0158

Ref 1/18/1977

Environm. Contam. Toxicol. 6, 279–292 (1977)

Archives of Environmental Contamination and Toxicology

© Springer-Verlag New York Inc. 1977

urrent Status of PCB Toxicity to Mink, and Effect on Their Reproduction[1]

hard J. Aulerich and Robert K. Ringer

Animal Project, Department of Poultry Science, Michigan State University, East Lansing, MI

Abstract. Experiments were conducted from 1968 to 1974 to investigate reproductive complications and mortality in mink fed Great Lakes coho salmon and to ascertain the effects of polychlorinated biphenyls (PCB's) on this fur bearer. The results of mink feeding trials indicated that coho salmon, as such, were not responsible for the loss of reproduction in the adult, or the kit mortality. Mink diets that contained other species of Great Lakes fish caused similar reproductive complications, but to a lesser degree.

Rancidity, mercury poisoning and chlorinated hydrocarbon pesticide contamination of the fish were all discounted as being responsible for the problem. The clinical signs and lesions noted in mink that died while receiving diets that contained Lake Michigan coho salmon were very similar to those observed in mink fed on rations that contained supplemental PCB's. These included anorexia, bloody stools, fatty liver, kidney degeneration, and hemorrhagic gastric ulcers. Analyses of tissues from mink that died when fed 30% Lake Michigan coho salmon or 30 ppm supplemental PCB diets showed similar PCB residues.

PCB toxicity experiments revealed that mink are very sensitive to these compounds and that the lethal dose varied inversely with the chlorine content of the PCB's although only Aroclor 1254 exerted a detrimental effect on reproduction when fed at a low level (2 ppm) for 8 months. The reproductive failure encountered in feeding mink Lake Michigan coho salmon and Aroclor 1254 was shown to be of a non-permanent nature.

The fisheries of the Great Lakes have for many years provided the mink ranching industries of the North Central United States and Canada with an abundant and inexpensive supply of fish for mink feeding. The utilization of Great Lakes fish as mink feed has, however, declined considerably since the early 1960's due to reports of reproductive complications and excessive kit mortality in mink fed these fish (Hartsough 1965). In the fall of 1967 an acute problem was evident as coho salmon, taken from tributaries of Lake Michigan during the spawning run, were fed to mink and caused a precipitous increase in newborn mink mortality. Breeding and whelping were reported as normal, but kit mortality reaching 80% was reported and appeared to depend upon the percentage of coho salmon fed, as

[1] Published with the approval of the *Michigan Agriculture Experiment Station* as Journal Article No. [illegible]

9229

well as the duration of feeding. The cause of the problem was not ascertain although rancidity, pesticide contamination, and mercury poisoning were pected (Aulerich, *et al.* 1971 and 1973). Because of the human health imp tions and the economic importance of the Great Lakes fisheries to the mink pet food industries this study was conducted to investigate the problem.

Experimental

The study consisted of a series of mink feeding experiments conducted between 1968 and 19 investigate the industry's observations relative to the adverse effects of Great Lakes fish on reproduction, to ascertain the cause of the problem, and to investigate the effects of the ca agent on mink.

The following general procedures were employed throughout the study. Specific procedure certain experiments are described in the text.

Composition of the basal diet was 15% fortified cereal,[2] 20% tripe, 5% beef liver, 15% horse 15% chicken, and 30% whole fish. The same basal diet, except where specified, was fed throug the study. Routine ranch procedures were employed in the feeding, care, and breeding of the an The mink were vaccinated as kits against canine distemper, botulism, and virus enteritis. Whe possible, the females were mated to males within their respective dietary groups. All matings verified by the presence of apparently normal motile spermatozoa in the vaginal smear after tion. The mated females were checked daily for young during the whelping period. Kits were on the day of birth and at 4 weeks of age.

Diets containing supplemental polychlorinated hydrocarbons were prepared by dissolv desired quantity of the compound in acetone and blending the solution with ground commerci cereal. The acetone was evaporated and the cereal-compound premix mixed with the other ingredients to yield a ration that contained the desired amount of the chlorinated compound

Preliminary Studies

The first phase of the study consisted of 3 mink feeding experiments designed to ascertain the and extent of the problem. In these trials, 201 mink were fed diets that contained 30% of species of Great Lakes or marine fish. As shown in Table 1, reproductive failure occurred females fed all diets that contained Lake Michigan coho salmon. Only 10 kits were whelped by mated females; eight were stillborn and the two that were born alive died within 24 hrs postp No teratism was observed. The reproductive performance of the females fed West Coast coho was not impaired. The average size of the litters whelped by females fed the rations that con Lake Erie cobo salmon, Lake Michigan bloater chub and Lake Michigan yellow perch, greatly reduced, although high kit mortality occurred on the diets that contained Lake Er salmon, Lake Michigan bloater chub and Lake Michigan yellow perch.

The average birth weight of the kits whelped by females fed the diet that contain Michigan bloater chub was significantly less ($P < 0.01$) than those whelped by females fed the ration (Table 2). The average body weights of 4-week-old kits nursed by females fed di contained West Coast coho salmon, Lake Erie coho salmon, Lake Michigan bloater chub an Michigan yellow perch were significantly less ($P < 0.01$) than those nursed by females fed the diet (Table 2).

