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THE EFFECTS OF TWO POLYCHLORINATED BIPHENYL MIXTURES (AROCLORS 1242 & 1016) ON MINK AND FERRETS

by

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ABSTRACT

The Effects of Two Polychlorinated Biphenyl Mixtures (Aroclors 1242 & 1016) on Mink and Ferrets.

By

Michael R. Bleavins

Aroclor 1242 or Aroclor 1016 were fed to mink and ferrets to investigate the chronic toxicity of these PCBs. Transplacental passage of Aroclor 1242 was also measured.

In mink, Aroclor 1242 was found to be more toxic than Aroclor 1016. Aroclor 1242 caused complete reproductive failure. The 20 ppm and 40 ppm Aroclor 1242 treatments resulted in the death of all mink. Two-thirds of the 10 ppm Aroclor 1242 mink also died. Aroclor 1016 impaired reproduction to a lesser degree than did Aroclor 1242. No outward sign of abnormalities beyond small size were found in the kits whelped and nursed by dams fed Aroclor 1016.

Ferrets were more resistant to the effects of either PCB mixture than mink. Feeding Aroclor 1242 resulted in complete reproductive failure, but was not fatal to adult ferrets. Placental transfer of PCBs was found to be greater in the mink than in the ferret.

The difference in higher substituted biphenyl isomer content and/or the levels of contaminants may be of major importance in evaluating the toxicity of these compounds.

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INTRODUCTION

In an effort to reduce the toxicity of polychlorinated biphenyls (PCBs) and yet take advantage of their uses in closed systems, Monsanto Co. introduced the PCB mixture Aroclor® 1016[1]. Aroclor 1016 is a distillation product of Aroclor 1254 (Hoopingarner et al., 1972). The more highly chlorinated isomers have been removed resulting in a mixture containing 1% or less of biphenyl molecules with more than four chlorine atoms. The Aroclor 1016 mixture is similar in chlorine content to the Aroclor 1242 mixture (41.3% vs. 42%) and is functionally comparable as a dielectric fluid (Kaley et al., 1976). However, Aroclor 1242 contains 9% biphenyls with five or more chlorines per molecule (Goldstein et al., 1975).

Mink (Mustela vison) are one of the most sensitive mammalian species to many toxins including diethylstilbestrol, PCBs, DDT, DDE, dieldrin, PBB, and other pesticides. This sensitivity makes the mink an excellent model for testing of the mechanisms and toxicity of various polychlorinated biphenyl mixtures.

Pilot studies conducted at the Michigan State University mink ranch, indicated that the European ferret (Mustela putorius furo) is more resistant to the effects of PCBs than its close relative the mink. The ferret was therefore selected as a comparison species in this study.

The PCBs Aroclor 1242 and Aroclor 1016 were fed to mink and ferrets to explore the relative toxicities of the two mixtures and to compare differences in species resistance.

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[1] Aroclor is a trade name for PCBs manufactured by Monsanto Co., St. Louis, MO.

REVIEW OF LITERATURE

The use of synthetic chemicals has grown rapidly in the last thirty years. This is especially true of the class of compounds known as the petrochemicals. Their versatility has propelled them into wide-spread and varied use. With the increase in petrochemical production, both in types of compounds and in the amounts produced, large quantities have been introduced into the biosphere. Recently it has become only too obvious that the effects of these substances in the environment is more serious than originally thought. Many of the man-made chemicals show little or no tendency to biodegrade and often have adverse toxicological and biological effects upon the plant and animal life exposed to them. Research has pointed out the dangers of DDT, DDE, dieldrin, and related pesticides. Current studies are further exposing the extreme toxicity of the contaminant tetrachlorodibenzodioxin (TCDD) in the herbicide 2, 4, 5-T. The polychlorinated biphenyls (PCBs) and other halogenated biphenyls make up another class of hydrocarbons prevalent in the world's ecosystem with the potential for harm.

Entrance of PCBs into the environment is possible by several routes. PCBs are transported throughout the ecosystem both aquatically and terrestially. Industrial leakage (Pichirallo, 1971; Heath et al., 1972), the flushing or intentional dumping of waste PCBs (Heath et al., 1972) and the weathering or destruction of materials containing PCBs (Pichirallo, 1971; Heath et al., 1972) are considered to be the primary sources of PCB pollution. PCB contamination of animal feeds has been traced to heat exchange fluids, hydraulic fluid, plastic wrappers, paints and sealants used on silo walls and feed troughs, and misused transformer fluid in herbicide spray (Anon., 1972). Their properties of lipid solubility, high flash points, and relative inertness to chemical and biological degradation have contributed to making PCBs among the most widespread of

chemical pollutants (Dustman et al., 1971). Even areas as distant from industrial activity as the Arctic of Greenland exhibit detectable levels of PCB in fat samples from native mammals (Clausen et al., 1974). The PCBs are poorly soluble in water, but readily so in fats and therefore concentrate in animal lipids (Heath et al., 1972) and may be found along with other organochlorine compounds such as DDT.

Although described by Schmidt and Schultz in 1881 (Peakall and Lincer, 1970), polychlorinated biphenyls were first introduced in 1929 for use in electrical transformers and condensers (Dustman et al., 1971) where their flame-resistant qualities were highly valued. Since World War II, the use of various PCBs has expanded dramatically. They were widely used as plasticizers (Anon., 1972), in marine antifouling paints and protective coatings (Rhee and Plapp, 1973), hydraulic fluids and cardboard cartons (Bailey et al., 1970), as "inert" ingredients for insecticides, as dust-allayers in detergents, in carbonless carbon paper, microscope immersion oil, and a wide range of plastic products (Anon., 1972).

Polychlorinated biphenyls are comprised of two covalently bonded benzene rings with chlorine substitution possible at any of the remaining carbons. There are ten possible sites for chlorine substitution; however, steric hindrance and electrostatic factors generally result in only four to eight of the available carbons being chlorinated (Jensen, 1970). PCBs were marketed as complex mixtures and were not obtainable as single compounds (Dustman et al., 1971). The mixtures were of many compounds of the same basic structure with different numbers of chlorine atoms in different positions. The physical properties of PCBs vary with chlorine content. Those mixtures with low chlorine content are fluid liquids while those with high chlorine content are highly viscous liquids or resins. In North America, PCBs were sold exclusively by Monsanto Co. and were marketed under the trade name of Aroclor. Aroclors are

designated by four digit numbers; the first two digits (12) following the trade name specify the compound contains chlorinated biphenyls while the last two digits give the approximate percent of chlorine by weight in the compound. This nomenclature was not followed when the Aroclor® 1016 mixture was placed on the market.

Chlorinated biphenyl contamination of animal species, including man is both numerous and world-wide. The following are a few of the many species affected: brook trout (Hutzinger et al., 1972), cormorants and pelicans (Anderson et al., 1969), pheasants (Dahlgren et al., 1971), fish and sea life (Jensen, 1966; Jensen et al., 1969; Koeman et al., 1971), bald eagles (Mulhern et al., 1970; Reichel et al., 1969), ducks (Friend and Trainer, 1970), swine (Platonow et al., 1972), mink (Aulerich et al., 1973), and humans (Biros et al., 1970).

PCBs, not being single entities, but rather complex mixtures made up of many isomers which can undergo biological degradation at different rates (Tucker et al ., 1975), appear to show greater toxicity in birds with an increased percentage of chlorine (Dustman et al., 1971). Commercial PCB mixtures which contain predominately mono- and dichlorobiphenyls readily undergo primary biodegradation by activated sludge microorganisms (Tucker et al., 1975). As the levels of tri-, tetra-, and pentachlorobiphenyl increase, the rate at which the mixture degrades is decreased. This resistance of more highly chlorinated biphenyls, particularly those containing five or more chlorines per molecule, explains in part their presence in biological and environmental samples. The apparent relationship between percent chlorination and toxicity and biodegradability, prompted Monsanto Co. to develop the Aroclor 1016 mixture. This PCB contains 41.3% chlorine with a very low percentage of the biphenyls (1%) having more than four chlorines per molecule (Goldstein et al., 1975). Arolcor 1016 as a dielectric fluid in sealed systems (capacitors and transformers) is

functionally similar to the Aroclor 1242 mixture (Kaley et al., 1976). Aroclor 1242 is a mixture of approximately 42% chlorine, but contains 9% biphenyls with five or more chlorines (Goldstein et al., 1975).

Fetotoxicity of Aroclor 1254 has been demonstrated by Villeneuve et al. (1971b) in the rabbit. Similar embryotoxic effects have been reported for the mink (Platonow and Karstad, 1973a; Aulerich and Ringer, 1977). In each of these studies, the dead fetuses born showed no malformations or indications of PCBs having a teratogenic effect. The hatchability of chicken eggs injected with Aroclor 1242 was reduced (Carlson and Duby, 1973). The period of organ formation in the bird appeared to be sensitive to low levels of PCBs since the majority of deaths occurred before organ formation was complete (Carlson and Duby, 1973).

Transplacental passage of polychlorinated biphenyls has been shown in cattle (Platonow and Chen, 1973), rats (Takagi et al., 1976; Ando, 1978), mice (Masuda et al., 1978a), rabbits (Grant et al., 1971a), rhesus monkeys (Allen and Barsotti, 1976), and humans (Masuda et al., 1978b). In each experiment and species, only a relatively small proportion of the mother's PCB body burden was involved. Much greater concentrations of PCB were contained in the maternal milk supply. In rats (Ando, 1978), the ratio of PCBs in milk to PCBs transferred through the placenta was calculated at 14.6:1. The transfer in this study was sufficient to significantly decrease the mother's tissue concentration of PCBs. Grant et al. (1971a) found that the larger the dose of PCB given to pregnant rabbits, the greater the residue in maternal and fetal tissues. Aroclor 1254 was also found to accumulate to a much greater extent than Aroclor 1221. The impaired growth and excessive early mortality of mink kits of normal weight at birth, suggest the presence of PCBs in the milk (Aulerich et al., 1973; Aulerich and Ringer, 1977).

PCBs were first shown to affect reproduction in mammals by Gilbert (1969) and later by Aulerich et al. (1971), both in mink fed PCB-contaminated fish. PCBs were later confirmed as the causative agent (Ringer et al., 1972) in the reproductive failure of mink.

Studies of prolonged oral consumption of various polychlorinated biphenyls have been conducted in mice (Kimbrough and Linder, 1974), rats (Wassermann et al., 1973), subhuman primates (Allen et al., 1974), chicks (Rehfeld et al., 1971), and mink (Aulerich and Ringer, 1977). Of these species, the mink ranks among the most sensitive, particularly with regards to embryotoxicity (Aulerich et al., 1973; Aulerich and Ringer, 1977).

The mink breeds once per year during the month of March. Both spermatogenesis and estrus are controlled by environmental light conditions (Bowness, 1957; Travis and Schiable, 1960; Holcomb et al., 1962; Aulerich et al., 1963; and Bostrom et al., 1968). Due to delayed implantation in this species, there is considerable variation as to length of gestation. Gestation averages about 50 days but varies between 40 to 70 days (Hansson, 1947). Litter size is from one to eight or more, averaging about four kits at birth. For a more detailed review of mink reproduction, see Hansson (1947) and Enders (1952).

The European ferret, a close relative of the mink, will breed several times per year. The first mating is generally in April (Ryland and Gorham, 1978) and whelping may continue well into September. As in the mink, spermatogenesis and estrus are under the influence of photoperiod (Williams, 1976). The ferret does not demonstrate delayed implantation and so exhibits a uniform gestation period of 41-42 days. Litter size ranges form 5-15 with larger litters often occurring.

