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The Reproductive Effects of Dietary Heptachlor in Mink (*Mustela vison*)

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Abstract. Adult female mink were fed diets containing 0 (control), 6.25, 12.5, and 25 ppm (μg/g) technical grade heptachlor prior to and throughout the reproductive period (181 days) to evaluate the effects of heptachlor consumption on reproduction and offspring viability and to assess the extent of placental and mammary transfer of heptachlor epoxide to mink offspring. Feeding 12.5 and 25 ppm resulted in significant reductions in feed consumption and body weights of female mink. Mortality was 0, 8, 67, and 100% for the control, 6.25, 12.5, and 25 ppm groups, respectively. All females in the 25 ppm group died within 88 days. Mink fed the two higher heptachlor diets displayed clinical signs indicative of central nervous system involvement just prior to death. Females were mated with males on the same dietary treatments. Whelping success rates were 67, 83, 27, and 0% for the control, 6.25, 12.5, and 25 ppm groups, respectively. High mortality in the 12.5 and 25 ppm groups accounted for the lack of reproductive success. Gestation length, litter size and birth weight of kits were not significantly affected by adult female consumption of 6.25 ppm heptachlor while kits whelped by females on the 12.5 ppm diet weighed significantly less than control kits at birth. Survival of kits in the 12.5 ppm group from birth to three weeks of age was also adversely affected. At three and six weeks of age, kit body weights in both the 6.25 and 12.5 ppm groups were significantly less than body weights in control kits. Examination of heptachlor epoxide concentrations in newborn and developing kits indicated both placental and mammary transfer of the chemical from the dams to the kits. The LC50 for the 181-day exposure period for female mink was 10.5 ppm heptachlor and the LOAEL, based on reduced kit growth, was 6.25 ppm.

Heptachlor (1,4,5,6,7,8,8-heptachlor-3_a,4,7,7_a-tetrahydro-4,7-methanoindene) is a chlorinated cyclodiene insecticide that was used extensively on a wide variety of agricultural crops in the 1960s and early 1970s (Dynamac Corp 1989). As with other chlorinated hydrocarbon compounds, heptachlor is persistent in the environment, existing primarily in its oxidized form as heptachlor epoxide (HE) (Nash and Harris 1973). Heptachlor and HE have been detected in several plant and animal species (Fox *et al.* 1964; Boddicker *et al.* 1971; Clark *et al.* 1980, 1983; Chawla *et al.* 1981; DeWeese *et al.* 1986) and in some instances have been associated with wildlife mortalities (Henny *et al.* 1983; Blus *et al.* 1984). Despite restrictions placed on the use of heptachlor by the US Environmental Protection Agency (EPA) in 1978 (US EPA 1978), contamination of human food products has occurred in Arkansas, Missouri, Oklahoma (Stehr-Green *et al.* 1986; Flora 1989) and Hawaii (Smith *et al.* 1984; Le Marchand *et al.* 1986).

In 1982, the milk supply on the island of Oahu, Hawaii was found to be contaminated with heptachlor (HE) as a result of dairy cattle consuming heptachlor-treated pineapple plants being used as a feed supplement (Le Marchand *et al.* 1986). Because animal studies have shown adverse reproductive effects from heptachlor exposure (Mestitzova 1967; Green 1970; Akay and Alp 1981) and because placental and mammary transfer of heptachlor epoxide and other chlorinated hydrocarbons have been documented in human studies (Curley *et al.* 1969; Curley and Kimbrough 1969; Takahashi *et al.* 1981; Takei *et al.* 1983; Mussalo-Rauhamma *et al.* 1988) there has been particular concern for pregnant women and nursing infants exposed to these chemicals (Le Marchand *et al.* 1986). Since the mink has been shown to be very sensitive to low concentrations of chlorinated hydrocarbons, particularly in terms of reproductive effects and offspring viability (Aulerich and Ringer 1977; Bleavins *et al.* 1980, 1984; Aulerich *et al.* 1985; Hochstein *et al.* 1988), it was considered to be an appropriate species for evaluating the subchronic toxicity of heptachlor. In addition, short-term studies conducted in our laboratory have shown mink to be sensitive to dietary heptachlor (Aulerich *et al.* 1990).

The objectives of the present study were to determine the subchronic toxicity of heptachlor to mink, the effect of dietary heptachlor on the reproductive performance of mink and on offspring viability, the placental and mammary transfer of HE to mink kits and whole-body HE concentrations in adult female mink fed heptachlor.

Materials and Methods

Sixty adult standard dark mink (*Mustela vison*) from stock raised at the Michigan State University Experimental Fur Farm were randomly di-

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vided into three treatment groups and a control group. Each group consisted of 12 females and three males. Mink were fed *ad libitum* a basal diet[2] supplemented with either 0 (control), 6.25, 12.5, or 25 ppm technical-grade heptachlor[3] for 181 days. The dietary concentrations selected were based on the results of a 28-day study in mink (Aulerich *et al.* 1990). The heptachlor-contaminated diets were formulated by dissolving the desired quantity of heptachlor (corrected for purity) in acetone and adding the solution to finely ground mink cereal to make a premix. After the acetone was evaporated, the heptachlor-cereal premix was combined with the basal diet to yield the nominal concentrations of heptachlor. Feed concentrations of heptachlor as determined by gas chromatographic analysis (see below) varied little from nominal concentrations. Samples from the control, 6.25, 12.5, and 25 ppm diets yielded concentrations of 0.01 ± 0.01, 5.15 ± 0.28, 11.98 ± 1.54, and 21.24 ± 4.15 ppm heptachlor, respectively.

