Effects of Hexachlorobenzene or Hexabromobenzene on Body and Organ Weights of Preweanling Rats after a Reciprocal Transfer between the Treated and Control Dams

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The effects of hexachlorobenzene (HCB) or hexabromobenzene (HBB) on body and organ weights of preweanling Wistar rat pups after a reciprocal transfer between control and HCB- or HBB-fed dams were studied. A significant increase in the liver weight over the control was observed on pups nursed by dams fed HBB- or HCB-treated diet. Based on body and organ weights, no significant difference ($p > 0.05$) was observed between pups born to HCB- or HBB-fed dams and nursed by dams and those nursed entirely by control dams. In the HBB groups, there was no significant difference between the control and treated pups in terms of brain, heart, kidney, and spleen weights. In the HCB groups, however, the treated pups generally had smaller brain, heart, kidney, and spleen than the controls. The results show that HCB- or HBB-treatment of the nursing mother had greater effects than the treatment of the natural mother. Furthermore, the results indicated that transmission of these compounds through milk had greater effects on pup liver weights than the placental transmission.

Hexachlorobenzene (HCB), a cereal grain fungicide, has been detected in various types of samples such as wild birds, fresh and marine fish, vegetable and animal fat, butter and cheese, human body and milk fat, and human serum (Vos et al., 1972). It has been reported to induce porphyria in infants (Cam, 1960) and young rats (Mendoza et al., 1975) that had been nursing on the milk from exposed mothers.

An HCB-related compound, hexabromobenzene (HBB), is a fire retardant used in plastics, textiles, and woods (Negishi et al., 1972; Raley, 1972; Mischutin, 1974; Pashin et al., 1974). Villeneuve and Khera (1975) reported that only a very small portion of HBB from the treated rat dams was transplacentally transmitted to the fetus. In addition, HBB was found not to be teratogenic at doses of up to 200 mg/kg (Khera and Villeneuve, 1975). Kitagawa et al. (1975) observed that 0.9 to 7 g/kg dosed po to mice did not cause severe toxicity in terms of body weight gain, food consumption, histopathology, and alkaline phosphatases or lactic dehydrogenases. Adult male rats fed ad libitum 10 to 160 ppm HBB in the diet did not show symptoms of intoxication, such as changes in levels of some esterases, hematological parameters, and body or organ weights (Mendoza et al., 1977b).

On the other hand, another brominated arylhydrocarbon, a mixture of polybrominated biphenyls (PBB), had been observed to be highly teratogenic to rats given by gavage 200 mg of PBB per animal at day 12 and 13 of gestation (Beaudoin, 1976). Strik (1973) reported that hexabromobiphenyl induced liver porphyria in the Japanese quail. A loss of weight, a decrease in milk production, hematomas, metritis, and deaths had been observed on dairy cows fed accidently PBB-contaminated protein concentrate (Jackson and Halbert, 1974). Liver abscesses, hyperkeratosis, and even deaths had been observed also on fetuses during parturition and on 6- to 18-month-old heifers and young bulls. These reports indicated that brominated arylhydrocarbons had similar effects as those observed with chlorinated hydrocarbons. Structurally, however, PBB is more related to polychlorinated biphenyl (PCB) than HBB or HCB.

In view of the susceptibility of human infants to porphyria induced by HCB and the chemical relationship of HBB to HCB, the present studies were initiated (a) to determine the effects of HCB or HBB on body and organ weights of suckling rats after a reciprocal transfer between litters of the treated and control dams immediately after parturition; (b) to determine the effects of the treatment of the nursing mother on the suckling rats; and (c) to compare pups born to and nursed by a control mother with pups born to a control mother but nursed by a treated mother.