MATERIALS AND METHODS

The mink used in these studies were from the Michigan State University experimental mink ranch. The European ferrets were obtained from Marshall Research Animals, North Rose, New York 14516, or were bred from this stock. All animals were housed and cared for at the Michigan State University experimental mink ranch. The mink and ferrets 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 experiments:

Standard ranch procedures were followed in the feeding, care, and breeding of the animals. The mink were vaccinated for canine distemper, botulism and virus enteritis in July. Ferrets were immunized against canine distemper in August.

In allotting 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 purchased ferrets arrived unidentified as to litter groups and so were randomly assigned to treatment groups.

The mink and ferrets were weighed individually at the start of the experiments and at specified intervals thereafter, except during the gestation period (March-May for mink, April-July for ferrets). Whenever possible, the females were mated with males within their respective dietary groups. Each female mink was given an opportunity to remate either the day after the original mating or eight days later. The female ferrets were mated beginning with their first cycle in April. Any females that recycled were remated up to three times. All matings were verified by the presence of motile spermatozoa in a vaginal smear taken after copulation. 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 four weeks of age.

The animals that died during the experiments were either sent to the MSU Animal Health Diagnostic Laboratory for necropsy or were necropsied by this author. Organ weights were recorded and selected tissues collected for PCB analysis and histopathologic examination. Liver, kidney, spleen, muscle, intestine, brain, and nerve tissues for histology were fixed in 10% neutral formalin and processed by routine histological procedures. Neural tissues were sectioned at 15 microns and all other tissues cut at 5 microns. Sections were stained with Harris's Hematoxylin and differentiated for ten seconds in 1% HCl and 80% ethyl alcohol. Each was then stained in 0.5% Eosin Y and differentiated in two changes of absolute ethanol. The slides were cleared in xylene and coverslipped using Flo-Texx®[1] liquid cover slip.

In the preparation of the experimental diets employed in these studies, the PCBs were dissolved in acetone and incorporated into 500 grams of commercial mink cereal. The acetone was evaporated off and the premix containing the PCBs was mixed with the other ingredients of the diet to yield a diet that contained the desired amount of either Aroclor 1242 or Aroclor 1016. The Aroclor 1242 used was of electrical grade, lot #KB-05-415 from Monsanto Company, St. Louis, Missouri. The Aroclor 1016 was supplied by Dr. Gil Veith, Environmental Protection Agency, Water Quality Laboratory, Duluth, Minnesota 55812. The composition of the basal (control) diet is shown in Table 1. After February 10th, 22 I.U. of Vitamin E per kilogram of feed was added to all diets and continued until July 1. Corn oil was added to the diet after April 20th at a level of one percent of the diet. This supplementation continued through lactation.

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[1] Lerner Laboratories, Stanford, CT 06902

Table 1. Composition of basal diet.

Ingredient	Weight (kg)	Percentage
Whole chicken	109.1	20.0
Commercial mink cereal[a]	90.9	16.7
Ocean fish scrap[b]	68.2	12.5
Beef tripe	18.2	3.3
Cooked eggs	15.9	2.9
Beef liver	36.4	6.7
Beef trimmings	18.2	3.3
Beef lungs	36.4	6.7
Powdered milk	5.9	1.1
Added water	146.4	26.8
Total	545.6	100.0

[a] XK-40 Grower, XK Mink Foods Inc., Thiensville, WI 53092

[b] Cod, Haddock, and Flounder, National Feed Co., New Holstein, WI 53061

EXPERIMENT 1. Effects of Aroclor 1242 and Aroclor 1016 on Growth, Reproduction, and Livability.

This experiment was started November 1, 1977 and continued through July 5, 1978. The purpose of this study was to determine the relative toxicities of Aroclor 1242 and Aroclor 1016, two PCB mixtures presently being utilized by industrial companies in sealed systems, to mink and ferrets.

PROCEDURE

One hundred five pastel mink were divided into five treatment groups and a control group. Each treatment group consisted of twelve females and three males, while the control group contained twenty-four females and six males. The mink were fed the basal diet supplemented with either 0 ppm (control), 5 ppm, 10 ppm, 20 ppm, or 40 ppm of Aroclor 1242 or 20 ppm Aroclor 1016. Forty-five ferrets were randomly assigned to three groups each containing twelve females and three males. Since the results of previous experiments in which PCBs were fed to mink showed no adverse effects on spermatogenesis (Aulerich and Ringer, 1977), only reproductive data pertaining to female mink and ferrets are reported in this study. Test matings of male ferrets fed 20 ppm of Aroclor 1242 to untreated ranch females showed normal litter size and weight.

Data were analyzed by Student's t-test between two means (Gill 1978a, 1978b) or the Bonferroni t statistic (Gill 1978a, 1978b) for comparisons of more than two means.

RESULTS

Aroclor 1242 produced 100% mortality in all adult mink that were fed diets at the 20 ppm and 40 ppm levels (Table 2). At 10 ppm a mortality rate of 66.7% occurred in males and females, while the 5 ppm diet was lethal only to one female. No mortality was noted in male mink fed the control, 5 ppm Aroclor

Table 2. Mortality of adult mink and ferrets and survival time of mink fed Aroclor 1242 that died during a 247-day study.

Dietary treatment	Mortality males No. died/ No. total	%	Mortality females No. died/ No. total	%	Survival time of mink that died (days + S.E.) Males	Females
MINK						
0 ppm PCB	0/6	0.0	3/24	12.5	----------	75 + 19.2 (38-102)
5 ppm Aroclor 1242	0/3	0.0	1/12	8.3	----------	151
10 ppm Aroclor 1242	2/3	66.7	8/12	66.7	156 + 25.3 (123-189)[a]	171 + 6.6 (142-190)
20 ppm Aroclor 1242	3/3	100.0	12/12	100.0	170 + 14.1 (147-192)	153 + 5.8 (133-190)
40 ppm Aroclor 1242	3/3	100.0	12/12	100.0	120 + 12.0 (96-132)	122 + 5.3 (89-138)
20 ppm Aroclor 1016	0/3	0.0	3/12	25.0	----------	92 + 4.9 (86-102)
FERRETS						
0 ppm PCB	0/3	0.0	0/12	0.0	----------	----------
20 ppm Aroclor 1242	0/3	0.0	0/12	0.0	----------	----------
20 ppm Aroclor 1016	0/3	0.0	1/12	8.3[b]	----------	----------

[a] Range in days

[b] Breech presentation, female died due to complications

1242, or 20 ppm Aroclor 1016 diets. Female mink fed the 20 ppm Aroclor 1016 diet showed a greater number of deaths than the control animals (25% vs. 12.5%), but a much lower mortality than those fed the same level (20ppm) of Aroclor 1242 (25% vs. 100%). As shown in Table 2, the mean survival time of mink fed Aroclor 1242 was, in general, inversely related to the level of dietary PCB.

All mink that died during the study were necropsied and observed for gross abnormalities. Necropsy revealed emaciation characterized by an almost complete absence of body fat in the mink fed Aroclor 1016 or 1242 diets. Gastric ulcers, as described by Aulerich et al. (1973), were noted in some mink fed the Aroclor 1242 diets. Control mink that died did not exhibit this extreme emaciation or incidence of gastric ulcers. Prior to death, the treated animals showed anorexia, weakness, and often lethargy. These symptoms become most obvious as the stress of winter compounded the PCB effects.

No mortality occurred in the ferrets, with the exception of a single female that was fed 20 ppm Aroclor 1016. This death was due to complications arising from a breech presentation at whelping, and was not attributed directly to the PCB treatment.

Reproduction was dramatically reduced in mink fed either of the PCBs (Table 3). Feeding Aroclor 1242 resulted in complete reproductive failure in those animals that survived long enough to be bred. The 40 ppm diet caused death in all animals prior to the breeding season (March). Eight females fed the 20 ppm Aroclor 1242 diet survived long enough to be mated, but died prior to the whelping period (late April to early May). Animals fed the 5 ppm and 10 ppm diets also failed to reproduce. The twenty-three females mated in these two groups (12 females fed 5 ppm and 11 fed 10 ppm) failed to demonstrate any outward signs of pregnancy prior to death or during the gestation period. Aroclor 1016 also reduced, but did not completely eliminate reproduction. Four of the nine mated females produced kits. The relative percentage of kits born

Table 3. Effect of PCBs on reproductive performance of mink and ferrets.

Dietary treatment	No. ♀ whelped/ No. ♀ mated	Whelped (%)	Average gestation (days)	No. whelped		Alive at birth (%)	Live kits/♀	
				Alive	Dead		Mated	Whelped
MINK								
0 ppm PCB	16/21	76.2	50.2	79	14	84.9[a][1]	3.8[a]	4.9[a]
5 ppm Aroclor 1242	0/12	0.0						
10 ppm Aroclor 1242	0/11	0.0						
20 ppm Aroclor 1242	0/8	0.0						
40 ppm Aroclor 1242	0/0	0.0						
20 ppm Aroclor 1016	4/9	44.4	51.3	25	5	83.3[a]	2.7[a]	6.3[a]
FERRETS								
0 ppm PCB	12/12	100.0	41.0	118	5	95.9[b]	9.8[b]	9.8[b]
20 ppm Aroclor 1242	0/12	0.0						
20 ppm Aroclor 1016	10/12[2]	83.3	41.2	87	4	95.6[b]	7.3[b]	8.7[b]

[1] Means within same column for each species with same superscript are not significantly different ($P < 0.05$).

[2] Does not include female that died during breech presentation.

alive was not affected by the reduced number of females that whelped.

The ferrets that received the control diet showed a whelping rate of 100%. The percentage of kits born alive was 95.9, with an average of 9.8 kits per female. Ferrets fed the Aroclor 1242 diet did not whelp. The females cycled, as evidenced by vulvar swelling, several times during the breeding season of March to August (Ryland and Gorham, 1978). Each was mated during three separate cycles but failed to exhibit outward signs of pregnancy. However, implantation sites were observed in two females when the study was terminated and the animals necropsied. As shown in Table 3, the reproductive performance of the female ferrets fed Aroclor 1016 was not significantly different from that of the control females.

The birth weight of mink kits whelped by dams fed Aroclor 1016 averaged numerically less than that of kits whelped by dams fed the control diet (Table 4). This difference, however, was not significant ($P < 0.05$). Four-week body weights were significantly lower for the kits nursed by dams fed Aroclor 1016. There was also a significant ($P < 0.01$) reduction in the biomass (average kit body weight gain between birth and four weeks of age x the average number of kits raised per lactating female) produced by female mink fed Aroclor 1016 when compared to control females (Table 4). The lactating control females produced 2.4 times the biomass of the lactating females fed Aroclor 1016. Mortality between birth and four weeks of age was 2.3 times greater in mink kits produced and nursed by females that consumed Aroclor 1016 than the control diet (56.0% vs. 24.1%).

Reproductive parameters, kit growth, and adult and kit mortality were not significantly affected in the ferrets fed the Aroclor 1016 diet (Table 4).

Hematocrit and hemoglobin values were not significantly different between the control and PCB-treated mink (Table 5). There was however, an inverse numeric relationship between hematocrit and hemoglobin values and dietary

Table 4. Average body weights and mortality of kits whelped and nursed by female mink and ferrets fed PCBs.

