United States
Environmental Protection
Agency

Environmental Research
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Duluth MN 55804

EPA-600/3-80-033
February 1980

Research and Development

Toxicity of the Polychlorinated Biphenyl Aroclor 1016 to Mink

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TOXICITY OF THE POLYCHLORINATED BIPHENYL AROCLOR 1016 TO MINK

by

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Contract No. 68-03-1187

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FOREWORD

Aroclor 1016 is a mixture of PCB's that was introduced almost 40 years after PCB's began to be commercially used. The mixture was made by redistilling Aroclor 1242 to remove some of the more highly chlorinated PCB's and make the product more environmentally acceptable. Because the mink industry has been especially affected by the release of PCB's into the environment and by their bioaccumulation in fish, the potential impact of Aroclor 1016 on mink needed to be determined. This study seeks to fulfill that need.

ABSTRACT

Effects of the PCB Aroclor 1016 on reproduction, growth, and survival of mink (*Mustela vison*) were investigated. Mink raised according to commercial mink-ranch procedures were fed diets that contained 0, 2, 10, and 25 ppm Aroclor 1016 for up to 18 months. Reproduction was not adversely affected, although kit growth and survival were suboptimum in some of the treated groups. No hematologic differences were observed between the treated and non-treated mink, but heart weight increased and kidney weight decreased in the older animals of two of the three PCB-treated groups. No consistent gross lesions associated with PCB toxicity were observed. The PCB residue in mink tissues was directly related to the quantity of Aroclor 1016 in the diet. Residues in mink kits suggest that Aroclor 1016 passes the placental barrier.

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TABLES

SECTION 1

INTRODUCTION

Polychlorinated biphenyls (PCB's) are chlorinated hydrocarbon compounds of varying chlorine content that have had wide industrial use since 1930 (Penning 1930). They have been used in the manufacture of plastics, paints, varnishes, resins, lubricants, synthetic and natural rubbers, waxes, asphalt, hydraulic fluids, allyl starch, and heat-transfer fluids. They have also been used for dust prevention, moisture proofing, sealing, and vapor suppression (Lichtenstein et al. 1969, Platonow et al. 1976).

Jensen (1966) first identified PCB's as environmental contaminants in 1966. Subsequent reports have demonstrated their toxicity to animals, including humans, and have confirmed their status as important environmental pollutants of the world ecosystem. Polychlorinated biphenyls tend to concentrate in animal tissues, and their low biodegradation rate suggests that, although they are not presently manufactured or sold in this country, the vast environmental burden of these compounds that has accumulated over the years will present a hazard for many years to come.

Aroclor®[1] 1016 is a PCB recently introduced as a possible substitute for other "more hazardous" Aroclors and for which few toxicological data are available. The objective of this study was to investigate the effects of Aroclor 1016 on reproduction, growth, and survival in mink (*Mustela vison*) to provide data for evaluating the relative toxicity of this chlorinated hydrocarbon compound. The mink was selected as a test animal because it is extremely sensitive to other Aroclors and is considered an excellent experimental animal for PCB investigations (Aulerich and Ringer 1977).

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[1] Trade name for PCB's previously manufactured by Monsanto Chemical Co., St. Louis, Mo.

SECTION 2

CONCLUSIONS

1. Mink are relatively tolerant of dietary Aroclor 1016.

2. Long-term feeding of up to 25 ppm Aroclor 1016 to mink did not adversely affect reproduction, but growth and survival of newborn kits were suboptimum.

3. No marked hematologic changes or clinical signs of PCB poisoning were observed in mink fed diets that contained up to 25 ppm Aroclor 1016 for 18 months. Increased heart and decreased kidney weights were noted in some of the PCB-treated animals, but were not observed consistently among the treated groups.

4. Residues of PCB in tissues from mink fed Aroclor 1016 were considerably lower than those from mink fed comparable levels of Aroclors 1242, 1248, and 1254. Residues in mink kits suggest that Aroclor 1016 is transferred across the placenta.

5. The greater tolerance of mink for Aroclor 1016 than for other Aroclors of similar chlorine content may be due to reduced absorption, greater excretion, or increased metabolism of Aroclor 1016.

SECTION 3

MATERIALS AND METHODS

This study consisted of an 18-month mink feeding experiment. On January 6, 1976, 60 standard (natural dark) ranch mink, approximately 8 months old, were allocated into four groups, each consisting of 3 males and 12 females. Littermates were divided among the groups to minimize the effects of genetics on reproduction and sensitivity to the PCB dietary supplement.

The animals were fed either a basic unsupplemented (control) diet[2] or the basic diet supplemented with 2, 10, or 25 ppm of Aroclor 1016. They were housed individually in suspended wire cages (61 x 76 x 46 cm) in an open-sided shed. Each cage was equipped with a nest box and two drinking cups. Routine mink-ranch procedures were followed in the feeding, care, and breeding of the animals.

The mink were immunized against canine distemper, botulism, and virus enteritis as kits. They were fed their respective diets *ad libitum*, except before the breeding season (March) when the animals were fed to "condition" them for optimum reproduction. Mating attempts were initiated on March 3, 1976, and whenever possible matings were made between mink within a dietary group. All matings were verified by the presence of apparently normal, motile spermatozoa in the vaginal smear after coition. Following a successful "sperm-checked" mating, each female was given another opportunity to mate, either the day after the first mating or 8 days later. The mated females were checked daily for young during the whelping period (April 24-May 15). Kits were counted and weighed on the day of birth and at 4 weeks of age.

Following the 1976 mink reproductive period, the adult breeder mink plus 20 kits (10 males and 10 females) whelped and nursed by females on each diet were retained on their respective diets, either through November 1976 (when 7 of the 10 kit males on each treatment were pelted) or through the next reproductive cycle (to June 28, 1977). In groups II and IV, where insufficient kits were produced to provide 20 weaned offspring, kits (two females in group II and four females and eight males in group IV) whelped and nursed by untreated females were used to fill the groups. Housing, feeding, care, and breeding of these animals were similar to those previously described.

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[2]The basic diet consisted of 25% commercial mink cereal, 20% chicken, 20% ocean fish (cod, haddock, and flounder mix), 15% beef tripe, 7.5% beef lungs, 7.5% beef trimmings, and 5% beef liver.