Mink were housed individually in wire cages (76 cm long × 61 cm wide × 46 cm high) suspended above the floor in an animal room. Routine mink farm procedures were followed in feeding, care and breeding of the mink. Water was provided *ad libitum* throughout the study. The photoperiod was regulated by timeclock to simulate natural light conditions. Temperature within the animal room approximated ambient temperature, except during cold periods when it was maintained above 0°C by thermostatically controlled electric heaters to prevent freezing of drinking water. Ventilation was provided by an exhaust fan and ceiling vents.

The mink were acclimated to the test facilities for seven days prior to receiving the test diets. The trial was initiated on January 2, 1989 and continued to July 2, 1989 (181 days). Body weights were measured weekly. Feed consumption was determined over a 2-day period each week, except during the reproductive period. Mink were observed daily for overt signs of toxicity including abnormal behavior and mortality. Gross necropsies were performed on all mortalities and body and organ (liver, kidneys, adrenal glands, spleen, heart, lungs and brain) weights were recorded.

After 42 days on treatment (February 13), females were mated to males within their respective dietary groups. Due to early mortality of males in the 25 ppm group, a small number of females in this treatment group were mated to males in the 12.5 and 6.25 ppm groups. Each female was given additional opportunities to mate following routine commercial mink farm breeding procedures. Successful matings were verified by the presence of motile spermatozoa in a vaginal aspiration taken after copulation. Females were checked daily for newborn young (kits) during the whelping period. During the later stages of pregnancy and during lactation, body weights of the females were not recorded, except at whelping and at three and six weeks post-whelping. Kit body weights were also determined at whelping and at three and six weeks (weaning) of age in the control, 6.25 and 12.5 ppm groups. No females in the 25 ppm group whelped and none of the mink in this group survived beyond 88 days on treatment.

Five kits were randomly chosen from the control and 6.25 ppm groups and euthanized (CO_2) at birth and at 3 and 6 weeks of age to determine whole-body HE concentrations for evaluation of placental, lactational and dietary transfer of HE. Only three, two, and two kits could be sampled in the 12.5 ppm group at birth and at 3 and 6 weeks of age, respectively, due to the low number of females whelping in this group. Kits euthanized at 3 and 6 weeks of age were taken from the same litters as those euthanized at birth. Maternal milk samples were collected from six randomly selected females from the control as well as the 6.25 ppm group between 30 and 35 days post-whelping (Jones *et al.* 1980) to determine HE concentrations.

[2]The basal diet consisted of 25% mink cereal, 20% chicken by-products, 20% ocean fish trimmings, 5% liver and 30% water. As fed, the diet contained 13.9% protein, 6.8% fat, 4.1% ash and 62.6% moisture (National Environmental Testing, Inc., Chicago, IL).

[3]Velsicol Chemical Corp., Memphis, TN; Technical-grade heptachlor; purity 72%, Lot No. 53714421.

At the end of the 181-day study, four, three, and four adult female mink were randomly selected from the control, 6.25 and 12.5 ppm groups, respectively, and euthanized (CO_2) to determine whole-body concentrations of HE. Similarly, nine and eight kits were euthanized on the 181st day from the control and 6.25 ppm groups, respectively, to evaluate the same parameter. All adults and kits were frozen after euthanization. While frozen, the mink were ground in a Hobart meat grinder to a hamburger-like consistency and refrozen for subsequent chemical analysis. Whole-body and milk HE concentrations were determined according to modifications of the procedure of Price *et al.* (1986).

A 50 g aliquot of whole ground mink carcass was frozen in liquid nitrogen and blended to a fine powder in a Waring blender. A 20 g portion was mixed thoroughly with 100 g anhydrous Na_2SO_4, packed into a 2.5 × 30 cm column between two layers (approximately 1 cm each) of Na_2SO_4, and the fat (containing the chlorinated pesticides) extracted by percolation with 300 ml of diethyl ether/petroleum ether (1:1). The solvent was collected and evaporated in a tared 400 ml beaker, and the total fat content determined. A 200 mg aliquot of fat was suspended in 1 ml hexane and chromatographed on a column of Silica Gel 60 (Alltech Associates, Inc., Deerfield, IL) according to the procedure of Price *et al.* (1986). Fractions SG-1, SG-2, and SG-3 were collected following elution with 15 ml hexane, 20 ml hexane, and 20 ml benzene, respectively, beginning the collection of SG-3 when the translucent layer reached within 1.5 cm of the glass wool support. Heptachlor, if present, eluted in SG-2, HE eluted in SG-3. For this study, in which residue free animals were exposed to heptachlor only, both SG-2 and SG-3 were combined for glc analysis.

Mink fetuses, which were too small to blend in a Waring blender, were weighed, minced with scissors, and mixed with three times their weight of anhydrous Na_2SO_4. They were then ground to a fine powder with a mortar and pestle, adding additional Na_2SO_4 if necessary to maintain a dry consistency. The mixture was quantitatively transferred to a 400 ml beaker and extracted three times with 100 ml of diethyl ether/petroleum ether (1:1) with gentle heating in a 60°C water bath. The organic extracts were combined in a 400 ml tared beaker and the fat recovered and fractionated on a silica gel column as above.