Dietary treatment	Kits: At birth No. alive	At birth Body wt. (g ± S.E.)	At 4 weeks No. alive	At 4 weeks Body wt. (g ± S.E.)	Avg. no. kits/ lactating ♀	Biomass[1] (g)	Mortality to 4 wks. (%)
MINK							
0 ppm PCB	79	9.0 ± 0.20[a2]	60	127.7 ± 2.33[a]	4.62[a]	548.4[a]	24.1
5 ppm Aroclor 1242	0*						
10 ppm Aroclor 1242	0*						
20 ppm Aroclor 1242	0*						
40 ppm Aroclor 1242	0†						
20 ppm Aroclor 1016	25	8.4 ± 0.25[a]	11	91.6 ± 9.94[c]	2.75[c]	228.8[c]	56.0
FERRETS							
0 ppm PCB	118	8.1 ± 0.13[b]	108	136.6 ± 3.05[b]	9.00[b]	1156.5[b]	8.5
20 ppm Aroclor 1242	0*						
20 ppm Aroclor 1016	87	8.5 ± 0.16[b]	77	142.9 ± 2.11[b]	7.70[b]	1034.9[b]	11.5

* All females failed to whelp.

† All females died prior to mating.

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

[2] Means within same column for each species with same superscript are not significantly different ($P < 0.05$).

Table 5. Hematocrit, hemoglobin, and mean corpuscular hemoglobin concentration values of adult mink and ferrets.

Dietary treatment	No. of animals	Hematocrit ± S.E.[3]	Hemoglobin ± S.E. (g%)	MCHC ± S.E. (g%)
MINK				
0 ppm PCB	27	52.0[a][4] ± 0.87	19.36[a] ± 0.406	37.22[a] ± 0.467
5 ppm Aroclor 1242	14	51.3[a] ± 1.21	18.68[a] ± 0.563	36.37[a] ± 0.648
10 ppm Aroclor 1242	5	48.9[a] ± 2.03	17.05[a] ± 0.943	34.84[a] ± 1.085
20 ppm Aroclor 1242	0[1]			
40 ppm Aroclor 1242	0[1]			
20 ppm Aroclor 1016	12	52.9[a] ± 1.31	19.29[a] ± 0.609	36.56[a] ± 0.700
FERRETS				
0 ppm PCB	15	59.0[b] ± 0.76	22.10[b] ± 0.328	37.48[b] ± 0.389
20 ppm Aroclor 1242	15	40.8[d] ± 0.76	15.09[d] ± 0.328	36.98[b] ± 0.389
20 ppm Aroclor 1016	14[2]	57.9[b] ± 0.78	21.62[b] ± 0.340	37.38[b] ± 0.403

[1] All animals died prior to termination of the study.

[2] Does not include one female that died during breech presentation.

[3] S.E. = $\sqrt{MS_E/r_i}$

[4] Means within the same column for each species with same superscript are not significantly different ($P < 0.05$).

Aroclor 1242 level. The data would indicate that the hematocrit and hemoglobin values for mink fed 20 ppm and 40 ppm Aroclor 1242 may have been significantly reduced prior to death. Aroclor 1016 had no effect on either of these parameters. The animals fed the 20 ppm Aroclor 1016 diet had hematocrit and hemoglobin values virtually identical to the control animals.

Ferrets fed the Aroclor 1242 diet showed significantly reduced hemoglobin and hematocrit values from the control animals. The ferret's greater resistance to the toxic effects of PCBs allowed the animals to survive longer at higher dietary PCB levels than the mink. The hematologic effects therefore could be measured at the conclusion of the study. The number of animals alive per treatment group was also higher, for the same reasons, and so allowed the standard error of the means to be smaller than was possible with the mink. The effects of Aroclor 1016 on the hematocrit and hemoglobin values of the ferret were not significant. This agrees well with the findings in the mink.

The white blood cell differentials of the control and PCB-treated animals were not significantly different for any cell type, except lymphocyte and monocytes (Table 6). Although not significantly reduced, there existed a definite trend for segmented neutrophil numbers to be reduced in animals exposed to Aroclor 1242. Mink fed 5 ppm Aroclor 1242 had significantly higher monocyte counts than the control animals. A similar increase was noted in the 10 ppm Aroclor 1242 treatment group, but was not significant. This was not found in the Aroclor 1016-treated animals.

Ferrets paralleled the mink with regard to white blood cell distribution. In the ferret, segmented neutrophils were significantly reduced in number and lymphocytes were significantly increased in those animals fed Aroclor 1242. There was a tendency for ferrets fed the Aroclor 1016 diet to likewise show a depressed neutrophil count and an increased lymphocyte count, although not a significant change at ($P < 0.05$). Monocyte numbers did not appear to increase

Table 6. White blood cell differential distribution[1] of female mink and ferrets fed control or PCB diets.

Dietary treatment	No. of animals	Basophil	Eosinophil	Juvenile	Band	Neutrophil	Lymphocyte	Monocyte
MINK								
0 ppm PCB	21	0.0^{a}[4] ± 0.05	1.1^{a} ± 0.35	0.0^{a} ± 0.00	8.0^{a} ± 0.87	58.5^{a} ± 11.27	28.6^{a} ± 3.00	3.7^{a} ± 0.56
5 ppm Aroclor 1242	11	0.1^{a} ± 0.07	0.8^{a} ± 0.48	0.0^{a} ± 0.00	7.0^{a} ± 1.21	36.2^{a} ± 15.58	49.6^{c} ± 4.14	6.3^{c} ± 0.77
10 ppm Aroclor 1242	4	0.0^{a} ± 0.11	0.3^{a} ± 0.80	0.0^{a} ± 0.00	9.3^{a} ± 2.00	33.3^{a} ± 25.83	50.0^{c} ± 6.87	7.3^{a} ± 1.28
20 ppm Aroclor 1242	0[3]	---	---	---	---	---	---	---
40 ppm Aroclor 1242	0[3]	---	---	---	---	---	---	---
20 ppm Aroclor 1016	9	0.0^{a} ± 0.07	1.8^{a} ± 0.53	0.0^{a} ± 0.00	5.1^{a} ± 1.33	49.4^{a} ± 17.22	40.7^{a} ± 4.58	3.0^{a} ± 0.86
FERRETS								
0 ppm PCB	12	0.5^{b} ± 0.16	3.0^{b} ± 0.55	0.0^{b} ± 0.30	6.7^{b} ± 1.55	46.6^{b} ± 3.09	39.0^{b} ± 3.30	4.3^{b} ± 0.46
20 ppm Aroclor 1242	12	0.3^{b} ± 0.16	1.3^{b} ± 0.55	0.0^{b} ± 0.30	8.9^{b} ± 1.55	27.9^{d} ± 3.09	56.1^{d} ± 3.30	5.5^{b} ± 0.46
20 ppm Aroclor 1016	11	0.4^{b} ± 0.16	3.5^{b} ± 0.58	0.8^{b} ± 0.31	7.1^{b} ± 1.62	34.7^{b} ± 3.23	49.0^{b} ± 3.44	4.5^{b} ± 0.48

[1] Expressed as percentage of total leukocyte counted.

[2] S.E. = $\sqrt{MS_{E/r_i}}$

[3] All animals died prior to termination of the study.

[4] Means within same column for each species with same superscript are not significantly different ($P < 0.05$).

to any noticable degree in the ferrets. Changes in the numbers of other white blood cells were not seen in the ferrets exposed to Aroclor 1016.

The extreme emaciation of animals dying from the effects of PCBs makes direct organ weight comparisons misleading. A truer index of relative organ weight changes can be made using the more consistent brain weight percentage rather than body weight percentage. There was no significant difference between brain weights of control and PCB-treated animals (Table 7). Therefore, organ weights are expressed as a percentage of brain weight.

No significant difference was found between heart weight of PCB-treated mink and control animals, except for the reduced heart weight in those mink fed 20 ppm Aroclor 1242 (Table 8). Ferrets did not show a reduction in heart weight when fed 20 ppm Aroclor 1242.

Kidney weights in mink were not affected by either Aroclor 1242 or Aroclor 1016 (Table 8). A trend toward increased kidney weight was seen in the higher dietary levels of Aroclor 1242. However, Aroclor 1242 caused a significant increase in kidney weight in the ferrets.

The liver weight of all animals fed Aroclor 1242 was significantly increased (Table 8). This enlargement is probably due to liver enzyme induction by the 1242 mixture. The Aroclor 1016-treated animals tended to have slightly larger livers than the controls, but this relationship was not significant.

Spleen weights were significantly greater in the 5 ppm and 10 ppm Aroclor 1242-treated mink, but not for the 20 ppm and 40 ppm Aroclor 1242 and 20 ppm Aroclor 1016-treated animals. The ferrets showed a significant increase in spleen weight to the 20 ppm Aroclor 1242 diet, but no such increase to the comparable level of Aroclor 1016.

No significant change was found in lung weight for any animals except for the mink fed the 40 ppm Aroclor 1242 diet. There was, however, a consistent

Table 8. Mean organ weights[1] of female mink fed a control diet or PCB supplemented feeds.

Dietary treatment	No. of animals	Organ				
		Heart	Kidney	Liver	Spleen	Lung
MINK						
0 ppm PCB	21	66.5[a][3] ± 1.93	51.3[a] ± 1.75	252.6[a] ± 12.34	27.0[a] ± 2.79	79.2[a] ± 6.67
5 ppm Aroclor 1242	11	67.2[a] ± 2.66	53.5[a] ± 2.42	393.8[c] ± 17.05	49.5[c] ± 3.85	110.9[a] ± 9.22
10 ppm Aroclor 1242	6	61.0[a] ± 3.61	47.0[a] ± 3.28	388.0[c] ± 23.08	42.0[c] ± 5.21	99.8[a] ± 12.48
20 ppm Aroclor 1242	9	56.4[c] ± 2.95	54.9[a] ± 2.68	330.2[c] ± 18.85	27.0[a] ± 4.25	119.9[a] ± 10.19
40 ppm Aroclor 1242	8	70.0[a] ± 3.13	60.0[a] ± 2.84	373.3[c] ± 19.99	35.0[a] ± 4.51	151.8[c] ± 10.81
20 ppm Aroclor 1016	10	69.9[a] ± 2.79	53.0[a] ± 2.54	291.0[a] ± 17.88	35.4[a] ± 4.04	94.5[a] ± 9.67
FERRETS						
0 ppm PCB	12	83.0[b] ± 3.50	71.8[b] ± 3.27	420.8[b] ± 36.74	66.5[b] ± 8.92	110.5[b] ± 4.44
20 ppm Aroclor 1242	12	81.8[b] ± 3.50	102.4[d] ± 3.27	786.6[d] ± 36.74	153.9[d] ± 8.92	118.4[b] ± 4.44
20 ppm Aroclor 1016	11	77.5[b] ± 3.65	74.7[b] ± 3.41	466.2[b] ± 38.37	74.6[b] ± 9.32	117.8[b] ± 4.64

[1] Expressed as percent of brain weight ± S.E.[2]

[2] S.E. = $\sqrt{MS_E/r_i}$

[3] Means within same column for each species are not significantly different from control mean ($P < 0.05$).

tendency for PCB-treated animals to have a larger mean lung weight than the control mink or ferrets.