All breeder mink that received Lake Michigan coho salmon canning by-products (head tails, viscera, and belly fat) in their diet died between the beginning of the breeding season (M and the end of the whelping period (May 15). Six adult mink fed Lake Michigan coho salmo

[2] Kel-blend No. 1009, *W. K. Kellogg Co.*, Battle Creek, MI.

e problem was not ascertai
l mercury poisoning were
e of the human health im
Lakes fisheries to the mink
investigate the problem.

s conducted between 1968 and
se effects of Great Lakes fish on
investigate the effects of the ca

hout the study. Specific procedu

)% tripe, 5% beef liver, 15% horse
pt where specified, was fed th
ding, care, and breeding of the
botulism, and virus enteritis. Wh
ective dietary groups. All matings
tozoa in the vaginal smear after co
the whelping period. Kits were co

rbons were prepared by dissolvi
solution with ground commercial
nd premix mixed with the other
nt of the chlorinated compound.

riments designed to ascertain the
ed diets that contained 30% of
1, reproductive failure occurred
on. Only 10 kits were whelped by the
rn alive died within 24 hrs postpar
the females fed West Coast coho salm
females fed the rations that con
Lake Michigan yellow perch,
e diets that contained Lake Erie
low perch.
ales fed the diet that contained
hose whelped by females fed the co
kits nursed by females fed die
Lake Michigan bloater chub and
those nursed by females fed the co

lmon canning by-products (heads,
inning of the breeding season (March
fed Lake Michigan coho salmon in the

Table 1. Reproductive performance of mink fed Great Lakes or marine fish as 30% of their diet

		Fish portion of diet	No. of females: Mated	No. of females: Whelped	No. of kits: Birth: Alive	No. of kits: Birth: Dead	No. of kits: 4 weeks alive
	1968	Ocean perch (control)	14[a]	13	48	9	46
	1967	L. Mich. coho salmon	8	0	0	0	0
	1968	Ocean whiting (control)	11[a]	10	38	2	31
	1968	West Coast coho salmon	12	11	58	7	47
	1967	L. Mich. coho salmon	12	5	2	7	0
	1968	L. Mich. coho salmon	12	0	0	0	0
	1968	L. Mich. coho salmon canning by-products	11[a]	0	0	0	0
	1969	Ocean whiting (control)	12	10	45	10	29
	1969	L. Mich. coho salmon	10[b]	1	0	1	0
	1969	L. Mich. bloater chub	12	7	10	12	5
	1969	L. Erie coho salmon	12	11[c]	32	17	7
	1969	L. Mich. yellow perch	12	10	31	7	15
	1969	L. Erie yellow perch	12	10	39	12	28

died before mating
died before mating
mated female died before whelping

Table 2. Average body weight (±S.E.) of live kits at birth and 4 weeks

		Fish portion of diet	At birth: No.	At birth: Body wt (g)	At 4 weeks: No.	At 4 weeks: Body wt (g)
	1968	Ocean whiting (control)	38	9.2 ± 0.38	31	162 ± 5.4
	1968	West Coast coho salmon	56	9.4 ± 0.17	47	138 ± 4.1[b]
	1968	L. Mich. coho salmon	2	5.3 ± 0.80	0	—
	1969	Ocean whiting (control)	45	8.2 ± 0.23	29	125 ± 3.8
	1969	L. Mich. bloater chub	10	7.0 ± 0.59[a]	5	91 ± 13.2[b]
	1969	L. Erie coho salmon	32	7.6 ± 0.20	7	86 ± 9.3[b]
	1969	L. Mich. yellow perch	36	8.3 ± 0.35	15	104 ± 7.2[b]
	1969	L. Erie yellow perch	39	8.2 ± 0.22	28	122 ± 3.9

ant difference from controls ($P < 0.05$) by t-test
ant difference from controls ($P < 0.01$) by t-test

first experiment also died during the later stages of the trial. The clinical signs and lesions [illegible] that died when fed diets that contained Lake Michigan coho salmon or its canning [illegible] consisted of anorexia, bloody stools, fatty infiltration, and degeneration of the liver and [illegible] hemorrhagic gastric ulcers. Antibiotic treatment and injections of iron, vitamins A, D, E, [illegible] complex vitamins did not arrest the mortality among the mink fed coho salmon. Bacterial [illegible] not reveal the presence of a pathogen, thus suggesting that the pathology was due to a [illegible] Histological examination confirmed the gross pathological observations but failed to elucidate [illegible] as to the causative agent.

Rancidity and mercury contamination data for the whole fish (Experiment 3, Table 2) [illegible] correlate the mink toxicity data and were discounted as a contributor to the problem (Aulerich [illegible] 1971). Chlorinated hydrocarbon pesticide contamination (total DDT and isomers or dieldrin [illegible] fish was inversely proportional to the number of kits whelped per female. This relationship, however, appeared spurious and may reflect, in part, the fat content of the fish, since feeding either DDT [illegible] isomers, or dieldrin to mink at levels far in excess of that contained in the fish failed to simulate the effects on the mink of feeding diets that contained Lake Michigan coho salmon (Aulerich and [illegible] 1970; Aulerich, *et al.* 1971).

In an effort to isolate the fraction of the coho salmon that contained the toxic factor(s) responsible for the mortality and impaired reproduction in mink, ground, whole, raw ocean fish and [illegible] Michigan coho salmon were subjected to an aceton-hexane extraction.[3] The supernatants and residues obtained from the fish were then recombined as described in Table 3, mixed with the other ingredients of the basal diet, and fed to mink from January 25 through June 30. The reproductive performance of the female mink fed these diets (as shown in Table 3) indicated that the toxic factor(s) present in the coho salmon was stored primarily in the fat and could be extracted with fat solvents.

Further analysis of the fish revealed that PCB contamination might be involved in the problem, since Lake Michigan coho salmon contained between 10 and 15 ppm PCB on analysis. An experiment was, therefore, conducted to ascertain the effects of PCB feeding on mink. In an exploratory feeding trial, a PCB-supplemented diet and a ration that contained Lake Michigan coho salmon were fed to adult breeder mink from January 1, 1971 to June 30, 1971 (Table 4). All mink that received the supplemental PCB's in their diet died between the beginning of the breeding season (March 1) and the end of the whelping period (May 15). Six of the 15 (3 male and 12 female) mink fed the 30% Lake Michigan coho salmon diet also died during the later stages of the experiment. The clinical signs and lesions of the mink that died when fed diets that contained coho salmon from Lake Michigan or supplemental PCB's were strikingly similar and consistent with those of the mink that died when fed the coho salmon diets in the previous feeding experiments.

