THE EFFECTS OF POLYCHLORINATED BIPHENYLS AND COHO SALMON ON MINK

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ABSTRACT

THE EFFECTS OF POLYCHLORINATED BIPHENYLS
AND COHO SALMON ON MINK

By

Susumu Iwamoto

Studies pertaining to reproductive complications and mortality in mink fed diets that contained Lake Michigan coho salmon or supplemental polychlorinated biphenyls (PCB's) were conducted at the Michigan State University Experimental Fur Farm over a four year period. Nine experiments dealing with various aspects of these two associated conditions were performed to investigate the nature of these disorders, their etiology and possible remedies.

In Experiment I, conducted to determine the effects of feeding high levels of supplemental PCB's or coho salmon on mink reproduction and viability, none of the females that received coho salmon or supplemental PCB's in the diet whelped. Anorexia, bloody stools, fatty degeneration of the liver and kidneys and hemorrhagic gastric ulcers were common clinical signs and lesions observed in the animals.

Experiment II was conducted to ascertain the effects of long-term, low-level consumption of PCB's on mink reproduction and viability, and to investigate the possibility of interactions between PCB's and some chlorinated hydrocarbon pesticide contaminants of Great Lakes fish. Mink growth data suggested that interactions occurred between PCB's and chlorinated hydrocarbon pesticides in which the action of PCB's

or certain chlorinated hydrocarbon pesticides may be potentiated in the presence of the other. Long-term consumption of PCB's by mink resulted in decreased body weight gains and increased heart, liver and kidney weights.

In Experiment III the acute toxicity values (LD_{50}) of some common PCB's for mink ranged from 500 mg/kg for Aroclor 1221 to 2250 mg/kg for Aroclor 1254.

Experiment IV was conducted to ascertain the effects of coho salmon and PCB's, fed alone or in combination with chlorinated hydrocarbon pesticides, on mink reproduction and viability. The results indicated that chlorinated hydrocarbon pesticides and PCB's do not appear to have a synergistic effect on mink reproductive performance, that the toxic factor present in Great Lakes fish is quite heat stable, and that PCB contamination of the fish is probably the primary cause of reproductive complications and mortality in mink fed these fish.

The results of Experiment V showed that Aroclor 1254 was much more detrimental to mink reproduction than Aroclors 1016, 1221 or 1242. The experiment also indicated that hematologic parameters were not a good indicator of the toxic effects of PCB's in mink.

In Experiment VI mink fed Aroclor 1254 readily accumulated the PCB's in the adipose tissue until a plateau level was reached. A 50 percent reduction in PCB residues in the adipose tissue was reached about 8 weeks after withdrawal of the PCB's.

Experiment VII was conducted to substantiate the results of previous experiments in which PCB's were implicated as the primary cause of reproductive problems associated with the feeding of Great Lakes fish to mink, and to evaluate acetone-hexane extraction of coho salmon as a practical method of removing PCB's from these fish to render them safe

for mink feed. The results confirmed the results of the previous experiments and showed that the toxic factor present in coho salmon is stored primarily in the fat and can be removed from the fish by acetone-hexane extraction.

The results of liver and kidney function tests performed on the mink in Experiment VIII indicated that impaired liver function was an early manifestation of PCB and coho salmon toxicity in mink.

The results of Experiment IX showed that reproductive failure attributed to the feeding of coho salmon and PCB's to mink was not of a permanent nature.

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Susumu Iwamoto

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

INTRODUCTION

Mink (*Mustela vison*) were first raised in captivity for the production of fur in the United States in 1866 (Kellogg *et al.*, 1948), and toward the end of the nineteen-twenties were exported from North America to Europe (Aitken, 1963) where they are raised extensively today. In 1972 the estimated world production of mink exceeded 22 million pelts (Anon., 1972a).

In the United States, the majority of mink ranches are concentrated in the upper Midwest where there is a suitable climate for raising mink, as well as abundant supplies of fish from the Great Lakes and meat by-products from packing plants to feed the animals (Travis and Schaible, 1960). In the United States 10 to 40 percent of the total mink diet is usually composed of fish (Travis and Schaible, 1960). For many years ranchers in the Midwest fed fish from the Great Lakes as a major protein source. The utilization of Great Lakes fish by the mink ranching industry, however, has constantly diminished since the mid 1960's due to reproductive complications when mink were fed these fish (Hartsough, 1965).

An acute problem was evident in 1968 when coho salmon (*Oncorhynchus kisutch*), taken from tributaries of Lake Michigan during the spawning run of 1967 and fed to mink before and during the breeding and whelping season, caused an unusually high incidence of kit mortality on several commercial mink farms. Kit mortality as high as 80 percent was reported (Aulerich *et al.*, 1971). The cause of the problem was demonstrated to

be due to the use of coho salmon in the diet. Microbiological toxins, thiaminase activity in the fish, rancidity, as well as mercury and chlorinated hydrocarbon pesticide contamination were all suspected as being responsible for the problem. None of these factors, however, were substantiated as being the cause of the disorder (Aulerich *et al.*, 1971).

Because of the tremendous human health implications, as well as the economic importance of Great Lake fishery to the mink ranching and pet food industries, this study was initiated to determine the factor or factors responsible for the reproductive failure and mortality associated with the feeding of Great Lakes fish to mink.

REVIEW OF LITERATURE

Coho Salmon-Mink Feeding Studies

A series of mink feeding experiments were conducted by Aulerich *et al.* (1970a, 1971, 1972 and 1973) in an effort to investigate the reproductive problems attributed to the feeding of Great Lakes fish to mink. In these trials, whole, raw, sexually mature coho salmon taken from tributaries of Lake Michigan were fed to mink at 30% of the diet from two months before breeding through gestation. Several other species of marine and Great Lakes fish were fed at the same level for comparison. In one study coho salmon canning by-products were used instead of whole, raw fish.

All of the 15 mink that received the Lake Michigan coho salmon canning by-products in their diet died between the beginning of breeding season and the end of the whelping period (Aulerich *et al.*, 1971). In another feeding trial four out of 10 females fed the diet that contained the ground, whole, Lake Michigan coho salmon also died; the rest failed to whelp (Aulerich *et al.*, 1971). Anorexia, bloody stools, fatty livers, and hemorrhagic gastric ulcers were common clinical signs and lesions observed in these mink. Antibiotic treatment and injections of iron, vitamins A, D and E, as well as the B-complex vitamins, had no beneficial effect in arresting the mortality. Reduced reproductive performance and/or excessive kit mortality were also observed in mink fed diets that contained Lake Michigan bloater chub, Lake Michigan yellow perch, and mature, whole, raw coho salmon from Lake Erie. Mink

rations that contained West Coast coho salmon and Lake Erie yellow perch did not impair reproduction nor result in excessive kit mortality (Aulerich *et al.*, 1971).

No correlation was found between the degree of oxidative rancidity or mercury contamination of the fish and the reproductive performance of the mink (Aulerich *et al.*, 1971). The level of pesticide residues (DDT and dieldrin) in the fish and the degree of reproductive decline and/or kit mortality was, however, directly related, but based on the results of previous studies by Aulerich and Ringer (1970b), chlorinated hydrocarbon pesticide residues were discounted as the cause of the problem.

These experiments demonstrated that coho salmon *per se* does not cause the reproduction and mortality problems, but that the disorder is associated with other species of Great Lakes fish and appears to be dependent upon the species of fish and its environment (Aulerich *et al.*, 1971).

Mink Reproduction

Mink have one breeding season per year, which extends from late February through March. Both spermatogenesis and estrus are controlled by environmental light conditions (Bowness, 1957; Travis and Schaible, 1960; Holcomb *et al.*, 1962; Aulerich *et al.*, 1963; and Bostrom *et al.*, 1968). Due to delayed implantation in this species there is a wide variation in the gestation period. Gestation averages about 50 days but varies between 40 and 70 days (Hansson, 1947).

Female mink may produce offspring by different males (superfecundation) and/or from two different ovulations more than a week apart (superfetation) (Travis and Schaible, 1960). Litter size may vary from 1 to

8, or more, but generally averages about 4 kits at birth. The average litter size at four weeks postpartum of Standard Dark and Pastel female mink at the Michigan State University Experimental Fur Farm in 1970 was 3.6 kits per female (Aulerich, 1970b).

Factors that Adversely Affect Mink Reproduction

Stress:

Animals are frequently under stress of one kind or another, caused by chilling, overheating, poor ventilation, disease, parasites, vaccination, medication, dehydration from water supply failure, improper handling, inadequate nutrition and unusual or excessive noises. All of these stresses may cause reproductive complications with mink (Schaible, 1969, and Travis, 1968).

Microorganisms:

Spoiled feed or toxic microorganism contamination, which may be the result of improper handling of feed, are frequent sources causing reproductive complications in mink. *Salmonella* and botulism organisms have been reported to cause infectious abortion in mink (Travis and Schaible, 1960).

Feed handling:

Improper handling and freezing of mink feed ingredients may result in loss in flavor, produce dangerous peroxides, and destroy essential vitamins (Schaible, 1969). A problem of this nature which caused heavy kit losses shortly after weaning was first described by McDermid and Ott (1947). The disease was termed "yellow fat" by McDermid and Ott (1947), but later referred to as fatty degeneration of the liver by Chaddock (1948), and steatitis by Hartsough and Gorham (1949).

This condition in mink is associated with the feeding of diets high in unsaturated fatty acids and low in vitamin E. The source of the highly unsaturated fat has been traced to rancidity in improperly stored fish and/or horsemeat. The use of vitamin E or antioxidants in the diet protects mink from the disease (Lalor *et al.*, 1951, and Mason *et al.*, 1951).

Sodium nitrite, frequently used in the preservation of fish, has been shown by Stout *et al.* (1968) to react with trimethylamine forming the toxic compound, dimethylnitrosamine, which causes mortality when fish treated with this compound are fed to mink.

Contaminants:

The infiltration of drugs, chemicals and hormones into mink feed ingredients can also cause severe reproductive problems. In recent years many new substances have been introduced in the agricultural area. These substances occasionally get into cereal or slaughter house by-products used in mink diets (Schaible, 1969). Diethylstilbestrol (DES), an estrogen-like compound previously used in poultry and livestock production to stimulate growth and enhance carcass quality, has been a common source of reproductive failure in mink. As little as 10 µg of DES/mink/day completely inhibits mink reproduction (Travis *et al.*, 1956).

Chlorinated hydrocarbon pesticides such as DDT and dieldrin are frequent contaminants of mink feed since they are common pollutants of many fresh water fish used for mink feeding (Hartsough, 1965).

Since 1965, the U.S. Bureau of Commercial Fisheries has been monitoring insecticide residues in Great Lakes fish. On the basis of the DDT and dieldrin levels in these fish, the rank of the Great Lakes, in the order of highest to lowest concentration of insecticides was:

Michigan, Ontario, Huron, Erie and Superior (Reinert, 1970). Among 12 Lake Michigan species of fish examined, bloater chubs had the highest whole fish averages for DDT (8.61 ppm) and dieldrin (0.23 ppm) (Reinert, 1970). Reinert (1970) also demonstrated that both the DDT and dieldrin concentrations in Great Lakes lake trout increased in direct proportion with total length of the fish; a 30-inch lake trout from Lake Michigan may be expected to contain between 20 and 30 ppm DDT. Analyses by the National Marine Fisheries Service, as reported by Aulerich *et al.* (1971) showed that Lake Michigan coho salmon contained 18.23 ppm total DDT and 0.12 ppm dieldrin residues, but Lake Erie coho salmon contained only 2.76 ppm total DDT and 0.03 ppm dieldrin residues.

The physiological effect of chlorinated hydrocarbon pesticides, such as DDT and dieldrin on reproduction in many birds (Rubin *et al.*, 1947; Dewitt, 1955; Genelly and Rudd, 1956; Albert, 1962; Azevedo *et al.*, 1965; Locke *et al.*, 1966; and Atkins and Linder, 1967), and mammals (Kitselman, 1953; Ball *et al.*, 1953; Bernard and Gaertner, 1964; Mestizova, 1966; and Ware and Good, 1967) has been well documented. In studies with mink, Gilbert (1969) reported that as little as 0.58 ppm DDE contamination of mink diets caused early kit mortality. Mink rations supplemented with 100 ppm DDT (Duby, 1970) or 100 ppm DDT plus 50 ppm DDD (Aulerich and Ringer, 1970b), however, had no adverse effect on mink reproduction or kits mortality.

The mercury content of the various fish fed to the mink in an experiment associated with this problem (Aulerich *et al.*, 1971) ranged from 0.08 ppm in ocean whiting to 0.36 ppm in the Lake Erie coho salmon, but no association was evident between the mercury content of the fish and the reproductive performance or mortality observed in the mink.

Since the Great Lakes fish-mink feed problem did not appear to be due to any of the above mentioned causes of reproductive complications, yet based on the clinical signs and lesions reported by Aulerich (1971) seemed to be due to some toxic substance in the fish, emphasis in the present study was placed on the influence of other contaminants of Great Lakes fish on mink.