Histologically, both mink and ferrets showed increased splenic entramedullary hematopoesis when exposed to Aroclor 1242 or Aroclor 1016. This stepped up production of blood cells outside of the bone marrow occurred in 50% of the 5 ppm Aroclor 1242 treatment mink and in 100% of the 10 ppm group. Mink fed the 20 ppm Aroclor 1016 diet also showed a high incidence (45.4%) of this condition. Splenic extramedullary hematopoesis was noted in only 11.1% of the mink on the basal diet. The ferrets exhibited a similar relationship between the two PCB mixtures and splenic hematopoesis. The 20 ppm Aroclor 1242 treatment group showed this condition in 93.3% of the animals; ferrets consuming the 20 ppm Aroclor 1016 diet had a 57.1% incidence. One third of the ferrets fed the basal diet had an increase in splenic extramedullary hematopoesis.

The mink receiving the 10 ppm Aroclor 1242 diet had a 40% incidence of periportal accumulations of extramedullary hematopoesis. This condition was not observed in any other mink, including the 20 ppm Aroclor 1016 group. The ferrets fed the 20 ppm Aroclor 1242 diet showed a 40% occurrance of this type of extramedullary hematopoesis, but the condition was also seen in 21.4% of the Aroclor 1016-treated ferrets. The occurrance of this type of extramedullary hematopoesis in the control animals was 6.7%.

Unlike the Aroclor 1254 mixture (Aulerich et al., 1973), the incidence of gastric ulcers was very low for mink fed the Aroclor 1016 diet. Only a single animal was found to have ulcers of the stomach lining and these were very small. The number and size of ulcers was greatest in the 20 ppm Aroclor 1242 treatment group with 50% of the twelve animals necropsied showing this condition. Three of the ten animals necropsied on the 40 ppm Aroclor 1242 diet had gastric ulcers, while 21.4% of the 5 ppm group had gastric ulcers. None of the eight animals necropsied on the 10 ppm Aroclor 1242 diet exhibited stomach ulcers.

One ferret fed the Aroclor 1242 diet exhibited gastric ulcers while none were seen in the Aroclor 1016-treated animals.

The body weights of adult mink fed the 40 ppm Aroclor 1242 diet were significantly lower than control animals after three months on treatment (Figure 1). By the fourth month of the study, the 20 ppm Aroclor 1242 animals also showed a significant reduction in body weight. The mink were not weighed again after March until the first of June to avoid injury and/or abortion in the bred females. During this three month period, mortality of the animals was high and so comparisons after March were not possible for the 10 ppm, 20 ppm, and 40 ppm Aroclor 1242 treatments. In June, the 5 ppm Aroclor 1242-treatment group was significantly reduced in body weight when compared to the control group. At this time of year, the control and 20 ppm Aroclor 1016 mink were in the early weeks of lactation, while all Aroclor 1242 mink failed to whelp. The added stress of caring for the young would tend to depress the mother's body weight and so the comparison to the 5 ppm Aroclor 1242 females underestimates the effects of this PCB mixture. No significant difference was observed between the 20 ppm Aroclor 1016 and the 5 ppm Aroclor 1242 treatment groups. The body weights of the control and 20 ppm Aroclor 1016 female mink dropped sharply between June and July due to the increasing demands of their growing kits. This reduction in body weight resulted in the 5 ppm Aroclor 1242 animals weighing significantly more than the control females. No significant difference was found between the 20 ppm Aroclor 1016 and 5 ppm Aroclor 1242-treated mink.

No significant change in body weight was seen in female ferrets fed either PCB mixture until after seven months on treatment (Figure 2). The 20 ppm Aroclor 1242 treatment group was significantly reduced in body weight when compared to the control or the 20 ppm Aroclor 1016 females. This reduction was in the spite of the females in the latter two groups caring for kits. By July,

Figure 1. Body weights of adult mink fed control or PCB diets expressed as a percent of initial body weight.

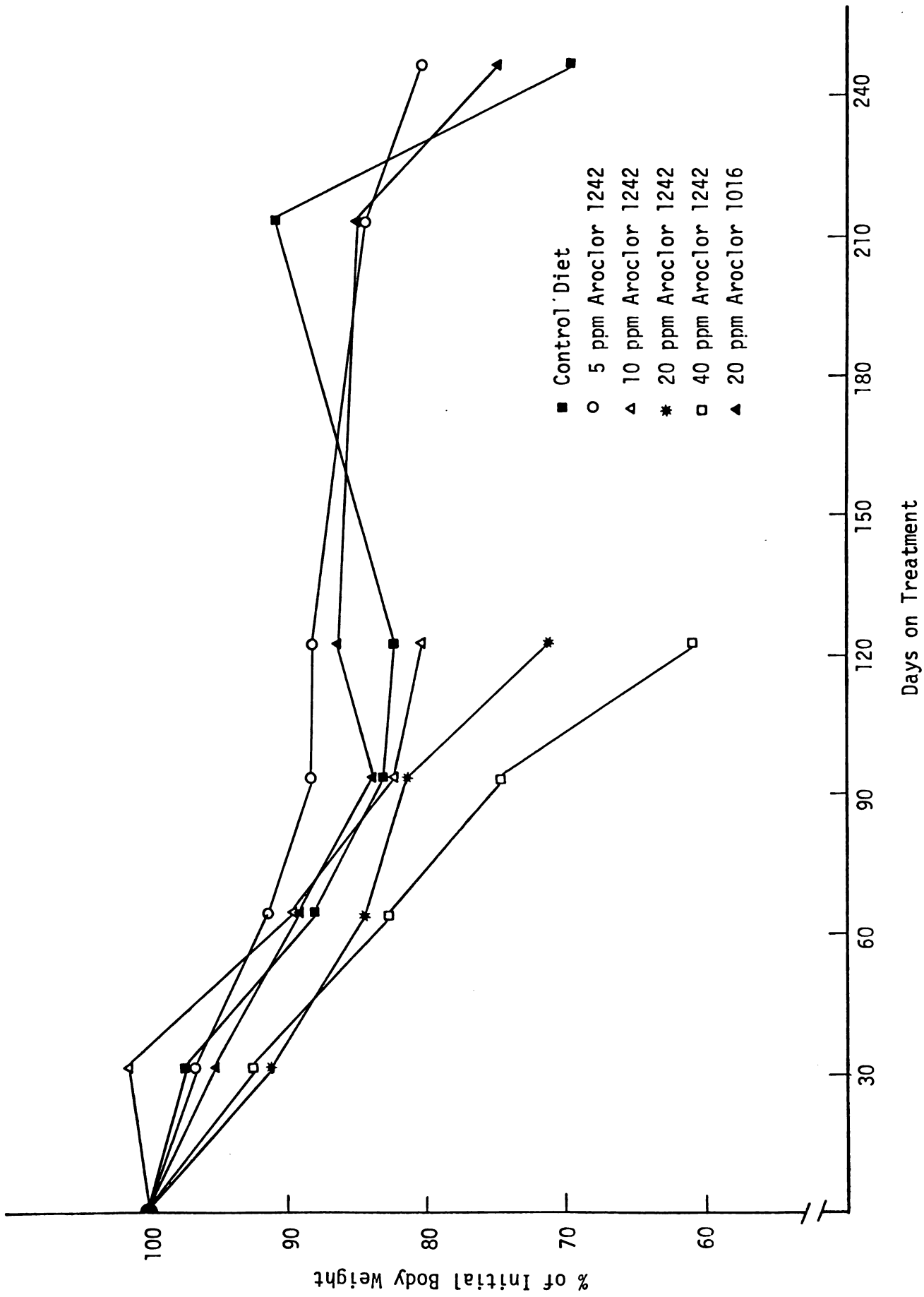
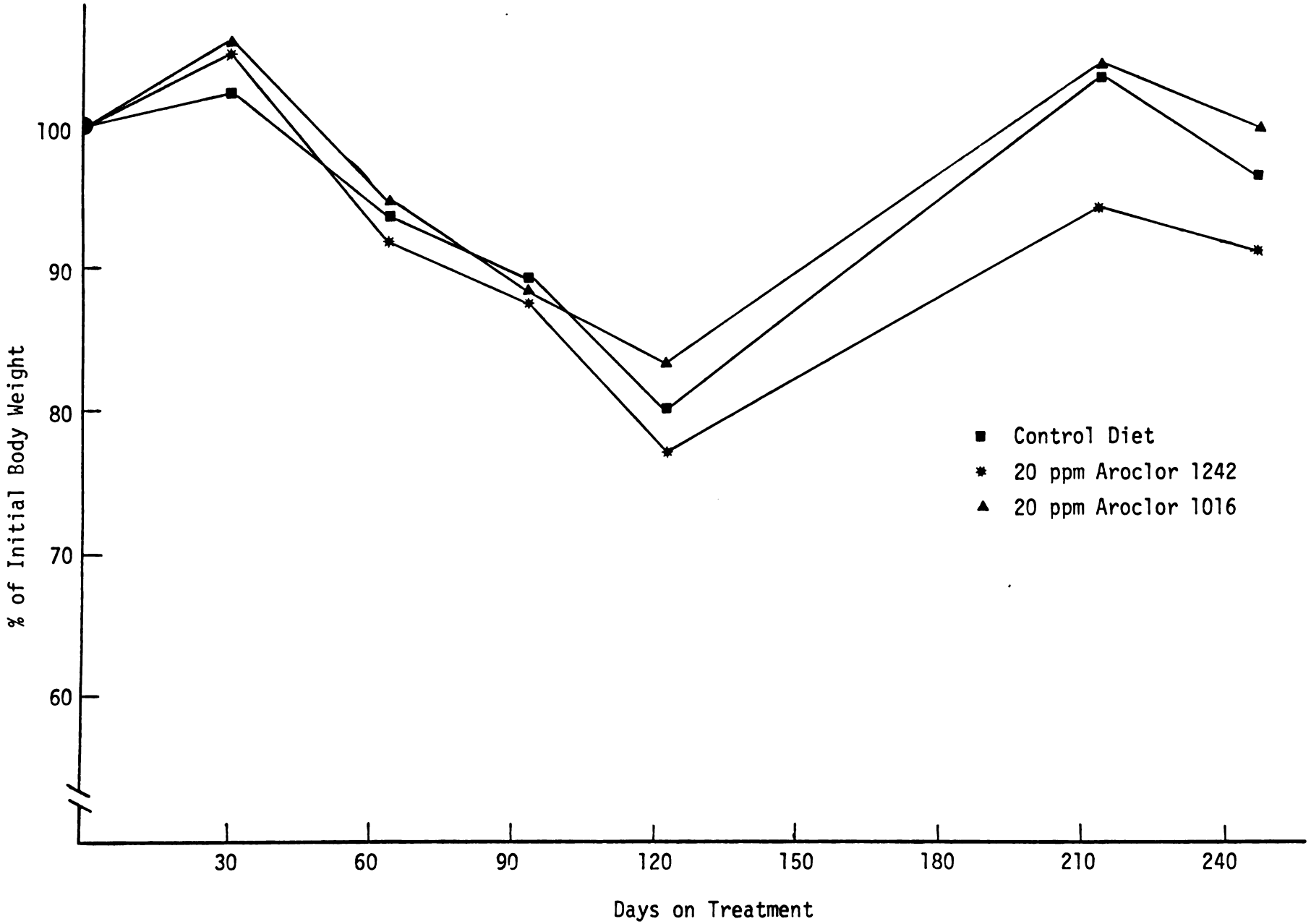

% of Initial Body Weight
100
90
80
70
60
30
60
90
120
150
180
210
240
Days on Treatment
Control Diet
5 ppm Aroclor 1242
10 ppm Aroclor 1242
20 ppm Aroclor 1242
40 ppm Aroclor 1242
20 ppm Aroclor 1016

Figure 2. Body weights of adult ferrets fed control or PCB diets expressed as a percent of initial body weight.