PCB residue analyses from selected tissues of control mink and mink that died while receiving diets that contained coho salmon or supplemental PCB's are summarized in Table 5. Although PCB's are known to accumulate in adipose tissue, no samples of body fat were available for analysis [illegible] the animals fed coho salmon or PCB's were emaciated at the time of death. Organ weights (expressed as a percentage of brain weight) of the mink that died and from sacrificed controls were not significantly different ($P > 0.05$).

Long-term Studies

Based on the results of the previous feeding trial, an experiment was conducted from August 25 [illegible] to June 1, 1972 to ascertain the effects of long-term, low-level consumption of PCB's on mink growth, reproduction, and viability, and to investigate the possibility of interactions between PCB's and other chlorinated hydrocarbon pesticide contaminants of Great Lakes fishes.

Thirty-five mink were divided into 5 groups of 6 females and 1 male per group and placed on dietary treatments shown in Table 6. The average body weight gains of the mink during the growth and furring periods (August 25 to December 22) are shown in Table 6. The results indicate

[3] For complete extraction procedures see, Iwamoto (1973).

:linical signs and lesions in the
salmon or its canning by-
eration of the liver and kidne
f iron, vitamins A, D, E, and
coho salmon. Bacterial cultu
: pathology was due to a to
ations but failed to elucidate

sh (Experiment 3, Table 2) d
utor to the problem (Aulerich
DDT and isomers or dieldrin)
female. This relationship, how
: fish, since feeding either DD
ed in the fish failed to simula
coho salmon (Aulerich and R

tained the toxic factor(s) respo
whole, raw ocean fish and
extraction.[3] The supernatants
ed in Table 3, mixed with the
through June 30. The reprodu
e 3) indicated that the toxic fact
ld be extracted with fat solvents
n might be involved in the pr
pm PCB on analysis. An exper
: on mink. In an exploratory f
Michigan coho salmon were f
e 4). All mink that received 3
e breeding season (March 1) and
12 female) mink fed the 30%
e experiment. The clinical sign
ho salmon from Lake Michig
hose of the mink that died when

and mink that died while rec
marized in Table 5. Although P
fat were available for analysis,
: of death. Organ weights (expr
from sacrificed controls were

was conducted from August 25,
sumption of PCB's on mink gro
teractions between PCB's and
fishes.
1 male per group and placed
t gains of the mink during the
vn in Table 6. The results indic

productive performance and mortality of female mink fed a control ration or experimental diets (Jan. 25

	% Mortality	No. of females		No. of kits			
				Birth			
		Mated	Whelped	Alive	Dead	Whelped/ mated	Alive at 4 weeks
I							
sting of 30% ocean fish with acetone and hexane supernatant and residue then the diet[a] (Control)	0	10	8	37	4	4.1	27
II							
the supernatant from the 30% acetone-hexane ae Michigan coho the supernatant from 30% ane extracted ocean fish diet	20	9	2	0	5	0.6	0
III							
the residue from the 30% acetone-hexane ae Michigan coho e residue from 30% ne extracted ocean fish diet	0	10	9	33	14	4.7	18

extraction procedures see: Iwamoto (1973)

4. Reproductive performance of control mink and mink fed on rations that contained coho or supplemental PCB's (Jan. 1 to June 30)

	Dietary treatment		
	Control 30% ocean fish mix	30% coho salmon	30% ocean fish mix plus 30 ppm PCB's[a]
males mated	12	12	11[b]
males whelped	11	0	0
s born			
	35	—	—
al	19	—	—
e kits at 4 weeks	29	—	—

PCB supplementation consisted of 10 ppm each of Aroclors® 1242, 1248, and 1254. Aroclor® ade name for polychlorinated biphenyls manufactured by *Monsanto Co.*, St. Louis, MO
female died before the mating season

Table 5. Average PCB residues in tissues from control mink and mink that died while receiving the diet that contained coho salmon or supplemental PCB's[a]

Dietary treatment	No. mink	Tissue					
		Brain	Liver	Kidney	Spleen	Lung	Muscle
30% ocean fish mix control	4	<0.01	<0.01	<0.01	<0.01	<0.01	N.D.[c]
30% Lake Michigan coho salmon	3	11.07 ±0.78	5.21 ±1.66	6.37 ±0.25	6.19 ±0.09	5.15 ±0.22	4.73 ±1.80
30% ocean fish mix plus 30 ppm PCB's[b]	12	11.00 ±1.43	4.18 ±0.58	4.47 ±0.42	4.79 ±0.39	4.78 ±0.53	4.88 ±0.54

[a] Analyses by M.S.U. Pesticide Research Center using the technique of Armour and Burke (1970). The PCB standards were composed of a mixture of the 3 Aroclors used. Quantitation was based on the area under the 4 major peaks

[b] PCB's consisted of 10 ppm each of Aroclors 1242, 1248, and 1254

[c] None detected

that 10 ppm of Aroclor[4] 1254 depressed body weight gains when fed continuously to growing mink. A dose-response curve for data on 0, 5, and 10 ppm of Aroclor 1254 at 4 months treatment yielded a regression equation of $Y = 207.5 - 107.4\ X$, where $X = \ln(\text{ppm}/5 + 1)$. This regression equation gave a significant dose-response ($P<.05$) (Ringer *et al.* 1972).

