Exhibit # 896

Title: Identification of the Factor(s) Responsible for Reproductive Failure and Mortality in Mink fed Coho Salmon and other Great Lakes Fish.

Cooperators: Fur Animal Project, Poultry Science Department, Michigan State University, East Lansing, Michigan
Mink Farmers' Research Foundation, Milwaukee, Wisconsin

Personnel: Dr. Richard J. Aulerich, Dr. Robert K. Ringer and Mr. Susumu Iwamoto

PROBLEM AND CURRENT SITUATION

Since the mid 1960's Great Lakes fish have been suspected of causing reproductive problems when fed to mink (Hartsough, 1965). In 1968 mink ranchers reported acute reproductive complications when coho salmon, taken from Lake Michigan, were fed to mink at 2 to 15 percent of the diet prior to and during the reproductive period. The adult animals were not adversely affected by the coho salmon. Breeding and whelping were reported as normal; however, kit mortality as high as 80 percent was reported and appeared to be in direct proportion to the duration and level of coho salmon fed.

Previous investigations concerning the nature and cause of the problem (Aulerich et al., 1971 a,b) have shown that:

1. Feeding coho salmon from Lake Michigan to mink at 30 percent of the diet for four months prior to whelping causes reproductive failure or kit mortality.

2. Coho salmon per se does not cause the reproductive problems of mortality.

3. The disorder is associated with the feeding of various species of Great Lakes fish to mink and appears to be dependent upon the species of fish and its environment.

✓ 4. Lake Michigan coho salmon canning by-products fed at 30 percent of the diet were lethal to adult breeders after three months of feeding.

5. There was no correlation between the degree of oxidative rancidity or thiaminase content of the fish and the performance of the mink.

✓ 6. Antibiotic treatments and injections of iron, vitamins A, D and E, as well as the B-complex vitamins had no beneficial effects on the malady.

7. Histopathological examination and bacterial cultures of tissues from the affected mink failed to reveal the cause of the pathological symptoms.

8. The clinical signs and lesions observed in mink fed coho salmon were anorexia, bloody stools, fatty degeneration of the liver and kidneys, and hemorrhagic gastric ulcers.

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9. A direct relationship was noted between the chlorinated hydrocarbon pesticide residue content of Great Lakes fish and the degree of reproductive complications encountered when these fish were fed to mink. No symptoms of DDT or dieldrin poisoning, however, were observed in the animals. The adverse reproductive effects that occurred in feeding Great Lakes fish to mink also could not be duplicated with diets that contained much higher levels of these pesticides than those found in the fish (Aulerich and Ringer, 1970; Duby, 1970).

10. The toxic effects encountered in feeding Lake Michigan coho salmon to mink have become more acute in recent years.

The implication that mercury contamination might be responsible for the reproductive complications and mortality observed in mink fed Great Lakes fish was investigated during 1971. Results of mink feeding experiments indicated that although methyl mercury was extremely toxic to mink it did not appear to be the causative agent responsible for the problem. The clinical signs and lesions associated with mercury poisoning in mink differed considerably from those in mink that died while receiving diets that contained Lake Michigan coho salmon. Analysis of the Great Lakes and marine fish fed during the study also showed no association between the mercury content of the fish and the reproductive performance of the animals.

Polychlorinated biphenyls (PCB's) are another contaminant of the Great Lakes fish that were recently suspected as a possible cause of this problem (Aulerich et al., 1972 and Ringer et al., 1972). PCB's are chemically similar to DDT. They are extremely stable, toxic compounds which accumulate in fatty tissues of animals exposed to them.

To ascertain the effects of these compounds on mink, a feeding trial was conducted during the 1971 mink reproductive period. A basal diet containing 30% ocean fish was supplemented with 30 ppm PCB's (10 ppm each of Aroclors R (1) 1242, 1248 and 1254) and fed to 15 (12 female and 3 male) adult breeder mink.

An unsupplemented basal diet and a ration that contained 30% Lake Michigan coho salmon (as a substitute for the ocean fish in the basal diet) were fed to similar groups of mink for comparison. The reproductive performance and mortality of the females are summarized in Table 1.

No female mink fed coho salmon or 30 ppm PCB supplementation whelped. All adult mink fed supplemented PCB's died by the end of the whelping period. Those females, on PCB's, that survived sufficient time to produce offspring showed no signs of whelping, but some showed evidence of fetal absorption on necropsy. Six adult mink fed the 30% coho salmon diet also died during the experimental period of January 1 to June 30, 1971.

The clinical signs and lesions associated with the PCB poisoning consisted of anorexia, bloody stools, fatty degeneration of the liver and kidneys

(1) Registered name for PCB's, Monsanto Company, St. Louis, Mo.