MATERIALS AND METHODS

Experimental Methods. Virgin Wistar rat females (Woodlyn Laboratories, Ltd., Guelph, Ontario, Canada), weighing approximately 250 g, were given water and fed ad libitum powdered rat cubes containing either 80 ppm HCB or HBB and 4% corn oil (Mazola) from 2 weeks before mating until the termination of the experiment. The control animals were fed the same rat diet with corn oil only. HCB, a microanalytical standard (BDH Chemicals, Ltd., Poole, England), and HBB, 98% purity (Aldrich Chemical Co., Inc., Milwaukee, Wis.) were used without further purification.

Females were mated and after insemination, they were individually caged. Five control and five HCB-fed dams and four control and four HBB-fed dams were used. Immediately after parturition, each litter was culled to ten pups and five randomly selected pups from a treated dam were exchanged with five pups from the control dam. The pups were not grouped according to sex. All pups were numbered to identify the adopted and natural pups.

Two age groups of pups were used: (a) 17-day-old group, consisting of 16- to 17-day-old pups and (b) 21-day-old group, consisting of 20- to 23-day-old pups. The pups of paired litters were weighed, sacrificed by decapitation, and bled thoroughly. The organs were immediately excised, rinsed with a cold 0.9% saline solution, blotted, and weighed.

Statistical Methods. The body, liver, kidney, heart, spleen, and brain absolute weights and their relative weights (organ weight/body weight) were analyzed separately for each variable within the HCB and HBB experiments.

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Table I. Labels Used to Identify Various Means and Treatment Combinations[a]

Nursing mother	17-day old		21-day old	
	Natural mother		Natural mother	
	Control	Treated	Control	Treated
Control	A	B	E	F
Treated	C	D	G	H

[a] Lines connecting letters indicated comparisons made between means.

The statistical model employed in this study was similar to the one used recently (Mendoza et al., 1977a) except the specific effects due to individual nursing and natural mothers were included. The data were analyzed without transformation assuming the following linear model:

$$y_{aijrstl} = \mu + A_a + f_{(a)i} + g_{(a)j} + (fg)_{(a)ij} + T_{(a)t} + R_{(ait)r} + S_{(ajt)s} + (RS)_{(aijt)rs} + e_{(aijrst)l}$$

where $y_{aijrstl}$ = response of pup l in a half litter born to mother s which was given treatment j and nursed by mother r which was given treatment i. The litter being born at time t and the response being observed at age a. (The response is defined as any one of the variables listed above). l = 1–5, t = 1–5 for HCB experiment or 1–4 for HBB experiment, j = 1 natural mother control or 2 natural mother treated, i = 1 nursing mother control or 2 nursing mother treated, a = 1 observed at 17 days old or 2 observed at 21 days old, $r = i$, and $s = j$. μ = grand mean or average of responses. A_a = effect of age at time of observation (17 or 21 days old). $f_{(a)i}$ = effect of treatment given to nursing mother. (The a enclosed in bracts indicates that this effect may vary with age at time of observation). $g_{(a)j}$ = effect of treatment j given to natural mother. $(fg)_{(a)ij}$ = interaction of treatment j given to the natural mother and treatment I given to the nursing mother. (The interaction measures whether the effect of natural mother treatment is the same at each level of the nursing mother treatment). This interaction is compounded with the effect of adoption. $T_{(a)t}$ = effect of time on experiment or effect of date of birth. $R_{(ait)r}$ = effect of the individual nursing mother. This effect is due to (1) variation in the lactating ability of individual mothers and (2) variations in the quantity of HCB or HBB transferred through the milk of individual mothers. $S_{(ajt)s}$ = effect of the individual natural mother. This effect is due to (1) variation in the genetic characteristics of the mother and (2) variations in the cross placental transfer of HCB or HBB in individual mothers. $RS_{(aijt)rs}$ = interaction of individual nursing mothers and individual natural mother effects. $e_{(aijrst)l}$ = random error.

