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Effects of Chronic Dietary Hexachlorobenzene Exposure on the Reproductive Performance and Survivability of Mink and European Ferrets[1]

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Abstract. Feeding adult mink and European ferrets diets that contained 1, 5, or 25 mg/kg added hexachlorobenzene (HCB) resulted in reduced reproductive performance as indicated by decreased litter size, increased percentage of stillbirths, increased kit mortality, and decreased early kit growth. Diets treated with 125 or 625 mg/kg HCB were lethal to adults of both species. In general, the mink were more sensitive to the toxic effects of HCB than were ferrets.

In a second experiment, the cross-fostering of mink kits whelped by untreated dams to females fed 2.5 mg/kg HCB, and *vice versa*, resulted in increased kit mortality when compared to untreated controls. The *in utero* exposure to HCB, however, resulted in higher kit mortality than exposure via the dam's milk.

The presence of hexachlorobenzene (HCB) in the world's ecosystems poses a significant risk to the inhabitants of these systems. As a pre-emergent fungicide and by-product of organochlorine synthesis (Mumma and Lawless 1975; Quinlivan and Chassemi 1977; Courtney 1979), this compound has been identified in the oceans in concentrations likely to result in serious problems (Morse 1975; Seltzer 1975).

Reduced reproductive success has been reported in rats fed HCB-treated diets (Mendoza *et al.* 1975; Grant *et al.* 1977; Kitchin *et al.* 1982) and an increased incidence of stillborn human infants was noted in Turkey following the consumption of HCB-contaminated bread by pregnant women (Courtney 1979). Hexachlorobenzene readily bioconcentrates in the food chain (Johnson *et al.* 1974; Lu and Metcalf 1975; Hansen *et al.* 1979; Niimi 1979). Tissue residues of this halogenated aromatic hydrocarbon are especially high in predatory mammals (Koss and Manz 1976) and birds (Vos *et al.*, 1969; Best 1973; Cromartie *et al.* 1975).

Since both the mink (*Mustela vison*) and European ferret (*Mustela putorius furo*) occupy a position at the apex of the food chain and represent species likely to be exposed to high concentrations of a chemical capable of biomagnification, such as HCB, they were selected to study the HCB-related effects on reproduction and survival in upper echelon carnivores. These two mustelids have also been shown to be among the more sensitive test animals available for studying the effects of other halogenated aromatic hydrocarbons (Bleavins *et al.* 1980; Ringer *et al.* 1981; Bleavins *et al.* 1982).

Materials and Methods

This study consisted of two experiments conducted at the MSU Experimental Fur Farm during 1981 and 1982.

Experiment 1

Seventy-eight adult standard dark mink were divided into five treatment groups and a control group, each consisting of ten females and three males. The same numbers of adult fitch-colored European ferrets were similarly assigned to five treatment groups and a

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7202

Table 1. Mean length of gestation and litter size of female mink and ferrets fed a control or HCB-supplemented diet in a chronic feeding trial

Dietary treatment	No. ♀♀ whelped/ no. mated	Gestation[a,b] (days)	No. kits whelped[b] Total	Alive	Dead
Mink					
0 ppm HCB	10/10	49.5 ± 1.23	6.4 ± 0.58	4.9 ± 0.72	1.5 ± 0.50
1 ppm HCB	8/10	48.6 ± 1.38	5.3 ± 0.65	4.3 ± 0.81	1.0 ± 0.56
5 ppm HCB	10/10	48.4 ± 1.23	5.9 ± 0.58	5.3 ± 0.72	0.6 ± 0.50
25 ppm HCB	8/10	47.5 ± 1.38	3.4 ± 0.65*	1.9 ± 0.81**	1.5 ± 0.56
Ferret					
0 ppm HCB	8/10	41.4 ± 0.35	8.8 ± 0.77	8.3 ± 0.82	0.5 ± 0.48
1 ppm HCB	8/10	41.6 ± 0.35	9.7 ± 0.77	9.6 ± 0.82	0.1 ± 0.48
5 ppm HCB	6/10	40.7 ± 0.40	11.3 ± 0.89	10.3 ± 0.95	1.0 ± 0.55
25 ppm HCB	6/10	41.7 ± 0.40	7.2 ± 0.89	4.7 ± 0.95*	2.5 ± 0.55**

[a] Based on date of final mating
[b] Mean ± SE
* Significantly different from control ($P < 0.05$)
** Significantly different from control ($P < 0.01$)

Table 2. Kit birth, three week, and six week body weights, and biomass produced by lactating female mink and ferrets fed a control or HCB-supplemented diet in a chronic feeding trial