The mink diets that contained the supplemental PCB were prepared by dissolving the desired quantity of Aroclor 1016[3] in acetone and blending the solution (only acetone in the control diet) with ground commercial mink cereal. The acetone was evaporated, and the cereal-PCB premix was mixed with the other dietary ingredients to yield a diet that contained the desired amount of the PCB.

The mink were weighed to the nearest 5 g at various time intervals (Tables 6 and 7), and blood samples were collected (by toenail clipping) for analysis. Hematocrits were measured in duplicate following centrifugation for 7 min at 11,500 rpm with an International (Model MB) microcapillary centrifuge. Hemoglobin content of the blood was determined either with an AO Spencer-Hb-meter[4] or by the cyanmethemoglobin method (Eilers 1967). Differential cell counts were made in duplicate on blood smears with Wright's stain (Davidson and Henry 1965).

The feeding trial was terminated on June 28, 1977. Necropsies were performed on all surviving mink (original animals plus the 10 females and 3 males of the F_1 generation), and their organ weights were recorded. Tissue samples from the animals were fixed in 10% neutral buffered formalin and were prepared for histopathologic examination according to routine laboratory procedures. Tissue sections were cut at 5 µ and stained with hematoxylin and eosin. The remainder of the tissues of the mink fed the experimental diets from January 6, 1976, to June 28, 1977, along with four newborn kits (April-May 1977) from each treatment group, were stored frozen for PCB residue analysis. The PCB residue analyses were made on a gas chromatograph according to the method described by Thompson (1977).

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[3]Supplied by Dr. Gilman D. Veith, Environmental Research Laboratory-Duluth, Duluth, Minn.

[4]American Optical Co., Buffalo, New York.

SECTION 4

RESULTS

The reproductive performance of the female mink fed the experimental diets is shown in Tables 1 and 2. Reproduction in the control groups (I) during 1976 and 1977 was considered satisfactory, as a litter average of 4.0 kits per mated female is normal. Although the average litter size per mated female during the 1976 reproductive period varied inversely with the amount of PCB added to the basic diet (Table 1), this trend was not repeated during 1977 (Table 2).

The length of gestation did not differ significantly between the control females and those fed diets that contained supplemental PCB. Gestation in mink is quite variable because of delayed implantation. The average gestation period for single-mated dark mink was reported to be 51.22 days by Bowness (1968).

Except for the reduced 4-week weights of kits whelped and nursed by the primaparous females in group IV during 1976 and 1977, the differences in kit weights shown in Tables 3 and 4 were probably not biologically significant.

Kit mortality at birth was unaffected by the addition of Aroclor 1016 to the diet of the dams, although kit mortality by 4 weeks was generally greater in the groups that received the PCB-supplemented diets than in the controls (Table 5).

Any significant differences in mink body weights between the controls and the PCB-treated animals occurred during the first few months of the feeding trial and were not evident during the latter part of the study (Table 6, Table 7).

No significant differences or trends were noted in the hematocrit or hemoglobin values, (Table 8) or in the differential blood cell counts (Table 9) from the mink on the various dietary treatments. These hematologic values are considered normal for mink and were in agreement with those reported by Jorgensen and Christensen (1966), Skrede (1970), Rotenberg and Jorgensen (1971), Fletch and Karstad (1972), and Asher et al. (1976).

The mean organ (liver, spleen, kidney, lung, adrenal, and heart) weights (expressed as a percentage of brain weight) of the mink that survived to the termination of the feeding trial are shown in Table 10. A significant increase in heart weight and reduction in kidney weight was observed in the older (2nd year) mink fed the diet supplemented with 2 and 25 ppm PCB. No consistent gross lesions that could be associated with PCB toxicity were observed in the mink that died during the study or those on which necropsies were performed at the termination of the feeding trial.

TABLE 1. REPRODUCTIVE PERFORMANCE OF FEMALE MINK FED A CONTROL DIET OR DIETS SUPPLEMENTED WITH AROCLOR 1016 FROM JANUARY 6, 1976, TO JUNE 30, 1976

Dietary treatment	Number of females		Average number of matings	Average gestation (days)	Number of kits at birth		Average number of kits whelped/female	
	Mated	Whelped			Alive	Dead	Mated	Whelped
I Basic diet (control)	12	10	2.1	54.1	43	4	3.9	4.7
II Basic diet plus 2 ppm PCB	10[a]	9	1.7	51.4	31	3	3.4	3.8
III Basic diet plus 10 ppm PCB	11[a]	5	1.5	49.6	22	3	2.3	5.0
IV Basic diet plus 25 ppm PCB	11	7	1.6	50.6	16	6	2.0	3.1

[a]One female died from injuries received during mating.

TABLE 2. REPRODUCTIVE PERFORMANCE OF FEMALE MINK FED A CONTROL DIET OR DIETS SUPPLEMENTED WITH AROCLOR 1016 FROM JANUARY 6, 1976, OR JULY 15, 1976, TO JUNE 28, 1977

Dietary treatment	Mink status	Number of females		Average number of matings	Average gestation (days)	Number of kits at birth		Average number of kits whelped/female	
		Mated	Whelped			Alive	Dead	Mated	Whelped
I Basic diet (control)	2nd yr[a]	12	10	1.9	47.7	44	5	4.1	4.7
	1st yr[b]	10	7	1.7	50.6	46	7	5.3	7.6
II Basic diet plus 2 ppm PCB	2nd yr	8	7	1.7	47.6	35	6	5.1	5.9
	1st yr	10	7	1.9	48.3	36	3	3.9	5.6
III Basic diet plus 10 ppm PCB	2nd yr	8	6	1.8	47.8	21	3	3.0	4.0
	1st yr	10	7	1.7	50.4	23	4	2.7	3.9
IV Basic diet plus 25 ppm PCB	2nd yr	11	7	1.7	48.3	43	3	4.2	6.6
	1st yr	10	9	2.0	48.9	44	4	4.8	5.3

[a]Females fed a control or PCB-supplemented diet from January 6, 1976, to June 28, 1977. Data pertain to only second reproductive period. Data for first reproductive period (March-June 1976) are presented in Table 1.

[b]Kit females whelped and nursed by mink fed the control or PCB-supplemented diets since January 6, 1976, or placed on the control or PCB-supplemented diets from weaning (July 15, 1976) through their first reproductive period (June 28, 1977).