The effects A_a, $f_{(a)i}$, $g_{(a)j}$, and $(fg)_{(a)ij}$ are considered fixed. All other effects and interactions are considered random. The random effects $R_{(ait)r}$, $S_{(ajt)s}$, and $(RS)_{(aijt)rs}$ are considered to be different in the treated and control groups: $V(R_{(ait)r}) = \sigma^2_{R_i}$; $V(S_{(ajt)s}) = \sigma^2_{S_j}$; and $V[(RS)_{(aijt)rs}] = \sigma^2_{R_iS_j}$. The labels shown in Table I will be used in describing the results of the comparison between various treatment combinations. The variance of any contrast among treatment means may be easily derived from the above model. Since an analysis of variance did not provide estimate of the appropriate variance components, all possible pairwise comparisons were made using paired t tests at each age with an estimate of error pooled over age groups. Variance estimates pooled over age groups were calculated for each nursing mother × treatment mother combination (Brownlee, 1965) and then averaged to obtain an effective standard error for the treatment means at each age. Furthermore, the average response at 17 days was compared to the average at 21 days using a t test.

The use of random effect terms in the model was in part intuitively explained by the fact that pups born in the same litter were more similar than pups born in different litters and pups nursed by the same mother were more similar than pups nursed by different mothers. The random effect terms allowed these factors to be taken into account in the analysis.

Table II. Mean Body Weights (g) of Rat Pups Nursed by the Control and Treated Dams[a]

	A	I	II	III
HCB group				
17-day-old	33.27	31.96	27.94	29.95
21-day-old	38.23	36.96	34.60	35.78
HBB group				
17-day-old	31.95	30.75	30.51	30.63
21-day-old	39.02	36.64	30.27	33.46

[a] A = mean body weight of pups born to and nursed by control dams; I = mean body weight of all pups nursed by control dams; II = mean body weight of all pups nursed by treated dams; III = grand mean or average of all body weights.

RESULTS AND DISCUSSION

Analyses of tissues from the preweanling rat pups (B, C, D, F, G, H), 21-day-old fetus, and 1-day-old pups showed that parent HCB or HBB were basically present. Therefore, it can be deduced that HCB or HBB were transmitted through the placenta and milk.

Table II shows the similarity of mean body weights of all pups nursed by control dams (A and I) and of all pups nursed by HCB- or HBB-fed dams (II). The mean weight of the control (A, nursed exclusively by control dams) was only slightly heavier than the overall grand mean (III). Although the mean weight of the 21-day-old pups was slightly higher than that of the 17-day-old pups in each treatment group, the difference between these weights was not statistically significant ($p > 0.05$) for either the HCB or HBB experiment. In a separate analysis, we found that 17- and 21-day-old rats were not statistically different from each other in terms of HCB accumulation and some esterase activities in various tissues (Mendoza et al., 1977a).

Various ways of expressing organ weights have been proposed. Organ weights have been presented as a ratio of the body or brain weight or relative weight and as an absolute weight (Gaunt et al., 1967; Feron et al., 1973). If absolute weights were used, the reduction or increase in the organ growth rate due to the treatment may not be detected. On the other hand, if the organ weight relative to the body weight was used alone, the interpretation of results could be misleading since the body weight is assumed to be synonymous to the body growth and the fatness or leanness of the animals was not taken into consideration.

Table III shows that the difference between the mean body weights of control and HCB-treated pups was not statistically significant (A and B vs. C and D, E and F vs. G and H) although the mean weights show that there was a trend for reduced body weights after HCB treatment.

Table III also shows that pups nursed by HCB-fed dams tend to have heavier livers than control pups (C vs. A and B, G vs. E and F) ($p < 0.05$). However, the difference between D and A or B and between H and E or F was not statistically significant ($p > 0.05$). The difference between liver mean weights of pups nursed by the same control (A vs. B, E vs. F) or treated (C vs. D, G vs. H) dams was also not significant. The results indicated that the effect of

Table III. Mean Body and Organ Weights (g) of Rat Pups Nursed by Either Natural or Adoptive Mothers that Were Fed on HCB or Control Diet[a]