Dietary treatment	Kit body weight[a] (g) No. kits	Birth	No. kits	3 week	No. kits	6 week	No. litters	Biomass[a,b]
Mink								
0 ppm HCB	49	9.0 ± 0.21	45	97 ± 3.4	45	245 ± 6.9	9	441 ± 55.5
1 ppm HCB	34	7.9 ± 0.25**	23	87 ± 4.7	19	229 ± 17.1	6	301 ± 67.9
5 ppm HCB	53	8.1 ± 0.20**	12	68 ± 6.2**	12	173 ± 20.0**	5	145 ± 74.4*
25 ppm HCB	15	7.6 ± 0.38**	2	36 ± 1.5**	2	71 ± 4.0**	1	59
Ferret								
0 ppm HCB	57[c]	9.0 ± 0.21	56	76 ± 2.9	42	257 ± 9.8	7	533 ± 60.6
1 ppm HCB	77	9.4 ± 0.18	68	91 ± 2.7**	59	301 ± 8.2**	8	689 ± 56.7
5 ppm HCB	62	7.9 ± 0.20**	49	87 ± 3.1*	42	263 ± 9.8	6	645 ± 65.4
25 ppm HCB	28	6.9 ± 0.30**	16	95 ± 5.5**	10	262 ± 20.0	6	350 ± 65.4

[a] Mean ± SE
[b] Biomass = average kit body weight gain between birth and three weeks of age × average number of kits per lactating female
[c] Does not include one female's litter that fell through the cage wire
* Significantly different from control ($P < 0.05$)
** Significantly different from control ($P < 0.01$)

control group. The animals were fed *ad libitum* a basal diet[3] with either 0 (control), 1, 5, 25, 125, or 625 mg/kg added HCB beginning January 20, 1981. The treated diets were prepared by mixing HCB with ground mink cereal to form a premix which was added in appropriate amounts to the other ingredients of the diet to yield the desired concentrations of HCB. Standard commercial farm procedures were followed in the care, feeding, and breeding of the animals. They were observed daily for signs of toxicity and gross necropsies were performed on those mink and ferrets that died prior to the termination of the study. During necropsy, livers were checked under ultraviolet light for fluorescence indicative of porphyria.

The females were mated to males within their respective treatment groups. Matings were confirmed by the presence of motile spermatozoa in vaginal smears taken immediately after copulation. Each mated female was given an opportunity for a second mating either the day after the initial mating or eight days later. The reproductive parameters shown in Tables 1 through 3 were monitored during the study. The kits were weaned at seven weeks of age and then placed on the untreated basal diet for the remainder of the study. The adults were maintained on their respective diets until termination of the study on December 16, 1981 (331 days for mink) and December 17, 1981 (332 days for ferrets).

[3] The basal diet, as fed, consisted of 20.0% whole chicken, 16.7% commercial mink cereal, 12.5% ocean fish scrap, 6.7% beef liver, 6.7% beef lungs, 3.3% beef tripe, 3.3% beef trimmings, 2.9% cooked eggs, 1.1% powdered milk, and 26.8% added water.

Experiment 2

Forty standard dark female mink (proven second year breeders) were randomly assigned to two groups of 20 animals each on March 1, 1982. The mink were cared for according to commercial mink farm procedures. Feed and water were provided *ad libitum* throughout the experiment. Thirty-nine of the 40 females were mated at least once between March 9–13, 1982, and were given an opportunity to remate the day following the initial mating. First matings were confirmed by the presence of motile spermatozoa in vaginal smears taken immediately following copulation.

The mink in both groups were fed the basal diet[3] prior to breeding. Beginning March 17, 1982, one group was continued on the basal diet for the duration of the experiment, while the other group of females received the basal diet treated with 2.5 mg/kg of HCB throughout pregnancy and lactation.

Each female was checked twice daily for the birth of kits during the whelping period. The kits were counted and weighed the day of birth and at 3 and 6 weeks of age. On days that mink from both the control and the HCB-treated groups whelped, their entire litters were transferred to females of the opposite treatment (cross-fostered). Prior to the switching of litters, the kits, nest boxes, and females' nasal region were dusted with talcum powder in an attempt to reduce the risk of rejection or the killing of the cross-fostered kits. Of 33 litters, fourteen litters were cross-fostered to yield the following treatment regimes:

1) Control: Eleven litters born to and raised by females fed the basal diet prior to breeding and throughout pregnancy and lactation.
2) Control → HCB: Seven litters born to females fed the basal diet, but raised by females fed the 2.5 mg/kg HCB-treated diet.
3) HCB → Control: Seven litters born to dams fed the 2.5 mg/kg HCB-treated diet, but raised by females fed the basal diet.
4) HCB: Eight litters born to and raised by females fed the 2.5 mg/kg HCB-treated diet throughout pregnancy and lactation.

The data from Experiments 1 and 2 were analyzed by either the Student's t-test (comparisons of two means) or the Dunnett's t-test (multiple comparisons to the control mean), as described by Gill (1978). Unless otherwise stated, the $P < 0.05$ level was used as the criteria for significant differences between treatment means.

