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thesis entitled

EFFECTS OF FEEDING FISH COLLECTED DOWNSTREAM FROM OAK RIDGE RESERVATION ON THE REPRODUCTIVE PERFORMANCE OF MINK

presented by

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has been accepted towards fulfillment
of the requirements for

M.S. degree in Animal Science

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November 15, 1995
Date

# EFFECTS OF FEEDING FISH COLLECTED DOWNSTREAM FROM OAK RIDGE RESERVATION ON THE REPRODUCTIVE PERFORMANCE OF MINK

By

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A THESIS

Submitted to
Michigan State University
in partial fulfillment of the requirments
for the degree of

MASTER OF SCIENCE

Department of Animal Science

1995

## ABSTRACT

## EFFECTS OF FEEDING FISH COLLECTED DOWNSTREAM FROM OAK RIDGE RESERVATION ON THE REPRODUCTIVE PERFORMANCE OF MINK

By

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Concentrations of radionuclides, metals, and organic compounds in water, sediment, and biota of the Clinch River and Watts Bar Reservoir on the Oak Ridge Reservation (ORR) have lead to concern with regard to environmental and human health. The objectives of the present study were to assess the effects of polychlorinated biphenyls and mercury in fish collected from the reservation on mink reproduction and to provide information to the Clinch River Environmental Restoration Program for evaluating the extent and degree of effects of ORR operations on wild piscivorous populations. Fish collected from downstream ORR containing 2.13 ppm total polychlorinated biphenyls (PCBs) and 0.35 ppm mercury were substituted for marine fish in mink (Mustela vison) diets at concentrations of 0, 25, 50, and 75%. The experimental diets were fed to adult mink beginning three months prior to breeding (December) and continuing until the young were weaned (June). No adverse effects on the reproductive performance of the adult mink or kit survival could be attributed to feeding the experimental diets.

## ACKNOWLEDGEMENTS

I would like to thank my major professor, Dr. Richard Aulerich, for his help and guidance throughout my graduate studies. I also extend my thanks to the remaining members of my graduate committee: Drs. Steven Bursian, Karen Chou, and James Render.

I am also greatful for the support and understanding that I received from my collegues at the MSU Experimental Fur Farm while learning mink husbandry: Christina Bush, Angelo Napolitano, Jeff Greenley and especially Phil Summer for listening and advising and for making even the most routine tasks enjoyable. And finally, a sincere thanks is extended to Ms. Carol Daniel for her friendship, patience and understanding, and for going above and beyond in helping me with my thesis.

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# INTRODUCTION

Operations and waste disposal activities at the Y-12 plant, the Oak Ridge National Laboratory (ORNL) and the Oak Ridge Gaseous Diffusion Plant, all located on the U.S. Department of Energy (DOE) Oak Ridge Reservation (ORR) in eastern Tennessee, have introduced a variety of airborne, liquid and solid wastes into the surrounding environment. Some of these wastes may affect off-site areas (areas beyond the ORR boundary) by entering local streams that ultimately drain into the Clinch River and Tennessee River systems. Concentrations of radionucleotides, metals and organic compounds in water, sediment and biota of the Clinch River and Watts Bar Reservoir suggest the presence of a variety of contaminants of possible concern with regard to the health of the environment and human population. The contaminants of particular concern are polychlorinated biphenyls (PCBs) and mercury (Hg). Thus, the DOE has initiated a comprehensive environmental restoration effort to eliminate releases of hazardous substances, pollutants and contaminants from the ORR.

Mink (Mustela vison) were identified in the Screening Level Risk Assessment for Off-Site Ecological Effects in Surface Waters Downstream from the U.S. Department of Energy Oak Ridge Reservation as a species being at risk (Suter, 1990). Since they also have been shown to be among the most sensitive, if not the single most sensitive, mammalian species to PCB toxicity (Aulerich and Ringer, 1977), they were the preferred animal model for this

study. Feeding studies conducted by Aulerich et al. (1971,1973), Hornshaw et al. (1983), and Heaton et al. (1995) have demonstrated the extreme sensitivity of mink to chlorinated hydrocarbon contaminants, especially PCBs, contained in fish taken from the Great Lakes. Research performed with mink has been instrumental in setting U.S. water quality standards for PCBs (Aulerich and Bleavins, 1981). Additional studies have shown this species to be similarly sensitive to other halogenated hydrocarbons, including polybrominated biphenyls (Aulerich and Ringer, 1979), hexachlorobenzene (Bleavins et al., 1984), and 2,3,7,8-tetrachlorobenzo-p-dioxin (Hochstein et al., 1988). The toxicosis of mercury has also been studied extensively in mink ( Aulerich et al., 1974; Wobeser et al., 1976). Numerous other toxicological studies with mink have been reported in the literature and summarized by Calabrese et al. (1992).