Nursing mother	17-day-old: Natural mother Control	17-day-old: Natural mother Treated	17-day-old: Effective standard error	21-day-old: Natural mother Control	21-day-old: Natural mother Treated	21-day-old: Effective standard error
Body						
Control	33.27 (A)	30.65 (B)	±3.47	38.23 (E)	35.69 (F)	± 2.84
Treated	28.35 (C)	27.52 (D)		36.25 (G)	32.95 (H)	
Liver						
Control	0.97	0.85	±0.17	1.26	1.24	±0.14
Treated	1.25	1.20		1.53	1.56	
Brain						
Control	1.34	1.29	±0.06	1.48	1.40	±0.05
Treated	1.22	1.17		1.39	1.35	
Heart						
Control	0.16	0.15	±0.01	0.18	0.18	±0.01
Treated	0.14	0.13		0.18	0.17	
Kidney						
Control	0.41	0.37	±0.03	0.44	0.43	±0.03
Treated	0.33	0.32		0.43	0.40	
Spleen						
Control	0.14	0.11	±0.03	0.15	0.19	±0.02
Treated	0.12	0.11		0.16	0.14	

[a] Asterisk indicates significantly different at $p < 0.05$. The line connecting two figures indicates the direction of comparison. Any comparisons shown in Table I but not shown in this table were not statistically significant, $p > 0.05$. Letters A to H (see Table I) apply also to means of organs.

Table IV. Mean Body or Organ Weights (g) of Rat Pups Nursed by Either Natural or Adoptive Mothers that Were Fed on HBB or Control Diet[a]

Nursing mother	17-day-old: Natural mother Control	17-day-old: Natural mother Treated	17-day-old: Effective standard error	21-day-old: Natural mother Control	21-day-old: Natural mother Treated	21-day-old: Effective standard error
Body						
Control	31.95 (A)	29.55 (B)	±1.37	39.02 (E)	34.26 (F)	± 2.37
Treated	29.91 (C)	31.11 (D)		29.70 (G)	30.84 (H)	
Liver						
Control	0.84	0.80	±0.08	1.26	1.14	±0.14
Treated	0.98	1.05		1.04	1.11	
Brain						
Control	1.38	1.35	±0.02	1.36	1.40	±0.04
Treated	1.34	1.35		1.38	1.36	
Heart						
Control	0.16	0.15	±0.01	0.18	0.17	±0.01
Treated	0.15	0.16		0.15	0.15	
Kidney						
Control	0.38	0.38	±0.02	0.44	0.42	±0.04
Treated	0.39	0.40		0.36	0.40	
Spleen						
Control	0.12	0.18	±0.03	0.16	0.15	±0.06
Treated	0.10	0.10		0.09	0.10	

[a] Asterisk indicates significantly different at $p < 0.05$. The line connecting two figures indicates the direction of comparison. Any comparisons shown in Table I but not shown in this table were not statistically significant, $p > 0.05$. Letters A to H (see Table I) apply also to means of organs.

HCB, if any, on liver weights of the treated pups (B and F) did not persist after the transfer from the treated mother to a control foster dam. HCB derived through placental transfer and ingestion of colostrum on the first day after parturition had been largely eliminated after the pups were removed from the HCB-fed dams (Mendoza et al., 1977a). Consequently, remission of HCB effects on the liver weight could be expected.

Table III further shows that the brains (17- or 21-day-old) or kidneys (17-day-old) of pups that remained with their control mothers were significantly heavier than those of pups nursed by the treated dams. The weights

Table V. Percentage of Liver to Body Weights of Rat Pups Nursed by Natural or Adoptive Mothers that Were on HCB, HBB or Control Diet[a]

Nursing mother	17-day-old			21-day-old		
	Natural mother		Standard error	Natural mother		Standard error
	Control	Treated		Control	Treated	
HCB group						
Control	2.91 (A)	2.78 (B)	0.30	3.27 (E)	3.44 (F)	0.25
Treated	4.47 (C)	4.49 (D)		4.25 (G)	4.74 (H)	
HBB group						
Control	2.61 (A)	2.72 (B)	0.15	3.24 (E)	3.37 (F)	0.26
Treated	3.27 (C)	3.36 (D)		3.52 (G)	3.61 (H)	