TABLE 3. AVERAGE BODY WEIGHT ($\pm$ S.E.) AT BIRTH AND FOUR WEEKS AND MORTALITY OF KITS WHELPED AND NURSED BY FEMALES FED A CONTROL DIET OR DIETS SUPPLEMENTED WITH AROCLOR 1016 FROM JANUARY 6, 1976, TO JUNE 30, 1976 (WEANING)

Dietary treatment	At birth		At 4 weeks		Kit mortality
	Number	Body weight (g)	Number	Body weight (g)	birth to 4 weeks (%)
I Basic diet (control)	43	9.7 $\pm$ 0.83	39	180.6 $\pm$ 5.46	9.3
II Basic diet plus 2 ppm PCB	31	9.1 $\pm$ 1.26	22	168.5 $\pm$ 5.42	29.0
III Basic diet plus 10 ppm PCB	22	10.6 $\pm$ 1.00	21	178.7 $\pm$ 5.71	4.5
IV Basic diet plus 25 ppm PCB	16	9.3 $\pm$ 1.22	11	132.3 $\pm$ 9.30[a]	31.3

[a]Significantly different ($P<0.01$) from control by Dunnett's t-test.

TABLE 4. AVERAGE BODY WEIGHT (± S.E.) AT BIRTH AND FOUR WEEKS AND MORTALITY OF FEMALE KITS WHELPED AND NURSED BY FEMALES FED A CONTROL DIET OR DIETS SUPPLEMENTED WITH AROCLOR 1016 FROM JANUARY 6, 1976, OR JULY 15, 1976, TO JUNE 28, 1977

Dietary treatment	Mink status	At birth		At 4 weeks		Kit mortality birth to 4 weeks (%)
		Number	Body weight (g)	Number	Body weight (g)	
I Basic diet (control)	2nd yr[a]	44	9.70 ± 0.27	43	153.7 ± 5.14	2.3
	1st yr[b]	46	9.52 ± 0.23	38	159.5 ± 3.27	17.4
II Basic diet plus 2 ppm PCB	2nd yr	35	8.75 ± 0.36	28	153.9 ± 3.72	20.0
	1st yr	36	8.43 ± 0.24[c]	25	143.6 ± 4.55[d]	30.1
III Basic diet plus 10 ppm PCB	2nd yr	21	10.95 ± 0.35[d]	14	177.7 ± 7.55[d]	33.3
	1st yr	23	8.69 ± 0.30[d]	17	159.4 ± 5.93	26.1
IV Basic diet plus 25 ppm PCB	2nd yr	43	9.15 ± 0.27	25	150.3 ± 4.53	41.9
	1st yr	44	9.47 ± 0.21	38	130.9 ± 4.88[c]	13.6

[a]Females fed control or PCB-supplemented diets from January 6, 1976, to June 28, 1977. Data pertain to only second reproductive periods (March-June 1977). Data for first reproductive period (March-June 1976) are presented in Table 3.

[b]Kit females whelped and nursed by females fed the same control or PCB-supplemented diets since January 6, 1976, or whelped by females fed non-PCB-supplemented diets and placed on the PCB-supplemented diets from weaning (July 15, 1976) through June 28, 1977.

[c]Significantly different (P<0.01) from control by Dunnett's t-test.

[d]Significantly different (P<0.05) from control by Dunnett's t-test.

TABLE 5. BIOMASS[a] OF MINK KITS FROM BIRTH TO FOUR WEEKS OF AGE PRODUCED BY LACTATING FEMALES FED THE CONTROL DIET OR DIETS SUPPLEMENTED WITH AROCLOR 1016

Dietary treatment	Mink reproductive status	Average number of kits/ lactating female	Average kit body weight gain (g) from birth to 4 weeks	Biomass (g)
I Basic diet[b] (control)	1st reproductive period	4.33	170.9	740.0
II Basic diet[b] plus 2 ppm PCB	1st reproductive period	2.75	159.4	438.4
III Basic diet[b] plus 10 ppm PCB	1st reproductive period	4.20	168.1	706.0
IV Basic diet[b] plus 25 ppm PCB	1st reproductive period	1.83	123.0	225.1
I Basic diet (control)	2nd reproductive period[c]	4.78	144.0	688.3
	1st reproductive period[d]	6.33	150.0	949.5
II Basic diet[c] plus 2 ppm PCB	2nd reproductive period[c]	5.60	145.2	813.1
	1st reproductive period[d]	5.00	135.2	676.0
III Basic diet[c] plus 10 ppm PCB	2nd reproductive period[c]	2.80	166.8	467.0
	1st reproductive period[d]	2.43	150.7	366.2
IV Basic diet[c] plus 25 ppm PCB	2nd reproductive period[c]	4.17	141.2	588.8
	1st reproductive period[d]	4.22	121.4	512.3

[a]Biomass = average kit body weight gain between birth and 4 weeks of age times the average number of kits raised per lactating female.

[b]Females fed experimental diets from January 6, 1976, to June 30, 1976.

[c]Females fed experimental diets from January 6, 1976, to June 28, 1977.

[d]Kits whelped and nursed by females fed the same diet since January 6, 1976, or whelped by non-PCB-treated females and fed the experimental diet (July 15, 1976, through June 28, 1977, as described in text).

TABLE 6. AVERAGE BODY WEIGHT (± S.E.) (IN GRAMS) OF MINK FED A CONTROL DIET OR DIETS SUPPLEMENTED WITH AROCLOR 1016 FROM JANUARY, 6, 1976, TO JULY 1, 1976

Dietary treatment	Sex	Date: 1/6/76	2/3/76	3/2/76	Four weeks post whelping or 6/2/76	7/1/76
I Basic diet (control)	F	884.6 ± 27.55 (12)[a]	895.8 ± 22.37 (12)	950.0 ± 30.81 (12)	853.8 ± 24.11 (12)	780.8 ± 21.62 (12)
	M	1,493.3 ± 166.04 (3)	1,398.3 ± 152.46 (3)	1,381.7 ± 149.33 (3)	1,533.3 ± 86.68 (3)	1,473.3 ± 54.90 (3)
II Basic diet plus 2 ppm PCB	F	889.2 ± 30.19 (12)	840.8 ± 27.83 (12)	874.2 ± 21.28 (12)	935.9 ± 51.97 (11)	772.5 ± 48.84 (10)
	M	1,740.0 ± 47.25 (3)	1,495.0 ± 7.64 (3)	1,426.7 ± 29.01 (3)	1,745.0 ± 25.00 (2)	1,645.0 ± 25.00[b] (2)
III Basic diet plus 10 ppm PCB	F	867.9 ± 30.46 (12)	855.4 ± 19.71 (12)	940.8 ± 30.62 (12)	890.5 ± 44.26 (11)	803.5 ± 34.16 (10)
	M	1,753.3 ± 99.57 (3)	1,666.7 ± 112.88 (3)	1,730.0 ± 153.7 (3)	1,770.0 ± 68.06 (3)	1,598.3 ± 31.18 (3)
IV Basic diet plus 25 ppm PCB	F	825.8 ± 28.30 (12)	801.7 ± 22.58 (12)	846.7 ± 29.99 (12)	868.2 ± 39.37 (11)	799.1 ± 29.46 (11)
	M	1,525.0 ± 51.96 (3)	1,456.7 ± 32.79 (3)	1,473.3 ± 113.34 (3)	1,683.3 ± 49.13 (3)	1,626.7 ± 23.98[b] (3)