Results

Experiment 1

Gas chromatographic analysis of the diets fed to the mink and ferrets revealed that HCB-concentrations were within ±24% of the nominal levels.

The reproductive performance of the mink and ferrets on the various dietary treatments is presented in Tables 1–3. All of the mink and ferrets fed the diet that contained the highest concentration of HCB (625 mg/kg), failed to survive to the breeding season and none of the mink that received the 125 mg/kg HCB diet whelped. Several female mink from the 125 mg/kg HCB-treatment group that died during April showed resorption sites in the uterus at necropsy indicating that, although these females were unable to maintain pregnancies, fertilization and implantation were not prevented. The female ferrets fed 125 mg/kg HCB failed to come into estrus, as determined by vulvar swelling, at any time during their normal breeding season (April to September). These animals were, therefore, never bred and could not be evaluated for other HCB-related reproductive effects.

Table 3. Mortality of offspring produced by female mink and ferrets fed a control or HCB-supplemented diet in a chronic feeding trial

Dietary treatment	Kit mortality (%)	
	To 3 weeks	To 6 weeks
Mink		
0 ppm HCB	8.2	8.2
1 ppm HCB	32.4	44.1
5 ppm HCB	77.4	77.4
25 ppm HCB	86.7	86.7
Ferret		
0 ppm HCB	1.8	26.3
1 ppm HCB	11.7	23.4
5 ppm HCB	21.0	32.3
25 ppm HCB	42.9	64.3

In the groups able to produce offspring, the length of gestation was not affected by dietary HCB (Table 1). However, in the mink fed 25 mg/kg HCB, a significant reduction in litter size was seen as well as a decrease in the number of kits born alive. No change was observed in the number of stillborn fetuses, although, because of the reduction in the number of viable offspring whelped, these stillborn fetuses constitute a greater percentage of the litter. The ferrets also did not show significant changes in litter characteristics until they received 25 mg/kg HCB in the diet. Although litter size was unchanged in ferrets fed 25 mg/kg HCB, the number of offspring born alive was lower than in the control and there was a higher incidence of stillborn kits.

As shown in Table 2, the mink kits born to dams consuming the 1, 5, and 25 mg/kg HCB-treated diets were smaller ($P < 0.01$) at birth than the kits whelped by untreated mothers. A similar relationship was seen in ferret kit birth weights, although only the 5 and 25 mg/kg treatment groups were significantly smaller than the controls. Mink kit body weights at three and six weeks of age in the 5 and 25 mg/kg HCB-treated groups were significantly ($P < 0.01$) lower than control kit body weights. At

Table 4. Mean estimated hexachlorobenzene consumption (mg) by adult mink and ferrets[a] in a chronic feeding trial

Dietary treatment	Male				Female			
	n	Mink	n	Ferret	n	Mink	n	Ferret
0 ppm HCB	3	0	2	0	9	0	10	0
1 ppm HCB	3	72	2	78	9	45	9	38
5 ppm HCB	3	359	3	392	10	223	9	191
25 ppm HCB	3	1,796	3	1,959	9	1117	8	955
125 ppm HCB[b]	3	1,818 ± 205.8	3	3,499 ± 1,454.3	10	2,649 ± 356.3	10	3,088 ± 512.1
625 ppm HCB[b]	3	2,629 ± 119.9	3	3,920 ± 686.0	10	1,546 ± 117.6	10	3,088 ± 230.1

[a] Based on daily food consumption values reported by Bleavins and Aulerich (1981)
[b] All animals on these dietary treatments died prior to termination of the study; HCB consumption values expressed as mean ± SE

three weeks of age, the ferret kits on all HCB treatments weighed significantly more than the untreated kits. By six weeks of age, only the 1 mg/kg HCB ferret kits continued to weigh more than the control kits. As shown in Table 2, a strong trend toward reduced biomass (Biomass = the average kit body weight gain between birth and three weeks of age × the average number of kits per lactating female) with increasing HCB concentrations was seen for the mink, although only the 5 mg/kg HCB-treated females showed a significantly lower biomass than the control mink. No significant difference was observed between the biomass produced by the control and HCB-treated ferrets.

Exposure of adult mink and ferrets to HCB caused a dose-related increase in kit mortality (Table 3). The percentage of kits dying before three weeks of age accounted for the greatest proportion of the mortality observed in the mink, with only occasional deaths occurring between the third and sixth week. Although not quantitated, empirical observation revealed that the majority of the kit deaths occurred within three days of birth. At three weeks of age, the percentage of kits dying was one-half to two-thirds lower in the ferrets than in the mink. The kit mortality to six weeks of age was still lower in the ferrets (except for the 0 mg/kg group) than in the mink kits raised by dams fed comparable dietary concentrations of HCB. A much greater portion of the kit mortality to six weeks of age occurred between three and six weeks post-partum in the ferrets than in the mink.