No clinical signs of PCB or chlorinated hydrocarbon poisoning (other than the reduced weight gains) were observed in the treated animals during the growth and furring periods. Considerable

Table 6. Effect of Aroclor 1254, alone or in combination with pesticides, on body weight gains of female mink

Dietary treatment	No. mink	Initial body wt. (g) (Aug. 25)	Body weight gain (g) ± S.E. from initial wt.			
			1 mo. (Sept. 24)	2 mo. (Oct. 25)	3 mo. (Nov. 25)	4 mo. (Dec. [illegible])
Basal diet (30% ocean fish mix; control)	6	1020	43 ± 17.4	203 ± 25.4	187 ± 46.2	2[illegible]
Basal diet plus 5 ppm PCB[a]	6	940	62 ± 22.4	153 ± 44.2	138 ± 42.7	12[illegible]
Basal diet plus 10 ppm PCB	6	923	43 ± 20.4	148 ± 32.0	132 ± 26.3	9[illegible]
Basal diet plus 10 ppm PCB and 10 ppm DDT	6	1052	43 ± 14.5	57 ± 14.6[c]	28 ± 21.5[c]	-15[illegible]
Basal diet plus 10 ppm PCB and 0.5 ppm dieldrin	6	940	108 ± 25.1	133 ± 26.7	115 ± 18.9	5[illegible]

[a] Aroclor 1254

[b] Significantly different ($P < 0.05$) from controls

[c] Significantly different ($P < 0.01$) from controls

[4] Aroclor is the trade name for polychlorinated biphenyls manufactured by *Monsanto Co.*, St. Louis, MO.

ink and mink that died while receiv

cy	Spleen	Lung	Muscle
1	<0.01	<0.01	N.D.[c]
7	6.19	5.15	4.73
5	±0.09	±0.22	±1.80
7	4.79	4.78	4.88
2	±0.39	±0.53	±0.54

the technique of Armour and Burke
Aroclors used. Quantitation was based

8, and 1254

s when fed continuously to growing
oclor 1254 at 4 months treatment
= ln (ppm-/5 + 1). This regression
972).
n poisoning (other than the reduced
growth and furring periods. Consid

pesticides, on body weight gains of

nt gain (g) ± S.E. from initial wt.

2 mo. (Oct. 25)	3 mo. (Nov. 25)	4 mo. (Dec.
203 ± 25.4	187 ± 46.2	2
153 ± 44.2	138 ± 42.7	1
148 ± 32.0	132 ± 26.3	9
57 ± 14.6[c]	28 ± 21.5[c]	-1
133 ± 26.7	115 ± 18.9	5

s manufactured by *Monsanto Co.*, St. Lou

7. Effect of Aroclor 1254 fed alone or in combination with pesticides, on mortality and reproduction of mink (August 25 to June 1) (6 females and 1 male/group)

	% Mortality	Females alive	Females whelped	Kits born Alive	Kits born Dead	Kits/female alive
	14	5	3	17	8	5.0
B[a]	29	4	0	0	0	0
PCB	71	1	0	0	0	0
PCB + 10 ppm DDT	43	4	0	0	0	0
PCB + 0.5 ppm dieldrin	100	0	0	0	0	0

1254

occurred on some diets after the animals had consumed the experimental ration for at least None of the mink fed the diet supplemented with 10 ppm of Aroclor 1254 plus 0.5 ppm of survived to the termination of the experiment, and only 1 of 6 mink survived on the diet that 10 ppm of Aroclor 1254.

mink fed the PCB-supplemented diets failed to produce offspring (Table 7). Those breeders during the gestation period exhibited various degrees of embryotoxicity when their uteri amined at necropsy. The PCB-treated mink that survived to June 1, 1972 were sacrificed and weighed. As shown in Table 8, continuous ingestion of Aroclor 1254, at 5 or 10 ppm, in enlargement of the liver, kidneys, and heart.

the results of the two previous experiments cast suspicion on PCB's as the primary cause of em, an additional feeding trial was conducted to further investigate and compare the effects PCB's and the toxic factor(s) present in Lake Michigan coho salmon on mink reproduction. in the treatments (Table 9) was a group of mink fed 5 ppm of Aroclor 1254 plus 6 ppm of 0.2 ppm of dieldrin. These levels approximated the residue values found in diets where 30%

9. Mean organ weights[a] of female mink fed a control ration or experimental diets (August 25, June 1, 1972)

treatment	Spleen	Kidney	Liver	Heart
trol)	27.4 ± 11.09 x[b]	44.5 ± 2.34 x	220.4 ± 29.36 x	65.8 ± 7.86 x
ppm PCB)[c]	47.6 ± 4.95 x	58.2 ± 4.63 y	440.8 ± 39.87 y	90.9 ± 16.04 y
ppm PCB)	39.6 ± 8.23 x	57.8 ± 1.59 y	421.3 ± 18.68 y	86.9 ± 10.4 y
ppm PCB ppm DDT)	51.0 ± 8.65 x	62.2 ± 4.63 y	579.5 ± 80.43 z	97.1 ± 19.9 y
ppm PCB ppm (n = 3)	30.9 ± 5.42 x	62.9 ± 5.06 y	466.6 ± 21.40 yz	87.9 ± 7.71 y

sed as % of brain weight ± S.E.
followed by the same letter(s) under each organ are not significantly different (P > 0.05)
1254

Lake Michigan coho salmon constituted the fish portion of the ration. Cooked coho salmon ([illegible] 1.05 kg/sq cm for 30 min) was included to test for the heat stability of the factor(s) caus[illegible] the reproductive problem.