100
90
80
70
60
% of Initial Body Weight
30
60
90
120
150
180
210
240
Days on Treatment
Control Diet
20 ppm Aroclor 1242
20 ppm Aroclor 1016

the Aroclor 1242 female ferrets no longer showed a significantly lowered body weight.

DISCUSSION

The results of this study indicate that mink are better able to tolerate Aroclor 1016 than Aroclor 1242. Diets supplemented with 20 ppm of Aroclor 1016 caused moderate reproductive complications in mink, while dietary levels of only 5 ppm of Aroclor 1242 resulted in complete reproductive failure. The adverse effects on reproduction caused by PCBs do not appear to be due to an effect on spermatogenesis. PCB-treated male mink have had acceptable levels of reproduction when mated to untreated females (Aulerich and Ringer, 1977). At dietary levels greater than 5 ppm, Aroclor 1242 resulted in considerable adult mortality in addition to reproductive failure. The ability of mink to reproduce when fed 20 ppm Aroclor 1016 in the diet for six months is in contrast to the results reported for other PCB mixtures (Platonow and Karstad, 1973a; Aulerich and Ringer, 1977).

In this study, mortality was limited to three female mink fed the diets supplemented with 20 ppm of Aroclor 1016; whereas, a comparable level of Aroclor 1242 resulted in 100% mortality of both male and female mink. The 71% adult mortality reported by Aulerich and Ringer (1977) in mink fed 10 ppm Aroclor 1254 for 10 months is similar to the 67% mortality noted in the mink that received 10 ppm dietary Aroclor 1242 for nine months in this study.

There was no difference in mortality rates between male and female mink fed diets supplemented with 10 ppm, 20 ppm, or 40 ppm of Aroclor 1242. However, in mink fed the 5 ppm Aroclor 1242 or the 20 ppm Aroclor 1016 diets, deaths were limited to female mink. Male mink have also been shown to survive longer than female mink when fed low levels (0.64-3.57 ppm) of Aroclor 1254 (Platonow and Karstad, 1973a).

The greater sensitivity of female mink to the effects of PCBs may be due to a number of factors. First, the amount of food consumed per unit of body weight is 31 percent greater in the female (Table 12) than the male. This results in the animal consuming a larger quantity of the PCB mixture per kilogram of body weight. Second, the smaller body size of the female also results in a larger surface area to volume ratio, thereby requiring a higher metabolic rate, especially during cold weather. Third, the winter months require the animals to deal with the problems of frozen food and water in addition to the cold weather. The mink are then forced to draw on their adipose tissue reserves. The female mink, having less stored fat due to a smaller body size, would exhaust their body fat at an earlier date than the male mink.

If it is assumed that female mink consume 150 g of feed per day (Schaible, 1970), the mean total consumption of Aroclor 1242 by the females that died (Table 9) while being fed diets that contained 5, 10, 20, and 40 ppm of this PCB was 113, 257, 460, and 729 mg, respectively. Using this assumed feed consumption and a mean body weight of 800 g, an LD_{50} for the females fed 10, 20, and 40 ppm Aroclor 1242 can be calculated as follows: LD_{50} = feed consumed per day x PCB concentration in the diet x mean days survived until 50 percent mortality ÷ body weight. Based on this formula, the calculated LD_{50} for females fed 10, 20, and 40 ppm Aroclor 1242 was 315, 521, and 833 mg/kg, respectively. This increasing LD_{50} value with respect to higher dietary concentrations may indicate differential absorption of the PCB mixture.

A comparison of the relative toxicity of Aroclors 1242 and 1016 with Aroclor 1254, a prevalent PCB mixture in the environment extremely toxic to mink (Aulerich and Ringer, 1977), can be made from the data presented in Table 10. At levels of ingestion of 5 and 10 ppm, mortality and reproduction results were similar for mink fed Aroclors 1242 and 1254. However, at a lower dietary

Table 9. Aroclor 1242 consumed per female prior to death.

Dietary treatment	No. of animals	Average no. of days on treatment	PCB consumed[a] (mg)
5 ppm	1	151.0	113.3
10 ppm	7	171.1	256.7
20 ppm	11[b]	153.2	459.6
40 ppm	11[c]	121.5	729.0

[a] Based on an assumed daily food consumption of 150 g/female.

[b] Does not include one animal that escaped during a severe snowstorm - later found dead.

[c] Does not include one animal that died due to fatty liver syndrome - tested as an outlier.

Table 10. Summary of mortality and reproduction in mink fed various levels of Aroclors 1254, 1242, and 1016.

Treatment level (ppm)	Period fed (days)	Mortality (no. dead/no. total)	Reproduction (kits/ ♀)
Aroclor 1254[a]			
0	280	1/7	5.0
0	297	0/8	4.1
2	297	1/8	0.3
5	280	2/7	0
10	280	5/7	0
Aroclor 1242			
0	247	3/30	4.9
2[a]	297	1/8	5.6
5	247	1/15	0
10	247	10/15	0
20	192[b]	15/15	0
40	138[c]	15/15	0
Aroclor 1016			
0	247	3/30	4.9
2[a]	297	0/8	4.5
20	247	3/15	6.3

[a] Data from Aulerich and Ringer, 1977.

[b] All mink died within 192 days on diet.

[c] All mink died within 138 days on diet; no females survived to whelping.

level of 2 ppm, Aroclor 1254 impaired reproduction while Aroclor 1242 did not have an adverse effect.

An estimated LC_{50} (dietary concentration lethal to 50 percent of the mink) can be calculated for Aroclors 1242 and 1254 from the data presented in Table 10. Using the procedure of Litchfield and Wilcoxon (1949) the LC_{50} for Aroclor 1242 fed to mink is 8.6 mg per kg diet when fed for 247 days. The slope of the dose-effect curve is 0.699. For Aroclor 1254 the LC_{50} is 6.65 ppm when fed for 280-297 days. The slope of this line is 2.627.

Although the effects of feeding Aroclor 1016 to mink were not as dramatic as those that occurred from the feeding of Aroclor 1242, some detrimental effects were observed. These included increased mortality among adult female mink in addition to reduced four-week body weights of kits. Higher kit mortality between birth and four weeks of age was also noted.

The growth rate of the nursing kits was expressed as the average biomass (Table 4) to account for the fact that kits raised by females with large litters may not receive as much milk per kit as those raised by females with smaller litters and, therefore, would weigh less at four weeks of age. Since almost all of the kit's weight gain up to four weeks of age is from the nourishment provided by the dam's milk, the reduced four week weights and increased mortality of kits nursed by dams fed Aroclor 1016 might be attributed to dam effects. PCBs have been shown to be excreted in milk (Platonow et al., 1971) and this may account for the poor growth and increased mortality of these mink kits. There may also be a reduction in the quantity of milk produced by the PCB-fed females, putting further stress on the growing kit. Long term, low-level feeding of PCBs has also been found to reduce weight gains and feed efficiency in growing swine and sheep (Hansen et al., 1976).

The ferrets were not as severely affected by either PCB mixture as were the mink. Mortality of adult ferrets was nonexistent (except for the breech

presentation). Reproduction was prevented in the ferrets fed 20 ppm Aroclor 1242, a level lethal to 100% of the adult mink. The presence of implantation/ absorption sites in two female ferrets, indicate that PCBs such as Aroclor 1242, are embryotoxic in this species.

The ferrets greater resistance to PCB toxicity may result from a difference in ability to metabolize and excrete the polychlorinated biphenyl isomers. Unfortunately, this hypothesis must await future study since the tissue residue data from this experiment were improperly analyzed by the testing laboratory.

The significant reduction of hematocrit and hemoglobin values in the ferrets exposed to Aroclor 1242 suggests a progressive anemia. This is supported by similar depressions of hematocrit and hemoglobin values reported for White Leghorn cockerels fed 100 ppm of Aroclor 1242 or 100 ppm or Aroclor 1254 by Iturri et al. (1974). Anemia has been described in monkeys (McConnell and Moore, 1979) and rats (Bruckner et al., 1973) exposed to halogenated aromatic hydrocarbons, including PCBs. The anemia was found to be most pronounced in animals in chronic toxicity studies. McConnell and Moore (1979) postulate that the anemic state is probably related to bone marrow depression rather than an increase in erythrocyte destruction. The mink in this study tended to exhibit depressed hematocrit and hemoglobin values and so would appear to be following a pattern similar to the ferrets. However, the greater sensitivity of the mink to the toxic effects of Aroclor 1242 may result in mortality prior to the onset of significant clinical signs.

The increase in lymphocytes and decrease in neutrophils found in the white blood cell differential counts of mink and ferrets consuming Aroclor 1242 diets is consistent with that seen in debilitating conditions (Davidsohn and Nelson, 1969). The emaciated state of the mink would strongly suggest an animal in poor health and physical condition, agreeing with the above diagnosis. Although in better condition than the mink, ferrets fed Aroclor 1242 were also in declining

health. The increase in monocyte counts in mink fed the 5 ppm Aroclor 1242 diet is in agreement with tetrachloroethane poisoning in humans (Davidsohn and Nelson, 1969).

The reduction in heart weight seen in mink fed 20 ppm Aroclor 1242 is not reproduced in any other mink treatment group or in the comparable treatment group for ferrets. However, a decrease in heart weight has been shown in Ring-necked pheasants (Dahlgren et al., 1972) and chickens (Iturri et al., 1974) fed PCBs. The significance of this weight change is not clear.

There was a trend toward increased kidney weight in mink fed the higher dietary levels of Aroclor 1242 and a significant increase in the kidney weight of ferrets consuming the Aroclor 1242 diet. A significant increase in kidney weight was observed by Iwamoto (1973) in mink fed 5 and 10 ppm of Aroclor 1254. Increases have also been noted in pheasants (Dahlgren et al., 1972) and other wild avian species (Prestt et al., 1970). Even though the kidneys constitute only a small proportion of the body's total weight, they receive a very high percentage of the resting cardiac output (Foulkes and Hammond, 1975). This results in the renal tissue being in contact with the circulating PCB levels to a greater degree than most other tissues of the body. These factors may play a role in the triggering of increasing kidney weight.

Both mink and ferrets fed Aroclor 1242 diets showed a significant increase in liver weight. Liver enlargement has been reported in numerous species exposed to PCBs, including the rat (Miller, 1944; Grant et al., 1971b), mink (Iwamoto, 1973), rabbit (Vos and Notenboom-Ram, 1972), chicken (Platonow and Funnell, 1972), and Ring-necked pheasant (Dahlgren et al., 1972). PCBs have been found to induce hepatic hydroxylating enzymes (Risebrough et al., 1968; Lincer and Peakall, 1970; Villeneuve et al., 1971a) as well as microsomal and other hepatic enzymes (Bickers et al., 1972). The induction of liver enzymes and other attempts to detoxify the PCBs probably accounts for the majority of

the liver's increase in weight on the Aroclor 1242 treatments. The Aroclor 1016 PCB mixture did not significantly increase liver weight in the mink or ferret. In studies conducted with rats, Aroclor 1016 produced significantly lower levels of liver enzyme induction that Aroclor 1242 or Aroclor 1254 (Goldstein et al., 1975). In this same study, no effect on liver weight was observed for Aroclor 1016-treated rats, but Aroclors 1242 and 1254 significantly increased liver weight. A similar study supported Aroclor 1016 as being a less potent inducer of microsomal oxidation enzymes (Bickers et al., 1972) than Aroclor 1254 in rats. The increased synthesis of some hydroxylating enzymes by the liver catalyzes the breakdown of steroid hormones (Risebrough et al., 1968). This metabolism of the steroid hormones may be a factor in the inability of the mink and ferret to reproduce normally when subjected to some PCB mixtures. However, one would expect steroid hormone catabolism to have an adverse effect on germ cell formation and development in the adult animals. Oogenesis and spermatogenesis in PCB-treated mink has been found to be comparable to control animals (Aulerich and Ringer, 1977). The embryotoxicity of PCBs appears to be of a more direct toxic nature. Although, ovulation and implantation occur in mink fed PCBs (Platonow and Karstad, 1973a) the females are unable to maintain pregnancy. As suggested by Goldstein et al. (1975), since both Aroclor 1016 and 1242 contain approximately the same percent chlorination, Aroclor 1242 may be a more potent inducer of hepatic enzymes than Aroclor 1016 because it contains higher concentrations of penta- and hexachlorinated biphenyls.