Based on the food consumption values reported by Bleavins and Aulerich (1981), the quantity of HCB consumed by the adult mink and ferrets on the various treatments was calculated (Table 4). Since no deaths directly attributable to the effects of HCB occurred in the animals fed the 0, 1, 5, and 25 mg/kg HCB diets, the values shown for these dietary treatments in Table 4 represent the estimated quantity of the chemical consumed prior to the termination of the study. The values reported for the 125 and 625 mg/kg HCB-treated animals denote the estimated quantity of the chemical required to cause death at these two levels of dietary exposure. The actual quantity of HCB consumed, however, may be slightly lower than reported, since food consumption declined in most animals for several days immediately preceeding death. The estimated quantity of HCB consumed by the male mink prior to death in the 125 and 625 mg/kg treatment-groups was below the nonlethal maximum dose tested (2,000 mg/kg of body weight or 3,600 mg for an average 1.8 kg male mink) during the preliminary LD_{50} trial (unpublished data). Unlike the acute exposure, the chronic effects included dramatic weight loss, anorexia, gastrointestinal hemorrhaging, bloody stool and death. No evidence of porphyria, as reported in certain other species treated with HCB, was observed in the mink or ferrets at necropsy. The male ferrets had a dietary intake of approximately 3,500 mg and 3,900 mg of HCB prior to death on the 125 and 625 mg/kg HCB diets, respectively. This quantity of chemical was greater than the lethal dose for the male mink which was approximately 1,800 and 2,600 mg of HCB for animals fed 125 and 625 mg/kg HCB, respectively. In both the male mink and ferrets, more HCB was ingested before death by animals consuming the 625 mg/kg HCB diet than by those eating the 125 mg/kg HCB-treated feed. Female mink fed the two highest dietary concentrations of HCB consumed an estimated 2,649 mg (125 mg/kg) and 1,546 mg (625 mg/kg) of the chemical. The amount of HCB consumed by the female ferrets was roughly equal for the two highest treatment levels (3,088 *vs* 3,031 mg) and generally was greater than the quantity consumed by female mink fed comparable concentrations of HCB.

The average survival time for mink and ferrets fed diets that contained 125 or 625 mg/kg HCB is shown in Table 5. Mink that received the 625 mg/kg HCB diet survived, on the average, less than

ble 5. Survival time of adult mink and ferrets[a] fed HCB-supplemented diets in a chronic feeding trial

Dietary treatment	Male No.	Male Mean ± S.E. (days)	Male Range (days)	Female No.	Female Mean ± S.E. (days)	Female Range (days)
Mink						
125 ppm HCB	3	67 ± 7.6	53-79	10	156 ± 21.0	84-266
625 ppm HCB	3	19 ± 0.9	18-21	10	18 ± 1.4	9-24
Ferret						
125 ppm HCB	3	121 ± 50.2	46-216	10	221 ± 36.6	39-332
625 ppm HCB	3	27 ± 4.7	18-34	10	42 ± 3.2	30-59

[a] No deaths attributable directly to HCB intoxication were observed in the 0, 1, 5, or 25 ppm treatment groups

Table 6. Gestation length and reproductive performance of female mink fed a control or HCB-supplemented diet in a cross-fostering study

Dietary treatment	No. ♀♀ whelped/ no. mated	Gestation[a,b] (days)	No. kits whelped[b] Total	Alive	Dead
Control	18/20	51 ± 0.8	6.4 ± 0.39	6.1 ± 0.43	0.3 ± 0.14
HCB	15/19	54 ± 0.9*	6.1 ± 0.43	5.8 ± 0.47	0.3 ± 0.16

[a] Based on date of final mating
[b] Mean ± S.E
* Significantly different from control ($P < 0.05$)

three weeks. In all instances, the ferrets survived longer than the mink on comparable dietary concentrations of HCB. Six of the female ferrets in the 125 mg/kg HCB-treatment group showed signs of hyperexcitability and abnormal aggressiveness for this species prior to death.

Experiment 2

As shown in Table 6, whelping success of 90 and 79% was obtained with female mink fed the control and 2.5 mg/kg HCB-treated diets, respectively. The HCB-treated females had significantly longer gestations than the controls; however, both were within the normal range of 40-60 days for mink (Travis and Schaible 1960). No difference was observed in total litter size or the average number of kits born alive vs dead between the two groups. Kit body weights at birth were comparable between the two groups, although female kits exposed to HCB *in utero* and via the milk weighed significantly more than control kits at three weeks of age and at six weeks of age. The male kits in the Control → HCB group were significantly heavier than their control counterparts (Table 7).

The kits whelped and nursed by the control females had a low mortality rate of 5% (Table 8). Kits in the Control → HCB treatment group showed an increased mortality rate of 13.6%, while a mortality rate of 22.5% was observed in the kits in the HCB → Control group. Litters whelped and nursed by females fed HCB had a kit mortality rate comparable to the HCB → Control group (20.5 *vs* 22.5%). No significant difference was noted in the biomass produced for any treatment.