It is known that fish, a major food item of mink, inhabiting aquatic systems downstream from the ORR contain elevated concentrations of PCBs and mercury. Therefore, the overall objective of this study was to assess the effects of these environmental contaminants in the fish on mink. These assessments will provide information for the Clinch River Environmental Restoration Program for evaluating the adverse effects of ORR operations on wild piscivorous populations.

## STUDY OBJECTIVES

The specific objectives of this study were to:

1. Determine concentrations of polychlorinated biphenyls and mercury contaminants in fish collected upstream and downstream from the ORR;
2. Determine the reproductive and other physiological effects in mink fed fish collected downstream from the ORR;
3. Examine mink for gross and histopathologic alterations of a toxicosis from contaminants found in fish from ORR which were fed to mink;
4. Determine concentrations of polychlorinated biphenyls and mercury in mink tissues;

## LITERATURE REVIEW

### OAK RIDGE NATIONAL LABORATORY

In 1942, the Army Corps of Engineers purchased 92 square miles of land under the guise (for security reasons) of establishing the Kingston Demolition Range. The area, located in eastern Tennessee, was given the name Clinton Engineer Works and was intended for the large-scale production of fissionable isotopes of uranium and plutonium needed for the atomic bomb. In November, 1942, construction of the headquarters for the Manhattan Engineer District and nerve center for the wartime atomic energy effort was begun. The first science facility, Clinton Laboratory (now known as Oak Ridge National Laboratory) was built in 1943. Its purpose was to serve as a pilot plant for the large plutonium-producing reactors and as a facility for research and development for large-scale production of plutonium. The major Oak Ridge facilities included two uranium production plants (Y-12 and K-25) and a laboratory (X-10). Late in 1944, a third plant, the thermal diffusion plant, was built to boost fissionable isotope production. By 1945, Oak Ridge employed a total of 82,000 people. When World War II ended, the population decreased and the Atomic Energy Commission began preparing long-range plans for Oak Ridge so that the community could continue to function as a major scientific center (Thompson, 1973; Kraus, 1976).

The Oak Ridge reactor was the world's second, and was in operation until 1963. The first batch of irradiated fuel slugs was taken from the reactor in 1943 and the first plutonium was

shipped from Clinton Laboratory in 1944. On August 6, 1945, the United States dropped the first atomic bomb of World War II on Hiroshima, Japan. The bomb used enriched uranium from the Clinton Laboratory. After the war, Clinton Laboratory shifted part of its wartime effort to peaceful research. Since the end of World War II, Oak Ridge has been a center for numerous projects including: demonstrating the safe production and chemical recovery of plutonium, developing safe methods for reprocessing nuclear fuels, producing radioisotopes for scientific research,operator training and development of nuclear power systems such as breeder and fusion reactors. For over 40 years, Oak Ridge produced nuclear weapons and has been a world center of study on the effects of radiation on the environment. Today, Oak Ridge consists of 24 divisions ( 16 major research divisions and 8 service divisions) located on 35,300 acres. It is a stable and progressive city with a worldwide reputation as a nuclear center of excellence and it has had a role in virtually every major scientific operation and activity in the atomic energy program (Thompson, 1973; Krause, 1976).

The many research and production projects conducted at Oak Ridge over the years have resulted in contamination of the environment of the ORR and adjacent areas with persistent and potentially toxic compounds, including PCBs and mercury. Because of concern for the effects these contaminants pose to human health and wild piscivorous populations, this study was conducted to provide information that could be used in an environmental risk assessment for the Clinch River Environmental Restoration Program.