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into the trial to determine if this compound, which is used to control sea lamprey in the Great Lakes, would have a detrimental effect on mink.

None of the mink employed in these experiments had previously received Great Lakes fish or supplemental PCB's in their diet. Standard ranch procedures were followed in feeding, breeding, and caring for the animals. The mink were vaccinated as kits for canine distemper, botulism, and virus enteritis. During the maintence and breeding season, the mink were fed to condition them for optimum reproduction. Whenever possible, the females were mated to males within their respective dietary groups. All matings were verified by taking a vaginal smear after copulation and observing it microscopically for the presence of normal appearing, motile sperm. The bred females were checked daily for young during the whelping period and the kits were counted, and weighed the day of birth.

Results and Discussion

Analyses of the Lake Michigan coho salmon used in the 1971 mink feeding trials showed PCB residues ranging from 10 to 15 ppm. Thus, a diet composed of 30% Lake Michigan coho salmon might be expected to contain approximately 3 to 5 ppm total PCB's.

Results of the PCB analyses of selected tissues from the mink that died in the 1971 experiment while receiving diets that contained either 30% Lake Michigan coho salmon or 30 ppm supplemental PCB's are summarized in Table 4, along with comparable data for control animals. As shown in Table 4, equivalent quantities of PCB's accumulated in tissues of mink that died while receiving the ration that contained Lake Michigan coho salmon and the basal diet supplemented with 30 ppm PCB's. The similarity is striking. The PCB residues in the brain of these mink averaged about 11 ppm and were approximately twice those obtained in other tissues.

The mean organ weights (expressed as a percent of brain weight) of control mink and the animals that died in the 1971 experiment on the diets that contained 30% coho salmon or 30 ppm PCB's are shown in Table 5. Analysis of variance of the organ weights showed no significant differences ($P < 0.05$) between the various dietary treatments.

The relative acute toxicity of PCB's to mink, as shown in Table 6, indicates that these compounds are apparently more toxic to mink than to rats (Tucker and Crabtree, 1970; Kimbrough, 1971), mice (Nishizumi, 1970) or birds (Tucker and Crabtree, 1970; Heath et al., 1970). Although some investigators (Risebrough and Brodine, 1970; Prestt et al., 1970) have reported a positive relationship between the percentage of chlorine (indicated by last two digits of Aroclor number) in PCB's and their acute toxicity, the toxicity of the Aroclors tested with mink in this study varied inversely with the percentage of chlorine in the compounds. Lichtenstein et al. (1969) noted a similar relationship in the toxicity of PCB's to flies, and Kimbrough (1971) reported that Aroclor 1254 was more toxic to rats than Aroclor 1260.

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The more commonly reported pathological effects of PCB exposure in mammals have been associated with liver damage (Bennett et al., 1936; Miller, 1944; and Peakall and Linder, 1970). To our knowledge, no previous accounts of gastric ulcers, as noted in this study, have been reported. Although the significance of the gastric ulcers has not been determined, the reproductive complications in mink fed Great Lakes fish may be attributable to hormonal imbalances resulting from PCB induced liver microsomal enzyme activity, as demonstrated in the rat by Stréet et al. (1969) or to embryo toxicity resulting from PCB passage across the placenta.

The average weight gains of female mink fed the control ration and the diets supplemented with PCB (Aroclor 1254) and chlorinated hydrocarbon pesticides in experiment I are shown in Table 2. Analysis of the data indicated a significant ($P < 0.01$) reduction in weight gains of the mink fed 10 ppm PCB's plus 10 ppm DDT after two months of feeding. A significant reduction in weight gain was also noted in the mink fed 10 ppm PCB plus 0.5 ppm dieldrin ($P < 0.01$), and 10 ppm PCB (P 0.05) after receiving these rations for four months. These observations suggest a potentiation in effect when PCB's are fed to mink in combination with chlorinated hydrocarbon pesticides. A synergistic effect of PCB's on DDT was demonstrated in insects by Tsao et al. (1953), and Lichtenstein et al. (1969) reported an interaction between Aroclors and DDT or dieldrin in which the toxicities of the pesticides were significantly increased.

The reproductive performance and mortality of the mink in experiment I are shown in Table 7. None of the females fed PCB supplemented diets whelped. All the animals fed the basal diet supplemented with 10 ppm PCB plus 0.5 ppm dieldrin died prior to the whelping season. High mortality also occurred in the group that received 10 ppm supplemental PCB. Although marked early reductions in weight gains occurred in the mink that received 10 ppm PCB plus 10 ppm DDT (see Table 2), only two of the seven mink died, and one death was not attributed to the dietary treatment.