Milk was extracted by lightly vortexing a 5 g sample with 15 ml acetone/hexane (1:1), the layers separated by centrifugation (2000 g), and the organic fraction dried through anhydrous Na_2SO_4 and transferred to a tared 50 ml conical tube. The milk was re-extracted consecutively with 15 ml hexane and 15 ml CH_2Cl_2. All three organic phases were combined and evaporated to dryness to determine the total fat content. The fat was taken up in hexane, quantitatively transferred to a silica gel column, and fractionated as above.

The combined chlorinated pesticide fractions (SG-2 and SG-3) recovered from silica gel chromatography were evaporated to dryness under a stream of N_2 and resuspended in an appropriate volume of ethanol/iso-octane (20:80). Heptachlor and heptachlor epoxide were determined by gas chromatography on a Shimadzu GC-4BM chromatograph with ^{63}Ni electron-capture detector. The compounds were chromatographed on a 3 mm × 1.8 m column packed with 1.5% SP2200/1.95% SP2401 (Supelco, Inc., Bellefonte, PA) at 200°C, with a N_2 carrier gas flow rate of 50 ml/min. Peak areas were integrated on a Spectra Physics SP2470 integrator and quantified by extrapolation from a 5 point standard curve (3.12, 6.25, 12.5, 25, and 50 pg).

Differences in adult female mink body weights and feed consumption between treatment groups and the control group were determined by one-way analysis of variance (ANOVA) followed by nonorthogonal designed contrasts using Bonferroni's *t* statistic (SAS Institute 1985). These parameters were not statistically analyzed for male mink due to the small number in each treatment group. The median lethal concentration (LC50) at 181 days for female mink was determined by the probit method using the computer software Toxstat (Gulley *et al.* 1989). Adult female body weights and kit body weights at birth and at 3 and 6 weeks post-whelping, length of gestation and litter size were also evaluated using Toxstat (Gulley *et al.* 1989). These data were analyzed for normality and homogeneous variance with the Chi-square test and Bartlett's test, respectively, before comparison of means.

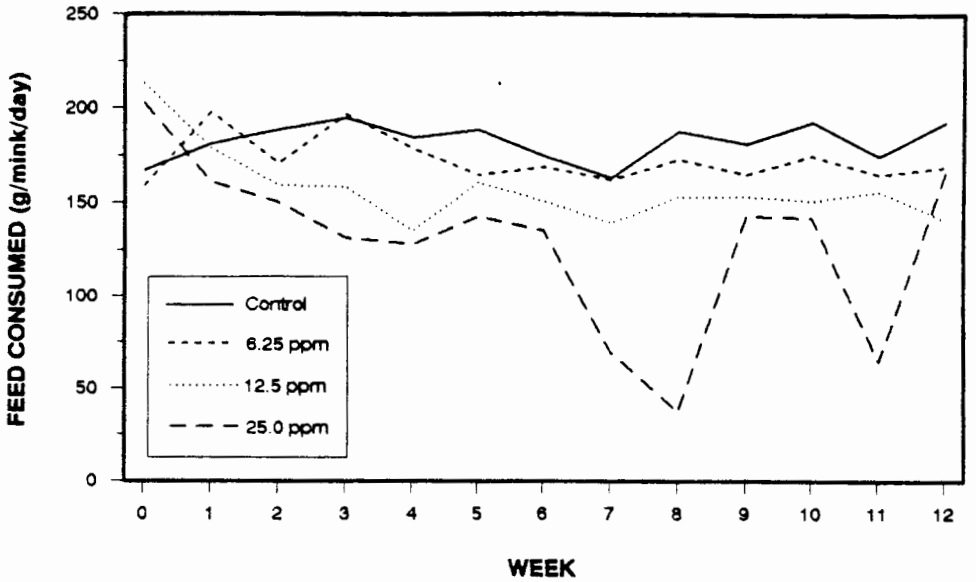

Fig. 1. The effect of dietary heptachlor on feed consumed by adult female mink during the first 12 weeks of the study. Feed consumption by females in the 6.25 ppm group was not significantly different from controls throughout the 12 week period; feed consumption in the 12.5 ppm group was significantly less than controls at weeks 3, 4, 10, and 12; feed consumption in the 25 ppm group was less than controls from weeks 2 through 8 and week 11. Significance ($p < 0.05$)