[a]Number of mink.

[b]Significantly different ($P<0.05$) from control by Dunnett's t-test.

TABLE 7. AVERAGE BODY WEIGHT (± S.E.) (IN GRAMS) OF MINK FED A CONTROL DIET OR DIETS SUPPLEMENTED WITH AROCLOR 1016 FROM JANUARY 6, 1976[a], OR JULY 15, 1976, TO JUNE 28, 1977

Dietary treatment	Mink status	Sex	Date: 7/15/76	8/15/76	9/14/76	10/14/76	11/13/77
I Basic diet (control)	2nd yr[b]	F	811.7 ± 22.92 (12)[c]	805.0 ± 28.54 (12)	894.2 ± 19.81 (12)	1,014.2 ± 23.31 (12)	1,043.3 ± 28.01 (12)
		M	1,506.7 ± 59.23 (3)	1,496.7 ± 77.52 (3)	1,570.0 ± 63.71 (3)	1,641.7 ± 60.55 (3)	1,745.0 ± 57.95 (3)
	1st yr[b]	F	631.0 ± 15.45 (10)	801.0 ± 15.59 (10)	1,012.0 ± 23.48 (10)	1,118.5 ± 27.60 (10)	1,184.0 ± 36.58 (10)
		M	896.0 ± 23.81 (10)	1,092.0 ± 23.61 (10)	1,364.0 ± 31.29 (10)	1,520.0 ± 37.20 (10)	1,593.3 ± 50.09 (9)
II Basic diet plus 2 ppm PCB	2nd yr	F	732.2 ± 52.30 (9)	802.8 ± 53.31 (9)	908.3 ± 47.65 (9)	1,016.7 ± 44.15 (9)	1,024.4 ± 52.90 (9)
		M	1,560.0 ± 10.0 (2)	1,557.5 ± 2.5 (2)	1,772.5 ± 2.5 (2)	1,872.5 ± 52.50 (2)	1,810.0 ± 70.00 (2)
	1st yr	F	704.0 ± 12.22[e] (10)	857.0 ± 10.25 (10)	1,059.5 ± 25.35 (10)	1,150.0 ± 31.69 (10)	1,134.0 ± 34.55 (10)
		M	985.6 ± 24.52 (9)	1,073.3 ± 26.52 (9)	1,342.8 ± 40.54 (9)	1,510.6 ± 53.55 (9)	1,457.8 ± 63.81[f] (9)
III Basic diet plus 10 ppm PCB	2nd yr	F	797.0 ± 27.85 (10)	792.0 ± 25.89 (10)	874.0 ± 36.87 (10)	979.0 ± 29.27 (10)	987.0 ± 38.12 (10)
		M	1,520.0 ± 52.92 (3)	1,440.0 ± 61.10 (3)	1,675.0 ± 77.51 (3)	1,833.3 ± 52.42 (3)	1,818.3 ± 73.41 (3)
	1st yr	F	703.0 ± 21.03[e] (10)	798.5 ± 24.54 (10)	997.5 ± 28.96 (10)	1,082.0 ± 31.74 (10)	1,030.0 ± 38.67 (10)
		M	1,080.0 ± 46.21[e] (10)	1,130.0 ± 28.56 (10)	1,397.0 ± 44.25 (10)	1,565.5 ± 44.08 (10)	1,627.0 ± 56.72 (10)
IV Basic diet plus 25 ppm PCB	2nd yr	F	783.6 ± 27.24 (11)	794.1 ± 26.91 (11)	865.5 ± 29.52 (11)	971.4 ± 28.39 (11)	1,036.4 ± 35.04 (11)
		M	1,680.0 ± 115.47 (3)	1,456.7 ± 51.72 (3)	1,625.0 ± 53.46 (3)	1,698.3 ± 91.04 (3)	1,753.3 ± 78.62 (3)
	1st yr	F	704.0 ± 28.88[e] (10)	764.0 ± 21.77 (10)	892.0 ± 24.67[e] (10)	928.0 ± 25.38[e] (10)	987.5 ± 15.87[e] (10)
		M	846.0 ± 37.75 (10)	1,050.0 ± 31.87 (10)	1,275.0 ± 36.46 (10)	1,372.0 ± 32.67[f] (10)	1,375.0 ± 51.76[e] (10)

continued

TABLE 7. (continued)