Discussion

The results of these experiments show mink and ferret to be sensitive to the toxic effects of HCB. The clinical signs of anorexia and eventual death are consistent with those seen in the rat (Villeneuve 1975). In addition, gastrointestinal hemorrhaging and bloody stools were observed in the mink and ferrets fed 125 and 625 mg/kg HCB. The high fat content of the basal diet (25%) probably resulted in excellent intestinal absorption of HCB by these carnivores. The quantity of HCB absorbed by the rat has been shown to be highly dependent on the fat content of the diet (Koss and Koransky 1975; Zabik and Schemmel 1980), with high fat rations dramatically increasing HCB absorption.

In general, the male mink and ferrets died earlier in the course of the experiment than females of the

Table 7. Birth, three week, and six week body weights of mink kits exposed to HCB in a cross-fostering study

Cross-fostering regime	Body weight[a] (g)					
	No.	Birth	No.	3 weeks	No.	6 weeks
Male						
Control	62	9.3 ± 0.21	31	104 ± 2.7	30	297 ± 11.4
Control → HCB	—	—	23	110 ± 3.2	23	340 ± 13.0*
HCB → Control	—	—	10	108 ± 4.8	10	309 ± 19.8
HCB	28	10.0 ± 0.31	11	112 ± 4.6	11	289 ± 18.9
Female						
Control	47	8.7 ± 0.23	27	94 ± 2.4	27	260 ± 8.3
Control → HCB	—	—	15	95 ± 3.2	15	274 ± 11.2
HCB → Control	—	—	21	97 ± 2.7	21	282 ± 9.5
HCB	59	9.1 ± 0.21	20	105 ± 2.7*	20	284 ± 9.7

[a] Mean ± S.E
* Significantly different from control ($P < 0.05$)

Table 8. Kit mortality and biomass produced by female mink fed a control and/or HCB-supplemented diet in a cross-fostering study

Cross-fostering regime	No. litters	No. kits	Kit mortality—%		Biomass[a,b]
			To 3 weeks	To 6 weeks	
Control	11	68	3.3	5.0	477 ± 57.8
Control → HCB	7	41	13.6	13.6	515 ± 72.4
HCB → Control	7	44	22.5	22.5	403 ± 72.4
HCB	8	43	20.5	20.5	377 ± 67.7

[a] Biomass = average kit body weight gain between birth and 3 weeks of age × average number of kits per lactating female
[b] Mean ± S.E

same species. The males appeared to exhibit a compounding of the toxic effects of HCB with the added stress and increased activity levels of these animals in preparation for and during the breeding period. This finding is in direct contrast to the events seen in the rat (Kuiper-Goodman *et al.* 1977; Richter *et al.* 1981), a species which readily develops porphyria following exposure to HCB. Also, in contrast to the rat, the mink and ferrets did not show signs of porphyria and so the toxic pathway leading to death may be unrelated to disruption of porphyrin synthesis. Gralla *et al.* (1977) reported that the dog also does not develop porphyria in response to HCB intoxication. They suggested that the dog may not possess the biosynthetic and excretory pathways which respond to HCB to result in porphyria.

The ferrets consistently lived longer than the mink fed comparable concentrations of HCB. As observed in previous studies with polychlorinated biphenyls (Bleavins *et al.* 1980), the ferret is quite sensitive to the effects of halogenated aromatic chemicals, but is notably more resistant than the mink. A differential sensitivity to HCB has also been reported in rodents, with the rat being less susceptible to the toxic effects than the guinea pig (Villeneuve and Newsome 1975).

The female ferrets consuming the 125 mg/kg HCB diet failed to exhibit signs of estrus during the normal breeding season (April to September). This condition may have been produced through the increased biotransformation of endogenous hormones by steroid hydroxylating enzymes induced by HCB. Levin *et al.* (1967) found an inhibition of the action of estradiol and estrone on the female reproductive tract due to the increased metabolism of estrogens. A similar condition may have been responsible for the anestrous state of the ferrets fed 125 mg/kg HCB in this experiment.

The maternal tissue stores of HCB act as a readily available reservoir of this chemical for continued exposure of the developing and growing offspring (Courtney and Andrews 1979). Transplacental passage of HCB from the mother to the fetus has been shown in rats (Villeneuve and Hierlihy 1975; Andrews and Courtney 1976; Courtney *et al.* 1976; Svendsgaard *et al.* 1979), mice (Courtney *et al.* 1976; Courtney and Andrews 1979; Svendsgaard *et al.* 1979), rabbits (Villeneuve *et al.* 1974), monkeys