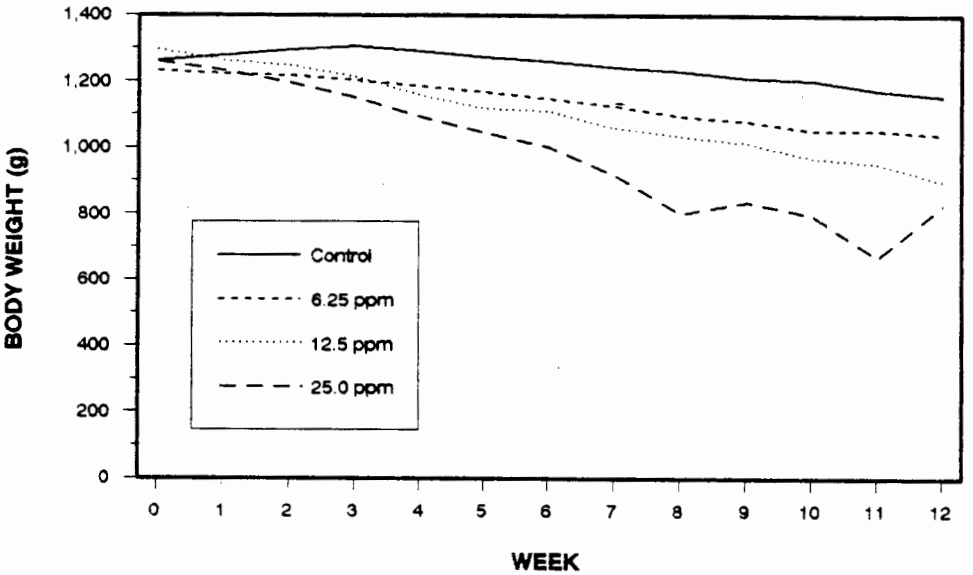

Fig. 2. The effect of dietary heptachlor on adult female mink body weights during the first 12 weeks of the study. Body weights of females in the 6.25 ppm group were not significantly different from controls throughout the 12 week period; body weights in the 12.5 ppm group were significantly less than controls from weeks 7 through 12; body weights in the 25 ppm group were significantly less than controls from weeks 4 through 12. Significance ($p < 0.05$)

ANOVA, followed by Bonferroni's t statistic, were conducted when the data were normally distributed and the variance of the control and treatment groups was homogeneous. If the data failed to fulfill the assumptions required for ANOVA, the Kruskal-Wallis nonparametric test was performed to compare group means. Relationships between whole-body HE concentrations (μg/g bw) and whole-animal HE body burdens (μg/animal) in adults and kits with dietary heptachlor concentrations were determined by linear regression analysis using a MacIntosh SE computer with the software program Stat-View 512 + (Brain Power Inc., Calabasas, CA). Statements of significance are based on $p < 0.05$.

Results

Feed consumption by female mink was similar for all groups during the acclimation period, designated as week 0 (Figure 1). During the subsequent 12 weeks, 6.25 ppm heptachlor had no significant effect on feed intake while 12.5 ppm heptachlor caused significant decreases at weeks 3, 4, 10 and 12 when compared to controls. Feed consumption by female mink fed 25 ppm heptachlor was significantly depressed from weeks 2 to 8 and at week 11. Heptachlor intake was 1.0, 1.7, and 3.1 mg heptachlor/kg/bw/day over the 12 week period for adult female mink fed diets containing 6.25, 12.5 and 25 ppm heptachlor, respectively.

Adult female mink body weights during the same 12 week period decreased in a dose-related manner (Figure 2). Although females fed 6.25 ppm heptachlor weighed less than controls, the difference was not significant. Females fed 12.5 ppm heptachlor weighed significantly less than control mink from weeks 7 through 12 while body weights of the 25 ppm group were significantly below those of controls from weeks 4 through 12. The body weights of females that whelped in the 12.5 ppm group were significantly reduced at whelping and 3 weeks post-whelping when compared to control dams, but not

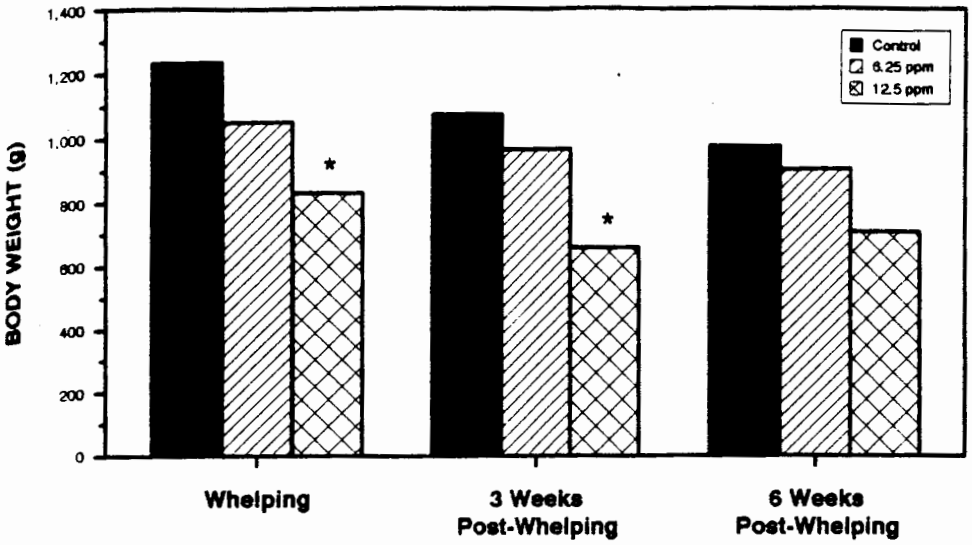

Fig. 3. The effect of dietary heptachlor on adult female mink body weights at whelping and at 3 and 6 weeks post-whelping. No females fed 25 ppm heptachlor whelped. Asterisk indicates significant difference ($p < 0.05$) from control

Table 1. Mortality and survival time of mink fed control and heptachlor-contaminated diets

Dietary heptachlor (ppm)	Mortality (no. died/no. total)	% mortality	Survival time of mink that died[a] (days)
Females			
0	0/12	0	—
6.25	1/12	8	178
12.5	8/12	67	88 ± 8 (34–107)
25	12/12	100	57 ± 4 (43–88)
Males			
0	0/3	0	—
6.25	0/3	0	—
12.5	1/3	33	90
25	3/3	100	38 ± 6 (31–51)

[a] Data expressed as mean ± standard error. Numbers in parenthesis are the range

at 6 weeks post-whelping (Figure 3). None of the 25 ppm female mink survived to the whelping period.