Data presented in Table 8 summarize the reproductive performance and mortality of the mink in experiment III. Supplementation of the control diet with 1 ppm PCB caused a slight reduction in reproduction; 5 ppm supplemental PCB resulted in a marked reduction in reproduction; and 10 ppm supplemental PCB totally inhibited reproduction and resulted in 31 percent adult mortality when fed only four months. Feeding 30 percent autoclaved coho salmon indicated that the toxic factor in the fish is quite heat stable. TFM fed at 1 ppm had no detrimental effects on reproduction or livability.

The results of these experiments and analyses clearly point out the sensitivity of mink to polychlorinated biphenyls and cast suspicion on PCB's as the primary factor responsible for this problem.

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Table 1. Reproductive performance and mortality of control mink and mink fed rations that contained coho salmon or supplemental PCB's from Jan. 1, 1971 to June 1, 1971.

	Dietary treatment		
	Basal diet[1] (control)	30% Lake Mich. coho salmon substituted for ocean fish in basal diet	Basal diet supplemented with 30 ppm PCB[2]
Females mated	12	12	11[3]
Females whelped	11	0	0
Kits born:			
alive	35	--	--
dead	19	--	--
Live kits at 4 wks.	29	--	--
% adult mortality June 1, 1971	0	40	100

(1)Basal diet:

Ingredients	Percentage
Fortified Cereal[a]	15
Tripe	20
Liver (beef)	5
Horsemeat	15
"Spent" chicken (b)	15
Ocean fish (c)	30

(a) Kel-blend #1002, W. K. Kellogg Co., Battle Creek, Mich.
(b) For description, see: Aulerich, R. J. and P. J. Schaible, 1966. Simple method of processing "Spent" chickens for mink feeding and their use during late growth and furring. Mich. Agr. Expt. Sta. Quart. Bul. 49(3):24-27.
(c) Cod, haddock and flounder mix.

(2) 10 ppm each of Aroclors 1242, 1248 and 1254.

(3) One female died prior to mating season.

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Table 2. Effect of Aroclor 1254, alone or in combination with pesticides, on body weight gains of female mink (experiment I).

Dietary treatment	No. Mink	Initial body wt. (gm) (Aug. 25)	Body weight gain (gm) ± S.E. from initial wt.			
			1 mo. (Sept. 24)	2 mo. (Oct. 25)	3 mo. (Nov. 25)	4 mo. (Dec. 22)
Basal Diet (30% ocean fish mix; control)	6	1029	43 ± 17.4	203 ± 25.4	187 ± 46.2	210 ± 35.1
Basal diet plus 5 ppm PCB(1)	6	980	62 ± 22.4	153 ± 44.2	138 ± 42.7	128 ± 43.7
Basal diet plus 10 ppm PCB	6	923	43 ± 20.4	148 ± 32.0	132 ± 26.3	92 ± 35.1*
Basal diet plus 10 ppm PCB and 10 ppm DDT	6	1052	43 ± 14.5	57 ± 14.6**	28 ± 21.5**	-15 ± 35.4**
Basal diet plus 10 ppm PCB and 0.5 ppm dieldrin	6	980	108 ± 25.1	133 ± 26.7	115 ± 18.9	57 ± 24.4**

(1) Aroclor 1254

* Significantly different ($P<0.05$) from controls.

** Significantly different ($P<0.01$) from controls.

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Table 3. Experimental design of experiment III

Group	Dietary Treatment
1	Basal diet (1) (control)
2	Substitution of 30% Lake Michigan coho salmon for 30% ocean fish in the basal diet
3	substitution of 30% autoclaved Lake Michigan coho salmon for 30% ocean fish in the basal diet.
4	Basal diet supplemented with 1 ppm PCB (2)
5	Basal diet supplemented with 5 ppm PCB
6	Basal diet supplemented with 15 ppm PCB
7	Basal diet supplemented with 5 ppm PCB plus 6 ppm DDT
8	Basal diet supplemented with 5 ppm PCB plus 6 ppm DDT plus 0.2 ppm dieldrin
9	Basal diet supplemented with 1 ppm TFM (3) (Sea lamprey larvicide)

(1) Basal diet:

Ingredients	Percentage
Fortified cereal (a)	15
Tripe	20
Liver (beef)	5
Horsemeat	25
"Spent" chicken (b)	15
Ocean fish (c)	30

(a) Kel-blend #1002, W. K. Kellogg Co., Battle Creek, Michigan
(b) For description, see: Aulerich, R. J. and P. J. Schaible, 1966. Simple method of processing "spent" chickens for mink feeding and their use during late growth and furring. Mich. Agr. Expt. Sta. Quart. Bul. 49(3):24-27.
(c) Cod, haddock and flounder mix.