Bailey *et al.* 1980), and humans (Siyali 1974). The so called "placental barrier" provides only limited protection to the developing fetus against exposure to certain chemicals that may be present in the pregnant female during gestation (Goodman *et al.* 1982). The placenta offers little resistance to the passage of small lipophilic molecules such as HCB. Svendsgaard *et al.* (1979) observed a six-fold increase in fetal concentrations of HCB in mice and rats that were multiple dosed during late gestation as compared to animals similarly dosed during early pregnancy. This phenomenon probably results from normal changes in placental permeability (Moya and Smith 1965). As the placental membranes become thinner and the flow rate of the umbilical circulation increases with advancing pregnancy, fetal contact with substances in the dam's blood is enhanced. Since most foreign substances cross the placenta by simple diffusion (Eckhoff 1972a, 1972b), changes in placental surface area available for transfer, thickness of placental membranes, and the rate of maternal blood flow with progressing gestation increases the rate of diffusion, resulting in near-term exposed fetuses possessing a greater concentration of HCB than those fetuses exposed during the early stages of development. The potential for chemically-induced fetotoxicities to be the result of placental and/or fetal biotransformation must also be considered (Goodman *et al.* 1982).

Although no morphological alterations were observed in the mink or ferret kits, HCB has been found to cause grossly observable teratogenic effects in rats (Khera 1974) and mice (Courtney *et al.* 1976), including cleft palates and kidney malformations.

Dietary exposure to HCB depressed reproduction in the rat (Mendoza *et al.* 1975; Grant *et al.* 1977). The females produced fewer litters and the number of pups surviving to weaning was reduced. Rats exposed to HCB also exhibited an increased incidence of stillbirths, as did women in Turkey consuming HCB-contaminated bread (Courtney 1979). Female mink fed 25 mg/kg HCB-treated diets produced fewer kits, but no increase was observed in the number of kits born dead per litter. However, due to a decrease in total litter size, the relative proportion of kits born dead was increased. Ferrets that received the 25 mg/kg HCB diet showed no change in total litter size, but demonstrated an elevated number of stillborns as was reported for other species.

Mink and ferret kits produced by females fed the HCB-treated diets in Experiment 1 were generally smaller at birth than the control kits. This is consistent with the effects noted in rats (Khera 1974; Grant *et al.* 1977), although exposure levels were much higher in the rodent studies. The reduced rat pup birth weights were first observed at 80 mg/kg HCB, while a depression in birth weight was observed at 1 mg/kg HCB in the mink and 5 mg/kg HCB in the ferrets. A reduced growth rate of offspring produced by HCB-treated dams has also been reported in rats (Grant *et al.* 1977) and monkeys (Bailey *et al.* 1980). Mink kits in the HCB-treatment groups exhibited lower three- and six-week body weights than control kits. These effects were seen with 5 mg/kg of maternal dietary HCB exposure which was considerably less than was required to produce a similar condition in the rat and monkey. In contrast to the mink, the three-week-old HCB-treated ferrets weighed significantly more than their control counterparts, although a corresponding increase in biomass was not observed.

The presence of HCB in the milk of lactating females has been shown for humans (Siyali 1973; Baaken and Seip 1976), Rhesus monkeys (Bailey *et al.* 1980), cattle (Fries and Marrow 1971), and rats (Grant *et al.* 1977). Siyali (1973) found HCB present in the breast milk samples of all the exposed women tested. High concentrations of HCB have been reported in weanling rats, compared to a relatively low dietary intake of the compound by their dams (Grant *et al.* 1977), which suggests that the excretion of HCB via the milk may be an important route of offspring exposure and of maternal excretion. This supposition is supported by the finding of Bailey *et al.* (1980) that nursing infant Rhesus monkeys were at greater risk than their mothers when the dams were exposed to HCB. Human infants are also sensitive to HCB when exposure was limited to that coming from their mothers. A 95% incidence of infant mortality (as compared to 10% mortality in adults) was reported for some villages in Turkey where nursing mothers consumed HCB-contaminated bread (Courtney 1979).

Offspring mortality attributed to HCB exposure showed a dose-dependent increase in both the mink and ferret. Even low level exposure to HCB resulted in poor kit survival. Reduced postnatal viability has also been observed in rats (Grant *et al.* 1977) and humans (Courtney 1979) exposed to HCB. Mortality to weanlings of 45.4% was reported for rat pups nursed by females fed diets that contained 100 mg/kg HCB (Kitchin *et al.* 1982), while 1 mg/kg dietary HCB exposure in adult mink resulted in 44.1% preweaning mortality in the kits. Even when taking into consideration the longer period of nursing in the mink than the rat (6 weeks *vs* 3 weeks), the mink must still be considered to be much more sensitive to the toxic effects of HCB. Ferret kit mortality in the 25 mg/kg HCB-treated group was 64.3%,

which was intermediate between the mortality observed at 100 mg/kg (45.4%) and 120 mg/kg (93.1%) HCB in rat pups (Kitchin *et al.* 1982). Thus, the ferret can also be classified as reproductively more sensitive to HCB than the rat. Grant *et al.* (1977) reported a 20 mg/kg HCB content in the maternal diet to be the no-effect level for reproduction in the rat, a level which had profound effects on the reproductive performance of both the mustelids tested in this study.