Polychlorinated biphenyls:

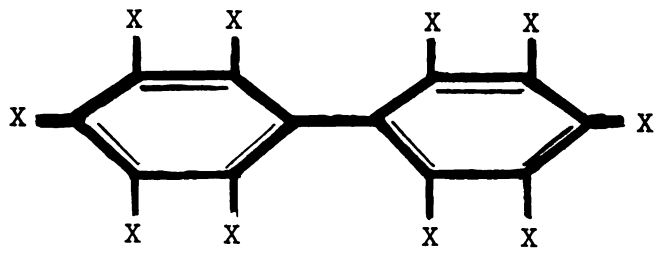
Polychlorinated biphenyls (PCB's) are chlorinated hydrocarbon compounds that have been employed in industry as dielectric fluids in transformers and capacitors, as well as for a multiplicity of less essential uses over the past two decades (Anon., 1972b). These compounds were not thought of as contaminants until environmental problems were attributed to them in Sweden (Jensen, 1966). Today, they are considered to be widespread environmental pollutants (Holden and Marsden, 1967; Holmes *et al.*, 1967; and Risebrough *et al.*, 1968).

PCB's are fat soluble and persistent compounds that tend to accumulate in oils and fats, and have been detected in milk, eggs, meat, fats, oil and cereal products (Anon., 1972b). They have directly or indirectly found their way into animal feed and animal products through water, paint, heat transfer fluids and plastic and cardboard food packing materials (Anon., 1972b).

Nisbet and Sarofim (1972) reported PCB levels in fish as high as 213 ppm from industrialized rivers in the U.S. They further reported PCB levels of 19, 20, 0.01 and 0.03 ppm in fish from Lake Ontario, Lake Michigan, and Atlantic Ocean and the Pacific Ocean, respectively. Analysis of Lake Michigan coho salmon used in the mink feeding trials concerned with this investigation showed that these fish contained 10

to 15 ppm PCB's (Aulerich *et al.*, 1971). Bache *et al.* (1972) found as high as 30.4 ppm PCB in trout from Cayuga Lake, New York.

The basic structure of PCB's is shown below. Any of the atoms appearing at the positions marked with an "X" can be substituted for by a chlorine atom (Jensen, 1970). The PCB's produced commercially contain



approximately 50 of the 210 different compounds which are theoretically possible when biphenyls are chlorinated. The most common mixtures contain between 40 and 60 percent chlorine (Anon., 1972b). In the United States these compounds are identified by the percent of chlorine by weight in the total product and sold under the trade name Aroclor^{R*} (Anon., 1972b). The last two digits of the Aroclor number indicate the percent chlorine.

Despite the recent interest in these compounds the LD_{50} of PCB's is rather poorly defined in comparison with the chlorinated hydrocarbon pesticides. Acute oral or dermal toxicity of chlorinated biphenyls on mice, rats, rabbits and guinea pigs have been studied by Miller (1944) and Tucker and Crabtree (1970). Sublethal effects of PCB's on mammals and birds were summarized by Peakall and Lincer (1970). The most striking pathological changes in the organs in mammals are alterations in the liver (Bennett *et al.*, 1938; Miller, 1944; Nishizumi, 1970; Koller and Zinkl, 1973; Grant *et al.*, 1971a; and Cecil *et al.*, 1973).

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*Trade name for PCB's manufactured by Monsanto Company, St. Louis, Missouri.

Like other organochlorine compounds, such as DDT, PCB's have been shown to have an adverse effect upon reproduction in birds, mice, rats and dogs (Deichmann and MacDonald, 1971). Pheasants given a capsule containing 50 mg of Aroclor 1256 weekly for 17 weeks produced fewer eggs than controls, and although a high percentage of the chicks piped the shell they did not hatch (Dahlgren and Linder, 1971).

In a study by the FDA (Anon., 1972c), weanling male and female rats were fed diets that contained Aroclor 1254 at levels of 0, 100 or 500 parts per million for 67 days and then pair-mated. Reproduction in the rats fed 100 ppm PCB was comparable to the controls in the numbers of litters produced and the number of pups per litter. However, 76.8 percent of the pups in the group fed 100 ppm PCB survived to weanling compared with 95.5 percent for the controls. The mean body weight of the rats fed 100 ppm PCB at weanling was 31.4 gm and that of the control group was 39.2 gm. Only two of the ten females fed 500 ppm Aroclor 1254 had litters and these pups died within 3 days after birth.

Platonow and Karstad (1972) reported on the effects of PCB's on mink. Feeding female mink rations that contained 3.57 ppm or 0.64 ppm of total PCB's resulted in either reproductive failure or total kit mortality within 24 hours postpartum. Adult female mink fed the ration that contained 3.57 ppm PCB all died by the 105th day, although clinical signs in animals were either absent or non-specific. The gross lesions observed at necropsy were emaciation, gastrointestinal and intra-abdominal hemorrhages, and a yellowish discoloration of the liver. Based on these results, Platonow and Karstad (1972) suggested that mink were among the more sensitive species to PCB poisoning.

METHODS

The animals used in these experiments were from the Michigan State University experimental mink ranch. The mink were confined in individual pens of uniform size and each pen was equipped with a water cup and nest box. Except where stated otherwise in the text, the following procedures pertain to all the experiments:

Standard ranch procedures were followed in feeding, breeding, and caring for the animals. The mink were vaccinated for canine distemper, botulism and virus enteritis each year in July.

In alloting the mink into various groups for experimentation, littermates were divided between groups in an effort to balance genetic differences in growth, reproduction and response to treatments.

The mink were weighed individually at the start of the experiments and at specified intervals thereafter, except during the gestation period (March-May). All matings were verified by taking a vaginal smear after copulation and observing it microscopically for the presence of normal appearing motile sperm. The females were checked daily for the birth of young during the whelping period. Kits were counted and weighed the day of birth and at 4 weeks of age.

Since the results of previous experiments in which PCB's and coho salmon were fed to mink showed no adverse effects on spermatogenesis (Aulerich, 1970), only reproductive data pertaining to female mink are reported in this study.

Mink kits found dead the day of birth or that died during the experiment, as well as tissue from the adult animals that died or that were sacrificed while receiving the various dietary treatments, were submitted to the M.S.U. Pesticide Research Center for PCB analysis. The tissue samples were stored in glass vials in a freezer (0°C) until processed for analysis.

The animals that died during the experiments were necropsied, organ weights recorded, and selected tissues collected for histopathologic examination. Routine histologic procedures were employed in preparing the tissue for histopathologic examination. The tissues were stained with hematoxylin and eosin.

In the preparation of the experimental diets employed in this study, the PCB's or pesticides were dissolved in acetone and incorporated into the experimental diet by blending the solution with a small quantity of ground commercial mink cereal. The acetone was evaporated off and the premix containing the PCB's or pesticide was mixed with the other ingredients of the diet to yield a ration that contained the desired amount of the various chlorinated compounds.

The composition and proximate analysis of the basal (control) diet is shown in Table 1.

All experimental data were subjected to analysis of variance. The differences among the dietary group means were tested using the Duncan's Multiple Range Test (Duncan, 1955).

Table 1. Composition of basal (control) diet

Ingredients	Percent
Supplemented cereal[1]	15.0
Tripe	20.0
Liver (beef)	5.0
Horsemeat	15.0
Chicken	15.0
Ocean fish[2]	30.0

[1]Kel-Centrate #1002, W. K. Kellogg Co., Battle Creek, Michigan.

[2]Mixture of cod, haddock and flounder.

Proximate analysis of basal diet ("as-fed" basis)

	Crude protein	Ether extract	N-free extract	Ash	Crude fiber	H_2O
Percent	11.16	7.14	7.71	2.41	0.89	70.69

EXPERIMENTS

The research reported in this thesis was conducted between 1971 and 1973. It consisted of the following experiments:

Experiment I. Effects of PCB's and Lake Michigan coho salmon on mink reproduction and viability

Experiment II. A. Effects of long-term, low-level consumption of PCB on mink

B. Investigation of interactions between PCB's and chlorinated hydrocarbon pesticides

Experiment III. LD_{50} of PCB's for mink

Experiment IV. Effect of various levels of PCB's and PCB's plus chlorinated hydrocarbon pesticides on mink reproduction and viability

Experiment V. Comparison of various PCB's on mink growth, reproduction, hematologic parameters and viability

Experiment VI. Rate of accumulation and depletion of PCB's in adipose tissue of mink

Experiment VII. Evaluation of acetone-hexane extraction as a practical method of removing toxic factors from Great Lakes fish

Experiment VIII. Investigation of the mode of action of PCB's in mink

Experiment IX. Impermanence of reproductive failure in mink fed PCB's or Lake Michigan coho salmon

Experiment I

This experiment was conducted to determine the effects of feeding high levels of supplemental PCB's or coho salmon on mink reproduction and viability.

Procedure

The experiment was conducted from January 1, 1971, to June 30, 1971. Thirty-six female (proven breeder) mink were allocated in three groups consisting of twelve females per group, and were fed a basal diet (Table 1) supplemented or modified as follows:

Group I - 1 Unsupplemented, unmodified basal (control) diet

Group I - 2 Basal diet except for the substitution of 30 percent ground, whole, raw, Lake Michigan coho salmon for 30 percent ocean fish

Group I - 3 Basal diet supplemented with 30 ppm PCB's (10 ppm each of Aroclors 1242, 1248 and 1254)

Results

No significant differences were noted between the mean body weights of the females on the three dietary treatments (Table 2) during the first three months of the feeding trial.

The reproductive performance of the mink is shown in Table 3. None of the females that received Lake Michigan coho salmon (Group I - 2) or supplemental PCB's (Group I - 3) in the diet whelped.

All the mink in Group I - 3 (30 ppm PCB) died between February 28 and May 2, 1971. Six of the 15 mink in Group I - 2 died between March

Table 2. Average body weight change (gm ± S.E.) of female mink fed a control ration or experimental diets (Experiment I)

Dietary treatment	Initial wt. (Dec. 27)	Weight change 1 mo. (Jan. 27)	Weight change 2 mo. (Feb. 25)
I - 1 (control) (n=12)	1067 ± 42.3	- 9 ± 17.6	-145 ± 24.0
I - 2 (30% coho salmon) (n=12)	1015 ± 41.9	-15 ± 21.8	-120 ± 22.5
I - 3 (30 ppm PCB[1]) (n=12)	976 ± 55.1	-32 ± 13.0	-155 ± 16.4

[1]The PCB supplementation consisted of 10 ppm each of Aroclors 1242, 1248 and 1254.

Table 3. Reproductive performance and mortality of female mink fed a control ration of experimental diets (Experiment I)

Dietary treatment	Adult females % mortality	Adult females No. mated	Adult females No. whelped	Kits No. born Alive	Kits No. born Dead	Kits Whelped/♀ mated	Kits No. alive at 4 wks.
I - 1 (control)	0	12	11	35	19	4.5	29
I - 2 (30% coho salmon)	25	12	0	-	-	-	-
I - 3 (30 ppm PCB[1])	100	11[2]	0	-	-	-	-

[1]The PCB supplementation consisted of 10 ppm each of Aroclors 1242, 1248 and 1254.

[2]One female died prior to the mating season.

10, 1971, and June 1, 1971, when the feeding trial was terminated. Anorexia, bloody stools, fatty degeneration of the liver and kidneys and hemorrhagic gastric ulcers (Figure 1) were common clinical signs and lesions observed in the animals that died in Groups I - 2 and I - 3. The clinical signs and lesions observed in these two groups of mink were strikingly similar. The gross and histopathologic lesions observed in the tissues from these mink are summarized in Table 4. Three of the six females in Group I - 3 that died during the latter stages of gestation had fetuses in their uteri. No fetuses were observed in the one female from Group I - 2 that died during the whelping period.

At necropsy, heart, liver, kidney, spleen and brain weights were recorded for the mink that died during the feeding trial. The mean organ weights (expressed as a percent of brain weight) for the female mink in Groups I - 2 and I - 3 are shown in Table 5, along with comparable data for seven control animals. Analysis of variance of the data presented in Table 5 showed no significant differences between the organ weights of the mink from the various dietary groups.

PCB tissue residue analyses from control mink and from the animals that died while receiving diets that contained coho salmon or supplemental PCB's are shown in Table 6. Similar PCB residue levels were noted in the tissues from mink fed the two experimental diets.

Discussion

The results of this experiment confirmed the findings of a study by Aulerich *et al.* (1971), who reported that reproductive failure occurred from feeding Lake Michigan coho salmon to mink.

Although no significant differences were observed in the weight gains of the mink on the various dietary treatments, these results may

Figure 1. Visceral surface of mink stomach showing a pyloric ulcer frequently observed in mink that died while receiving diets supplemented with PCB's (Experiment I).