The data presented in Table 9 show the sensitivity of the reproductive physiology of fe[illegible] to Aroclor 1254. Five ppm of Aroclor 1254 markedly reduced reproduction when fed only 4 [illegible] while 15 ppm of this PCB totally inhibited reproduction and resulted in 31% adult mortality. [illegible] 1254 did not exert any apparent adverse effects on spermatogenesis, since matings between [illegible] fed the non-PCB supplemented basal diet and PCB-treated males resulted in acceptable repro[illegible] The toxic factor(s) present in Lake Michigan coho salmon was heat stable and duplication, in [illegible] several contaminants (PCB's, DDT, and dieldrin) known to be present in the salmon did not [illegible] reproduce the effects of feeding Lake Michigan coho salmon.

Chronic Studies

In an effort to compare the chronic effects of Aroclor 1254 with other PCB's, a long-term [illegible] mink feeding trial involving Aroclors 1016, 1221, 1242, and 1254 was conducted. Each of 4 [illegible] supplemented with 2 ppm of a different PCB and control ration were fed to 8 female and 2 male [illegible] from August 8, 1972 through June 1, 1973, or until the kits were 4 weeks of age. Body weights [illegible] hemoglobin and hematocrit values of the animals were obtained at approximately monthly [illegible] during the experiment. No significant differences ($P > 0.05$) were noted in body weight gains, he[illegible] bin levels or hematocrit values of the mink on the various treatments. As shown in Table 10, Aro[illegible] 1254 was the only PCB that had an adverse effect on reproduction. Only 2 of 7 mated females [illegible] and produced only 1 live kit which weighed considerably less than the average weight of the [illegible] whelped by the females on the other dietary treatments.

An investigation of the acute toxicity of 3 Aroclors (1221, 1242, and 1254) to mink was co[illegible] using 81 adult mink not previously fed Great Lakes fish nor exposed to any supplemental PCB [illegible] chlorinated hydrocarbon pesticides. The study revealed that the oral (gavage) and intraper[illegible] lethal dose for 50% of the animals (LD_{50}) ranged from 500 to 4000 mg/kg and varied inversely with [illegible] chlorine content (last 2 digits of the Aroclor number) of the compounds (see Table 11). Intraper[illegible] injections of the PCB's were considerably more toxic to the mink than oral administration of [illegible] compounds.

Table 9. Effect of Aroclor 1254 fed singly and in combination with other chlorohydrocarbon [illegible] reproductive parameters (January 1 to May 10) (12 females and 4 males/group)

Dietary treatment	% Mortality	Females alive	Females whelped	Kits born		Kits/female alive
				Alive	Dead	
30% ocean fish (control)	6	11	11	56	10	**6.0**
30% raw Lake Michigan coho salmon	19	11	0	0	0	**0**
30% cooked Lake Michigan coho salmon	6	12	0	0	0	**0**
1 ppm PCB[a]	6[b]	10	8	35	8	**4.3**
5 ppm PCB	0	12	3	3	6	**0.8**
15 ppm PCB	31	8	0	0	0	**0**
5 ppm PCB + 6 ppm DDT	0	12	4	5	6	**0.9**
5 ppm PCB + 6 ppm DDT + 0.2 ppm dieldrin	19	10	5	15	7	**2.2**

[a] Aroclor 1254
[b] One female escaped

Reproductive performance and mortality of female mink fed a control ration or experimental diets from supplemental PCB (Aug. 8, 1972 to June 1, 1973 or until the kits were 4 weeks of age)

	Adult females			Kits				
				No. born				
	% Mortality	No. mated	No. whelped	Alive	Dead	Whelped/female mated	Alive at 4 wks.	Avg. wt. (g ± S.E.) at birth
	0	8	8	28	5	4.1	18	9.9 ± 0.32
B	0	8	8	28	8	4.5	16	9.2 ± 0.33
B	12	7	7	43	1	6.3	37	9.6 ± 0.22
B	12	7	7	35	4	5.6	32	9.3 ± 0.27
B	12	7	2	1	1	0.3	0	5.4

In an effort to determine the persistency of the reproductive failure produced by feeding PCB's from Michigan coho salmon to mink, 11 adult females that received a ration which contained 30% Michigan coho salmon and 3 females that had been fed a diet supplemented with 5 ppm of Aroclor 1254 from January 1 to June 1, 1972 and failed to whelp were placed on the basal (control) diet June 2, 1972 through June 1, 1973. The females were mated to control males during March, 1973. The reproductive performance of the females (summarized in Table 12) indicated that previous exposure to PCB's (or the toxic factor(s) in Lake Michigan coho salmon) does not permanently impair reproduction in mink.

Discussion

The results of the first 3 experiments of this study demonstrated that reproductive failure occurs in mink from feeding Lake Michigan coho salmon as 30% of the diet. The causative agent, however, did not appear to be coho salmon as such, nor to be confined to the coho salmon, as other species of Great Lakes fish fed to mink at 30% of the diet also produced adverse reproductive effects, but to a lesser degree. More acute reproductive complications occurred in mink that

11. Acute toxicity (LD_{50}) of oral and intraperitoneal administration of PCB's to mink

	LD_{50} (mg/kg)	
	Intraperitoneal[a]	Oral[b]
Aroclor 1221	>500 <750	>750 <1000
Aroclor 1242	1000	>3000
Aroclor 1254	>1250 <2250	4000

[a] Lethal dose for 50% of the animals within 4 days
[b] Lethal dose for 50% of the animals within 14 days

Table 12. Reproductive performance of female mink fed a control diet following failure to whelp the previous year while receiving a ration that contained either coho salmon or supplemental PCB's

Dietary treatment Jan. 1, 1972 to June 1, 1972	Dietary treatment June 2, 1972 to June 1, 1973	No. of females		Avg. litter size/female mated
		Mated (Mar. 1973)	Whelped (May 1973)	
30% Lake Michigan coho salmon	Basal (control) diet	11	7	3.5
5 ppm supplemental Aroclor 1254	Basal (control) diet	3	3	4.3

were fed diets which contained fish from Lake Michigan than in rations that contained similar species of fish from Lake Erie or the West Coast. The toxic factor(s) appeared to be concentrated in the canning by-products of the coho salmon, since feeding of the by-products to adult mink for about 3 months was more toxic than feeding the whole, raw fish.