Dietary treatment	Mink status	Sex	Date 12/18/76	1/15/77	2/15/77	3/15/77	6/1/77
I Basic diet (control)	2nd yr	F	997.5 ± 22.07 (12)	905.8 ± 31.03 (12)	944.2 ± 41.72 (12)	920.4 ± 38.16 (12)	884.6 ± 31.34 (12)
		M	701.7 ± 65.19 (3)	1,640.0 ± 63.70 (3)	1,676.7 ± 126.79 (3)	1,486.7 ± 147.45 (3)	
	1st yr	F	1,038.5 ± 32.61 (10)	1,022.5 ± 33.46 (10)	1,033.0 ± 34.18 (10)	1,033.0 ± 42.01 (10)	913.5 ± 43.63
		M	1,581.7 ± 147.45 (3)	1,540.0 ± 60.48 (3)	1,418.3 ± 49.55 (3)	1,465.0 ± 50.08 (3)	
II Basic diet plus 2 ppm PCB	2nd yr	F	986.7 ± 52.71 (9)	877.8 ± 48.49 (9)	845.0 ± 48.01 (9)	843.9 ± 40.58 (9)	885.0 ± 62.66 (8)
		M	1,907.5 ± 177.5 (2)	1,930.0 ± 270.00 (2)	1,935.0 ± 270.00 (2)	1,770.0 ± 290.00 (2)	
	1st yr	F	1,040.5 ± 40.88 (10)	1,032.5 ± 36.81 (10)	1,022.5 ± 38.49 (10)	1,013.5 ± 36.53 (10)	950.0 ± 24.66 (10)
		M	1,591.7 ± 49.08 (3)	1,543.3 ± 40.48 (3)	1,396.7 ± 14.45 (3)	1,380.0 ± 61.10 (3)	
III Basic diet plus 10 ppm PCB	2nd yr	F	910.5 ± 38.87 (10)	854.5 ± 56.79 (10)	910.6 ± 46.81 (8)	976.3 ± 34.17 (8)	921.3 ± 52.77 (8)
		M	1,726.7 ± 86.65 (3)	1,748.3 ± 130.15 (3)	1,660.0 ± 181.48 (3)	1,743.3 ± 146.22 (3)	
	1st yr	F	992.0 ± 46.35 (10)	917.0 ± 43.35 (10)	890.0 ± 46.27 (10)	980.0 ± 35.65 (10)	923.0 ± 32.81 (10)
		M	1,638.3 ± 40.83 (3)	1,530.0 ± 211.09 (3)	1,395.0 ± 220.96 (3)	1,335.0 ± 76.54 (3)	
IV Basic diet plus 25 ppm PCB	2nd yr	F	958.6 ± 6.53 (11)	926.8 ± 27.60 (11)	889.6 ± 28.95 (11)	879.6 ± 26.92 (11)	882.7 ± 45.56 (11)
		M	1,673.3 ± 65.68 (3)	1,675.0 ± 48.22 (3)	1,606.7 ± 76.87 (3)	1,538.3 ± 100.61 (3)	
	1st yr	F	971.5 ± 23.98 (10)	989.0 ± 39.75 (10)	990.5 ± 33.65 (10)	957.0 ± 31.62 (10)	896.5 ± 34.40
		M	1,563.3 ± 372.28 (3)	1,670.0 ± 221.89 (3)	1,567.5 ± 297.50 (2)	1,610.0 ± 270.0 (2)	

[a]Average body weights of mink (2nd-yr animals) from January 6, 1976, to June 2, 1976, as shown in Table 6.

[b]Mink placed on control or experimental diets January 6, 1976. Average body weight data for these mink from January 6, 1976, to June 2, 1976, are shown in Table 6.

[c]Number of mink.

[d]Mink whelped and nursed by females fed the same control or PCB-supplemented diets from January 6, 1976, to June 2, 1976, or whelped by females fed non-PCB-supplemented diets and placed on PCB-supplemented diets July 15, 1976.

[e]Significantly different ($P<0.01$) from control by Dunnett's t-test.

[f]Significantly different ($P<0.05$) from control by Dunnett's t-test.

TABLE 8. HEMATOCRIT AND HEMAGLOBIN VALUES FOR MINK FED A CONTROL DIET OR DIETS SUPPLEMENTED WITH AROCLOR 1016 FROM JANUARY 6, 1976, OR JULY 15, 1976, TO JUNE 28, 1977

			Hematocrit (% ± S.E.)			Hemoglobin (gm % ± S.E.)		
Dietary treatment	Mink status	Sex	Four weeks post whelping or 6/2/76	Four days post whelping 1977	6/28/77	Four weeks post whelping or 6/2/76[a]	Four days post whelping 1977[a]	6/28/77[b]
I Basic diet (control)	2nd yr[c]	F	53.8 ± 0.92 (12)	45.5 ± 1.44 (10)	52.5 ± 1.11 (12)	17.6 ± 0.26 (12)	14.9 ± 0.49 (10)	16.7 ± 0.52 (12)
		M	59.7 ± 1.71 (3)		40.6 ± 5.55 (3)			14.8 ± 1.44 (3)
	1st yr[d]	F		48.7 ± 0.84 (7)	52.4 ± 0.83 (10)		16.0 ± 0.33 (7)	16.0 ± 0.56 (10)
		M			51.8 ± 1.38 (3)			15.7 ± 0.46 (3)
II Basic diet plus 2 ppm PCB	2nd yr	F	54.3 ± 0.54 (11)	46.7 ± 1.51 (7)	52.3 ± 2.27 (8)	18.3 ± 0.30 (11)	15.4 ± 0.51 (7)	16.1 ± 0.86 (8)
		M	55.5 ± 0.50 (2)		48.8 ± 2.75 (2)			15.4 ± 0.90 (2)
	1st yr	F		46.0 ± 1.30 (7)	54.2 ± 0.78 (10)		15.7 ± 0.65 (7)	16.7 ± 0.29 (10)
		M			54.1 ± 1.49 (3)			16.6 ± 0.27 (3)
III Basic diet plus 10 ppm PCB	2nd yr	F	53.6 ± 1.15 (11)	47.3 ± 1.20 (6)	50.6 ± 1.11 (8)	18.0 ± 0.33 (11)	16.0 ± 0.34 (6)	16.8 ± 0.55 (8)
		M	56.3 ± 1.24 (3)		52.3 ± 4.13 (3)			16.4 ± 1.00 (3)
	1st yr	F		49.1 ± 1.29 (7)	53.8 ± 1.20 (10)		16.5 ± 0.40 (7)	17.5 ± 0.40 (10)
		M			52.6 ± 0.83 (3)			16.2 ± 0.20 (3)
IV Basic diet plus 25 ppm PCB	2nd yr	F	51.7 ± 1.16 (11)	44.9 ± 1.33 (7)	50.5 ± 1.12 (11)	17.0 ± 0.35 (11)	14.9 ± 0.61 (7)	16.4 ± 0.37 (11)
		M	56.0 ± 1.53 (3)		52.8 ± 2.32 (3)			17.4 ± 0.93 (3)
	1st yr	F		48.9 ± 0.34 (9)	49.7 ± 1.49 (10)		16.5 ± 0.16 (9)	15.8 ± 0.43 (10)
		M			57.3 ± 2.74 (2)			18.4 ± 0.0 (2)

[a]Hemoglobin determined with an AO Spencer Hb-meter.

[b]Hemoglobin determined by the cyanmethemoglobin method.

[c]Mink placed on control or experimental diets January 6, 1976.