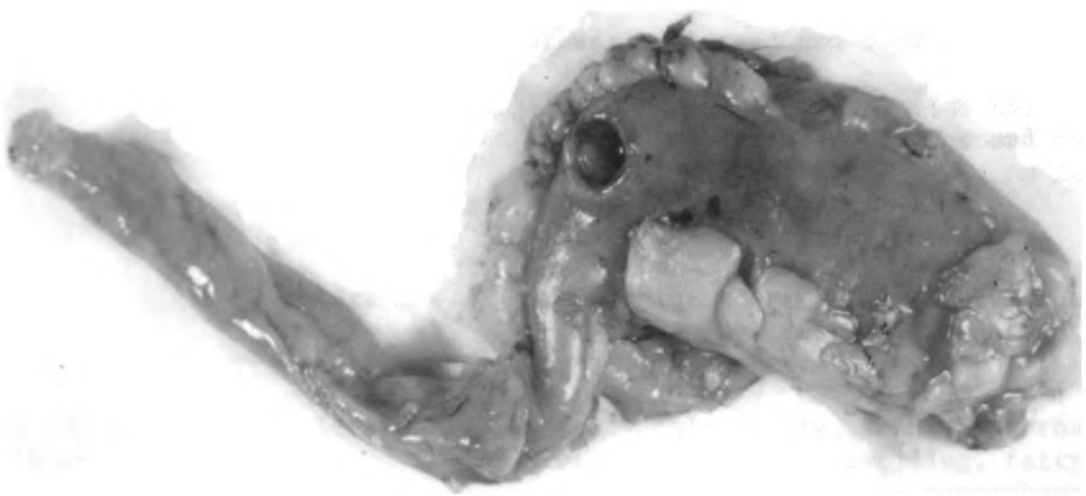

Figure I

Table 4. Gross and histologic lesions commonly observed in tissues from mink that died while receiving diets that contained Lake Michigan coho salmon or supplemental PCB's (Experiment I)

Dietary treatment	Tissue	Pathologic observations
I - 2 (30% coho salmon)	Liver	Gross: pale, fatty Histologic: cloudy swelling and fatty degeneration
	Stomach	Gross: gastric ulcers (pyloric)
	Cervical lymph nodes	Gross: enlarged and hard
	Kidneys	Histologic: cloudy swelling and fatty degeneration
	Lungs	Histologic: diffuse hyperemia
	Adrenals	Histologic: cortical hyperplasia and hypertrophy, cortical adenomas, medullary atrophy
I - 3 (30 ppm PCB's[1])	Liver	Gross: pale, fatty, focal hemorrhages Histologic: cloudy swelling, fatty degeneration, diffuse hemorrhages
	Stomach	Gross: gastric ulcers (pyloric)
	Kidneys	Gross: pale Histologic: cloudy swelling, hydropic degeneration, diffuse hemorrhages
	Lungs	Gross: pale Histologic: diffuse hyperemia
	Adrenals	Histologic: cortical hyperplasia, cortical adenomas
	Brain	Histologic: focal cortical chromophilic areas
	Spleen	Histologic: amyloidosis

[1]The PCB supplementation consisted of 10 ppm each of Aroclors 1242, 1248 and 1254.

Table 5. Mean organ weights[1] of female mink fed a control ration or experimental diets (Experiment I)

Dietary treatment	Organ			
	Heart	Liver	Kidneys	Spleen
I - 1 (control) (n=7)	75.8 ± 4.75	399.5 ± 48.68	65.3 ± 3.09	35.5 ± 5.92
I - 2 (30% coho salmon) (n=3)	76.3 ± 18.51	383.8 ± 19.04	67.6 ± 5.76	32.3 ± 11.78
I - 3 (30 ppm PCB[2]) (n=11)	72.9 ± 4.23	397.2 ± 24.1	70.2 ± 3.94	28.3 ± 6.45

[1]Expressed as percent of brain weight ± S.E.

[2]The PCB supplementation consisted of 10 ppm each of Aroclors 1242, 1248 and 1254.

Table 6. Average PCB content of tissues (ppm ± S.E.) from mink that died or were sacrificed[1] (Experiment I)

Dietary treatment	Tissue						
	Brain	Liver	Kidney	Spleen	Lung	Muscle	Heart
I - 1 (control) (n=4)[2]	<0.01	<0.01	<0.01	<0.01	<0.01	N.D.[3]	N.D.
I - 2 (30% coho salmon) (n=4)[4]	8.91 (±2.19)	11.43 (±6.22)	6.37 (±0.19)	6.19 (±0.07)	6.84 (±1.70)	6.24 (±1.98)	3.13 (±0.70)
I - 3 (30 ppm PCB[5]) (n=12)[4]	11.00 (±1.43)	4.95 (±0.57)	4.47 (±0.41)	4.79 (±0.39)	4.48 (±0.57)	4.88 (±0.54)	3.26 (±0.54)

[1]Analyses by M.S.U. Pesticide Research Center

[2]Sacrificed

[3]None detected

[4]Died

[5]The PCB supplementation consisted of 10 ppm each of Aroclors 1242, 1248 and 1254.

be misleading, since the animals were fed to condition them for optimum reproduction during this period, i.e., lean animals were "full-fed" while obese mink received restricted amounts of feed prior to and during the mating season.

If it is assumed that an adult female mink consumes 150 g of feed per day (Schaible, 1969), the average total intake of PCB's by the mink in Group I - 3 that died while receiving the diet that contained 30 ppm PCB's (for an average of 106 days) would have been about 477 mg. Since the Lake Michigan coho salmon used in this experiment contained PCB residues ranging from 10 to 15 ppm (Aulerich *et al.*, 1971), the diet fed to the mink in Group I - 2 (30% coho salmon) contained approximately 3 to 5 ppm of PCB's. The females in Group I - 2 that died and whose tissues were analyzed for PCB content survived an average of 68 days on the experiment and, thus, would have consumed approximately 51 mg of PCB's. The PCB residue levels shown in Table 6 do not reflect the variation in PCB consumption of the mink in Groups I - 2 and I - 3. This, however, may be due to differences in the toxicity and metabolism of the PCB's fed to the mink in Groups I - 2 and I - 3.

Studies by Bennett *et al.* (1938), Miller (1944), Grant *et al.* (1971a) and Cecil *et al.* (1973) have demonstrated degenerative liver changes, as well as an increase in liver size and lipid content in rats exposed to PCB's. Vos and Notenboom-Ram (1972) observed a similar increase in the weight of liver of rabbits exposed to Aroclor 1260. Although no significant increase in liver weights was noted in the mink that died while receiving diets that contained PCB's in this experiment, fat accumulation and other degenerative changes (as shown in Table 4) were evident.

The results of this experiment support the supposition that PCB's may be, at least in part, responsible for the reproductive failure and mortality observed when Lake Michigan coho salmon are fed to mink.

Experiment II

This experiment was started on August 24, 1971, to ascertain the effects of long-term, low-level consumption of PCB's on mink reproduction and viability and to investigate the possibility of interactions between PCB's and some chlorinated hydrocarbon pesticide contaminants of Great Lakes fishes.

Procedure

In this experiment, 30 natural dark female mink kits approximately three and one-half months of age were allocated into five groups and placed on the following dietary treatments.

Group II - 1 Unsupplemented, unmodified basal (control) diet

Group II - 2 Basal diet supplemented with 5 ppm Aroclor 1254

Group II - 3 Basal diet supplemented with 10 ppm Aroclor 1254

Group II - 4 Basal diet supplemented with 10 ppm Aroclor 1254 plus 10 ppm DDT

Group II - 5 Basal diet supplemented with 10 ppm Aroclor 1254 plus 0.5 ppm dieldrin

Individual mink body weights were recorded at the beginning of the feeding trial and at monthly intervals throughout the experiment (except during the gestation period).

Results

The average initial body weights and average weight gains of the mink on the various dietary treatments during the growth period are shown in Table 7.

Table 7. Average body weight change (gm ± S.E.) of female mink fed a control ration or experimental diets (Experiment II)

Dietary treatment	Initial wt. (Aug. 25)	Weight change 1 mo. (Sep. 24)	2 mo. (Oct. 25)	3 mo. (Nov. 25)	4 mo. (Dec. 22)	5 mo. (Jan. 24)	6 mo. (Feb. 24)
II - 1 (control) (n=6)	1020 a[2] (±60.8)	43 a (±17.4)	187 b (±29.6)	187 b (±46.2)	210 c (±35.1)	65 a (±65.3)	-192 a (±87.4)
II - 2 (5 ppm PCB)[1] (n=6)	940 a (±31.7)	62 b (±22.4)	153 b (±44.3)	138 ab (±42.7)	128 bc (±43.7)	80 a (±44.2)	-173 a (±45.0)
II - 3 (10 ppm PCB) (n=6)	923 a (±32.9)	43 a (±19.6)	148 b (±32.0)	132 ab (±26.3)	92 b (±35.1)	43 a (±31.7)	-155 a (±41.3)
II - 4 (10 ppm PCB + 10 ppm DDT) (n=6)	1052 a (±34.2)	43 a (±14.5)	57 a (±14.8)	45 a (±21.5)	-15 a (±35.4)	-30 a (±41.5)	-248 a (±46.7)
II - 5 (10 ppm PCB + 0.5 ppm dieldrin) (n=6)	940 a (±55.9)	108 b (±17.2)	133 b (±26.7)	102 ab (±18.9)	57 ab (±24.4)	-25 a (±26.9)	-252 a (±44.2)

[1]Aroclor 1254

[2]Values followed by same letter(s) under each date are not significantly different (P>0.05)

Five ppm supplemental PCB's (Group II - 2) in the ration did not significantly affect the weight gains of the mink. Ten ppm PCB's alone (Group II - 3) or in combination with 0.5 ppm dieldrin (Group II - 5) resulted in significant differences ($P<0.05$) in body weight gains after four months feeding. The mink fed the ration that contained 10 ppm PCB's plus 10 ppm DDT (Group II - 4), however, showed a significant reduction in weight gains after only two months feeding and weighed less than their initial body weights after 4 months on the diet.

No clinical signs of PCB or chlorinated hydrocarbon poisoning (other than the reduced weight gains) were observed in the treated animals during the growth period (Aug. 25 to Dec. 22). Considerable mortality, however, occurred on some diets during the maintenance and reproductive periods (after the animals had consumed the experimental rations for at least 160 days). None of the animals fed the ration supplemented with 10 ppm PCB plus 0.5 ppm dieldrin survived to the termination of the experiment (June 1, 1972), and only 1 of 6 mink survived on the diet that contained 10 ppm PCB. Although DDT in combination with PCB appeared to reduce mortality (Table 8), none of the females that survived to term on the rations that contained supplemental PCB whelped.

The PCB treated mink that survived to June 1, 1972, were sacrificed, their organs weighed, and selected tissues from those in Groups II - 2 and II - 3 were analyzed for PCB content. The PCB tissue residues and mean organ weights from these animals are shown in Tables 9 and 10. The mean weights of the kidneys, liver and heart of the PCB treated mink were significantly ($P<0.05$) greater than the controls.

Table 8. Reproductive performance and mortality of female mink fed a control ration or experimental diets (Experiment II)

Dietary treatment	Adult females			Kits			
	% mortality	No. mated	No. whelped	No. born Alive	No. born Dead	Whelped/♀ mated	No. alive at 4 wks.
II - 1 (control)	17	5	3	17	8	5.0	8
II - 2 (5 ppm PCB)[1]	33	4	-	-	-	-	-
II - 3 (10 ppm PCB)	83	1	-	-	-	-	-
II - 4 (10 ppm PCB + 10 ppm DDT)	33	4	-	-	-	-	-
II - 5 (10 ppm PCB + 0.5 ppm dieldrin)	100	1	-	-	-	-	-

[1] Aroclor 1254

Table 9. Average PCB content of tissue (ppm ± S.E.) from mink that died or were sacrificed[1] (Experiment II)

Dietary treatment	Tissue							
	Brain	Liver	Kidney	Spleen	Lung	Muscle	Heart	Adipose tissue
II - 1 (control) (n=1)[3]	N.D.[2]	N.D.	N.D.	N.D.	N.D.	N.D.	N.D.	--
II - 2 (5 ppm PCB)[4] (n=5)[5]	0.62 (±0.07)	36.39 (±8.04)	14.91 (±0.93)	2.88 (±0.71)	10.03 (±0.88)	13.30 (±3.10)	4.10 (±0.28)	139.59 (±7.88)
II - 3 (10 ppm PCB) (n=2)[3]	1.51	50.71	23.19	5.25	11.12	17.43	7.23	--

[1]Analyses by M.S.U. Pesticide Research Center

[2]None detected

[3]Died

[4]Aroclor 1254

[5]Sacrificed

Table 10. Mean organ weights[1] of female mink fed a control ration or experimental diets (Experiment II)

Dietary treatment	Spleen	Kidney	Liver	Heart
II - 1 (control) (n=4)	27.4 ± 11.09 a[2]	44.5 ± 2.34 a	220.4 ± 29.36 a	65.8 ± 7.86 a
II - 2 (5 ppm PCB)[3] (n=6)	47.6 ± 4.95 a	58.2 ± 4.63 b	440.8 ± 39.87 b	90.9 ± 16.04 b
II - 3 (10 ppm PCB) (n=6)	39.6 ± 8.23 a	57.8 ± 1.59 b	421.3 ± 18.68 b	86.9 ± 10.4 b
II - 4 (10 ppm PCB + 10 ppm DDT) (n=4)	51.0 ± 8.65 a	62.2 ± 4.63 b	579.5 ± 80.43 bc	97.1 ± 19.9 b
II - 5 (10 ppm PCB + 0.5 ppm dieldrin) (n=3)	30.9 ± 5.42 a	62.9 ± 5.06 b	466.6 ± 21.40 b	87.9 ± 7.71 b

[1]Expressed as percent of brain weight ± S.E.

[2]Values followed by the same letter(s) under each organ are not significantly different (P>0.05)

[3]Aroclor 1254

Discussion

Although no significant differences in body weight gains were observed in the short-term feeding trials (Experiments I and IV) involving PCB's, long-term administration of PCB's, as noted in this experiment, resulted in significant body weight changes among the various dietary treatments. The lack of significant differences in weight gains during the fifth and sixth months of the experiment may be due to the feeding regime employed to condition the animals for breeding. Based on these results it would appear that body weight changes are not a sensitive measurement of PCB exposure in mink.