## POLYCHLORINATED BIPHENYLS

### General Introduction

Polychlorinated biphenyls (PCBs) are molecules that have multiple chlorines attached to a biphenyl nucleus. Their general formula is C12HxCly where x=0-9 and y=10-x. The biphenyl molecule is made up of two connected rings of six carbon atoms each. Any or all of the 10 available sites can have chlorine atoms, with 209 different PCB compounds possible (Abramowicz, 1990). Approximately 25 congeners account for 50-75% of the total mass of PCBs found in the environment (McFarland and Clarke, 1989). The extensive use of PCBs in many industrial applications is due to the unique physical and chemical properties of these compounds. PCBs are resistant to acids, bases and other chemical agents, they have remarkable thermal stability, stability to oxidation and hydrolysis, low solubility in water, low flammability, high electric resistivity, favorable dielectric constants, and low vapor pressure at ambient temperature (DeVoogt and Brinkman, 1989).

### Production of PCBs in the United States

Commercial production of PCBs began in the United States in 1929. Since that time, approximately 1.5 million metric tons of PCBs have been produced in 10 countries. The Swan Chemical Company was the first U.S. company to produce PCBs. The company was purchased in the mid 1930s by Monsanto Industrial Chemicals which began producing various chlorinated biphenyl mixtures under the trade name Aroclor (De Voogt and Brinkman, 1989).

Chlorinated biphenyl mixtures were initially produced for dielectric fluids in transformers. In time, their utilization in lubricants and heat-transfer systems caused an increase in their demand. The production and sale of PCBs reached a peak in 1970 but were dramatically reduced in 1971 when, after much public and scientific concern, Monsanto voluntarily reduced its PCB production. By 1977 the company had ceased all production of PCBs. The estimated cumulative production of PCBs in the U.S. from 1930 to 1975 was 1400 million pounds. (DeVoogt and Brinkman, 1989).

There are many trade names under which commercial PCB mixtures have been sold. In the United States and Great Britain, the mixtures were sold under the name of Aroclor. In other countries they were sold as Clophen (Germany), Fenchlor (Italy), Kanechlor (Japan) and Phenochlor (France).

The Aroclor mixtures produced in the United States had a chlorine content of 21,32,42,48,54,60 or 61% by weight. The mixtures were designated by a four digit number, the first two digits, "12" (except for Aroclor 1016), represented the 12 carbons of the biphenyl skeleton. The second two digits indicated the percentage of chlorine in the mixture. Thus, Aroclor 1254 had 12 carbons and contained 54% chlorine. Since July, 1979, through authority of the Toxic Substance Control Act (TSCA), the manufacture, importation, distribution and further processing of PCBs in the U.S. has been banned (Hooper et al., 1990).

## Production Process

The production of PCBs involved chlorination of biphenyl and separation and purification of chlorinated biphenyl fractions. For the production of PCBs, a chlorinator was charged with proper quantities of biphenyl and a catalyst, such as ferric chloride or iron filings. Anhydrous chlorine was allowed to flow through the mixture and the charge was circulated using a pump. The mixture was heated above the melting point of the biphenyl and the hydrogen chloride produced by the chlorination process was discharged. The chlorination process typically took 12-36 hours. The crude Aroclor products were then treated with a 0.3% alkali mixture and purified to remove the catalyst, color, and hydrogen chloride by vacuum distillation. Commercial PCBs were produced as technical grade liquids and as liquid mixtures ready for application (DeVoogt and Brinkman, 1989). The resulting products ranged from light oily fluids (di, tri, and tetra-chlorobiphenyls) to heavy, honey-like oils (penta-chlorobiphenyl) to greases and waxes (more highly chlorinated biphenyls) (Abramowicz, 1990). During certain chemical production processes, accidental production of PCBs has been recognized. It is estimated that 50 tons of PCBs are produced annually as by-products of industry in the United States (Callahan et al., 1984; DeVoogt and Brinkman, 1989).