In Experiment 2, an elevated degree of kit mortality was seen in all groups exposed to HCB during the course of a cross-fostering study. Mortality of kits exposed to HCB via the milk (Control → HCB) was higher than in the control, but lower than in the group exposed only *in utero* (HCB → Control). The kits exposed to HCB *in utero* showed a mortality level comparable to the animals that received HCB insult both during gestation and nursing. Thus, at the low dietary concentration of HCB used in this study (2.5 mg/kg), *in utero* exposure constituted a more serious threat to offspring survival than exposure via the dam's milk. This phenomenon occurs even though the quantity of HCB received by a nursing animal via the dam's milk may be many times greater than the amount that passes across the placenta during fetal development (Grant *et al.* 1977; Bleavins *et al.* 1982). The cross-fostering of rat pups born to untreated dams to females fed 0.5% (5,000 mg/kg) HCB in their diet, resulted in the death of all pups (DeMatteis *et al.* 1961). These effects were seen at exposure levels much higher than can be tolerated by mink expected to produce viable offspring. Preweaning kit mortality comparable to that seen in the 2.5 mg/kg HCB-treated groups was not observed in rats until maternal HCB exposure reached 60 mg/kg (Kitchin *et al.* 1982). The improved survival of mink kits whelped by dams fed 2.5 mg/kg HCB in Experiment 2 (79.5%) compared to that observed at 1 mg/kg HCB in Experiment 1 (45.1%) may be the result of better maternal care provided by the second year females (Experiment 2) than by the yearling dams (Experiment 1). The difference might also be attributable to the length of dietary exposure to the chemical, and/or fluctuations in environmental factors from year to year.

References

Andrews JE, Courtney KD (1976) Inter- and intralitter variation of hexachlorobenzene (HCB) deposition in fetuses, Abstract 87. Toxicol Appl Pharmacol 37:128

Baaken AF, Seip M (1976) Insecticides in human breast milk. Acta Pediat Scand 65:535

Bailey J, Knauf V, Mueller W, Hobson W (1980) Transfer of hexachlorobenzene and polychlorinated biphenyls to nursing infant Rhesus monkeys: Enhanced Toxicity. Environ R 21:190

Best SM (1973) Some organochlorine pesticide residues in wildlife of the northern territory, Australia, 1970–71. Aust J Biol Sci 26:1161

Bleavins MR, Aulerich RJ (1981) Feed consumption and food passage time in mink (*Mustela vison*) and European ferrets (*Mustela putorius furo*). Lab Anim Sci 31(3):268

Bleavins MR, Aulerich RJ, Ringer RK (1980) Polychlorinated biphenyls (Aroclors 1016 and 1242): Effect on survival and reproduction in mink and ferrets. Arch Environ Contam Toxicol 9:627

Bleavins MR, Breslin WJ, Aulerich RJ, Ringer RK (1982) Excretion and placental and mammary transfer of hexachlorobenzene in the European ferret (*Mustela putorius furo*). J Toxicol Environ Health 10:929

Courtney KD (1979) Hexachlorobenzene (HCB): A review. Environ Res 20:225

Courtney KD, Andrews JE (1979) Mobilization of HCB during gestation. Toxicol Lett 3:357

Courtney KD, Copeland MF, Robbins A (1976) The effects of pentachloronitrobenzene, hexachlorobenzene, and related compounds on fetal development. Toxicol Appl Pharmacol 35:239

Cromartie E, Reichel WL, Locke LN, Belisle AA, Kaiser TE, Lamont TG, Mulhern BM, Prouty RM, Swineford DM (1975) Residues of organochlorine pesticides and polychlorinated biphenyls and autopsy data for bald eagles, 1971–72. Pestic Monit J 9:11

DeMatteis F, Prior BE, Rimington C (1961) Nervous and biochemical disturbances following hexachlorobenzene intoxication. Nature 191:363

Eckhoff G (1972a) Transplacental passage of drugs and o exogenous compounds: A review—Part I. Iowa St Univ Vet 1:25

Eckhoff G (1972b) Transplacental passage of drugs and other exogenous compounds: A review—Part II. Iowa St Univ Vet 2:98

Fries GF, Marrow GS (1971) Hexachlorobenzene excretion into milk of cows. J Anim Sci 39:155

Gill JL (1978) Design and Analysis of Experiments in the Animal and Medical Sciences, Volume I. Iowa St Univ Press, Ames, Iowa pp. 409

Goodman DR, James RC, Harbison RD (1982) Placental toxicology. Fd Chem Toxicol 20:123

Gralla EJ, Fleischman RW, Luthra YK, Hagopian M, Baker JR, Esber H, Marcus W (1977) Toxic effects of hexachlorobenzene after daily administration to beagle dogs for one year. Toxicol Appl Pharmacol 40:227