As shown by the data presented in Table 7, there appeared to be an interaction between PCB's and the chlorinated hydrocarbon pesticides on the growth of the mink. In a study by Lichtenstein *et al.* (1969) it was found that adding PCB and DDT together produced 100% mortality in flies at concentrations that produced only slight mortality when the compounds were tested alone. In this experiment and Experiment IV (Table 12) adult mink mortality was not increased by the addition of DDT to the PCB's but in both experiments the addition of dieldrin to the PCB's resulted in increased mortality. Dahlgren *et al.* (1972) suggested that effects of PCB's and dieldrin together are additive, not synergistic. Whether or not reproduction would be influenced by the interaction or potentiation posted in the growth of the mink was not ascertained, as none of the PCB-treated females whelped.

The differences in the PCB tissue residues of the mink in Experiments I (Table 6) and II (Table 9) are marked and may be attributed to the different levels of PCB's fed, duration of feeding, as well as the fact that the PCB tissue residues in Experiment II were from sacrificed animals whereas those in Experiment I were from mink that died while

receiving diets that contained PCB's. Dahlgren *et al.* (1972) stated that brain levels of PCB were generally higher in birds that died than in birds that were sacrificed, as was also observed in mink in Experiment II (Table 9) in this study.

PCB's are fat soluble and persistent compounds that tend to accumulate in oils and fats (Anon., 1972b) and residues are usually found to be most abundant in the tissues with the greatest concentration of adipose tissue (Grant *et al.*, 1971a, Curley *et al.*, 1971, and Platonow *et al.*, 1972).

The significant differences in the organ (heart, kidneys, and liver) weights of the PCB-treated and control mink observed in Experiment II (Table 10) and the relative similarity in organ weight of the mink on the various treatments in Experiment I (Table 5) might be due to the variation in the length of the two feeding trials, the longer exposure to PCB in Experiment II being manifested by an increase in the organ weights as was reported in rats by Bennett *et al.* (1938), Grant *et al.* (1971a), Miller (1944), Cecil *et al.* (1973) and in rabbits by Vos and Notenboom-Ram (1972).

Experiment III

This experiment was conducted during December, 1971, in an attempt to determine the LD_{50} (lethal dose for 50% of the animals) of some common PCB's (Aroclors 1221, 1242 and 1254) for mink.

Procedure

Fifty adult mink not previously fed Great Lakes fish nor exposed to any supplemental PCB's or chlorinated hydrocarbon pesticides were allocated to the experiment.

The PCB's were administered intraperitoneally (IP) since oral administration of the compounds resulted in vomiting shortly after they were administered.

Results

The acute toxicity values (lethal dose for 50 percent of the animals within 96 hours after administration) of Aroclors 1221, 1242 and 1254 for mink are given in Table 11. The toxicity of the PCB's varied inversely with the chlorine content (last two digits of the Aroclor number) of the compounds. The values ranged from 500 mg/kg for Aroclor 1221 to as high as 2250 mg/kg for Aroclor 1254.

Discussion

Lewin and McBlain (1972) reported an acute IP LD_{50} (5 days) toxicity of PCB's in mice of 880 to 2840 mg/kg for Aroclor 1254.

Although some investigators (Risebrough and Brodin, 1970, and Prestt *et al.*, 1970) have reported a positive relationship between the percentage of chlorine in PCB's and their relative toxicity, the toxicity of the Aroclors tested with the mink in this study varied inversely with the percentage of chlorine in the compounds.

Table 11. Acute toxicity (LD_{50})[1] of PCB's when administered IP to mink (Experiment III)

PCB	LD_{50}
Aroclor 1221	> 500 < 750 mg/kg
Aroclor 1242	1000 mg/kg
Aroclor 1254	> 1250 < 2250 mg/kg

[1]Lethal dose for 50% of the animals within 96 hours.

Experiment IV

This experiment consisted of a series of dietary treatments involving PCB's, chlorinated hydrocarbon pesticides, and coho salmon fed to mink in an attempt to ascertain the effects of these compounds on mink reproduction and viability.

Procedure

The experiment was conducted from January 1, 1972, to May 10, 1972. Eight groups consisting of twelve adult female (proven breeder) mink per group were placed on the following dietary treatments:

Group IV - 1 Unsupplemented, unmodified basal (control) diet

Group IV - 2 Substitution of 30 percent 1971 Lake Michigan coho salmon for 30 percent ocean fish in the basal diet

Group IV - 3 Same as Group IV - 2 except that the coho salmon was cooked prior to substitution

Group IV - 4 Basal diet supplemented with 1 ppm Aroclor 1254

Group IV - 5 Basal diet supplemented with 5 ppm Aroclor 1254

Group IV - 6 Basal diet supplemented with 15 ppm Aroclor 1254

Group IV - 7 Basal diet supplemented with 5 ppm Aroclor 1254 plus 6 ppm DDT

Group IV - 8 Basal diet supplemented with 5 ppm Aroclor 1254 plus 6 ppm DDT plus 0.2 ppm dieldrin

The fish fed to the mink in Group IV - 3 were autoclaved at 120°C and 15 p.s.i. for 30 min. The diet fed to the mink in Group IV - 8 was formulated in an attempt to duplicate the PCB, DDT, and dieldrin content in the 30 percent Lake Michigan coho salmon fed to the animals in Group IV - 2.

Results

The reproductive performance and mortality of the mink on the various dietary treatments are summarized in Table 12. Supplementation of the control diet with 1 ppm PCB (Aroclor 1254) caused a slight reduction in reproduction; 5 ppm PCB resulted in a marked reduction in reproduction; and 15 ppm supplemental PCB totally inhibited reproduction and resulted in 31 percent adult mortality when fed for four months. Reproductive failure also occurred on the diets that contained 30 percent autoclaved coho salmon (Group IV - 3).

The average body weight change of mink fed the various dietary treatment in Experiment IV is shown in Table 13. There were no significant differences in mink weight gains among the dietary treatments.

Table 14 shows the mean organ weights (expressed as a percent of brain weight) of the mink in Group IV - 6 that died during the experiment, as well as the PCB content of their tissues.

Discussion

The reproductive performance of the females fed coho salmon in this experiment was similar to those fed coho salmon in Experiment I - 2, as none of the animals whelped. The reproductive failure of the females fed the autoclaved coho salmon (IV - 3) demonstrated that the toxic factor present in the fish is quite heat stable. Supplementing the basal diet with equivalent amounts of PCB, DDT and dieldrin, as contained in the diet fed to the mink in Group IV - 2, did not impair reproduction to the extent noted in the animals in Group IV - 2. The reproductive performance and mortality data of mink fed the diet that contained 5 ppm PCB (IV - 5) and 5 ppm PCB plus DDT (IV - 7) or DDT and dieldrin (IV - 8) indicated that PCB's are the major source of

Table 12. Reproductive performance and mortality of female mink fed a control ration or experimental diets (Experiment IV)

Dietary treatment	Adult females			Kits			
	% mortality	No. mated	No. whelped	No. born Alive	No. born Dead	Whelped/♀ mated	No. alive at 4 wks.
IV - 1 (control)	8.3	11	11	56	10	6.0	46
IV - 2 (30% raw coho salmon)	8.3	11	0	0	0	0	0
IV - 3 (30% cooked coho salmon)[1]	0	12	0	0	0	0	0
IV - 4 (1 ppm PCB)[2]	8.3	12	8	35	8	3.6	18
IV - 5 (5 ppm PCB)	0	12	3	3	6	0.8	2
IV - 6 (15 ppm PCB)	33.0	11	0	0	0	0	0
IV - 7 (5 ppm PCB + 6 ppm DDT)	0	11	4	5	6	1.0	0
IV - 8 (5 ppm PCB + 6 ppm DDT + 0.2 ppm dieldrin)	8.3	11	5	15	7	2.0	0

[1]Autoclaved at 120°C and 15 p.s.i. for 30 min.

[2]Aroclor 1254

Table 13. Average body weight change (gm ± S.E.) of female mink fed a control ration or experimental diets (Experiment IV)

Dietary treatment	Initial weight (Jan. 1)	Weight change 1 mo. (Feb. 3)	Weight change 2 mo. (Mar. 1)
IV - 1 (control) (n=12)	1034 ± 78.6	-38 ± 33.5	-200 ± 47.8
IV - 2 (30% coho salmon) (n=12)	996 ± 45.4	-58 ± 10.3	-130 ± 19.9
IV - 3 (30% autoclaved coho salmon) (n=12)	1028 ± 27.7	-60 ± 13.8	-142 ± 28.7
IV - 4 (1 ppm PCB)[1] (n=12)	1070 ± 49.5	-72 ± 51.3	-181 ± 30.7
IV - 5 (5 ppm PCB) (n=12)	979 ± 31.9	-33 ± 19.4	-179 ± 19.3
IV - 6 (15 ppm PCB) (n=12)	1058 ± 33.9	-91 ± 26.7	-219 ± 30.5
IV - 7 (5 ppm PCB + 6 ppm DDT) (n=12)	961 ± 60.5	-66 ± 24.5	-168 ± 32.6
IV - 8 (5 ppm PCB + 6 ppm DDT + 0.2 ppm dieldrin) (n=12)	1031 ± 45.3	-68 ± 15.5	-189 ± 29.8

[1]Aroclor 1254

Table 14. Mean organ weight[1] and mean PCB content[2] (ppm ± S.E.) of tissue from mink that died while receiving diets that contained 15 ppm supplemental PCB[3] (Experiment IV)

Dietary treatment	Tissue							
	Spleen	Kidney	Liver	Heart	Brain	Lung	Muscle	Adipose tissue
				Mean organ weight				
IV - 6 (15 ppm PCB) (n=5)	38.7 (±3.16)	66.0 (±4.00)	374.9 (±44.15)	90.8 (±14.69)				
				Mean PCB content				
IV - 6 (15 ppm PCB) (n=3)	5.37 (±1.26)	16.03 (±3.69)	50.51 (±5.42)	15.41	3.44 (±0.22)	15.10 (±3.56)	19.21 (±2.64)	233.39 (±14.07)

[1]Expressed as percent of brain weight ± S.E.

[2]PCB analyses by the M.S.U. Pesticide Research Center

[3]Aroclor 1254

reproductive complications in mink and that the pesticides (DDT and dieldrin) did not appear to have a synergistic effect on mink reproductive performance, as was suggested by mink growth in Experiment II.

Many pesticides are known to stimulate the synthesis of the hydroxylating liver enzymes which aid in the digestion of foreign compounds (Morello, 1965). These enzymes, however, also catabolize endogenous substances, such as the steroid hormones, which may influence sexual function by disturbing the metabolism of the sex hormones (Peakall, 1967). Like the organochlorine insecticides, PCB's have also been shown to induce hepatic hydroxylating enzymes (Risebrough *et al.*, 1968; Lincer and Peakall, 1970; and Villeneuve *et al.*, 1971a).

Following the administration of high dosages of PCB's to boars, Platonow *et al.* (1972) noted lowered levels of dehydroepiandrosterone and estrogen in the excreted urine and also suggested that PCB compounds have a deleterious effect upon reproductive activity. Studying the effects of PCB's and DDT on the estrus cycle of mice, Orbert *et al.* (1972) reported single injections of DDT or PCB's increased the length of the cycle, resulting in less frequent periods of sexual receptivity in the female, thereby causing a decline in the reproductive capacity of the animal.

In a study by Villeneuve *et al.* (1971b) it was demonstrated that Aroclors 1221 and 1254 administered orally to female rabbits during gestation crossed the placental barrier. Grant *et al.* (1971b) and Villeneuve *et al.* (1971b) reported Aroclor 1254 induced abortion and was fetotoxic to rabbits.

Undoubtedly the adverse physiological effects on reproduction induced by PCB's, DDT and dieldrin could also account for the reproductive impairment observed in mink fed these compounds.

Based on the results of the experiments conducted in this study, adult mink mortality on diets that contained PCB's was in general directly proportional to the total intake of these compounds. Assuming the mink that died in Experiments II, IV and V had consumed 150 g of feed per day (Schaible, 1969) and weighed 700 g, the oral lethal dose (LD_{100}) of Aroclor 1254 was calculated to be about 470 mg/kg, and the oral LD_{50} was approximately 350 mg/kg (Figure 2). An oral LD_{50} of Aroclor 1254 for rats was reported by Tucker and Crabtree (1970) to be 500 to 1000 mg/kg. According to Miller (1944), two oral doses of 69 mg of 42 percent chlorinated biphenyl a week apart were fatal to guinea pigs.

Although it may be inexpedient to compare the toxicity of compounds under different experimental conditions, it would appear from these results that mink are quite sensitive to PCB's.

Experiment V

This long-term feeding trial was conducted to investigate and compare the effects of four different PCB's fed at a low level on growth, reproduction, hematologic parameters, and viability of mink.