Adult female mink mortality for the 181-day exposure period was 0, 8, 67, and 100% for the control, 6.25, 12.5, and 25 ppm groups, respectively (Table 1). The median lethal concentration (LC50) for the 181-day period was 10.5 ppm heptachlor for female mink. All females in the 25 ppm group died within 88 days, with an average consumption of 177 mg heptachlor prior to death. Female mortalities in the 12.5 ppm group occurred through day 107 and the single mortality in the 6.25 ppm group occurred on day 178. All male mink in the control and 6.25 ppm groups survived the 181-day trial, whereas one male in the 12.5 ppm group died on day 90 and all males in the 25 ppm group died within 51 days.

Mink fed 12.5 and 25 ppm heptachlor generally displayed clinical signs indicative of central nervous system involvement prior to death, such as hyperexcitability and seizures. Hindlimb ataxia was observed in several animals prior to more pronounced neurological effects. Bloody stools were observed frequently in mink fed 25 ppm heptachlor. Nearly all mink stopped eating for one to two weeks before death. Ultimately, the animals became listless and then convulsed just prior to dying. Fatty livers, ulcerations and bloody mucus in the stomach were observed at necropsy in mink fed 12.5 and 25 ppm heptachlor.

The effects of dietary heptachlor consumption on reproduction are presented in Table 2. All female mink fed 25 ppm heptachlor died prior to the whelping period (mid-April to early May). At necropsy, no fetuses were observed in the uteri of the five bred females in the 25 ppm group. Seven of 11 bred females fed 12.5 ppm heptachlor also died prior to whelping. Of these, two females contained fetuses in their uteri. Three of the four remaining mink successfully whelped. Feeding 6.25 ppm heptachlor had no adverse effect on female reproductive performance. Gestation length and litter size were not significantly affected by heptachlor consumption (Table 2), however, the percentage of stillborn kits was increased in the 12.5 ppm group relative to the control and 6.25 ppm groups (Table 2). A single morphologic anomaly was observed in a kit from the 12.5 ppm group. The front legs and tail were considerably shortened and the kit survived for only one day. Feeding male mink 6.25 and 12.5 ppm heptachlor had no effect on sperm motility or morphology. Male mink in the 25 ppm group died before they could be mated.

Consumption of 6.25 ppm heptachlor by female mink prior to breeding and during gestation had no significant effect on kit birth weights while kits whelped by dams in the 12.5 ppm group weighed significantly less than kits from control mothers at whelping (Table 3). At 3 and 6 weeks of age, kit body weights in both the 6.25 and 12.5 ppm groups were significantly below those of control kits. Kit survival from birth to 3 weeks of age was reduced in the 12.5 ppm group compared to control and 6.25 ppm kits, whereas survival from 3 to 6 weeks of age was comparable for all groups.

In utero exposure to 6.25 and 12.5 ppm heptachlor resulted in HE body burdens of 8.9 and 24.8 μg/animal and whole-body concentrations of 0.86 and 3.08 μg/g bw (ppm), respectively, in kits at birth (Table 4). The relationships between heptachlor concentration in feed consumed by dams prior to breeding and during gestation and HE body burdens and whole-body HE concentrations in newborn kits were linear, with coefficients of determination (R^2) of 0.70 and 0.77, respectively. HE body burdens continued to increased in the 6.25 and 12.5 ppm kits

Table 2. The effect of dietary heptachlor on reproductive performance in adult female mink

Dietary heptachlor (ppm)	Number females whelped/number females mated	Gestation length[a,b] (days)	Litter size[b]	No. kits whelped		Kits alive at birth (%)
				Alive	Dead	
0	8/12 (67)[c]	60.8 ± 1.9	6.2 ± 0.2	44	6	88
6.25	10/12 (83)	58.6 ± 2.8	6.2 ± 0.6	59	3	95
12.5	3/11 (27)[d]	51.0 ± 2.1	5.7 ± 0.3	12	5	71
25	0/5 (0)[d]	—	—	—	—	—

[a] Based on the date of the final successful mating
[b] Data expressed as mean ± standard error
[c] Number in parentheses is % of mated females that whelped
[d] Early mortality prevented breeding of other mink in these groups

Table 3. The effect of dietary heptachlor on body weight and survival of kit mink

Dietary heptachlor (ppm)	Kit body weight (g)[a]			Kit survival (%)	
	Birth	3 weeks	6 weeks	Birth to 3 weeks	3 weeks to 6 weeks
0	11.0 ± 0.3 (41)	128.3 ± 2.3 (36)	311.7 ± 9.7 (31)	92	100
6.25	9.8 ± 0.3 (59)	99.2 ± 4.7 (45)[b]	253.3 ± 18.2 (35)[b]	83	88
12.5	8.5 ± 0.4 (12)[b]	62.7 ± 7.5 (5)[b]	158.3 ± 51.0 (3)[b]	45	100