[d]Mink whelped and nursed by females fed the same control or PCB-supplemented diets from January 6, 1976, to June 2, 1976, or whelped by females fed non-PCB-supplemented diets and placed on PCB-supplemented diets July 15, 1976.

TABLE 9. DIFFERENTIAL BLOOD CELL COUNTS (JUNE 28, 1977) FOR MINK FED A CONTROL DIET OR DIETS SUPPLEMENTED WITH AROCLOR 1016 FROM JANUARY 6, 1976, OR JULY 15, 1976, TO JUNE 28, 1977

Dietary treatment	Mink status	Sex	Eosinophils	Basophils	Neutrophils Band	Neutrophils Mature	Lymphocytes	Monocytes
	2nd yr	F	3.6 ± 0.83 (10)	0.9 ± 0.18 (10)	1.0 ± 0.33 (10)	66.5 ± 2.14 (10)	24.0 ± 1.55 (10)	3.9 ± 0.60 (10)
I Basic diet (control)		M	2.7 ± 1.45 (3)	0.0 ± 0.0 (3)	1.3 ± 1.33 (3)	54.7 ± 4.69 (3)	36.7 ± 4.36 (3)	4.7 ± 1.85 (3)
	1st yr	F	3.1 ± 0.57 (9)	1.0 ± 0.44 (9)	0.2 ± 0.15 (9)	71.1 ± 3.27 (9)	19.8 ± 2.72 (9)	4.8 ± 0.98 (9)
		M	7.3 ± 1.27 (3)	0.7 ± 0.33 (3)	0.3 ± 0.33 (3)	50.0 ± 3.00 (3)	35.7 ± 7.86 (3)	6.0 ± 2.65 (3)
	2nd yr	F	2.9 ± 0.72 (8)	0.6 ± 0.26 (8)	0.6 ± 0.38 (8)	66.1 ± 3.34 (8)	23.9 ± 3.27 (8)	5.9 ± 0.93 (8)
II Basic diet plus 2 ppm PCB		M	7.5 ± 1.50 (2)	1.0 ± 1.00 (2)	2.0 ± 0.0 (2)	49.5 ± 2.50 (2)	35.5 ± 2.50 (2)	4.5 ± 1.90 (2)
	1st yr	F	3.0 ± 1.06 (10)	0.9 ± 0.18 (10)	1.0 ± 0.47 (10)	64.4 ± 2.89 (10)	25.7 ± 1.73 (10)	5.1 ± 0.71 (10)
		M	6.0 ± 1.00 (3)	1.0 ± 0.58 (3)	0.0 ± 0.0 (3)	43.7 ± 4.83 (3)	43.0 ± 4.04 (3)	6.3 ± 0.89 (3)
	2nd yr	F	4.1 ± 1.27 (8)	0.8 ± 0.41 (8)	1.0 ± 0.63 (8)	58.9 ± 3.79 (8)	30.6 ± 3.91 (8)	4.4 ± 0.53 (8)
III Basic diet plus 10 ppm PCB		M	3.3 ± 0.88 (3)	0.7 ± 0.67 (3)	0.3 ± 0.33 (3)	55.0 ± 2.65 (3)	33.0 ± 1.53 (3)	7.3 ± 0.89 (3)
	1st yr	F	2.1 ± 0.60 (10)	0.3 ± 0.30 (10)	0.8 ± 0.39 (10)	67.4 ± 4.00 (10)	24.0 ± 3.44 (10)	5.3 ± 0.54 (10)
		M	5.3 ± 0.93 (3)	1.3 ± 0.97 (3)	1.0 ± 1.00 (3)	51.0 ± 4.04 (3)	37.0 ± 5.13 (3)	4.3 ± 0.67 (3)
	2nd yr	F	2.5 ± 0.84 (11)	0.6 ± 0.28 (11)	2.0 ± 0.49 (11)	68.3 ± 2.66 (11)	22.6 ± 2.20 (11)	3.8 ± 0.50 (11)
IV Basic diet plus 25 ppm PCB		M	5.0 ± 2.65 (3)	1.3 ± 0.33 (3)	1.3 ± 0.67 (3)	60.7 ± 1.64 (3)	27.7 ± 1.44 (3)	4.0 ± 2.08 (3)
	1st yr	F	1.7 ± 0.67 (10)	0.6 ± 0.16 (10)	0.6 ± 0.34 (10)	70.4 ± 3.00 (10)	23.1 ± 2.43 (10)	3.6 ± 0.48 (10)
		M	8.0 ± 3.00 (2)	0.5 ± 0.50 (2)	3.0 ± 3.00 (2)	66.0 ± 2.00 (2)	17.0 ± 3.0 (2)	6.0 ± 0.0 (2)

TABLE 10. MEAN ORGAN WEIGHTS[a] OF MINK FED A CONTROL DIET OR DIETS SUPPLEMENTED WITH AROCLOR 1016 FROM JANUARY 6, 1976, OR JULY 15, 1976, TO JUNE 28, 1977