The significant increase in spleen weight seen in mink fed 5 ppm and 10 ppm Aroclor 1242 and the ferrets fed 20 ppm Aroclor 1242 is in contrast to the findings in some other species. The rat (Grant et al., 1971b), chicken (Flick et al., 1965), and pheasant (Dahlgren et al., 1972) were found to have a reduction in spleen weight when exposed to PCBs. An increase in spleen size is however, not unexpected since enlargement of the spleen is usually

accompanied by poor health (Raab, 1974), especially in chronic conditions.

The high incidence of splenic extramedullary hematopoesis in adult mink and ferrets fed Aroclor 1242 or Aroclor 1016 diets indicates a need for erythrocytes. As stated in the pathologists'[1] report:

> *The frequent finding of periportal accumulations of extramedullary hematopoesis and splenic extramedullary hematopoesis presumably connotates increased demand for marrow cells. These findings are frequent in young animals but usually confined to the spleen.*

The spleen serves as the principal backup organ for the bone marrow, but because of the abundance of bone marrow space, reactivation of extramedullary sites rarely takes place in adult life (Erslev and Gabuzda, 1974). Extramedullary hematopoesis often indicates inappropriate rather than compensatory formation of blood cells (Erslev and Gabuzda, 1974). The PCBs appear to be initiating or accentuating a depression in blood cell numbers.

All PCB-treated mink showed a nearly continuous decline in body weight as the study progressed. The angle of the descending line for the Aroclor 1242 treatment groups became sharper as the dietary concentration increased. Aroclor 1016 treatment resulted in a line that closely paralleled the 5 ppm Aroclor 1242 treatment group. It was only during the latter stages of lactation that the body weights of mink fed the 20 ppm Aroclor 1016 deviated greatly from the mink fed 5 ppm Aroclor 1242. All mink are normally at their maximal body weight in the fall of the year due to the deposition of fat in preparation for winter. The control animals showed a more typical pattern of body weight changes. As the stresses of winter, frozen food and water, as well as the cold continued, the animals showed a reduction in body weight. When the weather begins to

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[1] Drs. T. G. Bell and G. A. Padgett, Department of Pathology, Michigan State University.

become less severe, the mink are once again able to increase their body weight. Nursing females decline in weight as lactation progresses and so begin to loose weight during June and July.

The control, Aroclor 1242 and Aroclor 1016 treatment groups of ferrets showed little differences in body weight between groups until March. At this time the Aroclor 1242 ferrets showed a relative reduction in body weight compared to the control and Aroclor 1016-treated groups. This was to continue for the rest of the study, in spite of the demands made by the growing kits whelped by the control and Aroclor 1016-treated females. Aroclor 1016-treated and control ferrets exhibited very similar weights throughout the course of the experiment. Unlike the PCB-treated mink, the ferrets fed the PCB diets were able to regain the weight they lost over the winter months when the weather began to warm.

Mink have been shown to metabolize PCBs, especially the lower chlorinated biphenyls (Platonow and Karstad, 1972). The early eluting peaks of the PCB mixture correspond to the lower chlorinated isomers (Hutzinger et al., 1974). It was the first eight eluting peaks that Platonow and Karstad (1972) found to be significantly reduced in the tissues of mink fed Aroclor 1254. Their studies with cattle and swine showed a less pronounced reduction in the early eluting peaks than were found in the mink. This finding has led them to postulate that a species' susceptibility to the toxic effects of PCBs may be related to the animals ability to metabolize the lower chlorinated biphenyls. The problem appears to be more complex than this indicates. The Aroclor 1016 mixture contains a greater concentration of the lower chlorinated biphenyl isomers than Aroclor 1242 (Appendix A) and yet was found to be less toxic to mink in Experiment I. The animal's ability to metabolize PCBs may however, be an important factor in determining sensitivity. Metabolism of PBBs, compounds similar in structure to PCBs (Appendix B), has been shown to potentiate the

toxicity of the compound (Aulerich and Ringer, 1979). A similar reaction may be involved in PCB toxicity. The animal's ability to metabolize the overall isomer content of a PCB mixture may be more important than the ability to metabolize the lower chlorinated biphenyls alone.

The difference in toxicity found between Aroclor 1016 and other PCB mixtures may be due to limited absorption of Aroclor 1016, a higher excretion rate of 1016, the removal of contaminants (Appendix C) such as chlorinated dibenzofurans (Bowes et al., 1975) and chlorinated dibenzodioxins (Porter and Burke, 1971) in the production of Aroclor 1016, or because Aroclor 1016 is metabolized to a greater extent. The degradation of the lower chlorinated biphenyls has been shown to be more rapid than for the more highly substituted biphenyls (Hutzinger et al., 1974; Tucker et al., 1975). The ability of mink and ferrets to metabolize and excrete PCBs may be dependent upon the percentage of the higher chlorinated biphenyls, as well as the overall percentage of chlorination of the PCB mixture.

EXPERIMENT II. Food Consumption of Mink and Ferrets.

This study was initiated to contrast the food consumption of mink and ferrets to more precisely evaluate the effect of PCBs on these species.

PROCEDURE

The experiment was conducted during February 1979 with the temperature inside the test building ranging from 11°C-14°C. Twelve mink (six males and six females) and twelve ferrets (six males and six females) were placed into individual cages with removable dropping pans. The animals were allowed to acclimate to the cages and building for ten days prior to the beginning of the study. Each animal was fed a weighed quantity of the basal diet each day. Any uneaten food was subtracted from the original feed weight. The food consumption for each mink and ferret was measured for four consecutive days and the average value calculated. The animals were weighed on the initial and final day of the study. No animal fluctuated by more than four percent of its original body weight during the experiment.

The mink and ferret food consumption values were then contrasted by the standard two mean "t" test (Gill, 1978a, 1978b).

RESULTS

No significant difference was found between the amount of food consumed per day by mink and ferrets of the same sex (Tables 11 and 12). Both species were noted to eat small quantities of food often rather than a large meal all at once. The animals wasted considerable amounts of food by carrying the feed and then dropping it through the wire of the cage. The wasted feed was recovered and corrected for in calculating food consumption.

Table 11. Mean food consumption of adult mink and ferrets.

Animal	Food consumption[1] (g)						
	#1	#2	#3	#4	#5	#6	Mean ± S.E.
Males							
Mink	203.1	251.1	249.0	189.2	220.8	189.8	217.2 ± 11.41[a2]
Ferret	211.5	219.5	254.5	243.1	230.5	258.3	236.2 ± 7.73[a]
Females							
Mink	149.5	143.6	129.4	104.3	155.3	125.0	134.5 ± 7.68[b]
Ferret	90.3	94.5	111.9	139.1	109.3	147.5	115.4 ± 9.50[b]

[1] Average daily food consumption.

[2] Means within same column for same sex are not significantly different ($P < 0.05$).

Table 12. Mean food consumption of adult mink and ferrets per unit of body weight.

Animal	Feed consumption/body weight (g/kg)						
	#1	#2	#3	#4	#5	#6	Mean ± S.E.
Males							
Mink	123.5	123.8	115.4	113.6	115.3	124.7	119.4 ± 2.09[a][1]
Ferret	109.2	108.4	109.6	134.5	143.2	136.7	123.6 ± 6.61[a]
Females							
Mink	158.2	141.5	148.3	140.9	177.5	165.6	155.3 ± 5.93[b]
Ferret	124.5	125.6	146.3	169.6	133.7	173.5	145.5 ± 8.84[b]

[1] Means within the same column for same sex are not significantly different ($P < 0.05$).

DISCUSSION

The hypothesis that the ferret's greater resistance to polychlorinated biphenyls may be due to a difference in the quantity of contaminated food consumed cannot be supported by these results. The mink and ferrets did not show any significant difference in food consumption or in food consumed per kilogram of body weight.

EXPERIMENT III. Food Passage Rate in the Mink and Ferret.

This experiment was designed to determine the rate of food passage in mink and ferrets and thereby confirm or deny this parameter as a factor in the differential species' resistance to PCBs.

PROCEDURE

The experiment was run during February 1979 with a temperature range of 11°C-14°C. Eighteen mink and eighteen ferrets (equal numbers of each sex) were brought indoors and placed into individual cages with removable dropping pans. An acclimation period of three days was used. All animals were fed, ad libitum, the basal diet to which ferric oxide was added at a concentration of one gram per kilogram of diet. The time at which each animal first ate the dyed food was recorded. All animals were observed and the time that dye began to appear in the feces noted. Passage time was then calculated as the time between ingestion of dyed food and the appearance of dye in the feces.

The mink and ferret food passage times were statistically tested by the standard "t" test between two means (Gill, 1978a, 1978b).

RESULTS

Male and female data were combined for both mink and ferrets since no sex difference in food passage time was found upon analysis by the two-sample "t" test.

No significant difference was found between the rate of food passage in the mink and ferret (Table 13). The time necessary for the food to pass through the digestive tract of the mink averaged 186.8 minutes and of the ferret averaged 181.8 minutes.

DISCUSSION

Since food passage time was not significantly different for the ferret or the mink, this also cannot be offered as an explanation for the ferrets' greater

Table 13. Food passage time of adult mink and ferrets as measured by ferric oxide dye marker technique.

Mink		Ferret	
Animal no.	Passage time (min.)	Animal no.	Passage time (min.)
FP 1003	155	IF 131	172
FP 1133	189	IF 81	190
FP 81	178	IF 21	179
FP 125	189	IF 41	168
FP 145	201	IF 11	148
FP 1057	144	IF 111	194
DP 483	215	GF 1	219
DP 493	180	IF 61	175
DP 553	225	HF 31	181
Male mean ± S.E.	186.2 ± 8.68		180.7 ± 6.53
FP 1092	171	IF 50	155
FP 1042	189	IF 32	161
FP 1060	138	IF 1770	159
FP 1064	185	IF 2	181
FP 1100	147	IF 60	198
FP 1022	221	IF 12	212
FP 138	222	IF 110	195
DP 494	235	IF 90	209
FP 122	179	IF 20	177
Female mean ± S.E.	187.4 + 11.19		183.0 ± 7.24
Overall mean±S.E.	186.8 ± 6.87*	Mean ± S.E.	181.8 ± 4.74*

* No significant difference.

resistance to the effects of PCBs. The rapid rate of food passage through the digestive tract of both species would suggest less time for absorption and therefore the possibility of lower sensitivity to PCBs than species having longer passage times such as the goat (Castle, 1956), the Ring-necked pheasant (Duke et al., 1968), the chicken, the dairy cow, and human (Travis and Schaible, 1960). This is however not the case as the extreme sensitivity of the mink demonstrates. The ferret too is quite sensitive, in comparison to rodent species, showing complete reproductive failure when fed a diet containing 20 ppm Aroclor 1242. The rate of food passage was found to be approximately three hours for the mink and ferret, both species being carnivorous. Frequent meals keep food in the alimentary tract much of the time and these animals are efficient at utilizing protein and fat (Travis and Schaible, 1960). The high lipid solubility of PCBs would tend to concentrate them in the fat portion of the ration - a principle component of the animal's usable diet. Further study is needed to determine the reason for the ferrets' ability to tolerate higher dietary polychlorinated biphenyl concentrations.