[a] Asterisk indicates significantly different at $p < 0.05$. The line connecting two figures indicates the direction of comparison. Any comparisons shown in Table I but not shown in this table were not statistically significant, $p > 0.05$.

of the brains and kidneys of pups born to HCB mothers but nursed by control dams were heavier than, but not significantly different ($p > 0.05$) from, those of pups that remained with the treated mothers (B vs. D). The spleens of pups nursed by HCB-fed dams were generally smaller than those of the pups nursed by the control dams. Although no significant differences were observed for other organs examined, organs of the pups (C, D, G, G) nursed by, HCB-fed dams tend to be consistently lighter than those that remained with their control mothers. These results were comparable to previous observations (Mendoza et al., 1975). Vos et al. (1972) observed that predator birds fed HCB-contaminated mice had enlarged livers and a significant decrease in the heart weight.

Now, considering HBB, Table IV shows that, in general, the body or organ weights of 17- and 21-day-old pups were not statistically different from each other regardless of the treatment. In other words, the amount of HBB derived through the placenta and milk had no significant effects on body and organ actual weights. The significant differences indicated in the table were so inconsistent that it is difficult to interpret them. It should be noted that pups nursed by the HBB-treated dams had heavier livers than those nursed by the control dams. Conversely, the former generally had smaller organs than the latter.

The difference between relative weights of the control and HCB-treated livers (A and B vs. C, E vs. G and H) shown in Table V was statistically significant. As can be expected, the difference between relative weights within the group nursed by the control or treated dams (A vs. B, C vs. D, E vs. F, G vs. H) was not significant. Previous reports indicated that the increase in the liver weight due to the HCB treatment was associated with microsomal enzyme induction (Medline et al., 1973; Grant et al., 1974) and smooth endoplasmic reticulum proliferation (Kuiper-Goodman et al., 1976). An increase in liver carboxylesterases of the suckling rat was also observed after feeding the dams with a diet containing 80 ppm HCB (Mendoza and Shields, 1976).

Like the HCB-treated pups, 17-day-old, HBB-treated pups had relative liver weights that were significantly heavier than those of the corresponding control pups (A vs. C and D, B vs. D) (Table V). In 21-day-old pups, there was a tendency toward heavier livers in HBB-treated pups, but no significant difference was observed between relative liver weights in either control or HBB-treated groups. Although adult male rats showed no ill effects after feeding on HBB-containing diets (Mendoza et al., 1977b), it seems that in terms of liver weights, suckling pups showed a certain degree of susceptibility to HBB. Strik (1973) reported that with hexabromobiphenyl treatment, there was an increase in the liver weight of the Japanese quail; the increase in the liver relative weight was magnified by the decrease in the body weight as the hexabromobiphenyl dose increased.

Further analysis showed no significant difference observed between relative weights of brain, heart, kidney, and spleen that could be attributed to the HCB or HBB treatment (data not shown), although there was a general tendency for growth retardation in terms of actual weights of the body and organs examined, except liver, of the pups nursed by the treated dams.

In summary, it could be stated that two statistical interpretations using either actual or relative weights were very similar. In general, pups nursed by the HCB- or HBB-fed dams had larger livers than the controls. Both HCB and HBB significantly increased the liver relative weights of pups nursed by treated dams. Results further show that pups (B and F) born to HCB- or HBB-fed dams but nursed by control dams did not show marked effects on body or organ weights (actual or relative) that could be attributed to placentally transmitted HCB or HBB. It can also be concluded that HCB or HBB obtained through milk had greater effects on suckling rats than that placentally transmitted. It is important, therefore, that milk should be free of these types of compounds when nursing the young. Further studies are in progress to determine the effects of HBB on esterases and other biochemical parameters on suckling, juvenile, and mature rats.