Procedure

This experiment was initiated on August 8, 1972. Fifty pastel mink kits approximately three months of age were allocated into five groups (consisting of 10 females per group) and were fed a basal diet supplemented or modified as follows:

Group V - 1 Unsupplemented, unmodified basal (control) diet

Group V - 2 Basal diet supplemented with 2 ppm Aroclor 1016

Group V - 3 Basal diet supplemented with 2 ppm Aroclor 1221

Group V - 4 Basal diet supplemented with 2 ppm Aroclor 1242

Figure 2. Relationship between consumption of Aroclor 1254 and mortality in mink. Calculation based on mortality data from Experimental groups II - 2, II - 3, IV - 4, IV - 6 and V - 5 and an assumed feed consumption of 150 g/day.

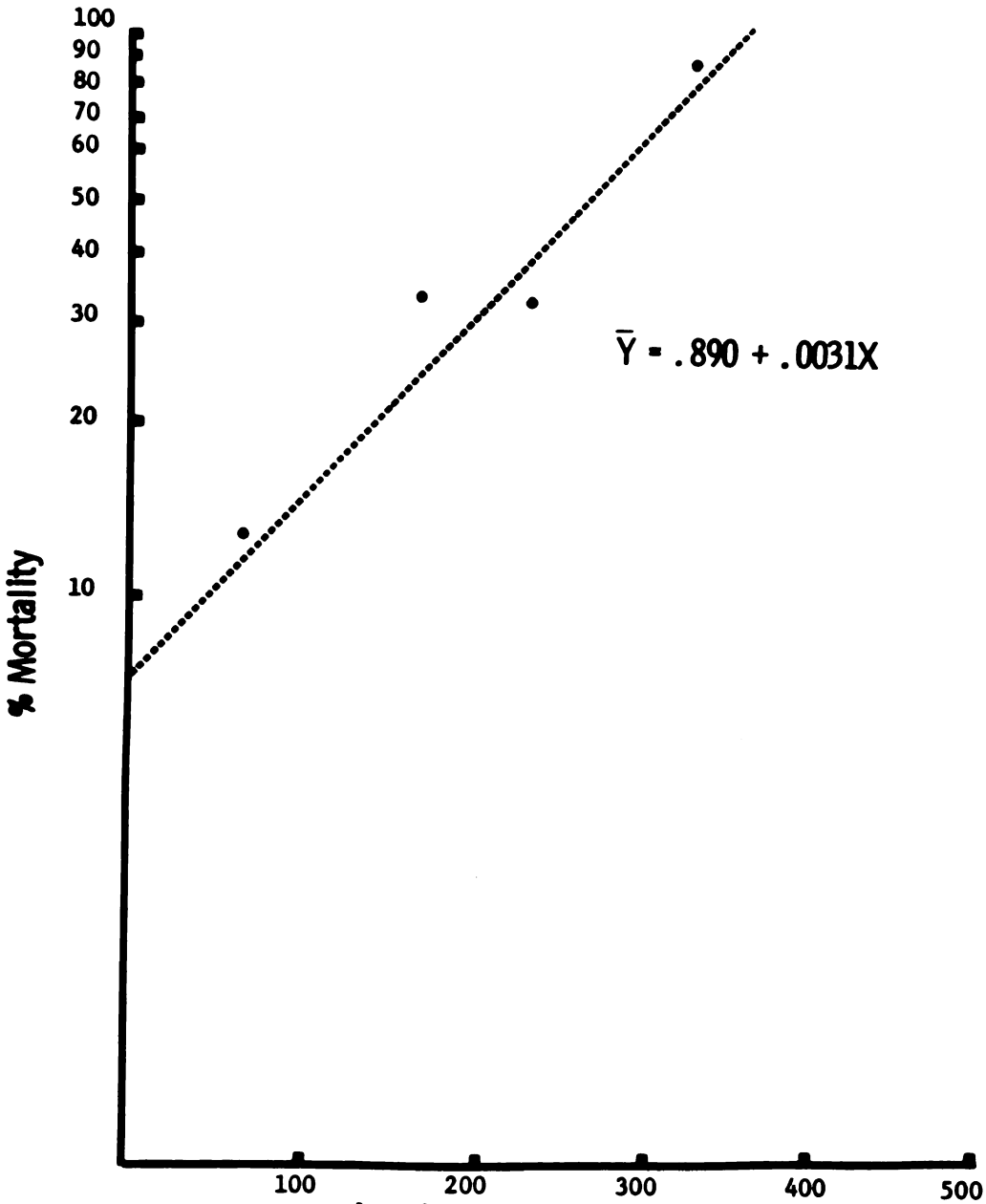
100
90
80
70
60
50
40
30
20
10
% Mortality
$\bar{Y}$ = .890 + .0031X
100
200
300
400
500
Aroclor 1254 consumption (mg.)

Figure 2

Group V - 5 Basal diet supplemented with 2 ppm Aroclor 1254

Females that did not whelp by May 30, 1973, were removed from the experimental diets; those that whelped were continued on the experimental rations until their kits were four weeks old.

Samples of blood were collected from the mink (by clipping a toenail) at the beginning of the trial and at monthly intervals thereafter (except during April). These samples were analyzed for hemoglobin content (acid hematin method, Dennington and Lucas, 1955) and hematocrit values.

On June 1, 1973, three female mink fed each of the diets in Experiment V were sacrificed, the organs weighed, and selected tissue collected for PCB residue analyses.

Results

The average initial body weights and average weight gains (through Feb. 19, 1973) of the female mink are shown in Table 15. Although the weight gains of the mink fluctuated considerably during the course of the experiment, no significant differences were noted except during the first month after the start of the feeding trial. After November, 1972, a trend toward reduced weight gains was observed in the groups fed supplemental PCB's while the control animals maintained a fairly constant body weight, except just prior to the breeding season (March) when all animals were fed to "condition" them for optimum reproduction (thin mink continued to be fed *ad libitum* but obese females received restricted amounts of feed).

The average hematocrit values for the mink are shown in Table 16. The hematocrit values for all the groups dropped considerably after the whelping period (April 24-May 15). The average hemoglobin content and

Table 15. Average body weight change (gm ± S.E.) of female mink fed a control ration or experimental diets (Experiment V)

Dietary treatment	Initial weight Aug. 7	Weight change 1 mo. (Sep. 1)	2 mo. (Oct. 1)	3 mo. (Nov. 17)	4 mo. (Dec. 15)	5 mo. (Jan. 22)	6 mo. (Feb. 19)
V - 1 (control) (n=8)	799 a (±41.6)	128 b[1] (±25.8)	225 a (±40.1)	209 a (±51.8)	213 a (±52.1)	204 a (±55.9)	233 a (±48.4)
V - 2 (2 ppm Aroclor 1016) (n=8)	780 a (±37.5)	126 b (±27.7)	243 a (±41.5)	288 a (±35.4)	173 a (±35.9)	139 a (±27.4)	145 a (±18.9)
V - 3 (2 ppm Aroclor 1221) (n=8)	803 a (±27.6)	76 a (±14.7)	216 a (±34.7)	263 a (±47.2)	177 a (±45.8)	130 a (±41.7)	116 a (±45.3)
V - 4 (2 ppm Aroclor 1242) (n=8)	753 a (±34.1)	88 ab (±18.8)	196 a (±37.0)	235 a (±36.2)	144 a (±44.7)	91 a (±54.5)	91 a (±48.4)
V - 5 (2 ppm Aroclor 1254) (n=8)	774 a (±21.1)	120 b (±28.1)	176 a (±29.9)	195 a (±20.1)	135 a (±11.9)	120 a (±21.4)	116 a (±25.0)

[1]Values followed by same letter(s) under each date are not significantly different (P>0.05)

Table 16. Hematocrit values for mink fed a control diet and rations

Dietary treatment	Initial (Aug. 7)	1 mo. (Sept. 5)	2 mo. (Oct. 2)	Average 3 mo. (Oct. 30)
V - 1 control (basal diet)	51.8 (±0.82)	56.0 (±0.22)	56.3 (±0.48)	57.5 (±0.58)
V - 2 2 ppm Aroclor 1016	51.1 (±0.81)	54.8 (±0.88)	54.6 (±0.91)	56.2 (±0.87)
V - 3 2 ppm Aroclor 1221	51.7 (±0.76)	55.5 (±0.81)	55.0 (±0.67)	56.8 (±0.59)
V - 4 2 ppm Aroclor 1242	51.5 (±1.06)	53.8 (±0.76)	54.1 (±0.74)	54.2 (±2.43)
V - 5 2 ppm Aroclor 1254	51.0 (±0.65)	55.0 (±1.06)	54.7 (±0.65)	56.5 (±0.66)

containing supplemental PCB's (Experiment V)

hematocrit values (% ± S.E.)					
4 mo. (Nov. 27)	5 mo. (Dec. 27)	6 mo. (Jan. 22)	7 mo. (Feb. 19)	8 mo. (Mar. 19)	10 mo. (May 15)
59.7 (±0.41)	55.4 (±0.80)	57.1 (±0.89)	57.9 (±0.88)	57.3 (±0.74)	52.4 (±2.06)
60.4 (±0.80)	58.1 (±0.54)	58.1 (±1.18)	56.5 (±0.78)	58.9 (±0.76)	54.2 (±1.21)
59.8 (±1.01)	56.4 (±0.70)	58.9 (±0.72)	54.8 (±2.83)	58.1 (±1.06)	51.3 (±2.46)
56.4 (±1.19)	53.7 (±2.92)	51.9 (±3.33)	56.4 (±1.33)	57.8 (±1.26)	52.6 (±1.47)
56.5 (±0.56)	54.8 (±1.10)	57.8 (±1.36)	57.1 (±1.02)	56.1 (±0.71)	52.5 (±1.06)

mean corpuscular hemoglobin concentration for the animals at monthly intervals are shown in Table 17.

The reproductive performance, adult mortality and average kit weights at birth are shown in Table 18. The one live kit whelped by the females fed Aroclor 1254 weighed much less than the average weight of the kits whelped by females on the other rations (Table 18). Three adult female mink on Experiment V died during the study, one each from Groups V - 3, V - 4 and V - 5. There were no statistical significant differences among the diet or organ weight (Table 19).

Discussion

Based on the previous mentioned assumption concerning feed consumption, the female mink in Group IV - 4 (1 ppm PCB; Experiment IV) consumed about 18 mg of Aroclor 1254 during the four months they were on experiments prior to whelping, which did not significantly impair reproduction. The females in Groups IV - 5 (5 ppm PCB; Experiment IV) and V - 5 (5 ppm PCB), however, consumed approximately 90 and 81 mg of Aroclor 1254, respectively, during the same period which severely impaired reproduction. As noted in the results of these experiments (Table 18), Aroclor 1016, 1221 and 1242 did not adversely affect reproduction, which is in agreement with the results of studies by Villeneuve *et al.* (1971a and 1971b). Koller and Zinkl (1973) also reported Aroclor 1221 was less toxic than Aroclor 1254 and did not induce enzyme activity in fetuses or placentas of rats and rabbits.

The reduced weight of the kits whelped by the only female that reproduced in Group V - 5 is in agreement with the work of Villeneuve (1971a), who reported a significant reduction in litter weights of rabbits fed Aroclor 1254 at 100 mg/kg/day.

Table 17. Average hemoglobin (Hb) content and mean corpuscular hemoglobin supplemental PCB's (Experiment V)

Dietary treatment		Initial (Aug. 7)	1 mo. (Sept. 5)	2 mo. (Oct. 2)	Average hemo- 3 mo. (Oct. 30)
V - 1 control (basal diet)	Hb	18.6±0.47	10.0±0.22	20.1±0.19	20.4±0.33
	MCHC	35.9±0.70	35.6±0.33	35.6±0.19	35.4±0.36
V - 2 2 ppm Aroclor 1016	Hb	18.6±0.49	19.5±0.20	19.4±0.24	20.6±0.34
	MCHC	36.3±0.53	35.8±0.35	35.6±0.32	36.7±0.25
V - 3 2 ppm Aroclor 1221	Hb	17.8±0.40	19.9±0.33	19.4±0.24	21.0±0.13
	MCHC	34.4±0.59	35.8±0.89	35.3±0.73	37.0±0.40
V - 4 2 ppm Aroclor 1248	Hb	18.6±0.24	19.3±0.25	19.6±0.36	20.4±0.93
	MCHC	36.1±0.41	35.9±0.20	36.2±0.33	37.3±0.39
V - 5 2 ppm Aroclor 1254	Hb	18.9±0.41	20.1±0.29	20.1±0.18	20.4±0.20
	MCHC	37.1±0.73	36.6±0.43	36.8±0.28	36.0±0.28

concentration (MCHC) for mink fed a control diet and rations containing

globin (g % ± S.E.) and MCHC (% ± S.E.)