The impaired growth and excessive early mortality noted in kits that were of "normal" weight at birth but nursed by females fed Great Lakes fish (Table 2 Experiment 3) would indicate that the toxic factor(s) present in the fish interferred with lactation or was conveyed to the kits *via* the milk.

The suspicion that PCB contamination of the Great Lakes fish may be responsible for the mortality and impaired reproduction and growth in mink fed diets containing these fish was supported by the results of the mink feeding trials involving PCB's. The PCB residue content of Lake Michigan coho salmon, the sensitivity of mink to PCB's, the similarity of the reproductive complications in mink fed coho salmon and PCB's, the clinical signs and lesions observed in the mink that died while receiving diets that contained coho salmon and PCB's, and the accumulation of comparable quantities of PCB residues in the tissues of these animals, strongly implicate PCB's as the factor responsible for the problems encountered in feeding Great Lakes fish to mink.

The greater toxicity of the coho salmon canning by-products may be attributable to its high fat content, as it was demonstrated in this study and reported by Jensen *et al.* (1970) and Berlin (1970) that PCB's tend to accumulate in body fat. According to Albros and Fishbein (1972) and Allen *et al.* (1974a) about 90% of ingested PCB's are absorbed from the gut and deposited primarily in the fatty tissue.

The degree of PCB toxicity to mink was directly proportional to the total intake of the compound (Table 13). Females fed 5 ppm of Aroclor 1254 for 7 months prior to breeding had a litter average of 0.8 which was comparable to the 0.3 litter average from females fed the same PCB at 2 ppm for 7 months prior to breeding.

If it is assumed that an adult female mink consumes about 150 g of feed per day (Schaible 1970) the total intake of PCB by the female mink fed 5 ppm of Aroclor 1254 in the diet, or 30% Lake Michigan coho salmon for 4 months prior to whelping, or 2 ppm of Aroclor 1254 for 9 months prior to whelping would have been about 90, 75, and 61 mg respectively; all of which resulted in near

complete reproductive failure. From these results and those reported by Platonow and Karstad (1973) it would appear that mink are more sensitive to PCB's than rats (Tucker and Crabtree 1970, Kimbrough 1971), mice (Nishizumi 1970), or birds (Tucker and Crabtree 1970, Heath *et al.* 1970).

In the experiment comparing the chronic toxicity of Aroclors 1016, 1221, 1242, and 1254, only 1254 was noted to have a detrimental effect on mink reproduction. These results, however, do not eliminate the possibility of adverse effects of the other Aroclors at higher levels. Studies by Iwamoto (1973) and Platonow and Karstad (1973) showed that ovulation and implantation does occur in mink treated with Aroclor 1254 but that gestation may not continue to term. Aroclor 1254 has also been reported to be fetotoxic in rabbits (Villeneuve *et al.* 1971b) and to transfer across the placenta in rabbits (Grant *et al.* 1971b) and cows (Platonow and Chen 1973). Cecil *et al.* (1974) observed no embryotoxicity from feeding hens Aroclors 1221 or 1268 but reported embryotoxic and teratogenic effects from Aroclors 1232, 1242, 1248, and 1254.

The exact mechanism by which PCB's disturb the reproductive performance of mammals is not well understood. It has been shown that these compounds enhance the hepatic metabolism of sex hormones (Peakall 1967, Risebrough *et al.* 1968, Lincer and Peakall 1970, Villeneuve *et al.* 1971a, Nowicki and Norman 1972, Platonow and Funnel 1971 and 1972, and Benthe *et al.* 1972). The resulting hypoestrogenism could cause hormonal imbalances leading to atrophy of the uterus, as reported by Koller and Zinkl (1973) in rabbits treated with Aroclor 1254.

The clinical signs and lesions observed in the mink that died during the study while receiving diets that contained coho salmon or supplemental PCB's were similar and comparable with those described for PCB-treated mink by Platonow and Karstad (1973). Liver enlargement, as noted in rats (Grant *et al.* 1971a, Allen and Abrahamson 1973, Cecil *et al.* 1973, Grant and Phillips, 1974, and Goldstein

Table 13. Summary of the effect of Aroclor 1254 on mink reproduction

Treatment level	Period fed	Reproduction offspring/female
[illegible] ppm	Jan–May	0
[illegible] ppm	Aug–Jun	0
[illegible] ppm + 10 ppm DDT	Jan–May	0
[illegible] ppm + 0.5 ppm dieldrin	Jan–May	0
[illegible] ppm	Aug–Jun	0
[illegible] ppm	Jan–May	0.8
[illegible] ppm + 6 ppm DDT	Jan–May	1.0[a]
[illegible] ppm + 6 ppm DDT + 0.2 ppm dieldrin	Jan–May	2.0[a]
[illegible] ppm	Aug–Jun	0.3
[illegible] ppm	Jan–May	4.3
[illegible] ppm		4.1
[illegible] ppm		5.0
[illegible] ppm		6.0

[a] All died before 4 weeks of age

et al. 1975), mice (Orberg and Lungberg 1974), swine (Hansen *et al.* 1975 monkeys (Allen *et al.* 1974b), Japanese quail (Cecil *et al.* 1973), pheasant (Dahlgren *et al.* 1972), chickens (Iturri 1974), and rabbits (Koller and Zinkl 197 exposed to PCB's was also observed in mink fed 5 ppm or more of Aroclor 125 for 9 months. However, except for the reduced growth rate and the hemorrhagi gastric ulcers, which have also been reported in PCB-treated monkeys (Allen *al.* 1974b) and swine (Hansen *et al.* 1975), the clinical signs and lesions assoc ated with PCB poisoning in mink appear to be rather non-specific.