EXPERIMENT IV. Nail Growth of Ferrets Fed a 20 ppm Aroclor 1242 Diet.

This study was undertaken in an attempt to reproduce the excessive nail growth seen in the ferrets fed Aroclor 1242 at 20 ppm of the diet in Experiment I.

PROCEDURE

The experiment was begun January 30, 1979 and was terminated October 23, 1979. Twelve ten week old ferrets were randomly divided into two groups each consisting of three males and three females. One group was fed the basal (control) diet and the other group fed the diet supplemented with 20 ppm Aroclor 1242. The animals were weighed and the nails on the right front foot measured at the start of the study, at periodic intervals during the study, and at the end of the experiment. Any animal dying during the study was necropsied. The females were mated during June and July and reproduction data recorded. Upon termination of the study, the animals were weighed and necropsied. The nails on the left hind foot were also measured when the study ended.

The right front foot from each animal was fixed in phosphate-buffered 10% formaldehyde solution. The second proximal toe from each foot was acidified in 10% formaldehyde solution for 48 hours. The toe was then decalcified for 16-18 hours in Decal[1]. During the decalcification process constant agitation of the solution was achieved by using a magnetic stirrer. Decalcified toes were washed for 3 hours in running tap water and processed by routine histological procedures. Sections were cut at approximately 5 microns. The variety of tissues (muscle, nail, decalcified bone, etc.) made exact 5 micron sections impossible. Sections were stained with Harris's Hematoxylin and differentiated for ten seconds in 1% HCl and 80% ethyl alcohol. Each was then stained in 0.5%

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[1] Scientific Products, Division of American Hospital Supply Corp., Evanston, IL.

Eosin Y and differentiated in two changes of absolute ethanol. The slides were cleared in xylene and coverslipped using Flo-Texx liquid cover slip.

RESULTS

The initial and final body weights were not significantly different for male ferrets fed the control diet and male ferrets fed the 20 ppm Aroclor 1242 supplemented diet (Table 14). Female ferrets fed the PCB diet weighed significantly less than females consuming the control diet. Upon termination of the study, all animals were in good flesh and appeared to be in excellent health. One female ferret fed 20 ppm Aroclor 1242, however, was found to have small white plaque lesions on her lungs, possibly from avian tuberculosis. No other gross lesions were noted in any of the remaining ferrets.

Reproduction was normal for the females fed the control ration. Each produced a litter of from 7 to 9 kits after a 41 day gestation period. The kits grew normally and no mortality was observed in these three litters. The female ferrets fed 20 ppm of Aroclor 1242 were mated and whelped normal size litters after 37 days (2 females) or 41 days (1 female). All kits were born dead or died within 24 hours. Each female was remated with the next estrus. Two females delivered kits and experienced the same 100 percent mortality. The litters, however, were much smaller (2 and 3 kits). The third female appeared to be pregnant, but failed to whelp. Possibly, the kits were reabsorbed by the mother or were eaten immediately after birth and prior to the female being checked for whelping.

The combined nail length of the right front foot was not significantly different at the initiation of the study between the control and 20 ppm Aroclor 1242 animals. There was a significant increase in right front foot nail length of the PCB ferrets, both male and female, after 235 days on treatment. The differences in length were more obvious in the males. The female probably

Table 14. Nail lengths of male and female ferrets fed 20 ppm Aroclor 1242 or a control diet for 235 days.

	No. of animals	Weight (g)		Combined nail lengths of right front foot[1] (mm)		Combined nail lengths of left hind foot[1] (mm)
		Initial	Final	Initial	Final	Final
MALES						
Control	3	717[a2]	1323[a]	27.7[a]	28.8[a]	33.2[a]
20 ppm Aroclor 1242	3	800[a]	1350[a]	29.7[a]	49.7[c]	58.3[c]
FEMALES						
Control	3	623[b]	865[b]	27.3[b]	30.0[b]	22.8[b]
20 ppm Aroclor 1242	3	612[b]	628[d]	27.8[b]	37.7[d]	25.0[b]
S.E.[3]		37.4	58.2	1.01	1.7	2.02

[1] Combined nail length = sum of the individual nail lengths on the foot.

[2] Means with same superscript within sexes are not significantly different ($P < 0.05$).

[3] S.E. = $\sqrt{MS_E/3}$

has a greater wearing down of the nails due to caring for the young, while the male remains relatively inactive throughout the year. The combined nail lengths of the left hind foot were greater in males fed Aroclor 1242 than in the male ferrets fed the control diet. This difference between the two groups was even more extreme than for the right front foot. The female left hind foot nail lengths were not significantly longer for PCB animals than the control females.

The ferrets consuming the PCB diet in this study showed hyperkeratosis in the epithelium of the hair and skin tissues. As stated by the pathologist[1], there was a failure in the maturation of the epithelium of the hair and nail beds. The nuclei of these cells were being retained, thereby resulting in a thick and irregular deposition of keratin. Also noted was nearly complete hypoplasia of the bone marrow in the toes of the PCB-treated ferrets. The lesions of the hair, skin, and bone marrow were evident, but less pronounced in the females than in the males fed Aroclor 1242.

DISCUSSION

The increase in nail lengths seen in ferrets fed Aroclor 1242 has not been reported in other species to the knowledge of this author. However, abnormal and excessive hoof growth has been observed in cattle exposed to polybrominated biphenyls (Jackson and Halbert, 1974; Wastell et al., 1978). Hyperkeratosis has also been reported in humans (Kuratsune et al., 1972) and rabbits (Vos and Beems, 1971) exposed to PCBs and cattle exposed to PBB (Wastell et al., 1978). In tests with rabbits, Vos and Beem (1971) found that Aroclor PCB mixtures caused

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[1] Dr. T. G. Bell, Department of Pathology, Michigan State University.

a far lesser degree of hyperkeratosis than Phenoclor® [2] or Clophen® [3] PCB mixtures. This difference in reaction may be due to a lesser level of contamination in the Aroclor mixtures (Hutzinger et al., 1974). In Rhesus monkeys treated with tetrachlorodibenzodioxin (TCDD), finger and toenails were often lost (McConnell et al., 1978). No change in nail length was reported in this study. Nail weakness was found in humans following accidental exposure to PCBs in Japan (Kuratsune et al., 1972), although no alteration of nail length or thickness was observed.

Hyperkeratosis in cattle can be caused by chlorinated napthalenes (Smith et al., 1972). Hutzinger et al. (1974) discuss the presence of chlorinated napthalenes in polychlorinated biphenyl mixtures, especially those mixtures manufactured outside the United States. The higher levels of chlorinated napthalenes in Phenoclors and Clophens than Aroclors may explain part of the difference noted in hyperkeratosis of rabbits as reported by Vos and Beem (1971). Ultraviolet light has also been shown to trigger biphenyl reactions which yield napthalenes and may indicate another potential source of these compounds. Poisoning by chlorinated napthalenes causes a sharp decline in vitamin A levels in the blood (Smith et al., 1972), possible by interferring with the conversion of carotene to vitamin A. Blood levels of vitamin A were not determined in this study.

The excessive nail growth seen in ferrets fed Aroclor 1242 may result from contaminants in the Aroclor mixture and/or effects yet unknown caused by the chlorinated biphenyls themselves.

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[2] Tradename of PCB mixture produced by Prodelec Co., France.

[3] Tradename of PCB mixture produced by Bayer Co., Germany.

Figure 3. Nail growth of right front feet of Aroclor 1242-treated (upper row) and control (lower row) ferrets (top view).

Figure 4. Nail growth of right front feet of Aroclor 1242-treated (upper row) and control (lower row) ferrets (bottom view).

EXPERIMENT V. Placental Transfer of PCBs.

To determine whether or not placental transfer of polychlorinated biphenyls occurs and if so, to what degree, pregnant mink and ferrets were injected with ^{14}C labeled Aroclor 1242.

PROCEDURE

Four pregnant female mink and four pregnant ferrets were selected. Animals were chosen that appeared to be in the final trimester of gestation for mink and day 37 of pregnancy for the ferrets. This approximation as to stage of pregnancy was necessary for the mink due to delayed implantation in this species. The mink were treated the last week of April and the ferrets on May 11, 1979. Each animal was anesthetised with 0.075 ml of Tilazol®[1] intramuscularly. The jugular vein was then exposed and 1μCi of ^{14}C-labeled Aroclor 1242[2] in propylene glycol (0.1 ml) was slowly injected into the bloodstream. After two hours, a blood sample was collected via cardiac puncture and the female sacrificed by cervical dislocation. Liver, kidney, urine, and amniotic fluid were taken from each female. The fetuses were then removed from the placenta, frozen, and later analyzed for ^{14}C activity. Kit tissues were dissected from the fetuses for individual tissues or the entire kit was homogenized using a Tekmar Tissue Homogenizer[3] and an aliquot used for radioactivity determination.

Tissues were then weighed to 0.1 g and solubilized in 1 ml of Unisol[4] overnight. On completion of digestion, 0.5 ml of water-free methanol was added and agitated to mix. Each sample then received 10 ml of Unisol-Complement[4].

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[1] Trade name for a 1-1 combination of tiletamine hydrochloride and zolaespam (a diazepinione tranquilizer). Parke Davis Co., Ann Arbor, MI 48106

[2] Produced by New England Nuclear, Boston, MA 02118, specific activity 31.1 mCi/mmol

[3] Tekmar Company, P.O. Box 37202, Cincinnati, OH 48222

[4] Isolab Incorporated, Akron, OH 44321

After capping and brief shaking, the samples were counted at 10°C using an Isocap/300 Liquid Scintillation System[5], model #6872. Each sample was allowed to cold and dark adapt for at least two hours prior to counting. Due to hemolysis of the maternal blood samples, it was necessary to use only the plasma and reduce the native coloration present with four drops of 30% hydrogen peroxide solution mixed with the finished radioactive cocktail. Each sample was counted for ten minutes. Following the initial counting, internal standards were made to determine the quenching effects in all samples.

The samples were corrected for quenching effects, background radiation and weight (or volume) of tissue used. The efficiency of the scintillation counter was then compensated for to give the value in disintegrations per minute (DPM). This figure was then expressed as the percentage of total DPM injected into the mother for each tissue.

RESULTS

No significant difference was found in the maternal plasma, urine, and amniotic fluid between mink and ferrets injected with ^{14}C-labeled PCBs (Table 15). Similarly, no difference was noted in the fat, kidney, or liver PCB concentrations of the two species. The radioactivity of each tissue or fluid, with the exception of plasma, was numerically less for ferrets than for mink. Ferrets showed less variability within species than the mink. The standard errors of the ferret's tissue and fluid means were consistently smaller, by at least one half, than the standard errors associated with mink tissues.