[a] Data expressed as mean ± standard error. Numbers in parentheses refer to sample size (i.e. number of kits alive at that time period)
[b] Significantly different ($p < 0.05$) from control value

Table 4. The effect of dietary heptachlor exposure on total body burden and whole-body concentration of heptachlor epoxide (HE) and percent fat in mink kits at birth and three and six weeks of age

Dietary heptachlor (ppm)	Sample size	HE body burden (μg/animal)[a]	Body weight (g)[a,b]	Whole-body HE concentration (μg/g bw)[a]	Body fat (%)[a]
Birth					
0	5	0[c]	10.3 ± 0.7	0[c]	1.4 ± 0.3
6.25	5	8.9 ± 2.3	10.4 ± 1.3	0.86 ± 0.17	1.4 ± 0.2
12.5	3	24.8 ± 7.4	7.9 ± 0.9	3.08 ± 0.70	1.5 ± 0.1
3 weeks					
0	5	0.1 ± 0.1	133.5 ± 3.8	0[c]	10.9 ± 0.5
6.25	5	59.7 ± 13.1	117.2 ± 12.9	0.48 ± 0.09	10.2 ± 1.0
12.5	2	53.8 ± 16.1	65.1 ± 16.2	0.82 ± 0.04	5.0 ± 1.2
6 weeks					
0	5	0[c]	310.0 ± 26.0	0[c]	11.2 ± 1.4
6.25	5	883.9 ± 199.9	272.8 ± 57.3	3.24 ± 0.22	9.5 ± 1.6
12.5	2	1075.2 ± 811.5	182.3 ± 78.0	4.89 ± 2.36	6.6 ± 2.7

[a] Data expressed as mean ± standard error
[b] Mean body weight represents only euthanized kit mink
[c] Trace (< 0.01 μg/g)

through lactation (birth to three weeks of age), but when expressed as a function of body weight, whole-body HE concentrations declined during this period when compared to concentrations at birth (Table 4). However, a significant positive relationship between whole-body HE concentrations in kits and the dams' dietary treatment level continued to three weeks of kit age ($R^2 = 0.87$). After three weeks of age, kits were provided with the same diet as their respective dams in addition to heptachlor exposure through nursing. Body burden levels and whole-body concentrations of HE at six weeks of age were considerably elevated over those at three weeks for kits in the 6.25 and 12.5 ppm groups (Table 4). Whole-body HE concentration in kits at six weeks of age was directly related to the dams treatment concentration ($R^2 = 0.76$). Milk samples obtained between 30 to 35 days postpartum from dams fed 6.25 ppm heptachlor had a mean HE concentration of 4.6 ppm as compared to trace amounts in the milk of control dams. The amount of fat in milk collected from control (15.8%) and 6.25 ppm (16.8%) dams was not significantly different.

Adult female mink in the 6.25 and 12.5 ppm groups euthanized at the end of the 181-day feeding period had whole-body HE concentrations of 8.87 and 13.83 μg/g bw, respectively (Table 5). HE concentrations in the body were directly related to the concentration of heptachlor in the diet ($R^2 = 0.77$). The

Table 5. The effect of dietary heptachlor on total body burden and whole-body concentration of heptachlor epoxide (HE) and percent body fat in adult female mink after 181 days

Dietary heptachlor (ppm)	Sample size	HE body burden (μg/animal)[a]	Body weight (g)[a,b]	Whole-body HE concentration (μg/g bw)[a]	Body fat (%)[a]
0	4	5.8 ± 1.4	1044 ± 71	0.01 ± 0.0	22.9 ± 1.7
6.25	3	8416.6 ± 839.3	948 ± 41	8.87 ± 0.73	18.0 ± 0.6
12.5	4	12690.0 ± 4332.0	861 ± 123	13.83 ± 2.95	13.5 ± 2.1

[a] Data expressed as mean ± standard error
[b] Mean body weight represents only euthanized adult female mink

Table 6. The effect of dietary heptachlor on total body burden and whole-body concentration of heptachlor epoxide (HE) and percent body fat in two to three-month-old mink kits euthanized at the end of the 181-day study period

Dietary heptachlor (ppm)	Sample size[a]	HE body burden (μg/animal)[b]	Body weight (g)[b]	Whole-body HE concentration (μg/g bw)[b]	Body fat (%)[b]
0	3	1.6 ± 1.4	720 ± 89	0[c]	12.3 ± 1.3
6.25	3	4338.6 ± 872.9	656 ± 131	6.62 ± 0.06	11.8 ± 1.2

[a] Three kits comprised each sample except for one sample in the control group which contained two mink
[b] Data expressed as mean ± standard error
[c] Trace (< 0.01 μg/g)

percent body fat of the euthanized adult females decreased with increasing amounts of heptachlor in the diet (Table 5). Kits between two and three months of age from the 6.25 ppm group (no kits remained in the 12.5 ppm group) showed continued accumulation of HE when euthanized at the end of the 181-day trial (Table 6) compared to concentrations observed at 6 weeks of age. There were no mortalities in kits from the control and 6.25 ppm groups during the period from 6 weeks of age to the end of the trial.