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Pharmacokinetics of Mirex in Goats. 2. Residue Tissue Levels, Transplacental Passage during Recovery

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Male and female goats were given 1 mg of mirex/kg of bodyweight for 61 weeks, followed by 10 mg of mirex/kg of bodyweight to the females for 4 weeks. Thereafter, mirex levels in adipose tissue and blood were determined at intervals over a 52-week recovery period for the males and a 34-week recovery period for the females. An additional group of female goats was given 1 mg of mirex/kg of bodyweight for 18 weeks and 10 mg of mirex/kg of bodyweight for 4 weeks thereafter. Mirex levels in adipose tissue and blood were determined in these goats at intervals over a 52-week recovery period. During the 34- and 52-week recovery periods, the mirex levels in adipose tissue decreased to half their original value in female goats and in male goats after a 52-week recovery period to about a third of their original value. The proportional decrease in mirex plasma concentrations was much greater than in adipose tissue. At the end of the recovery period on autopsy, mirex was also present in brains and livers of the adult goats and in fetuses of early gestational age.

Mirex [dodecachlorooctahydro-1,3,4-metheno-2*H*-cyclobuta[*cd*]pentalene] is a very stable chemical that is used as an insecticide particularly in the control of fire ants. At one time it was also used as a flame retardant. The accumulation of this chemical in goats has previously been reported (Smrek et al., 1977). In that study male and female goats were dosed with 0 or 1 mg of mirex/kg of body weight. During the dosing period the goats were bred twice. A steady state in adipose tissue was not reached during a 61-week dosing period. At the end of the study the adipose tissue levels were lower in female than in male goats but reproduction did not noticeably affect mirex adipose tissue levels. This paper reports the results of a 34–52-week recovery period after dosing of the goats was discontinued.

METHODS

The methods of dosing and maintaining the goats as well as the chemical analyses of the biological specimens have been outlined in detail in a previous paper (Smrek et al., 1977). Briefly, 15 female and 10 male goats were divided into groups of five. All goats were weighed at biweekly intervals throughout the experiment. One group of five males and five females were kept as controls. One group of five males and five females were dosed with 1 mg of mirex/kg of bodyweight per day for 61 weeks. Following this the five females were given 10 mg of mirex/kg of body weight for 4 weeks, and then dosing was stopped. The group of dosed female goats and the controls were bred twice during the dosing period. Dosing was started at the onset of the first pregnancy. Blood and adipose tissue biopsies were obtained from these goats at intervals during the recovery period as indicated in Tables I and II. The female goats were killed 34 weeks after dosing was stopped, and autopsies were performed. At the time of the autopsies the goats were in early pregnancy and fetal material for chemical analysis was also collected. The male goats were killed 52 weeks after dosing was stopped.

An additional group of five female goats was given 1 mg of mirex/kg of bodyweight for 18 weeks and then 10 mg of mirex/kg of bodyweight for 4 weeks. In this group, dosing was started on the first postpartum day after the first breeding cycle. The schedule for adipose tissue biopsies and collection of blood for this group of goats is given in Table III. The goats were killed after a 52-week recovery period. The male and female control goats were killed with the experimental goats. Since dosing was discontinued earlier in the female goats which were allowed to recover for 52 weeks, it was possible to kill all female goats within a 1-week period.

The serial adipose tissue samples were obtained by open biopsy from the rump as described previously (Smrek et al., 1977). At autopsy, all tissues from major organs were fixed in 4% formaldehyde for microscopic examination. All tissue sections were stained with hematoxylin and eosin. Tissues were also collected for chemical analysis from brain, liver, superficial and deep sucutaneous adipose, and omental fat. These tissues were stored frozen until chemical analysis was done.

RESULTS AND DISCUSSION

The bodyweight of the female goats fluctuated but showed no particular trend. The male goats continued to gain weight throughout the experiment (Figure 1).

The results of the chemical analysis of the adipose tissues, brain, liver, and plasma are given in Tables I–IV. In the female goats (Tables I and III), the mirex plasma and adipose tissue levels declined very slowly during the

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