## Applications

Depending on their application, the uses of PCBs have been divided into two categories: open and closed systems. Open-ended systems are those from which PCBs can not be re-collected. Open systems include use of PCBs in plasticizers, carbonless copy paper,

lubricants, inks, laminating agents, paints, adhesives, waxes, additives in cement and plaster, casting agents, sealing liquids, fire retardants, immersion oils and pesticides. The use of PCBs in open systems can lead to environmental contamination and it was for this reason that most countries decided to terminate the open-ended use of PCBs during the years 1971 through 1973. In 1973, the use of PCBs was restricted to four closed systems. The closed systems included the use of PCBs in cooling liquids in transformers, dielectric liquids in capacitors, heat-conducting fluids in heat-exchangers and fire- or heat-resistant corrosion-free hydraulic fluids in mining equipment and vacuum pumps (DeVoogt and Brinkman, 1989). At present, some closed systems in which PCBs are used are not considered closed as these systems are known to leak to some extent (UNEP, 1985), and thus, PCBs from many sources may lead to environmental contamination (DeVoogt and Brinkman, 1989).

## PCBs in the Ecosystem

In spite of the ban on PCB production in this country, the persistence of PCBs in the environment and their concentration in the biological food chain have caused much concern. The extensive application of these chemically and thermally stable compounds has resulted in widespread contamination. It is estimated that several million pounds have been released into the environment (Abramowicz, 1990).

Polychlorinated biphenyls are toxic, mutagenic and teratogenic agents with bioaccumulation and bioconcentration ability. They are a global environmental health hazard as they have a detrimental impact on nearly every member of the biota, appearing in the tissues of most living creatures as well as in the air, water, soils and sediments over most of

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the earth, and are thus of ecological and medical concern ( Hooper et al., 1990). The environmental burden in the air, water, sediment, soils and biota was estimated by the National Research Council (1979) to be 82 million kg. The Great Lakes have received considerable attention with regards to PCB contamination because of substantial food-chain concentrations. Although the ban of PCB production may eventually result in a decrease to trivial levels in systems such as the Great Lakes, differences in mobility, biodegrability, waste storage and continued use of PCBs suggests that we may have to cope with this pollutant for many years to come.

The chemical and physical properties that make PCBs so commercially desirable also make them serious environmental pollutants. Their stability, along with the ease at which they are taken up by living organisms and accumulated at high levels in the food chain has resulted in environmental problems. Their nonpolar nature makes them a good insulating coolant in electrical equipment because of the high dielectric constant, but it is their nonpolarity that also causes them to be highly lipophilic and to bioaccumulate. The chlorine in PCBs is electronegative and stabilizes the biphenyl molecule. This results in a heat-resistant fluid with long- term stability, making them ideal for oils and hydraulic fluids but also recalcitrant to physical and biological degredation. Highly substituted biphenyls are less volatile, less soluble in water, and more chemically stable than the lesser substituted congeners.

## Fate of PCBs

The highest biological activities in a water column are at the air-water and water-sediment boundaries. Accumulation of PCBs at these surfaces mediates their bioaccumulation by

bacteria and plankton, resulting in the introduction of chlorinated biphenyls into the food web (Young et. al.,1977).

Once PCBs enter a freshwater ecosystem, they have numerous abiotic interactions with the ecosystem. Because PCBs are highly nonpolar, they tend to accumulate and localize in sediments (Young et. al., 1977; Steen et. al., 1978). They can be removed from sediments by being transported downstream or by diffusional loss to the water column. PCBs in the water column can be removed by transport downstream and are subject to photodegredation and volatility losses (Baxter and Sutherland, 1984). Biotic interactions occur in freshwater and because of the nonpolar nature of PCBs, they accumulate in the lipids of aquatic biota. The lipophilic nature of PCBs and resistance to breakdown, allow them to biomagnify, (Sanders and Chandler, 1972) impacting organisms at every level of the food chain.

## Bioaccumulation of PCBs

There are three physiochemical properties that control PCB bioaccumulation; the degree of chlorination, water solubility and PCB stereochemistry. The higher chlorinated penta-and hexachloro biphenyl isomers accumulate to the greatest extent and thus have a higher bioaccumulation rate. The higher chlorinated PCBs also have half-lives in organisms that are related exponentially to their degree of chlorination (Kalmaz and Kalmaz, 1979). The bioaccumulation of PCBs from water by aquatic organisms is correlated with the lipophilicity of PCBs (Veith et al., 1980).