Discussion

Diets containing 25 ppm heptachlor (dose equivalent to 3.1 mg/kg bw/day) fed to female mink caused a reduction in feed intake and a concomitant decrease in body weight within 28 days on treatment. In a 28-day feeding trial with male mink, Aulerich *et al.* (1990) also reported decreased feed consumption at dietary concentrations of 25 ppm (3.11 mg/kg bw/day) heptachlor, but no significant body weight loss. They observed no effects on these parameters in male mink fed 12.5 ppm (1.79 mg/kg bw/day) heptachlor. Female mink fed 12.5 ppm (1.7 mg/kg bw/day) heptachlor in the present study had decreased feed intake after 21 days on treatment, but experienced no reduction in body weight until day 49. Feeding 6.25 ppm (1 mg/kg bw/day) heptachlor to female mink had no adverse effect on feed consumption or body weight. Similarly, rats fed 0.1 to 10 ppm heptachlor for 21 days displayed no decrease in feed intake or body weights (Polin, personal communication).

Consumption of 25 ppm (3.1 mg/kg bw/day) heptachlor was highly toxic to male and female mink causing 100% mortality within 88 days. The earliest deaths occurred in two male mink after only 31 and 32 days on treatment, and 87% of all mink in this group died by day 57. Aulerich *et al.* (1990) observed no mortality in male mink fed 25 ppm (3.11 mg/kg bw/day) heptachlor for 28 days. They also reported no deaths in males fed 12.5 ppm (1.79 mg/kg bw/day) heptachlor, whereas the same dietary concentration caused one female mink mortality after 34 days in the present study. However, most of the mortalities for mink fed 12.5 ppm (1.7 mg/kg bw/day) heptachlor came later in the trial, with seven of eight female deaths occurring between 86 to 107 days and one male death on day 90. Consumption of 6.25 ppm (1 mg/kg bw/day) heptachlor caused a single death in a female on the 178th day of the trial. Assuming the death of this female was due to heptachlor toxicity, the lowest-observed-adverse-effect level (LOAEL) in female mink for the 181-day study period (26 weeks) was 6.25 ppm (1 mg/kg bw/day). In comparison, the LOAEL for female mice fed heptachlor for 80 weeks was 18 ppm (2.3 mg/kg bw/day) and the no-observed-adverse-effect level (NOAEL) was 9 ppm (1.2 mg/kg bw/day) (NCI 1977).

Heptachlor, like other chlorinated cyclodiene insecticides, is a central nervous system stimulant (Murphy 1986). Consumption of 12.5 (1.7 mg/kg bw/day) and 25 ppm (3.1 mg/kg bw/day) heptachlor by adult mink generally caused hyperexcitability, loss of hindlimb coordination, and tonic-clonic seizures prior to death. Similar signs were reported in rats following acute exposure to much higher doses of heptachlor (200 mg/kg bw) and HE (100 mg/kg bw) (Hrdina *et al.* 1974). Cats exhibited seizure-type behavior within 20 to 40 minutes after receiving an intravenous dose of 2 to 10 mg heptachlor/kg bw (Joy 1976). Aulerich *et al.* (1990) observed no neurotoxic effects in male mink fed 12.5 (1.79 mg/kg bw/day) and 25 ppm (3.11 mg/kg bw/day) heptachlor for 28 days. However, several mink fed 100 ppm (6.19 mg/kg bw/day) exhibited neurological aberrations consistent with those observed at 12.5 (1.7 mg/kg bw day) and 25 ppm (3.1 mg/kg bw/day) dietary heptachlor in this study.

Fatty infiltration of the liver was observed at necropsy in nearly all mink fed 12.5 (1.7 mg/kg bw/day) and 25 ppm (3.1 mg/kg bw/day) heptachlor. Aulerich *et al.* (1990) also reported liver steatosis in male mink fed diets containing 100 ppm (6.19

mg/kg bw/day) heptachlor. Fatty livers have been noted in rats following acute (Pelikan 1971; as cited in Dynamac 1989) and chronic (Witherup *et al.* 1955) oral exposure to heptachlor.

The effects of heptachlor consumption prior to breeding and during gestation on reproductive performance were difficult to assess due to high mortality in the 12.5 and 25 ppm groups before the whelping period. No fetuses were observed in the uteri of the five bred females fed 25 ppm heptachlor, whereas two of seven females in the 12.5 ppm group contained fetuses at time of death. Reproductive performance was not adversely affected in mink fed 6.25 ppm (1 mg/kg bw/day) heptachlor. In contrast, a dietary level of 5 ppm heptachlor (0.25 mg/kg bw/day) fed to male and female rats for two generations caused a decrease in pregnancy rates. The first generation pregnancy rate was 72% versus 94% in the controls, while none of the second generation heptachlor-treated females became pregnant (Green 1970). Male and female mice consuming 50 ppm (7.5 mg/kg bw/day) heptachlor in the diet for 10 weeks also failed to reproduce (Akay and Alp 1981).

In utero exposure of kits to heptachlor from dams fed 6.25 ppm had no adverse effect on kit body weights or survival rates at birth. However, feeding 12.5 ppm (1.7 mg/kg bw/day) heptachlor to adult female mink did cause a decrease in kit birth weights and survival rates compared to controls. Gestation length was also slightly reduced relative to control females. However, this value was well within the reported range for mink (Sundqvist *et al.* 1989). No difference was observed in litter size of females fed 6.25 (1 mg/kg bw/day) and 12.5 ppm (1.7 mg/kg bw/day) heptachlor. However, marked decreases in litter size have been reported by Mestitzova (1967) in rats fed considerably higher concentrations (6 mg/kg bw/day) of heptachlor.