The acute toxicity (LD_{50}) trials indicated that mink were considerably mor sensitive to PCB's injected intraperitoneally than when the compounds wer administered by gavage. Undoubtedly a large portion of the orally administere PCB's passed unabsorbed from the animals which may account for the variatio in toxicity associated with the route of administration.

The inverse relationship observed between the LD_{50} of the PCB's for min and the chlorine content of the compounds was not in agreement with th toxicity reported in birds (Prestt *et al.* 1970) and man (Risebrough and Brodin 1970) (which indicated a positive relationship between the percentage of chlorin in PCB's and their toxicity) and was not supported by the mink reproductive dat in this study. Of Aroclors 1221, 1242, 1016 (41 percent chlorine), and 1254 fed at ppm of the diet, only Aroclor 1254 had an adverse effect on reproductior According to Curley *et al.* (1971) and Weigel and Smith (1974), PCB's wit higher number of chlorine per molecule are retained in tissues for longer period of time than those with lower percent chlorination. Goldstein *et al.* (197 suggested that this preferential retention of the higher chlorinated PCB's migh account for their greater biological effectiveness which may explain the impai ment of reproduction in mink observed with Aroclor 1254 but not with the othe lesser chlorinated PCB's.

Acknowledgements. This study was supported by grants from the *National Marine Fisheries Servi U.S. Department of Commerce;* the *Michigan Department of Natural Resources;* the *Mink Farm Research Foundation;* and the *Department of Health, Education and Welfare/Public Health Servi Food and Drug Administration.* The authors express their appreciation to the *Oregon and Pennsylv nia Fish Commissions,* the *Michigan Department of Natural Resources and the National Mari Fisheries Service, U.S.D.C.,* for providing fish used in these feeding trials. The PCB's were furnish by *Monsanto Company.* We are also indebted to Dr. Robert Reinert for the pesticide analyses. D Matthew Zabik for the PCB analyses and Mr. Susumu Iwamoto for technical assistance.

References

Albros, P. W., and P. Fishbein: Intestinal absorption of polychlorinated biphenyls in rats. Bu Environ. Contam. Toxicol. **8**, 26 (1972).

Allen, J. R., and L. J. Abrahamson: Morphological and biochemical changes in the liver of rats fe polychlorinated biphenyls. Arch. Environ. Contam. Toxicol. **1**, 265 (1973).

Allen, J. R., L. A. Carstens, and D. A. Barsotti: Residual effects of short-term, low-level exposure nonhuman primates to polychlorinated biphenyls. Toxicol. Appl. Pharmacol. **30**, 440 (1974a).

Allen, J. R., D. H. Norback, and I. C. Hsu: Tissue modifications in monkeys as related to absorptio distribution, and excretion of polychlorinated biphenyls. Arch. Environ. Contam. Toxicol. **2**, (1974b).

Armour, J. A., and J. A. Burke: A method for separating polychlorinated biphenyls from DDT and its analogs. J. Ass. Offic. Anal. Chem. **53**, 761 (1970).

Aulerich, R. J., and R. K. Ringer: Some effects of chlorinated hydrocarbon pesticides on mink. Amer. Fur Breeder **43**, 10 (1970).

Aulerich, R. J., R. K. Ringer, H. L. Seagran, and W. G. Youatt: Effect of feeding coho salmon and other Great Lakes fish on mink reproduction. Can. J. Zool. **49**, 611 (1971).

Aulerich, R. J., R. K. Ringer, and S. Iwamoto: Reproductive failure and mortality in mink fed on Great Lakes fish. J. Reprod. Fert., Suppl. **19**, 365 (1973).

Berthe, H. F., A. Schmoldt, and H. Schmidt: Induktion Mikrosomaler Leberenzyme nach eimaliger gabe van Polychlorierten Biphenylen (PCB) und anschliebender stress-situation. Arch. Toxicol. **29**, 97 (1972).

Berlin, M.: PCB-effect on mammals. Proc. PCB-Conference, Wenner-Gren Center, Stockholm, Sweden, pp. 44–50 Sept. 29 (1970).

Cecil, H. C., S. J. Harris, J. Bitman, and G. F. Fries: Polychlorinated biphenyl-induced decrease in liver vitamin A in Japanese quail and rats. Bul. Environ. Contam. Toxicol. **9**, 179 (1973).

Cecil, H. C., J. Bitman, R. J. Lillie, and G. F. Fries: Embryotoxic and teratogenic effects in unhatched fertile eggs from hens fed polychlorinated biphenyls (PCB's). Bul. Environ. Contam. Toxicol. **11**, 489 (1974).

Curley, A., V. W. Burse, M. E. Grim, R. W. Jennings, and R. E. Linder: Polychlorinated biphenyls: Distribution and storage in body fluids and tissues of Sherman rats. Environ. Res. **4**, 481 (1971).

Dahlgren, R. B., R. J. Bury, R. L. Linder, and R. F. Reidinger, Jr.: Residue levels and histopathology in pheasants given polychlorinated biphenyls. J. Wildl. Mangt. **36**, 524 (1972).

Goldstein, J. A., P. Hickman, V. W. Burse, and H. Bergman: A comparative study of two polychlorinated biphenyl mixtures (Aroclor 1242 and 1016) containing 42% chlorine on induction of hepatic porhyria and drug metabolizing enzymes. Toxicol. Appl. Pharmacol. **32**, 461 (1975).

Grant, D. L., W. E. J. Phillips, and D. C. Villeneuve: Metabolism of a polychlorinated biphenyl (Aroclor 1254) mixture in the rat. Bul. Environ. Contam. Toxicol. **6**, 102 (1971a).