(2) Aroclor 1254.

(3) 3-trifluoromethyl-4-nitrophenol

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Table 4. Average PCB residues in tissues from control mink and mink that died while receiving diets that contained coho salmon or supplemental PCB's(a)

Dietary Treatment	No. mink	PCB tissue residue (ppm) ± S.E.						
		Brain	Liver	Kidney	Spleen	Lung	Muscle	Heart
30% Ocean fish Mix (control)	4	<0.01	<0.01	<0.01	<0.01	<0.01	N.D.(c)	N.D.
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	2.84 ± 1.21
30% Ocean fish mix plus 30 ppm PCB's(b)	12	11.00 ± 1.43	4.38 ± 0.58	4.47 ± 0.42	4.79 ± 0.39	4.78 ± 0.53	4.88 ± 0.54	3.26 ± 0.49

(a) Analyses by M.S.U. Pesticide Research Center.

(b) 10 ppm each of Aroclors 1242, 1248 and 1254.

(c) None detected.

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Table 5. Mean organ weights[a] of control mink and mink that died on rations that contained coho salmon or supplemental PCB's.

	Dietary treatment		
	Controls 30% Ocean fish mix	30% Coho salmon	30% Ocean fish mix plus 30 ppm PCB's[b]
No. Mink	7	3	11
Organ			
Heart Mean wt. + S.E.	75.8 ± 4.75	76.3 ± 18.51	72.9 ± 4.23
Liver Mean wt. + S.E.	399.5 ± 48.68	383.8 ± 19.04	397.2 ± 24.1
Kidney Mean wt. + S.E.	65.3 ± 3.09	67.6 ± 5.76	70.2 ± 3.94
Spleen Mean wt. + S.E.	35.5 ± 5.92	32.3 ± 11.78	29.3 ± 6.45

(a) Expressed as percent of brain weight

(b) 10 ppm each of Aroclors 1242, 1248 and 1254.

Table 6. Acute toxicity (LD_{50} per 48 hours) of several PCB's when administered as a single IP injection to mink.

PCB	LD_{50}
Aroclor 1221	>250 < 500 ppm
Aroclor 1242	>500 < 1,000 ppm
Aroclor 1254	about 1,000 ppm

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Table 7. Effect of Aroclor 1254, fed alone or in combination with pesticides, on mortality and reproduction of mink (Aug. 25 to May 10) (6 females and 1 male/group)

Dietary treatment	% mortality	Females alive	Females whelped	Kits born Alive	Kits born Dead	Kits/female alive
Control	14	5	3	17	8	5.0
5 ppm PCB(1)	29	4	0	0	0	0
10 ppm PCB	71	1	0	0	0	0
10 ppm PCB + 10 ppm DDT	43	4	0	0	0	0
10 ppm PCB + 005 ppm dieldrin	100	0	0	0	0	0

(1) Aroclor 1254.

Table 8. Effect of Aroclor 1254 fed singly and in combination with hydrocarbon pesticides on reproductive parameters (Jan. 1 to May 10) (12 females and 4 males/group)

Dietary greatment	% mortality	Females alive	Females whelped	Kits born Alive	Kits born Dead	Kits/female alive
Control (ocean fish)	6	11	11	56	10	6.0
Raw coho salmon	19	11	0	0	0	0
Cooked coho salmon	6	12	0	0	0	0
1 ppm PCB(1)	6(3)	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
1 ppm TFM(2)	0	12	12	67	4	5.9

(1) Aroclor 1254
(2) 3-trifluoremethyl-4-nitrophenol
(3) One female escaped.

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EXHIBIT A

RESEARCH PROPOSAL

Title: PCB implications in reproductive failure and mortality in mink fed Great Lakes fish.

Cooperators: Fur Animal Project, Poultry Science Department, Michigan State University, East Lansing, Michigan

Mink Farmers' Research Foundation, Pittsville, Wisconsin

Personnel: Dr. R. J. Aulerich, Dr. R. K. Ringer and Mr. S. Iwamoto

Problem and Current Situation:

Reproductive complications and mortality in mink attributed to the use of coho salmon and other Great Lakes fish in mink diets have forced ranchers to abandon these fish as a source of mink feed. The loss of this fishery has had tremendous economic consequences for the Midwest mink ranching industry, which now must rely heavily on fish shipped in from the coast for use in mink rations.