Bioaccumulation is also affected by the ability of PCBs to pass through biological membranes. Therefore, there is an optimal steric configuration for PCB bioaccumulation.

The stereochemistry of PCB molecules affects the strength of adsorption of PCBs to membrane surfaces. The most nonplanar molecules are the most strongly absorbed while those PCBs with planar aromatic rings are weakly absorbed (Veith et al., 1980).

Biota can acquire PCBs from three sectors of the environment; atmosphere, water and food. Terrestrial organisms acquire PCBs by absorption of PCBs in the atmosphere through the lung walls, absorption of PCBs in the atmosphere through the epidermis, and absorption of PCBs derived from food and/or water through the gastrointestinal tract. Aquatic organisms acquire PCBs by absorption of PCBs in the water through the gills, absorption of PCBs in the water through the epidermis and consumption of contaminated food.

The main route of uptake of PCBs by aquatic organisms is via absorption through the gills since the gills represent the active membrane surface for water exchange (Phillips, 1980). Once absorbed through the gills, PCBs are partitioned into the blood and then transported from the blood to the tissues (Kenaga, 1975). The assimilation of PCBs from ingested food occurs by partition across the lipoprotein membranes lining the gut into the bloodstream (Walker, 1975). The PCBs associated with benthic organisms are derived from contaminated sediment, and are directly related to the sediment PCB concentration (Nimmo et al., 1971).

Inert organochlorines with high octanol/water partition coefficients (Kow) can biomagnify ( the concentration of the chemical in the organism reaches a level that exceeds that in the diet of the organism). This results in food-chain accumulation, in which the concentration of a chemical increases with every step up the food chain (Clark et al., 1988; Connelly and Pederson, 1988). PCBs are transported to the intestinal wall in association with lipid molecules. They separate from the lipid and diffuse as a single molecule through the

intestinal wall. At the other side of the intestinal wall, the chemical is reassociated with lipoproteins and resynthesized triglycerides (Vetter et al., 1985).

Biomagnification occurs as a result of food ingestion. Gobas et al. (1993) conducted studies with humans and found that the concentration of PCBs in the blood of mothers was four times higher than that in the cord blood of the fetuses. However, on a lipid-weight basis, PCB concentrations in the mothers and the fetuses were approximately equal because lipid concentrations in the cord blood were three to four times lower than that in maternal blood (Fomon et al., 1970; Gobas et al., 1989). After birth, no biomagnification occurred but when the infants were exposed to the mother's milk, the PCB lipid-based concentration in the infants rose to exceed those in the milk (biomagnification).

## Biodegradation of PCBs

Numerous aerobic and anaerobic bacteria have been identified as being capable of PCB degradation ( Sayler et. al., 1978; Klages and Lingens, 1980). Aerobes oxidatively attack PCBs, breaking open the carbon ring and destroying the compound. Anaerobes leave the biphenyl ring intact while removing the chlorines. Anaerobic dechlorination degrades highly chlorinated compounds into less chlorinated derivatives. The two types of bacteria can work together to biologically destroy all PCB mixtures (Abramowicz, 1990). Certain fungi may also aerobically degrade PCBs to lower chlorinated compounds.

Besides microbial degradation, some PCBs may be susceptible to photochemical reactions or biochemical degradation. Most PCBs that accumulate in aquatic sediments are shielded from photolysis (Larsson, 1984). Photochemical dechlorination may yield products that are

more readily degraded by microorganisms than the original compounds or it can also lead to the formation of toxic polychlorinated dibenzofurans (Baxter and Sutherland, 1984).

## Toxic Effects of PCBs in the Ecosystem

Toxic effects of PCB contamination to the aquatic biota appear to be sublethal and chronic. In wildlife, physiological and developmental effects are the most sensitive endpoints for PCB toxicity. Growth retardation, immune system suppression ( Friedman and Sklan, 1989), elevated rates of disease, wasting syndrome, subcutaneous, pericardial and peritoneal edema, hepatic porphyria, congenital malformations (Fox et al., 1991;Gilbertson et al., 1991), altered hormone, retinol and vitamin A concentrations (Government of Canada, 1991; McFarland and Clarke, 1989), impaired calcium metabolism, thyroid alterations ( Jeffries and French, 1972; Hurst et al., 1974) and behavioral changes ( McArthur et al., 1983) have all been documented.

