechanism of its oxidation to PCP and further to TCBQ are the subjects of further studies.

It seemed evident that the ingestion of guar gum, especially in combination with γ-linolenic acid or DHA, accelerates the metabolism of liver and increases the excretion of chemically stable lipophilic environmental pollutants and thereby decreases their accumulation in the body. We suggest that the decrease in fat tissue affects the excretion of these pollutants. We conclude that the excretion of HCB in milk was so fast that the guar gum-induced acceleration of its excretion from the dams was masked.

ABBREVIATIONS USED

DDT, 1,1,1-trichloro-2,6-*bis*(*p*-chlorophenylethane); DHA, docosahexaenoic acid; HCB, hexachlorobenzene; HCBP, hexachlorobiphenyl; PCB, polychlorinated biphenyl; PCDD, polychlorinated dibenzo-*p*-dioxin; PCDF, polychlorinated dibenzofuran; PCP, pentachlorophenol; PECB, pentachlorobenzene; TCBQ, tetrachlorobenzoquinone; TCDD, 2,3,7,8-tetrachlorodibenzo-*p*-dioxin.

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