Grant DL, Phillips WEJ, Hatina GV (1977) Effect of hexachlorobenzene on reproduction in the rat. Arch Environ Contam Toxicol 5(2):207

Hansen LG, Dorn SB, Beamer PD (1979) Residues and effects from feeding high concentrations of hexachlorobenzene to broiler cockerels. Poultry Sci 58(1):81

Johnson JL, Stalling DL, Hogan JW (1974) Hexachlorobenzene (HCB) residues in fish. Bull Environ Contam Toxicol 11:393

Khera KS (1974) Teratogenicity and dominant lethal studies on hexachlorobenzene in rats. Fd Cosmet Toxicol 12:471

Kitchin KT, Linder RE, Scotti TM, Walsh D, Curley AO, Svendsgaard D (1982) Offspring mortality and maternal lung pathology in female rats fed hexachlorobenzene. Toxicol 23:33

ss G, Koransky W (1975) Studies on the toxicology of hexachlorobenzene. I. Pharmacokinetics. Arch Toxicol 34:203

Koss G, Manz D (1976) Residues of hexachlorobenzene in wild mammals of Germany. Bull Environ Contam Toxicol 15:189

Kuiper-Goodman T, Grant DL, Moodie CA, Korsrud GO, Monro IC (1977) Subacute toxicity of hexachlorobenzene in the rat. Toxicol Appl Pharmacol 40:529

Levin W, Welch RW, Conney AH (1967) Effect of chronic phenobarbital treatment on the liver microsomal metabolism and uterotropic action of estradiol-17β. Endocrin 80:135

Lu P, Metcalf RL (1975) Environmental fate and biodegradability of benzene derivatives as studied in a model aquatic ecosystem. Environ Health Perspect 10:269

Mendoza CE, Grant DL, Shields JB (1975) Body burden of hexachlorobenzene in suckling rats and its effect on various organs and on liver porphyrin accumulation. Environ Physiol Biochem 5:460

Morse RW (1975) Assessing potential ocean pollutants. National Academy of Sci, Washington, DC

Moya F, Smith E (1965) Placental transport of drugs and anesthetics. Anesthesiol 26:465

Mumma CE, Lawless EW (1975) Task 1—Hexachlorobenzene and hexachlorobutadiene pollution from chlorocarbon processes. EPA 530-3-75-003. U.S. Environmental Protection Agency, Washington, DC

Niimi AJ (1979) Hexachlorobenzene (HCB) levels in Lake Ontario salmonids. Bull Environ Contam Toxicol 23:20

Quinlivan SC, Chassemi M (1977) Sources, characteristics, and treatment and disposal of industrial wastes containing hexachlorobenzene. J Hazard Mater 1:343

Richter E, Renner G, Bayerl J, Wick M (1981) Differences in the biotransformation of hexachlorobenzene (HCB) in male and female rats. Chemosphere 10(7):779

Ringer RK, Aulerich RJ, Bleavins MR (1981) Biological effects of PCBs and PBBs on mink and ferrets—A review. In: Khan, MAQ (ed) Halogenated Hydrocarbons: Health and Ecological Effects. Pergamon Press, Elmsford, NY pp. 329–343

Seltzer RJ (1975) Ocean pollutants pose potential danger to man. Chem Engr News 53:19

Siyali DS (1973) Polychlorinated biphenyls, hexachlorobenzene and other organochlorine pesticides in human milk. Med J Australia 2:815

Siyali DS (1974) Placental barrier reduces pesticide intake to fetus. Med J Australia 1:285

Svendsgaard DJ, Courtney KD, Andrews JE (1979) Hexachlorobenzene (HCB) deposition in maternal and fetal tissues of the rat and mouse. II. Statistical quantification of HCB in tissues. Environ Res 20:267

Travis HF, Schaible PJ (1960) Fundamentals of Mink Ranching. Cir Bull #229, Mich St Univ, E Lansing, Michigan, p. 101

Villeneuve DC (1975) The effect of food restriction on the redistribution of hexachlorobenzene in the rat. Toxicol Appl Pharmacol 31:313

Villeneuve DC, Hierlihy SL (1975) Placental transfer of hexachlorobenzene in the rat. Bull Environ Contam Toxicol 13(4):489

Villeneuve DC, Newsome WH (1975) Toxicity and tissue levels in the rat and guinea pig following acute hexachlorobenzene administration. Bull Environ Contam Toxicol 14:297

Villeneuve DC, Panopio LG, Grant DL (1974) Placental transfer of hexachlorobenzene in the rabbit. Environ Health Perspect 4:112

Vos JG, Breeman HA, Benschop H (1969) The occurrence of fungicide hexachlorobenzene in wild birds and its toxicological significance. Pest Prog 7:142

Zabik ME, Schemmel R (1980) Influence of diet on hexachlorobenzene accumulation in Osborne Mendel rats. J Environ Pathol Toxicol 4:97

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