Epidemiological data for the Great Lakes suggest humans and wildlife may exhibit subtle, chronic effects due to PCB exposures. Wildlife, unlike humans, do not recognize or avoid contaminated food supplies and thus may receive greater dietary exposure to contaminants. Predators high on the food chain, such as fish-eating birds and mammals, are exposed to greater concentrations of PCBs than animals lower in the food chain due to biomagnification and bioaccumulation. Small birds and mammals have high metabolic rates, eat more per unit body weight per day and are thus exposed to greater concentrations of PCBs in vivo at a faster rate than larger species.

## Absorption and Metabolism of PCBs

PCBs are readily absorbed by passive diffussion from the gastrointestinal tract, and transported by blood to all tissues with little to no elimination. Initial tissue distribution is proportional to the rate of blood flow to the tissues and the tissue volume. Initially the highest concentrations are seen in highly perfused tissues such as the liver and large volume muscles. Eventually equilibrium is reached for all tissues, the concentration at equilibrium being determined by the lipid content of that tissue. Thus, there are higher concentrations of PCBs in adipose tissue and lower concentrations in blood and liver. When the PCB concentration in the liver is reduced by metabolism and excretion, more PCBs will partition from the blood to the liver to reestablish the tissue/blood ratio. Additional PCBs will partition from all other tissues into the blood to reestablish tissue/bood ratios. PCBs that are cleared from the liver are cleared from all other tissues as well, and those PCBs not metabolized will concentrate in the adipose tissue (Lutz et al., 1977).

It is possible that PCBs can be excreted through the small intestine wall and excreted in the feces. Williams et al. (1965) found that dieldrin injected intravenously into rats with the bile duct cannulated excreted the organochlorine compound into the feces, suggesting excretion from the gut wall. This suggests that PCBs may be partially excreted in an unchanged state into the gastrointestinal tract.

Many foreign compounds such as drugs, food additives, pesticides, and industrial chemicals are metabolized to more polar derivatives (Parke, 1968). PCBs must be transformed into more polar metabolites for excretion. Because commercial mixtures of PCBs are complex, it is difficult to obtain information on the quantitative and qualitative

aspects of PCB metabolism.

Yamamoto et al. (1973) studied the metabolism of PCBs and found that a major metabolite of 2,4,3',4'-tetrachlorobiphenyl was a 5-hydroxyderivative and they attributed the acute toxicity of 2,4,3',4'-tetrachlorobiphenyl to the production of the phenolic metabolite within the body. Although no evidence, other than for 2,5,2',5'-tetrachlorobiphenyl, has been found for the formation of epoxides during the metabolism of PCBs, Brodie et al. (1971) reported that halogenobenzenes injected intraperitoneally into rats induced massive necrosis of the centrolobular regions of the liver. They suggested that the epoxide produced in aromatic hydroxylation as a labile intermediate could be responsible for the hepatic necrosis. This hypothesis could also extend to metabolically-induced PCB toxicity.

## MFO Induction by PCBs

The mixed-function oxidase (MFO) enzymes are major components of the biological defense of living organisms against chemical stresses in the environment. These enzymes work by adding oxygen to lipophilic, endogenous and foreign compounds, catalyzing their biotransformation to more water soluble and readily excreted products. The cytochrome P-450 system is capable of hydroxylating, epoxidating and dealkylating xenobiotics. Because MFO enzymes are present in a wide variety of organisms, including humans, and since they play a central role in detoxification, they are good non-specific biomarkers of exposure to xenobiotic chemicals (Ionnides et. al., 1984). Exposure to significant quantities of mixed inducers such as PCBs can result in induction of isozymes which may activate other contaminants (Ionnides et al., 1984) resulting in the formation of free radicals. Free radicals

may damage cells or organ systems and /or alter the rate and patterns of normal biosynthesis and metabolism of essential biomolecules such as retinoids (Parke, 1968) and steroid hormones (Wood et al., 1983) resulting in secondary effects on growth, reproduction and disease susceptibility.