4 mo. (Nov. 27)	5 mo. (Dec. 27)	6 mo. (Jan. 22)	7 mo. (Feb. 19)	8 mo. (Mar. 19)	10 mo. (May 15)
20.9±0.20	20.4±0.32	20.2±0.32	20.3±0.35	20.5±0.51	18.2±0.96
34.9±0.34	36.8±0.34	35.4±0.20	35.1±0.18	35.8±0.15	34.8±0.46
20.8±0.27	20.9±0.34	20.4±0.43	20.1±0.32	20.6±0.29	19.6±0.36
34.4±0.31	36.0±1.17	35.0±0.18	35.5±0.32	35.0±0.27	36.3±0.43
21.4±0.53	20.5±0.28	20.5±0.29	19.2±1.11	20.7±0.36	18.3±0.34
35.8±0.66	36.3±0.66	34.7±0.34	35.0±0.46	35.7±0.21	36.2±1.73
20.1±0.48	20.0±1.02	18.2±1.21	19.8±0.55	20.4±0.53	18.2±0.64
35.6±0.39	37.5±0.36	35.0±0.34	35.1±0.36	35.3±0.13	34.9±0.25
20.5±0.34	20.3±0.33	20.4±0.41	20.1±0.43	19.9±0.30	17.7±0.52
36.1±0.44	37.1±0.27	35.4±0.28	35.2±0.65	35.5±0.24	33.7±0.48

Table 18. Reproductive performance and mortality of female mink fed a control ration or experimental diets (Experiment V)

Dietary treatment	Adult females			Kits				
	% mortality	No. mated	No. whelped	No. born Alive	No. born Dead	Whelped/♀ mated	No. alive at 4 wks.	Average wt. (g ± S.E.) at birth
V - 1 (control)	0	8	8	29	5	4.3	29	9.9 ± 0.32
V - 2 (2 ppm PCB Aroclor 1016)	0	8	8	28	8	4.5	28	9.2 ± 0.33
V - 3 (2 ppm PCB Aroclor 1221)	12	7	7	43	1	6.3	43	9.6 ± 0.22
V - 4 (2 ppm PCB Aroclor 1242)	12	7	7	35	4	5.6	35	9.3 ± 0.27
V - 5 (2 ppm PCB Aroclor 1254)	12	7	2	1	1	0.3	1	5.4

Table 19. Mean organ weight[1] of mink fed a control diet or experimental rations (Experiment V)

Dietary treatment	Organ			
	Spleen	Kidney	Liver	Heart
V - 1 (control) (n=5)	35.7 (±3.38)	58.6 (±2.84)	260.4 (±18.11)	68.8 (±1.26)
V - 2 (2 ppm PCB Aroclor 1016) (n=3)[2]	30.1 (±3.15)	58.9 (±4.21)	280.7 (±22.54)	61.8 (±4.74)
V - 3 (2 ppm PCB Aroclor 1221) (n=3)[3]	27.5 (±1.28)	58.5 (±2.37)	331.6 (±20.45)	65.2 (±2.36)
V - 4 (2 ppm PCB Aroclor 1242) (n=3)[2]	29.6 (±1.26)	57.2 (±2.81)	276.6 (±22.07)	70.9 (±5.71)
V - 5 (2 ppm PCB Aroclor 1254) (n=4)[3]	31.9 (±3.32)	52.8 (±2.47)	313.9 (±14.64)	65.3 (±1.99)

[1]Expressed as percent of brain weight ± S.E.

[2]Sacrificed

[3]One mink died, the rest were sacrificed

Koller and Zinkl (1973) reported that hematological values in rats and rabbits were not altered by exposure to PCB's, which is consistent with the results of this experiment.

Experiment VI

This experiment was conducted to determine the rate of PCB accumulation and depletion in adipose tissue of mink.

Procedure

The experiment was conducted from August 8, 1972, through December 26, 1972. Nine natural dark male mink kits were fed a basal ration supplemented with 5 ppm Aroclor 1254 from August 8, 1972, through October 17, 1972. From October 18, 1972, through December 26, 1972, the mink were fed a basal (control) diet that contained no supplemental PCB's. Three males were fed the basal (unsupplemented) diet from October 3, 1972, through December 26, 1972, and served as controls.

At biweekly intervals the mink were anesthetized (CI-744)* and an incision made in the inguinal area. A sample (1 g) of adipose tissue was removed and the incision stitched closed. The biopsies were taken alternately from each side of the inguinal area.

The fat samples were stored frozen (0°C) in glass vials prior to analysis for PCB residues by the Michigan State University Pesticide Research Center.

Results

The average PCB residues recovered from the adipose tissue samples are shown in Table 20 and Figure 3. Mink fed 5 ppm Aroclor 1254 in

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*Experimental anesthetic, Parke, Davis & Company, Detroit, MI.

Table 20. Average PCB residues in adipose tissue of mink fed a control diet or a ration that contained 5 ppm supplemental Aroclor 1254 (Experiment VI)

Dietary treatment	No. mink	Avg. PCB[1] residue (ppm ± S.E.) in adipose tissue										
		Aug. 8	Aug. 21	Sept. 5	Sept. 19	Oct. 3	Oct. 17	Oct. 31	Nov. 14	Nov. 28	Dec. 11	Dec. 26
Basal diet supplemented with 5 ppm Aroclor 1254	9	1.4 ±0.77	40.8 ±5.71	72.4 ±6.27	99.1 ±9.06	125.8 ±6.35	128.9 ±7.82					
Removed from PCB supplemental diet Oct. 17, 1973, and fed basal (control) diet through Dec. 26, 1973	9							118.2 ±7.03	94.0 ±5.19	76.6 ±5.04	65.7 ±5.08	61.1 ±5.76
Basal (control) diet	3					N.D.[2]	0.2 ±0.21	0.2 ±0.15	0.1 ±0.08	N.D.	N.D.	N.D.

[1]Aroclor 1254

[2]None detected

Figure 3. Accumulation and depletion of PCB in adipose tissue of mink fed 5 ppm Aroclor 1254 (Experiment VI).

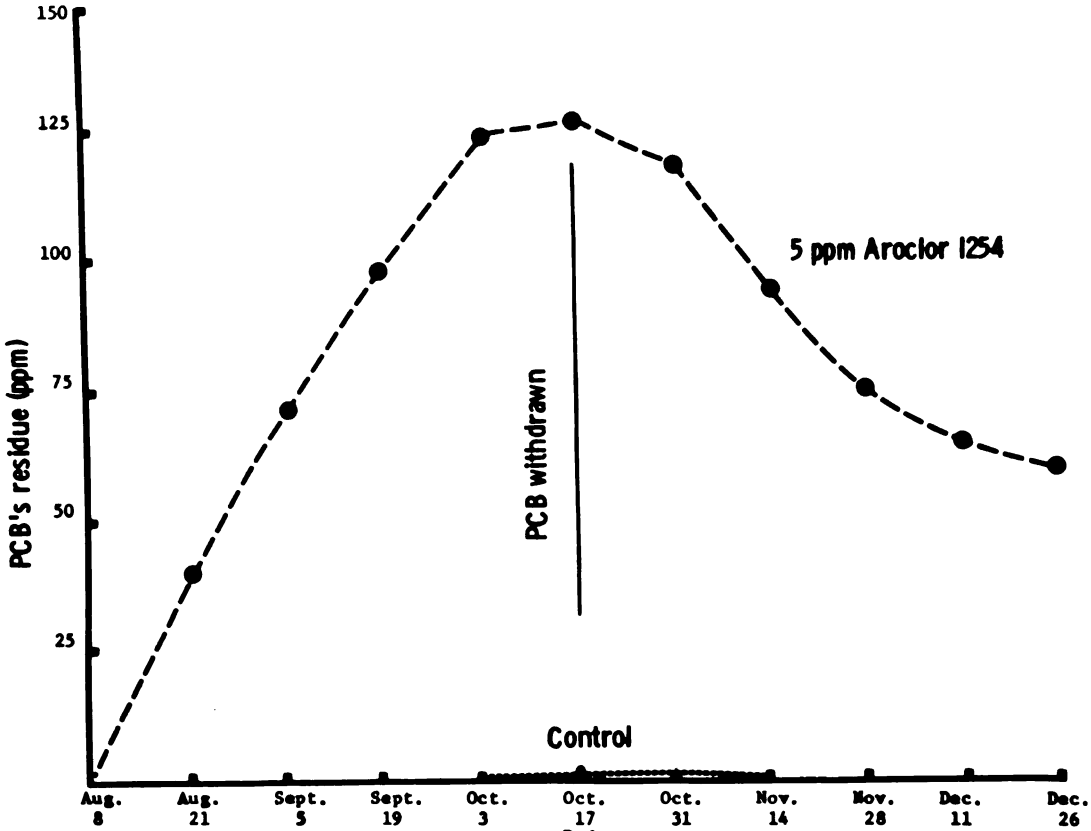
150
125
100
75
50
25
PCB's residue (ppm)
5 ppm Aroclor 1254
PCB withdrawn
Control
Aug. 8
Aug. 21
Sept. 5
Sept. 19
Oct. 3
Oct. 17
Oct. 31
Nov. 14
Nov. 28
Dec. 11
Dec. 26
Date

Figure 3

the diet rapidly accumulated PCB residues in the fat during the first 8 weeks. At about the eighty week a plateau of approximately 125 ppm in PCB residue was reached.

Upon withdrawal of the PCB's from the diet (Oct. 18, 1972) the residues in the adipose tissue decreased. A 50% reduction in PCB tissue residues was reached about the eighth week after withdrawal.

Discussion

Curley *et al.* (1971) suspected that the PCB levels in adipose tissue of rats fed Aroclor 1254 for 8 weeks were not representative of steady state values because after 240 days on the diet the animals stored substantially more PCB in their adipose tissue. Although the mink in this experiment accumulated approximately 125 ppm of PCB in the adipose tissue when the level plateaued at 8 weeks, the mink in Experiment IV that received 15 ppm Aroclor 1254 stored about 250 ppm of PCB in their adipose tissue.

The PCB tissue residue depletion pattern was similar to that reported for the chicken by Scott *et al.* (1971).

Experiment VII

This experiment was conducted to substantiate the results of previous experiments in which PCB's were implicated as the primary cause of reproductive problems associated with the feeding of Great Lakes fish to mink, and evaluate acetone-hexane extraction of coho salmon as a practical method of removing PCB's from these fish to render them safe for mink feed.

Procedure

The experiment was initiated January 25, 1973. Thirty female mink (proven breeders) were randomly divided into three groups consisting of 10 mink per group. They were fed a basal mink ration (Table 1)

supplemented or modified as follows:

Group VII - 1 Basal diet consisting of 30% ocean fish extracted with acetone and hexane and the supernatant and residue then added back to the diet (control)

Group VII - 2 Substitution of the supernatant from the equivalent of 30% acetone-hexane extracted Lake Michigan coho salmon for the supernatant from 30% acetone-hexane extracted ocean fish in the basal diet

Group VII - 3 Substitution of the residue from the equivalent of 30% acetone-hexane extracted Lake Michigan coho salmon for the residue from 30% acetone-hexane extracted ocean fish in the basal diet

The fish used in these diets were extracted in 15 kg quantities by mixing in a 1:1 ratio (by volume) with acetone for 24 hrs. The acetone fish mixture was then put through a press to remove the supernatant from the residue. The supernatant (extract) was mixed with 5 kg of commercial mink cereal and the acetone evaporated off under a hood and the cereal-extract mixture was saved. The residue from the acetone extraction was then mixed for 24 hours with an equal volume of hexane and the mixture was pressed to separate the supernatant from the residue. The residue was air-dried and the supernatant was combined with 5 kg of cereal and the hexane evaporated from the mixture under a hood. The acetone and hexane extracted portions of the fish (combined with the cereal) and the residue from the extractions were then incorporated with the other dietary ingredients (Table 1) to provide the diets outlined above.

Result

The reproductive performance of the female mink is shown in Table 21. The extraction process with acetone and hexane did not adversely affect reproduction as evidenced by an average of 4.1 kits whelped per female mated when ocean fish was extracted and used in the diet. The mink fed the diet that contained the coho salmon acetone and hexane extract (Group VII - 2) showed impaired reproduction, as only five kits were whelped. These kits were dead at birth or died within 24 hours postpartum. The diet that contained the residue from the acetone-hexane of the coho salmon (Group VII - 3) supported satisfactory reproduction.

Discussion

The results of this experiment indicate that the toxic factor(s) present in coho salmon is stored primarily in the fat and can be removed from the fish by acetone-hexane extraction.

As previously discussed (Experiment II) PCB's are fat soluble compounds which are stored predominantly in adipose tissue. In addition to removing PCB's from the fish, the acetone-hexane extraction procedure should have also removed other potentially harmful chlorinated hydrocarbon contaminants, such as DDT and dieldrin, since the acetone-hexane extraction employed in this experiment is similar to the procedure used by the M.S.U. Pesticide Research Center to remove PCB from tissues for residue analysis (Zabik, 1972).

Experiment VIII

This experiment was conducted to ascertain the mode of action of PCB's and the factor(s) present in Lake Michigan coho salmon responsible for mortality and reproductive complications in mink.

Table 21. Reproductive performance and mortality of female mink fed a control ration or experimental diets (Experiment VII)

Dietary treatment	Adult females			Kits			
	% mortality	No. mated	No. whelped	No. born Alive	No. born Dead	Whelped/♀ mated	No. alive at 4 wks.
VII - 1 Ocean fish ext. + ocean fish res. (control)	0	10	8	37	4	4.1	37
VII - 2 Coho salmon ext. + ocean fish res.	20	9	2	0	5	0.6	0
VII - 3 Ocean fish ext. + coho salmon res.	0	10	9	33	14	4.7	33

Procedure

The experiment was conducted from January 19, 1973, to May 31, 1973. Thirty-six female mink were allocated into two groups, and were fed a basal diet (Table 1) supplemented or modified as follows:

Group VIII - 1 Substitution of 30% Lake Michigan coho salmon for 30% ocean fish in the basal diet

Group VIII - 2 Basal diet supplemented with 5 ppm Aroclor 1254

Three of the 18 females in each group were placed on each of the dietary treatments at the beginning of the experiment. At 10 day intervals, three additional females from each group received the experimental diets. The mink were weighed and blood and urine samples were collected from the animals at 10 day intervals. Beginning March 1 the females were mated with males fed the basal (control) diet. The urine samples were analyzed for pH, protein, glucose and ketones using Labstix^{R}* reagent strips. Specific gravity was determined with a refractometer.** The blood samples were used to monitor liver and kidney function and were analyzed for serum alkaline phosphatase (SAP),*** serum glutamic-pyruvic transaminase (SGPT)† and blood urea nitrogen (BUN).††

Results

The urine analyses (Table 22) showed no significant changes in the parameters measured in the mink on either dietary treatment during the

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*Ames Company, Division Miles Lab., Inc., Elkhart, IN.