Although concentrations of 6.25 ppm heptachlor in the present study did not affect the reproductive capacity of female mink, it did have an adverse effect on postnatal survival of kits within individual litters. Kit survival was only 25% in one litter from the 6.25 ppm group during the period from birth to 3 weeks of age, while survival in another litter was 29% from 3 to 6 weeks of age. However, overall kit survival to 3 weeks of age was 83% for the 6.25 ppm (1 mg/kg bw/day) group. In a similar study conducted by Green (1970), diets containing 5 ppm (0.25 mg/kg bw/day) heptachlor fed to rats prior to mating and during gestation resulted in 16% survival of newborns to 3 weeks of age. In the present study, 45% of the kits from dams fed 12.5 ppm (1.7 mg/kg bw/day) group survived to 3 weeks of age, indicating that rat pups are more sensitive to heptachlor than mink kits. In contrast, Witherup *et al.* (1976, as cited in WHO 1984) reported only slightly decreased pup survival in rats at 2 and 3 weeks of age in second generation litters exposed to 10 ppm heptachlor. Postnatal growth, as assessed by body weight gain, was decreased in kits from the 6.25 and 12.5 ppm groups. In contrast, there was no growth retardation in suckling rats from dams fed 10 ppm heptachlor over three generations (Eisler 1968).

The presence of HE in kits whelped by dams fed 6.25 and 12.5 ppm heptachlor confirms the work of previous researchers concerning its ability to cross the human placenta (Curley *et al.* 1969; Polishuk *et al.* 1977). Transplacental transfer of other chlorinated hydrocarbons has also been demonstrated in mink (Bleavins *et al.* 1981, 1982, 1984). The concentration of HE in kits at birth increased with respect to the dams' treatment level, with kits whelped by dams fed 12.5 ppm heptachlor having 3.6 times greater HE concentrations than kits in the 6.25 ppm group. Although total body burdens of HE increased in kits from the heptachlor-treated groups during the lactational period (birth to 3 weeks of age), whole-body HE concentrations declined. This suggests that the growth rate of kits exceeded the accumulation of HE residues derived from nursing, as kits are entirely reliant upon the dam's milk during this period. A comparison of HE body burdens at birth with those at 3 weeks of age for kits in the 6.25 ppm group indicates that transfer of HE via lactation is greater than through the placenta. HE body burden results for kits in the 12.5 ppm group were less convincing. However, this may be due to lower production of milk by dams in this group as they were considerably underweight. Milk samples collected from dams in the 6.25 ppm group 4–5 weeks after whelping, contained a mean HE concentration of 4.6 ppm (27.6 ppm, fat basis). Although this result was obtained after three weeks of kit age, it does reflect the potential for considerable uptake of HE via lactation. Heptachlor residues up to 5.0 ppm (fat basis) have been reported in milk for human consumption from Oahu, Hawaii (Le Marchand *et al.* 1986).

Kits were provided the same diets as their dams after reaching three weeks of age. The exponential increase of total body burdens and whole-body HE concentrations in the 6.25 and 12.5 ppm kits from 3 to 6 weeks of age is due to the increased intake of heptachlor from this dietary supplementation. Total body burdens and whole-body concentrations in kits (2–3 months of age) euthanized at the end of the 181-day study demonstrate a continued accumulation of HE. Body burdens of HE increased nearly fivefold, and whole-body concentrations doubled compared to values at 6 weeks of kit age.

Whole-body concentrations of HE in adult female mink from the 6.25 and 12.5 ppm groups euthanized at the end of the 181-day study period were 8.87 and 13.83 μg/g bw, respectively. Rats fed 1 ppm heptachlor for the same duration had a whole-body HE concentration of 0.24 μg/g bw (Polin, personal communication). Using the regression of whole-body HE concentrations in adult female mink on dietary heptachlor concentrations of 0, 6.25 and 12.5 ppm, a concentration of 1 ppm fed to female mink would predict a whole-body HE concentration of 1.64 μg/g bw. This indicates that mink accumulate HE residues to a greater extent than rats.

The results of this study indicate that consumption of 6.25 ppm dietary heptachlor for approximately 120 days prior to whelping did not affect the ability of female mink to produce viable offspring. However, postnatal exposure of young mink in this group to low levels of HE through lactation and/or dietary heptachlor resulted in reduced growth and survival compared to control kits. These findings are supported by large increases in total body burdens and whole-body concentrations of HE in kits during this period.

Diets supplemented with 25 ppm heptachlor were highly toxic, causing 100% mortality within 88 days on treatment. Feeding 12.5 ppm heptachlor caused early mortality (67%) in this group, thereby precluding an accurate assessment of reproductive performance. However, the reproducing females in the 12.5 ppm group produced an increased number of stillborn kits and there was a reduction in postnatal survival and growth of the kits. Using postnatal body weight gain and survival as endpoints, the LOAEL is 6.25 ppm (1 mg/kg bw/day) heptachlor. Depending on body weight, 25 μg of HE in mink kits at whelping may be lethal and continued exposure to HE from

dams fed 12.5 ppm heptachlor during the lactational period is also detrimental to survival.

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