Dietary treatment	Mink status	Sex	Number	Organ					
				Liver	Spleen	Kidney	Lung	Adrenal	Heart
I Basic diet (control)	2nd yr[b]	F	12	344.2 ± 25.35	32.1 ± 4.85	57.4 ± 1.95	82.4 ± 3.66	1.25 ± 0.09	66.6 ± 1.64
		M	3	722.3 ± 184.81	78.2 ± 22.20	83.1 ± 3.49	114.1 ± 4.84	1.60 ± 0.40	101.0 ± 9.37
	1st yr[c]	F	10	319.9 ± 8.88	37.5 ± 8.23	56.9 ± 2.32	83.6 ± 3.41	1.24 ± 0.10	71.1 ± 3.81
		M	3	462.0 ± 12.77	46.7 ± 2.36	67.8 ± 4.62	109.9 ± 3.28	1.00 ± 0.10	78.7 ± 3.18
II Basic diet plus 2 ppm PCB	2nd yr	F	8	300.0 ± 34.23	38.2 ± 6.89	52.6 ± 3.03	74.1 ± 3.00	1.35 ± 0.13	80.8 ± 4.21[d]
		M	2	651.8 ± 265.75	60.1 ± 25.4	63.3 ± 0.71[d]	126.7 ± 10.7	1.00 ± 0.30	110.3 ± 22.20
	1st yr	F	10	277.6 ± 15.13	27.0 ± 2.48	50.0 ± 2.03	72.7 ± 3.57	0.96 ± 0.06	74.4 ± 3.21
		M	3	393.6 ± 25.58	29.4 ± 6.57	58.8 ± 4.27	100.8 ± 11.24	0.93 ± 0.18	88.6 ± 11.57
III Basic diet plus 10 ppm PCB	2nd yr	F	8	303.9 ± 14.03	29.4 ± 3.16	50.5 ± 1.59	76.0 ± 2.68	1.18 ± 0.03	69.4 ± 1.34
		M	3	611.8 ± 213.95	71.5 ± 21.62	74.6 ± 2.12	114.2 ± 11.62	1.37 ± 0.04	121.9 ± 24.77
	1st yr	F	10	313.8 ± 16.53	28.1 ± 2.56	52.3 ± 1.42	89.3 ± 6.93	1.06 ± 0.04	75.1 ± 3.02
		M	3	426.3 ± 52.51	42.2 ± 11.84	74.9 ± 3.93	117.2 ± 8.51	1.07 ± 0.04	89.2 ± 3.17
IV Basic diet plus 25 ppm PCB	2nd yr	F	11	380.2 ± 37.23	33.2 ± 5.12	52.6 ± 1.48	80.6 ± 2.19	1.26 ± 0.10	78.7 ± 3.49[d]
		M	3	454.7 ± 19.56	38.9 ± 15.16	59.1 ± 2.01[d]	101.2 ± 10.19	1.07 ± 0.09	92.1 ± 7.81
	1st yr	F	10	316.1 ± 16.09	33.6 ± 3.12	53.5 ± 1.37	80.9 ± 2.75	1.20 ± 0.09	69.5 ± 2.50
		M	2	397.5 ± 57.70	41.3 ± 8.70	64.9 ± 3.85	110.0 ± 0.14	1.20 ± 0.30	76.9 ± 4.50

[a]Expressed as % of brain weight ± S.E.

[b]Mink fed control or PCB-supplemented diets from January 6, 1977 to June 28, 1977.

[c]Mink whelped and nursed by females fed the same control or PCB-supplemented diets since January 6, 1976 or whelped by females fed non-PCB-supplemented diets and placed on PCB-supplemented diets from July 15, 1976 through June 28, 1977.

[d]Significantly different (P<0.01) from control by Dunnett's t-test.

The PCB residue in the tissues of mink fed the experimental diets over the 18-month period and in newborn kits whelped by females in each dietary group was directly related to the quantity of Aroclor 1016 in the diet (Table 11). The PCB residues were greatest in the adipose tissue.

TABLE 11. AVERAGE PCB RESIDUES (ppm)[a] IN TISSUES FROM CONTROL MINK AND MINK FED DIETS SUPPLEMENTED WITH AROCLOR 1016 FROM JANUARY 6, 1976, TO JUNE 28, 1977, AND IN NEWBORN KITS WHELPED BY THESE FEMALES

Dietary treatment	Number of mink per pooled sample	Sex	Tissue: Brain	Liver	Heart	Kidney	Skeletal muscle	Adipose tissue	Kits
	3	F	N.D.[b]	0.018	0.017	N.D.	N.D.	N.D.	0.006[c]
I	3	F	N.D.	N.D.	N.D.	N.D.	0.007	N.D.	
Basic diet	3	F	N.D.	0.031	N.D.	N.D.	N.D.	N.D.	
(control)	3	F	0.011	N.D.	N.D.	N.D.	N.D.	0.138	
	3	M	N.D.	N.D.	N.D.	N.D.	N.D.	0.072	
	2	F	0.032	0.365	0.033	0.059	0.026	1.938	0.040
II	2	F	0.032	0.137	0.066	0.060	0.033	2.272	
Basic diet	2	F	0.044	0.306	0.048	0.047	0.063	2.675	
plus 2 ppm PCB	2	F	0.047	0.175	0.051	0.089	0.056	0.949	
	2	M	0.049	0.070	0.041	0.052	0.040	1.474	
	2	F	0.056	0.214	0.055	0.218	0.201	4.513	0.149
III	2	F	0.136	0.626	0.096	0.352	0.212	7.621	
Basic diet	2	F	0.832	0.296	0.020	0.172	0.190	5.606	
plus 10 ppm PCB	2	F	0.089	0.393	0.122	0.116	0.177	4.129	
	3	M	0.170	0.529	0.097	0.399	0.320	4.109	
	3	F	0.231	0.903	0.053	0.201	0.443	7.805	0.254
IV	3	F	0.177	0.517	0.145	0.287	0.318	7.308	
Basic diet	3	F	0.203	0.882	0.245	0.417	0.253	6.757	
plus 25 ppm PCB	2	F	0.147	0.714	0.042	0.216	0.315	9.651	
	3	M	0.139	0.610	0.348	0.419	0.378	8.517	

[a]Analysis by Pesticide Analytical Lab, Pesticide Research Center, Michigan State University, East Lansing, MI 48824.

[b]N.D. none detected; detection limits for Aroclor 1016 = 0 ppb.

[c]Pooled sample of four kits per dietary treatment.

SECTION 5

DISCUSSION

The results of this study indicate that mink can ingest up to 25 ppm of Aroclor 1016 in their diet for 18 months without total reproductive failure. These findings are in sharp contrast to those reported from feeding mink Aroclor 1254 (Aulerich and Ringer 1977) or meat from cows fed Aroclor 1254 (Platonow and Karstad 1973). As little as 2 ppm Aroclor 1254 in the diet of mink from August to June, or 5 ppm from January to June resulted in nearly complete reproductive failure, and higher concentrations of the compound caused complete reproductive failure and death of adults (Aulerich and Ringer 1977). Similar effects, though somewhat less toxic than those noted with Aroclor 1254, have also been found from feeding mink various concentrations of Aroclor 1242 (Ringer, unpublished data).

Food consumption was not measured in this study, but if one assumes that an adult female mink consumes about 150 g of food per day (Schaible 1971), the total intake of PCB by the female mink that received 25 ppm supplemental Aroclor 1016 in the diet for almost 18 months would have been over 2,000 mg, or 20 times the amount of Aroclor 1254 that resulted in reproductive failure in previous trials (Aulerich and Ringer 1977).