**Sargent-Welch Scientific Co., Detroit, MI.

***Sigma 104^{R}, Sigma Chemical Co., St. Louis, MO.

†Sigma 505^{R}, Sigma Chemical Co., St. Louis, MO.

††Urograph^{R}, Warner Chilcott Lab., Morris Plains, NJ.

Table 22. Urine analysis values at 10-day intervals for mink fed a diet that contained 30% coho salmon or 5 ppm Aroclor 1254 (Experiment VIII)

Dietary treatment	Parameters	Days on dietary treatment				
		Initial	10 days	20 days	30 days	40 days
VIII - 1 (30% Lake Mich. coho salmon)	pH[1]	5.0	5.0	5.0	6.0	6.0
	Specific gravity	--	1.109	1.063	1.083	1.088
	Protein[1] (mg/100 ml)	30.0	30.0	38.3	39.2	58.0
	Glucose[1]	trace amounts				
	Ketones[1]	trace amounts				
	Blood[1]	values varied from trace to moderate amts.				
VIII - 2 (5 ppm Aroclor 1254)	pH[1]	5.0	6.0	5.7	6.0	6.0
	Specific gravity[2]	1.037	1.066	1.059	--	--
	Protein[1] (mg/100 ml)	50.0	30.0	22.5	31.0	--
	Glucose[1]	trace amounts				
	Ketones[1]	trace amounts				
	Blood[1]	values varied from trace to moderate amts.				

[1]Determined by Labstix[R] reagent strips, Ames Co., Elkhart, IN.

[2]Determined by refractometer - TS meter, Sargent-Welch Scientific Co., Detroit, MI.

course of the experiment. The results of the blood analyses are shown in Table 23. No changes were noted in the BUN or SAP values for the animals fed either diet during the trial. The BUN values noted for the mink were within the 10 to 30 mg/100 ml concentrations usually considered as normal in the dog (Bernstein, 1965). The SGPT values, however, increased in both the PCB and coho salmon fed mink in direct proportion to the length of time the animals were fed the experimental diets.

Although the last three females in each group were placed on the dietary treatments during the mating period (March 1-24), only one female from Group VIII - 1 whelped, producing one kit. This female had received the experimental ration containing 30 percent coho salmon for 67 days prior to whelping. Two females from Group VIII - 2 (5 ppm PCB) whelped seven kits. Five of the kits, however, died prior to weaning. The females that whelped in Group VIII - 2 had received the PCB supplemented ration for an average of 58 days prior to whelping.

Discussion

As noted in the results, all tests conducted to monitor kidney function showed no adverse effects on the kidneys of the mink fed either coho salmon or PCB. These results support the findings of Koller and Zinkl (1973) who reported no changes in BUN levels of rabbits treated orally with 300 mg of Aroclors 1221, 1242 or 1254 weekly for 14 weeks.

The use of SAP values as a diagnostic test of liver function has been described by Bodansky *et al.* (1933), Bessey *et al.* (1946), Bloom (1957) and Kaneko and Corneluis (1970). Hoe (1961b) reported that increased SAP levels were associated with hepatic neoplasms, hepatitis and fatty degeneration of the liver. Although no changes were observed in the SAP levels in the mink during this experiment, the values were

Table 23. Average serum alkaline phosphatase (SAP), serum glutamic-pyruvic transaminase (SGPT) and blood urea nitrogen (BUN) levels at 10-day intervals for mink fed a diet containing 30% coho salmon or 5 ppm Aroclor 1254 (Experiment VIII)

Dietary treatment	Analysis	Days on dietary treatment				
		Initial	10 days	20 days	30 days	40 days
VIII - 1 30% Lake Mich. coho salmon	SAP[1] (sigma units/ml)	1.87	1.56	1.89	2.05	--
	SGPT[2] (units/ml)	46.5	57.0	79.0	104.7	114.0
	BUN[3] (mg/100 ml)	26.7	21.7	15.0	25.0	22.5
VIII - 2 5 ppm Aroclor 1254	SAP[1] (sigma units/ml)	1.86	1.87	1.83	1.49	1.72
	SGPT[2] (units/ml)	48.0	49.9	79.4	125.8	33.3
	BUN[3] (mg/100 ml)	17.5	15.8	22.0	17.0	20.0

[1]Sigma 104 procedure, Sigma Chemical Co., St. Louis, MO.

[2]Sigma-Frankel procedure, Sigma Chemical Co., St. Louis, MO.

[3]Urograph procedure, Warner-Chilcott Labs., Morris Plains, NJ.

considerably greater than the normal levels reported for the dog (0.05-0.55 Sigma units/ml) by Van Vleet and Albert (1968), but within the normal range (0.8-2.3 Sigma units/ml) for man (Anon., 1963).

The use of serum transaminase activity was first employed for the diagnosis of hepatic necrosis by Wroblewski and La Due (1955). According to Hoe (1961a), transamination activity, especially SGPT, can play an important part in the diagnosis of active liver cell damage and necrosis before any abnormalities are shown by other liver function tests. In this experiment the SGPT values of the mink in both Groups VIII - 1 and VIII - 2 doubled within 30 days after the animals were placed on the dietary treatments. These elevated SGPT levels (see Table 23) were in the range (50-400 units/ml) of values indicative of moderate liver necrosis in dougs (Kaneko and Cornelius, 1970). Vos and Notenboom-Ram (1972) observed microscopic liver lesions and elevated serum transaminase levels in rabbits treated with Aroclor 1260. Koller and Zinkl (1973) reported elevated SGPT and serum glutamic-oxalacetic transaminase (SGOT) in rabbits treated with Aroclors 1242 and 1254 but not with Aroclor 1221.

The similarity in results obtained with these liver and kidney function tests in rabbits and mink suggest that the mode of action of PCB's in these species are similar. The results of this trial also support the theory that the toxic factor contained in Great Lakes fish and responsible for the reproductive failure and mortality in mink is PCB's.

Experiment IX

This experiment was conducted to ascertain whether or not the reproductive failure attributed to the feeding of coho salmon and PCB's to mink was of a permanent nature.

Procedure

Eleven adult females that received a ration that contained 30 percent whole, raw, Lake Michigan coho salmon (Group IV - 2) and three adult female that were fed a diet supplemented with 5 ppm PCB 1254 (Group IV - 5) during 1972 and failed to whelp were placed on a basal (control) diet (Table 1) on June 2, 1972, and retained on this ration through the gestation period in 1973. These females were mated with males (fed the basal diet) during March 1973.

Results

The reproductive performance of the females during 1973 is shown in Table 24. All three of the females previously fed 5 ppm Aroclor 1254 whelped and the average litter size (4.3) was comparable to that obtained from control animals in previous experiments. Seven out of the 11 females previously fed the coho salmon diet whelped. The average litter size per female mated was 3.5. Excessive early kit mortality was not noted in either group.

Discussion

Based on the results obtained in Experiment VI which showed a 50 percent reduction of PCB residues from adipose tissue eight weeks after PCB withdrawal from the diet, one would expect a one year withdrawal period, as employed in this experiment, would eliminate almost all PCB residues from the mink's bodies. The lesions and cellular changes ascribed to PCB exposure in mink, as discussed in the previous experiments, were also apparently not irreparable.

These results of this experiment further suggest that PCB contamination of Great Lakes fish is probably the primary cause of the reproductive and mortality problems associated with the feeding of these fish to mink

and indicated that PCB (Aroclor 1254) exposure does not permanently impair reproduction in mink.

Table 24. 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 (Experiment IX)

Dietary treatment Jan. 1, '72- Jun. 1, '72	Dietary treatment Jun. 2, '72- Jun. 1, '73	No. ♀ mated (March 1973)	No. ♀ whelped (May 1973)	Avg. litter size/♀ mated
IX - 1 30% Lake Mich. coho salmon	Basal (control) diet	11	7	3.5
IX - 2 5 ppm supplemental Aroclor 1254	Basal (control) diet	3	3	4.3

CONCLUSIONS

This study consisted of a series of experiments conducted to investigate reproductive complications and mortality in mink associated with the use of Great Lakes fish in mink diets and to isolate the factor(s) present in the fish that are responsible for this problem. The following conclusions are based on the results of these experiments.

1. Feeding Lake Michigan coho salmon at 30% of the diet to mink resulted in reproductive failure and/or mortality.

2. PCB's are extremely toxic to mink.

3. The clinical signs and lesions (anorexia, bloody stools, fatty degeneration of the liver and kidneys and hemorrhagic gastric ulcers) observed in mink fed PCB's and Lake Michigan coho salmon were strikingly similar.

4. Lethal dose studies (based on IP injections of PCB's) indicated that the toxicity of PCB's to mink varied inversely with the chlorine content of the compounds. PCB feeding experiments, however, showed that Aroclor 1254 was much more detrimental to mink reproduction than Aroclors 1016, 1221, or 1242.

5. Mink growth data suggested that interactions exist between PCB's and chlorinated hydrocarbon pesticides in which the action of PCB's or certain chlorinated hydrocarbon pesticides may be potentiated in the presence of the other.

6. Long term consumption of PCB's by mink resulted in decreased body weight gains and increased organ (heart, liver and kidney) weights.

7. Mink readily accumulated Aroclor 1254 in adipose tissue until a plateau level was reached. The depletion rate from the adipose tissue was 50% in 8 weeks.

8. Impaired liver function was an early manifestation of PCB and coho salmon toxicity in mink.

9. The toxic factor(s) (PCB's?) present in Lake Michigan coho salmon are quite heat stable.

10. Acetone-hexane extraction of Lake Michigan coho salmon removed the toxic factor(s) (PCB's?) and rendered the fish safe for use as mink feed.

11. The reproductive failure in female mink produced by the feeding of PCB's or Lake Michigan coho salmon is not of a permanent nature.

12. The results of the experiments conducted in this study indicated that the toxicity and impaired reproduction encountered in feeding coho salmon and other Great Lakes fish to mink is probably due to PCB residues in the fish.

LITERATURE CITED

LITERATURE CITED

Aitken, F. C. 1963. *Feeding of Fur-Bearing Animals*. Tech. Comm. No. 23. Commonwealth Agricultural Bureau. Farnham Royal, Bucks, England.

Albert. T. F. 1962. The effect of DDT on the sperm production of the domestic fowl. Auk 79:104-107.

Anon. 1963. The colorimetric determination of phosphatase acid, alkaline prostatic in serum or other fluids at 400-420 mμ. Tech. Bul, No. 104. Sigma Chemical Co., St. Louis, MO.

Anon. 1972a. World production of mink pelts in 1971. Vara Paladjur. 43:104.

Anon. 1972b. Agriculture responsibility concerning polychlorinated biphenyls (PCBs). U.S.D.A. Ad Hoc Group on PCBs, Office of Science and Education, U.S.D.A., Washington, D.C.

Anon. 1972c. Polychlorinated biphenyls and the environment. International Task Force on PCBs, Washington, D.C.

Ambrose, A. M., H. E. Christensen, D. J. Robbins, and L. J. Rather. 1953. Toxicological and pharmacological studies on chlordane. Arch. Ind. Hyg. Occup. Med. 7:197-210.

Atkins, T. D., and R. L. Linder. 1967. Effects of dieldrin a reproduction of penned hen pheasants. J. Wildl. Manage. 31(4):746-753.

Aulerich, R. J. 1970. (personal communication)

Aulerich, R. J., L. C. Holcomb, R. K. Ringer and P. J. Schaible. 1963. Influence of photoperiod on reproduction in mink. Mich. Agr. Expt. Sta. Quart. Bul. 46(1):132-138.

Aulerich, R. J., R. K. Ringer, P. J. Schaible and H. Seagran. 1970a. An evaluation of processed Great Lakes fishery products for feeding mink. Feedstuffs 42(42):48-49.

Aulerich, R. J., and R. K. Ringer. 1970b. The effects of some chlorinated hydrocarbon pesticides on mink. Progress Report to Mink Farmers. Research Foundation, Milwaukee, Wisc.

Aulerich, R. J., R. K. Ringer, H. L. Seagran, and W. G. Youatt. 1971. Effects of feeding coho salmon and other Great Lakes fish on mink reproduction. Can. J. Zool. 49:611-616.

Aulerich, R. J., R. K. Ringer and S. Iwamoto. 1972. Reproductive failure and mortality in mink fed coho salmon and other Great Lakes fish. Proc. Int. Symp. Environment and Reproduction in Mammals and Birds, Edinburgh, Scotland, March 27-30, 1972 (in press).