Grant, D. L., D. C. Villeneuve, K. A. McCully, and W. E. J. Phillips: Placental transfer of polychlorinated biphenyl in the rabbit. Environ. Physiol. **1**, 61 (1971b).

Grant, D. L., and W. E. J. Phillips: The effect of age and sex on the toxicity of Aroclor 1254, a polychlorinated biphenyl, in the rat. Bul. Environ. Contam. Toxicol. **12**, 145 (1974).

Hansen, L. G., C. S. Byerly, R. L. Metcalf, and R. F. Bevill: Effect of a polychlorinated biphenyl mixture on swine reproduction and tissue residue. Amer. J. Vet. Res. **36**, 23 (1975).

Harrsough, G. R.: Great Lakes fish now suspect as mink food. Amer. Fur Breeder **38**, 25 (1965).

Heath, R. G., J. W. Spann, J. F. Kreitzer, and C. Vance: Effects of polychlorinated biphenyls on birds. Proc. XV Int. Orn. Congr. The Hague, Netherlands, 20 pp. Aug. (1970).

Hurn, S. J.: The effects of various chlorinated hydrocarbons on the cardiovascular physiology and hematology of the domestic fowl. Ph.D. Thesis, Department of Poultry Science, *Michigan State University*, East Lansing, MI 145 pp. (1974).

Iwamoto, S.: The effects of polychlorinated biphenyls and coho salmon on mink. M.S. Thesis, Dept. of Poultry Sci., *Michigan State University*, East Lansing, MI 73 pp. (1973).

Jensen, S., A. G. Johnels, T. Odsjo, M. Olsson, and G. Otterlind: PCB-occurrence in Swedish wildlife. Proc. PCB Conference Wenner-Gren Center, Stockholm, Sweden pp. 30–42 Sept. 29 (1970).

Kimbrough, R. D.: Proc. Interagency Meeting of Polychlorinated Biphenyls (PCB's). *U.S. Dept. Health, Education, and Welfare*, 111 pp. Aug. 5 (1971).

Kolier, L. D., and J. G. Zinkl: Pathology of polychlorinated biphenyls in rabbits. Amer. J. Pathol. **70**, 363 (1973).

Lincer, J. L., and D. B. Peakall: Metabolic effects of PCB in the American kestrel. Nature **228**, 783 (1970).

Koshizumi, M.: Light and electron microscope study of chlorobiphenyl poisoning. Arch. Environ. Hlth, **21**, 620 (1970).

Nowicki, H. G., and A. W. Norman: Enhanced hepatic metabolism of testosterone, 4-androstene-3, 17-dione, and estradiol-17B in chicken pretreated with DDT or PCB. Steroids **19**, 85 (1972).

Orberg, J., and C. Lundberg: Some effects of DDT and PCB on the hormonal system in the mature mouse. Environ. Physiol. Biochem. **4,** 116 (1974).

Peakall, D. B.: Pesticide-induces enzyme breakdown of steroids in birds. Nature **216,** 505 (1967).

Platonow, N. S., and H. S. Funnell: Anti-androgenic-like effect of polychlorinated biphenyls in cockerels. Vet. Rec. **88,** 109 (1971).

Platonow, N. S., and H. S. Funnell: The distribution and some effects of polychlorinated biphenyls (Aroclor 1254) in cockerels during prolonged feeding trials. Can. J. Comp. Med. **36,** 89 (19[illegible]).

Platonow, N. S., and N. Y. Chen: Transplacental transfer of polychlorinated biphenyl (Aroclor 1254) in a cow. Vet. Rec. p. 69 Jan. 20 (1973).

Platonow, N. S., and L. H. Karstad: Dietary effects of polychlorinated biphenyls on mink. Canad. J. Comp. Med. **37,** 391 (1973).

Prestt, I., D. J. Jefferies, and N. W. Moore: Polychlorinated biphenyls in wild birds in Britain and their avian toxicity. Environ. Pollut. **1,** 3 (1970).

Ringer, R. K., R. J. Aulerich, and M. Zabik: Effect of dietary polychlorinated biphenyls on growth and reproduction of mink. 164th Nat'l Meeting, *Amer. Chem. Soc.* 12, 149 Aug. 28–31 (19[illegible]).

Risebrough, R. W., P. Reiche, D. B. Peakall, S. G. Herman, and M. N. Kirven: Polychlorinated biphenyls in the global ecosystem. Nature **220,** 1098 (1968).

Risebrough, R. W., and V. Brodine: More letters in the wind. Environment **12,** 16 (1970).

Schaible, P. J.: Nutrition and feeding, Sect. III. In: The Blue Book of Fur Farming. Editorial Service Co., Milwaukee, WI (1970).

Tucker, R. K., and D. C. Crabtree: Handbook of toxicity of pesticides to wildlife. *U.S. Dept. of Interior, Bur. Sport Fisheries and Wildlife.* Res. Publication No. 84 (1970).

Villeneuve, D. C., D. L. Grant, W. E. J. Phillips, M. L. Clark, and D. J. Clegg: Effects of PCB administration on microsomal enzyme activity in pregnant rabbits. Bul. Environ. Contam. Toxicol. **6,** 120 (1971a).

Villeneuve, D. C., D. L. Grant, K. Khera, D. J. Clegg, H. Baer, and W. E. J. Phillips: The fetotoxicity of a polychlorinated biphenyl mixture (Aroclor 1254) in the rabbit and in the rat. Environ. Physiol. **1,** 67 (1971b).

Weigel, W. W., and C. Smith: Metabolism of a polychlorinated biphenyl (Aroclor 1016) in rats. Fed. Proc. (Abstr.) **33,** 499 (1974).

Manuscript received September 14, 1976; accepted January 15, 1977.