Although the effects of feeding Aroclor 1016 to mink were not as dramatic as those that occurred from feeding other Aroclors, some detrimental results attributed to Arcolor 1016 were observed in this study. The reduced 4-week weights of the kits nursed by primaparous females fed the 25 ppm PCB-supplemented diet (Table 3, Table 4) and the excessive kit mortality between birth and 4 weeks that occurred on most of the PCB-supplemented diets suggest that lactation may have been adversely affected quantitatively or qualitatively, or both. Polychlorinated biphenyls are excreted in milk (Platonow et al. 1971, Fries et al. 1972). Up to 4 weeks of age, almost all the kits' weight gains can be attributed to the nourishment provided by the dam's milk. Biomass (average gain in kit body weight between birth and 4 weeks of age times the average number of kits raised per lactating female) provides for a comparison of the lactational performance between the groups and suggests that lactation was affected on the higher PCB-supplemented diets (Table 5). Suboptimum kit growth and an excessively high mortality have been reported in mink kits nursed by females fed Great Lakes fish contaminated with PCB's (Aulerich et al. 1973).

The lower body weights recorded during the early part of the study of the 1st-year mink fed the diet supplemented with 25 ppm PCB (Table 7) might be due to reduced palatability of the food. As the study progressed, the body weight differences between this group and the control were not significant. In other feeding trials, in which PCB's have been noted to suppress weight gains in mink (Ringer et al. 1972), the reduction in body weights became more pronounced as the study progressed.

No alterations were observed in the hematological characteristics measured in this study, or in mink fed Aroclors 1016, 1221, 1242, or 1254 at 2 ppm for 10 months by Iwamoto (1973). Platonow and Karstad (1973), however, reported disseminated intravascular coagulation and multiple hemorrhages in the lungs, brain, spleen, and liver of mink fed beef contaminated with Aroclor 1254 at a concentration of 3.6 ppm.

The reduced kidney weights of mink fed diets supplemented with Aroclor 1016 were in contrast to the increased kidney, liver, and heart weights in mink fed Aroclor 1254 (Aulerich and Ringer 1977). Goldstein et al. (1975) compared the effects of feeding rats Aroclors 1242 and 1016 and found that Aroclor 1242 increased liver weight, whereas Aroclor 1016 had no affect on liver weight.

The PCB residues of the newborn kits (Table 11) suggest that Aroclor 1016 passes the placental barrier in mink. Polychlorinated biphenys are known to be excreted via the milk (Platonow et al. 1971, Fries et al. 1972), and it is possible that the newborn kits may have nursed before their collection for PCB residue analysis. Studies by Villeneuve et al. (1971) and Platonow and Chen (1973), however, have demonstrated placental transfer of Aroclor 1254 in rabbits and cattle.

The tissue residues of Aroclor 1016 in the adults (Table 11) were considerably lower than those reported for mink fed comparable levels of Aroclors 1242, 1248, and 1254 by Platonow and Karstad (1973) and Aulerich and Ringer (1977). This result is in agreement with the report of Kaley et al. (1976) that Aroclor 1016 accumulates more slowly and to a lesser extent in rat tissues than Aroclor 1242. This difference, as well as differences in the toxicity of these Aroclors to mink may therefore be due to limited absorption of Aroclor 1016, a higher excretion rate of Aroclor 1016, or increased metabolism of Aroclor 1016.

According to Curley et al. (1971) and Weigel and Smith (1974), PCB's with a higher number of chlorine atoms per molecule are retained in tissues for longer periods of time than those with a lower percentage of chlorination. Aroclor 1016 contains 41.3% chlorine (Goldstein et al. 1975), but only about one-tenth the level of the more resistant pento- and hexachlorobiphenyls as Aroclor 1242, which contains 42% chlorine (Kaley et al. 1976). In studies comparing the effects of Aroclors 1254 (54% chlorine) (Bickers et al. 1972) and 1242 (Goldstein et al. 1975) with Aroclor 1016, it was found that Aroclors 1242 and 1254 produced a manyfold increase in some drug-metabolizing enzymes, whereas Aroclor 1016 produced a maximum increase of only 40-50% in most enzymes. Thus, metabolism of PCB's appears to be dependent upon not only the percentage of chlorination, but also upon the percentage of chlorination of the various homologs, which could account for the higher tolerance of mink for Aroclor 1016 observed in this study than for Aroclor 1254 observed in previous studies.

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TECHNICAL REPORT DATA
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1. REPORT NO. EPA-600/3-80-033	2.	3. RECIPIENT'S ACCESSION NO.
4. TITLE AND SUBTITLE Toxicity of the Polychlorinated Biphenyl Aroclor 1016 to Mink		5. REPORT DATE February 1980 issuing date
		6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) R. J. Aulerich and R. K. Ringer		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS Michigan State University East Lansing, Michigan 48824		10. PROGRAM ELEMENT NO. 2BH154
		11. CONTRACT/GRANT NO. 68-03-1187
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory - Duluth, MN Office of Research and Development U.S. Environmental Protection Agency Duluth, MN 55804		13. TYPE OF REPORT AND PERIOD COVERED
		14. SPONSORING AGENCY CODE EPA/600/03
15. SUPPLEMENTARY NOTES		

16. ABSTRACT

Effects of the PCB Aroclor 1016 on reproduction, growth, and survival of mink (Mustela vison) were investigated. Mink raised according to commercial mink-ranch procedures were fed diets that contained 0, 2, 10, and 25 ppm Aroclor 1016 for up to 18 months. Reproduction was not adversely affected, although kit growth and survival were suboptimum in some of the treated groups. No hematologic differences were observed between the treated and non-treated mink, but heart weight increased and kidney weight decreased in the older animals of two of the three PCB-treated groups. No consistent gross lesions associated with PCB toxicity were observed. The PCB residue in mink tissues was directly related to the quantity of Aroclor 1016 in the diet. Residues in mink kits suggest that Aroclor 1016 passes the placental barrier.

17. KEY WORDS AND DOCUMENT ANALYSIS

a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group
Toxicity of PCBs to mink Mink Aroclor 1016	PCBs Toxicity Testing	06 T
18. DISTRIBUTION STATEMENT RELEASE TO PUBLIC	19. SECURITY CLASS *(This Report)* UNCLASSIFIED	21. NO. OF PAGES 32
	20. SECURITY CLASS *(This page)* UNCLASSIFIED	22. PRICE

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

U.S. GOVERNMENT PRINTING OFFICE 1980-657-146/5594