There was significantly less radioactivity in the ferret fetuses than in the mink fetuses exposed to ^{14}C-labeled PCBs (Table 16). No significant difference was found in the individual fetal tissues examined, although fetal

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[5] Searle Analytic Inc., Des Plaines, IL 60018

Table 15. Maternal Concentrations[1] of PCB Two Hours After Intravenous Injection.

	n	Fluid			Tissue		
		Plasma	Urine	Amniotic	Fat	Kidney	Liver
Mink	(4)	0.24^{a}[2] ± 0.033	3.45^{a} ± 0.554	0.07^{a} ± 0.049	2.42^{a} ± 0.295	0.87^{a} ± 0.144	2.84^{a} ± 0.458
Ferret	(4)	0.31^{a} ± 0.013	1.87^{a} ± 0.215	0.02^{a} ± 0.003	2.23^{a} ± 0.108	0.65^{a} ± 0.061	1.65^{a} ± 0.214

[1] Concentration expressed as percent of initial dose x 10^{-1} ± S.E. per gram of tissue or ml of fluid.

[2] Means with the same superscript are not significantly different ($P < 0.05$).

Table 16. Fetal Concentrations[1] of PCB Two Hours After Maternal Intravenous Injection.

	n	Whole kit		n	Tissue				
		Last trimester	First trimester		Fat	Kidney	Liver	Brain	Intestine
Mink	(4)	0.19^{a}[2] ± 0.017	0.01 ± 0.002	(2)	0.37^{a} ± 0.141	0.24^{a} ± 0.027	0.95^{a} ± 0.542	0.11^{a} ± 0.007	0.21^{a} ± 0.045
Ferret	(4)	0.12^{c} ± 0.004	---	(4)	0.20^{a} ± 0.019	0.22^{a} ± 0.011	0.18^{a} ± 0.023	0.12^{a} ± 0.012	0.13^{a} ± 0.021

[1] Concentration expressed as percent of initial dose x 10^{-1} per gram of tissue or ml of fluid.

[2] Means with the same superscript are not significantly different ($P < 0.05$).

ferret tissues tended to contain less radioactivity than fetal mink tissues. Due to delayed implantation in the mink, it was difficult to estimate the stage of pregnancy of the females. For this reason, kits were not only taken in the final trimester of development, but unintentionally also taken during the first trimester. These first trimester kits contained significantly less radioactivity than the final trimester mink kits.

DISCUSSION

Transplacental passage of PCBs from mother to fetus has been shown in mice (Masuda et al., 1978a), rats (Takagi et al., 1976; Ando, 1978), rabbits (Grant et al., 1971a; Villenevue et al., 1971b), cattle (Platonow and Karstad, 1973b), rhesus monkeys (Allen and Barsotti, 1976), and humans (Masuda et al., 1978b). The results of this experiment demonstrates that placental transfer of PCBs also occurs in the mink and ferret.

Takagi et al. (1976) found the average placental transfer of PCB from dam to fetus to be less than 0.28% of the PCB absorbed by the mother in oral feeding trials in rats. This is in general agreement with the 0.11%[1] of maternal dose observed in the mink fetuses and 0.07% in the ferret fetuses in this study. In the species previously tested, placental transfer accounts for only a small proportion of the PCB level found in the young. A much greater quantity of PCBs are entering the growing offspring via the milk. Ando (1978) found the transfer of hexachlorobiphenyl to be fourteen times greater by lactation than by placental transfer in the rat. The transfer of hexachlorobiphenyl through the milk was sufficient to significantly decrease the mother's tissue concentrations of this compound. In humans, the transfer of PCBs via the milk has been shown to be of much greater importance than placental transfer (Masuda et al.,

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[1] % of initial dose per gram of fetus x average weight of fetus (6 g).

1978b). The higher kit mortality and reduced biomass produced by female mink fed 20 ppm Aroclor 1016 in Experiment I suggest that PCBs are being excreted in the milk of these animals. Earlier studies conducted with mink (Aulerich and Ringer, 1977) reported similar results in females fed PCB-contaminated fish. The milk of mink intraperitoneally injected with ^{14}C-labeled Aroclor 1242 was found to contain PCBs (unpublished data), although the levels were not quantified. The ferrets fed Aroclor 1016 in Experiment I were able to produce a kit biomass comparable to the control animals; kit mortality was likewise comparable to the untreated females. This may indicate a lower PCB concentration in the milk or some other as yet unexplained species difference.

The difference in PCB residue levels seen between last trimester and first trimester mink kits is not unexpected. Placental permeability increases with advancing gestation (Nalbandov, 1958) as does the surface area of the placenta. The villous membrane becomes thinner and the flow rate of the umbilical circulation is greater as pregnancy progresses (Moya and Smith, 1965). These factors cause the fetus to be more intimately in contact with substances in the mother's blood during the latter stages of pregnancy. Most foreign substances have been shown to cross the placenta by simple diffusion (Eckhoff, 1972a, 1972b) and so these changes in the surface area available for transfer, the thickness of placenta membranes and maternal blood flow, would increase the rate of diffusion. This would result in the fetuses near term having greater concentrations of PCBs than the fetuses in the early stages of development.

The mink and ferret both have deciduate placentas with a close association established between maternal and fetal tissues. The ferret has an endotheliochorial zonodiscoidalis placental type (Enders, 1952) during the first part of gestation, but an endotheliochorial bidiscoidal placenta in the latter stages of pregnancy (Enders, 1957). The mink exhibits an endotheliochorial zonary

placenta (Enders, 1957) throughout pregnancy. The mink placenta, being more truly zonary than the ferret placenta, has a slightly greater surface area between mother and fetus for transplacental exchange. The mink placenta forms a nearly continous band while the bidiscoidal placenta of the ferret has areas between the disc shaped regions without uterine contact. The greater area for diffusion in the mink placenta than the ferret placenta may explain the significantly higher concentrations of PCBs found in whole kit samples.

CONCLUSIONS

1. Mink can tolerate higher levels of Aroclor 1016 than of Aroclor 1242 in regards to livability and reproductive efficiency. Ferrets were also better able to withstand the effects of Aroclor 1016 than the effects of Aroclor 1242, although mortality differences were not seen.

2. The ferret is more resistant to the effects of either PCB mixture than the mink.

3. Aroclor 1016 reduced the "biomass" produced by mink females consuming this diet and caused increased kit mortality.

4. Food consumption and food passage times were the same for mink and ferrets.

5. Ferrets fed 20 ppm of Aroclor 1242 in the diet exhibited excessive toe nail growth.

6. Placental transfer of Aroclor 1242 was found to occur to a greater degree in the mink than in the ferret. Mink kits early in development did not show nearly as high a PCB concentration as near-term kits.

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APPENDICES

APPENDIX A

Molecular composition of four PCB mixtures[a].

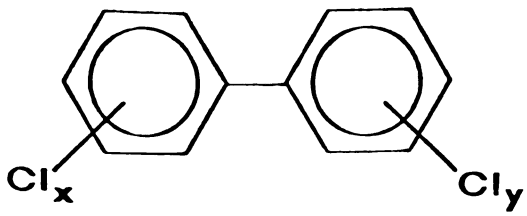
Molecular formula	Presence (%) in Aroclor			
	1221	1016	1242	1254
$C_{12} H_{10}$	11	<0.1	<0.1	<0.1
$C_{12} H_9 Cl$	51	1	1	<0.1
$C_{12} H_8 Cl_2$	32	20	16	0.5
$C_{12} H_7 Cl_3$	4	57	49	1
$C_{12} H_6 Cl_4$	2	21	25	21
$C_{12} H_5 Cl_5$	<0.5	1	8	48
$C_{12} H_4 Cl_6$	ND[b]	<0.1	1	23
$C_{12} H_3 Cl_7$	ND	ND	<0.1	6
$C_{12} H_2 Cl_8$	ND	ND	ND	ND

[a] Data from Hutzinger et al. (1974)

[b] None detected, <0.01% = ND.

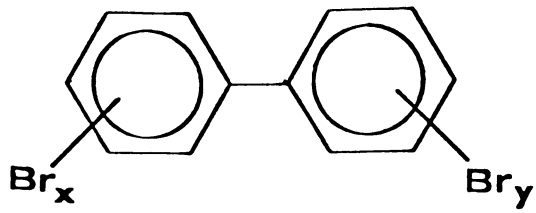
APPENDIX B

Structure of PCBs and related compounds.



PCB

(polychlorinated biphenyls)



PBB

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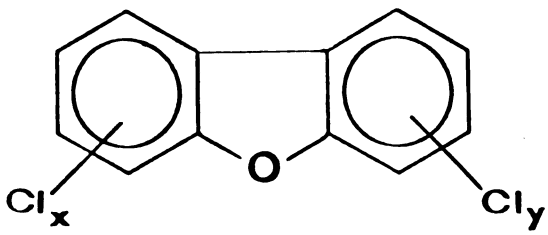
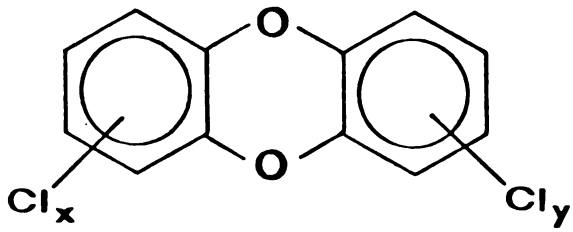


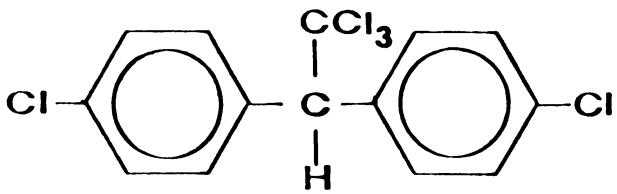
PCDF

(polychlorinated dibenzofurans)

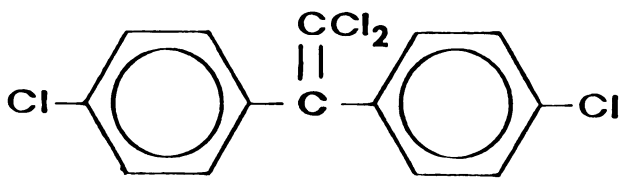


PCDD

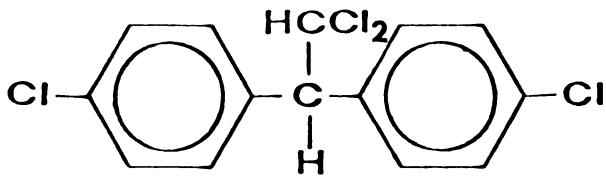
(polychlorinated dibenzodioxins)



DDT



DDE



DDD

APPENDIX C

Chlorinated dibenzofuran concentrations[1] in several PCB mixtures[2].

PCB	4-Cl	5-Cl	6-Cl	Total
Aroclor 1248	0.5 (25)	1.2 (60)	0.3 (15)	2.0
Aroclor 1254	0.1 (6)	0.2 (12)	1.4 (82)	1.7
Aroclor 1254	0.2 (13)	0.4 (27)	0.9 (60)	1.5
Aroclor 1260	0.1 (10)	0.4 (40)	0.5 (50)	1.0
Aroclor 1260	0.2 (24)	0.3 (38)	0.3 (38)	0.8
Aroclor 1016	N.D.[3]	N.D.	N.D.	---
Clophen A-60	1.4 (16)	5.0 (58)	2.2 (26)	8.6
Phenoclor DP-6	0.7 (5)	10.0 (74)	2.9 (21)	13.6

[1] Expressed as μg/g. Values in parentheses represent quantity as percent of total dibenzofuran.

[2] Data from Hutzinger et al., 1974.

[3] N.D. = none detected (<0.00 1 μg/g).