Because of the importance of the Great Lakes fishery to the mink industry, an investigation of this problem was undertaken by the Michigan State University Fur Animal Project. Mink feeding experiments (Aulerich et al., 1971a, b) have confirmed ranchers' reports of the detrimental affects of Great Lakes fish on mink reproduction and livability and more recent studies (Aulerich et al., 1972a, b) have indicated that polychlorinated biphenyl (PCB) contamination of the fish is suspected to be the cause of the problem. The results of feeding trials, tissue and feed analyses, necropsy examinations and toxicity experiments strongly suggested that PCB's were the primary causative agent responsible for the reproductive complications and mortality associated with the feeding of Great Lakes fish to mink. PCB's were also noted to be considerably more toxic to mink than to other species (rats, mice and birds).

Additional experiments, however, should be conducted to substantiate the supposition that PCB's are implicated in the disorder and to investigate methods for removal of PCB's from the fish or treatments that would render PCB contaminated fish safe for animal feeding.

Objectives - The specific objectives of the proposed study are to:

1. Substantiate the results of the previous study (see 1972 Research Progress Report to the Mink Farmers' Research Foundation) in which PCB's were implicated as the primary cause of the problem associated with the feeding of Great Lakes fish to mink.

2. Investigate possible interactions between PCB's and chlorinated hydrocarbon pesticides.

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3. Evaluate hexane extraction of coho salmon as a practical method of removing PCB's (and chlorinated hydrocarbon pesticides) from the fish to render them safe for animal feed.

4. Ascertain whether or not the reproductive complications encountered in female mink fed PCB's or Lake Michigan coho salmon are of a permanent nature.

5. Determine the mode of action of PCB's in causing reproductive failure and early kit mortality.

Procedure:

To meet the first three objectives of the study, eight groups of adult mink (consisting of 12 females and three males per group) will be fed a basal mink ration supplemented or modified as follows:

Group	I	Basal diet (control).
Group	II	Substitution of Lake Michigan coho salmon for ocean fish in the basal diet.
Group	III	Basal diet supplemented with 2.5 ppm PCB (Aroclor(R)(1) 1254.
Group	IV	Basal diet supplemented with 2.5 ppm PCB plus 6 ppm DDT.
Group	V	Basal diet supplemented with 6 ppm DDT.
Group	VI	Basal diet supplemented with 2.5 ppm PCB plus 0.2 ppm dieldrin.
Group	VII	Substitution of the supernatant from the equivalent of 30% hexane extracted Lake Michigan coho salmon for the supernatant from 30% hexane extracted ocean fish in the basal diet.
Group	VIII	Substitution of the residue from the equivalent of 30% hexane extracted Lake Michigan coho salmon for the residue from 30% hexane extracted ocean fish in the basal diet.

In addition to the above treatments, the female mink that received the ration that contained 30% raw Lake Michigan coho salmon and the diet that contained 5 ppm supplemental PCB from January 1 to June 1, 1972 in the previous study (see 1972 Research Progress Report to the Mink Farmers' Research Foundation) will be retained on basal (control) diet through the 1973 breeding and whelping season to determine whether or not the reproductive impairment associated with the dietary treatments involving PCB's and coho salmon is of a permanent nature.

PCB analyses will be conducted on mink milk and kits found dead or sacrificed at birth from the various dietary treatments in an effort to ascertain if PCB's are passed through the placenta and as a result are embryo toxic, and/or if they are passed from the female to her kits through the milk.

The mink in Groups I-VIII will be placed on experiment January 1, 1973 and fed their respective diets until June 15, 1973. Littermates will be allocated, as far as possible to different dietary treatments in order to equalize the effect of genetics on reproduction. Routine ranch procedures

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will be followed in mating and caring for the animals. The mink will be vaccinated for botulism, virus enteritis and distemper before being placed on the experiment. Only animals free of Aleutian disease (as ascertained by the "Iodine Agglutination Test") will be used in the study.

The adult mink will be weighed at the beginning of the experiment, at whelping, and at the termination of the study (June 15, 1973). Kit weights and litter size will be recorded at day of birth and at 2 and 4 weeks of age.

Any adult mink that die during the experiment will be necropsied. PCB, DDT and dieldrin analyses of tissues and diets will be conducted by the M.S.U. Pesticide Research Center.

The hexane extractions of the fish used in the diets fed to the mink in Groups VII and VIII will consist of a composite of 3 extractions and condensations for each lot of fish.

Cooperative Agreement:

Michigan State University will supply the mink facilities, feed, labor, equipment, materials, and technical personnel to conduct the experiment. The Mink Farmers' Research Foundation will supply the necessary funds to assist in conducting the experiment per the attached memorandum of agreement.

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