Aulerich, R. J., R. K. Ringer, and S. Iwamoto. 1973. Why Great Lakes fish are unsafe to use as a feed for mink. U.S. Fur Rancher 52(3):12.

Azevedo, J. A., Jr., E. G. Hunt, and L. A. Woods, Jr. 1965. Physiological effects of DDT on pheasants. Calif. Fish, Game 51(4): 276-293.

Bache, C. A., J. W. Serum, W. D. Youngs and D. J. Lisk. 1972. Polychlorinated biphenyl residues. Accumulation in Cayuga Lake trout with age. Sci. 177:1191-1192.

Ball, W. L., K. Kay, and J. W. Sinclair. 1953. Observations on toxicity of aldrin. 1. Growth and estrus in rats. Arch. Ind. Hyg. Occup. Med. 7:292-300.

Bennett, G. A., C. K. Drinker and M. F. Warren. 1938. Morphological changes in the livers of rats resulting from exposure to certain chlorinated hydrocarbons. J. Ind. Hyg. Toxicol. 20:97-123.

Bernard, R. F., and R. A. Gaertner. 1964. Some effects of DDT on reproduction in mice. J. Mammal. 45(2):272-276.

Bernstein, L. M. 1965. *Renal Function and Renal Failure*. The Williams and Wilkins Co., Baltimore, Md.

Bessey, O. A., O. H. Lowry and M. J. Brock. 1946. A method for the rapid determination of alkaline phosphatase with five cubic millimeters of serum. J. Biol. Chem. 164:321-329.

Bitman, J., H. C. Cecil, S. J. Harris and G. F. Fries. 1968. Estrogenic activity of O, P'-DDT in the mammalian uterus and rat oviduct. Sci. 162:371-372.

Bloom, F. 1957. The diagnosis and treatment of liver diseases of the dog. N. Am. Vet. 38(1):17-27.

Bodansky, A., L. F. Hallman and R. Bonoff. 1933. Phosphatase studies. II. Determination of serum phosphatase. Factors influencing the accuracy of the determination. J. Biol. Chem. 101:93-104.

Bostom, R. E., R. J. Aulerich, R. K. Ringer, and P. J. Schaible. 1968. Seasonal changes in the testes and epididymides of the ranch mink. Mich. Agr. Expt. Sta. Quart. Bul. 50(4):538-558.

Bowness, R. E. 1957. Influence of light on mink production. Nat. Fur News 28(11):18.

Cecil, H. C., S. J. Harris, J. Bitman, and G. F. Fries. 1973. Polychlorinated biphenyl-induced decrease in liver vitamin A in Japanese quail and rats. Bull. Environ. Contam. Toxicol. 9(3): 179-185.

Chaddock, T. T. 1948. Fatty degeneration of liver in mink. Vet. Med. 43:197-199.

Curley, A., V. W. Burse, M. E. Grim, R. W. Jennings and R. E. Linder. 1971. Polychlorinated biphenyls: distribution and storage in body fluids and tissue of sherman rats. Environ. Res. 4:481-495.

Dahlgren, R. B., and R. L. Linder. 1971. Effects of polychlorinated biphenyls on pheasant reproduction, behavior, and survival. J. Wildl. Manage. 35(2):315-319.

Dahlgren, R. B., R. L. Linder, and C. W. Carlson. 1972. Polychlorinated biphenyls: their effects on penned pheasants. Environ. Health Persp. 1:89-101.

Deichmann, W. B., and W. E. MacDonald. 1971. Organochlorine pesticides and human health. Food Cosmet. Toxicol. 9:91-103.

Denington, E. M., and A. M. Lucas. 1955. Blood technics for chickens. Poultry Sci. 34:360-368.

DeWitt, J. B. 1955. Effects of chlorinated hydrocarbon insecticides upon quail and pheasants. J. Agric. Food Chem. 3(3):672-676.

Duby, R. T. 1970. Pesticides vs. reproduction still a puzzle. Amer. Fur Breeder 43(4):15.

Duncan, D. B. 1955. Multiple range and multiple F tests. Biometrics 11:1-42.

Genelly, R. E., and R. L. Rudd. 1956. Effects of DDT, toxaphene, and dieldrin on pheasant reproduction. Auk 73:529-539.

Gilbert, F. F. 1969. Physiological effects of natural DDT residues and metabolites on ranch mink. J. Wildl. Manage. 33(4):933-943.

Grant, D. L., W. E. J. Phillips and D. C. Villeneuve. 1971a. Metabolism of a polychlorinated biphenyl (Aroclor 1254) mixture in the rat. Bull. Environ. Contam. Toxicol. 6(2):102-112.

Grant, D. L., D. C. Villeneuve, K. A. McCully and W. E. J. Phillips. 1971b. Placental transfer of polychlorinated biphenyls in the rabbit. Environ. Physiol. 1:61-66.

Hansson, A. 1947. The physiology of reproduction in mink with special reference to delayed implantation. Acta Zool. 28:1-136.

Hartsough, G. R., and J. R. Gorham. 1949. Steatitis (yellow fat) in mink. Vet. Med. 44:345-346.

Hartsough, G. R. 1965. Great Lakes fish now suspect as mink food. Amer. Fur Breeder 38(10):25.

Hoe, C. M. and D. G. Harvey. 1961a. An investigation into liver function tests in dogs. Part 1. Serum transaminases. J. Small Anim. Pract. 2:22-31.

Hoe, C. M. and D. G. Harvey. 1961b. An investigation into liver function tests in dogs. Part 2. Tests other than transaminase estimation. J. Small Anim. Pract. 2:109-127.

Holcomb, L. C., P. J. Schaible, and R. K. Ringer. 1962. The effects of varied lighting regimes on reproduction in mink. Mich. Agr. Expt. Sta. Quart. Bul. 44(4):666-678.

Holden, A. V., and K. Marsden. 1967. Organochlorine pesticides in seals and porpoises. Nature 216:1274.

Holmes, D. C., J. H. Simmons, and J. O'G. Tatton. 1967. Chlorinated hydrocarbons in British wildlife. Nature 216:227.

Jensen, S. 1966. Report of a new chemical hazard. New Sci. 32:612.

Jensen, S. 1970. PCB as contaminant of the environment-history. National Swedish Environmental Protection Board PCB Conference, Wenner-Cren Center, Stockholm, Sweden, Sept. 29, 1970.

Kaneko, J. J., and C. C. Cornelius. 1970. *Clinical Biochemistry of Domestic Animals*, 2nd ed. Academic Press, New York.

Kellogg, C. E., C. F. Bassett, and R. K. Enders. 1948. Mink raising. Circ. No. 801. U.S. Dept. of Agriculture, Washington, D.C.

Kitselman, C. H. 1953. Long term studies on dogs fed aldrin and dieldrin in sublethal dosages, with reference to the histopathological finding and reproduction. J. Am. Vet. Med. Assoc. 123 (916):28-30.

Koller, L. D., and J. G. Zinkl. 1973. Pathology of polychlorinated biphenyls in rabbits. Am. J. Pathol. 70(3):363-373.

Lalor, R. J., W. L. Leoschke and C. A. Elvehjem. 1951. Yellow fat in the mink. J. Nutr. 45:183-188.

Lewin, V., and W. A. McBlain. 1972. Acute intraperitoneal toxicity of DDT and PCB's in mice using two solvents. Bull. Environ. Contam. Toxicol. 8(4):245-250.

Lichtenstein, E. P., K. R. Schulz, T. W. Fuhremann, and T. T. Liang. 1969. J. Econ. Entomol. 62(4):761-765.

Lincer, J. L., and D. B. Peakall. 1970. Metabolic effects of PCB in the American kestrel. Nature 228:783-784.

Locke, L. N., N. J. Chura, and P. A. Stewart. 1966. Spermatogenesis in bald eagles experimentally fed a diet containing DDT. Condor 68(5):497-502.

Mason, K. E., and G. R. Hartsough. 1951. "Steatitis" or "yellow fat" in mink, and its relation to dietary fats and inadequacy of vitamin E. J. Am. Vet. Med. Assoc. 119:72.

McDermid, A. M., and G. L. Ott. 1947. "Yellow fat" as observed in mink. J. Am. Vet. Med. Assoc. 110:34.

Mestizova, M. 1966. On reproduction studies and the occurrence of cataracts in rats after long-term feeding of the insecticide heptachlor. Experientia 23(1):42-43.

Miller, J. W. 1944. Pathologic changes in animals exposed to a commercial chlorinated diphenyl. Public Health Rep. 59:1085.

Morello, A. 1965. Induction of DDT-metabolizing enzymes in microsomes of rat liver after administration of DDT. Can. J. Biochem. 43(3):1289-1293.

Nisbet, I. C. T. and A. F. Sarofim. 1972. Rates and routes of transport of PCBs in the environment. Environ. Health Persp. 1:21-38.

Nishizumi, M. 1970. Light and electron microscope study of chlorobiphenyl poisoning in mouse and monkey liver. Arch. Environ. Health 21:620-632.

Orberg, J., N. Johansson, J. E. Kihlstrom, and C. Lundberg. 1972. Administration of DDT and PCB prolong oestrous cycle in mice. Ambio. 1(4):148-149.

Peakall, D. B. 1967. Pesticide-induced enzyme breakdown of steroids in birds. Nature 216(4):505-506.

Peakall, D. B., and J. L. Lincer. 1970. Polychlorinated biphenyls another long-life widespread chemical in the environment. Bioscience 20:958-964.

Platonow, N. S., and L. Karstad. 1972. Distribution, metabolism and some effects of polychlorinated biphenyls (Aroclor 1254) in mink. Can. Fed. Biol. Soc., 15th Ann. Congr., Quebec.

Platonow, N. S., R. W. Liptrap, and H. D. Geissinger. 1972. The distribution and excretion of polychlorinated biphenyls (Aroclor 1254) and their effect on urinary gonadal steroid levels in the boar. Bul. Environ. Contam. Toxicol. 7(6):358-365.

Presst, I., D. J. Jefferies, and N. W. Moore. 1970. Polychlorinated biphenyls in wild birds in Britain and their avian toxicity. Environ. Pollut. 1:3-26.

Reinert, R. E. 1970. Pesticide concentrations in Great Lakes fish. Pestic. Monit. J. 3(4):233-240.

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

Risebrough, R. W., and V. Brodine. 1970. More letters in the wind. Environment 12(1):16.

Rubin, M., H. R. Bird, N. Green, and R. H. Carter. 1947. Toxicity of DDT to laying hens. Poult. Sci. 26:410-413.

Schaible, P. J. 1969. Nutrition and feeding, Sec. III. In the blue book of fur farming. Editorial Service Co., Inc., Milwaukee, Wisc.

Scott, M. L., D. V. Vadera, P. A. Mullenhoff, G. L. Ramsey, and R. W. Rice. 1971. Results of experiments on the effects of PCB's on laying hen performance. Proc. 1971 Cornell Nutr. Conf.

Stout, F. M., J. Adair and J. E. Oldfield. 1968. Hepatotoxicosis in mink associated with feeding toxic herring meal. Amer. Fur Breeder 41(6):12-14.

Travis, H. F., C. F. Bassett, R. G. Warner and J. K. Loosli. 1956. Influence of diethylstilbestrol on growth and reproduction of mink (abstr.). Fed. Proc. 15:175.

Travis, H. F., and P. J. Schaible. 1960. Fundamentals of mink ranching. Cir. Bul. 229, Michigan State University, East Lansing, Mich.

Travis, H. F., and P. H. Harman. 1968. Sonic booms and farm raised mink. Nat. Fur News 41(6):18-20.

Tucker, R. K., and D. G. Crabtree. 1970. Handbook of toxicity of pesticides to wildlife. U.S. Dept. of the Interior, Bur. of Sport Fisheries and Wildlife. Resources Publ. No. 84.

VanVleet, J. F., and J. O. Alberts. 1968. Evaluation of liver function tests and liver biopsy in experimental carbon tetrachloride intoxication and extrahepatic bile duct obstruction in the dog. Am. J. Vet. Res. 29(11):2119-2131.

Villeneuve, D. C., D. L. Grant, W. E. J. Phillips, M. L. Clark, and D. J. Clegg. 1971a. Effects of PCB administration on microsomal enzyme activity in pregnant rabbits. Bull. Environ. Contam. Toxicol. 6:120-128.

Villeneuve, D. C., D. L. Grant, K. Khera, D. J. Clegg, H. Baer, and W. E. J. Phillips. 1971b. The fetotoxicity of a PCB mixture (Aroclor 1254) in the rabbit and in the rat. Environ. Physiol. 1:67-71.

Vos, J. G., and E. Notenboom-Ram. 1972. Comparative toxicity study of 2, 4, 5, 2', 4', 5-hexachlorobiphynyl and a polychlorinated biphenyl mixture in rabbits. Toxicol. Appl. Pharmacol. 23: 563-578.

Ware, G. W., and E. E. Good. 1967. Effects of insecticides on reproduction in the laboratory mouse. Toxicol. Appl. Pharmacol. 10(1):54-61.

White, A. G. 1961. *Clinical Disturbances of Renal Function*. W. B. Saunders Co., Philadelphia.

Wroblewski, F., and J. S. LaDue. 1955. Serum glutamic oxalacetic transaminase activity as an index of liver cell injury a preliminary report. Ann. Internal Med. 43:345-360.

Zabik, M. J. 1972. (personal communication)