Though usually beneficial, biotransformation can lead to reactive intermediates that are more toxic than the parent compound leading to chemical-induced toxicities including mutagenesis, carcinogenesis, teratogenesis, and neurotoxicity. Many carcinogenic xenobiotics depend on their conversion by cytochrome P-450 to the carcinogenic metabolite (Conney, 1982).

Animals having a "normal" aromatic hydrocarbon receptor (Ah receptor) are more responsive to the inductive effects of various polycyclic aromatic hydrocarbons. When the receptor is defective, the animals are non-responsive to the inductive effects of aromatic hydrocarbons (Poland et al., 1979).

## Toxic Equivalency Factors

Polyhalogenated aromatic hydrocarbons (PHAHs) are a group of lipophilic and chemically stable environmental contaminants and include the polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs) and polychlorinated biphenyls (PCBs). The most toxic known PHAH is 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) which binds to the aromatic hydrocarbon receptor (Ah receptor) through which its toxic effects are proposed to be mediated. Some of these effects include reproductive failure, teratogenesis, carcinogenesis and immunotoxicity (Ahlborg et al., 1994).

Much of the toxicity caused by PCBs has been attributed to specific congeners that resemble TCDD and it is believed that they exert a number of common toxic responses similar to TCDD because of the common mechanism of binding to the Ah receptor (Giesy et al., 1994a). Environmental samples of dioxin-like compounds usually exist as a complex mixture of congeners, therefore in order to simplifiy risk assessment and regulatory control, the concept of toxic equivalents (TEQs) has been introduced. Toxic equivalency factors (TEFs) are estimates of the relative potency of individual congeners expressed relative to TCDD (Safe, 1990; Giesy et al., 1994b). TEFs are based on effects such as lethality, deformities, or enzyme induction. TEFs can be used to calculate concentrations of TEQs that are contributed by individual congeners. The TEFs are summed and expressed as a total equivalent concentration of TCDD. The TEQ is determined by multiplying individual congener concentrations with their corresponding TEFs. Evaluation of congeners contributing to the TEQ reveals that congeners 77, 156, 105, 157 and 114 ( IUPAC congener identification numbers) are the dominating congeners in the lesser-chlorinated Aroclor mixtures (Ahlborg et al., 1994).

## Toxic and Biological Responses to PCBs

PCB mixtures and individual congeners elicit toxic and biologic responses in organisms which include: 1. a wasting syndrome (progressive weight loss which is not related to decreased food consumption); 2. skin disorders ( acneform eruptions or chloracne, alopecia, edema, hyperkeratosis and blepharitis due to hypertrophy of the Meibomian glands); 3. hyperplasia of the epithelial lining of the extrahepatic bile duct, the gall bladder and urinary

tract; 4. lymphoid involution ( thymic and splenic atrophy with humoral and/or cell-mediated immunosuppression and/or associated bone marrow and haematologic dyscrasias); 5. hepatomegaly and liver damage( necrosis, hemorrhage and intrahepatic bile duct hyperplasia); 6. porphyria ( disordered porphyrin metabolism of the cutanea tarda type); 7. endocrine and reproductive disfunction (altered plasma concentrations of steroid and thyroid hormones with menstrual irregularities, reduced conception rate, early abortion, excessive menstrual and postconceptional haemorrhage, anovolution in females, and testicular atrophy and decreased spermatogenesis in males); 8. teratogenesis ( cleft palate and kidney malformations); 9. carcinogenesis (Safe et al., 1982).

Numerous studies have shown that PCBs administered as a single dose are less toxic than the same amount administered over a long period of time. There is usually a latent period of time between the time of exposure and the onset of signs of toxicity. Some disorders are manifested after several months of exposure, such as porphyria, while other clinical signs may occur within days of exposure, such as thymic atrophy. Because PCBs have such a long half-life, the synptoms of chronic toxicity can develop even after exposure has ceased.

## Carcinogenicity of PCBs

It appears that virtually all PCB congeners are stable and not readily converted by biotransformation enzymes into reactive intermediates that could potentially cause damage to DNA. Although there is some evidence that some PCBs can covalently bind to DNA and cause genotoxic effects in some in vitro systems (Morales and Matthews, 1979), short-term

administration of PCBs to mice failed to initiate carcinogenesis in the skin (Oesterle and Demi, 1984; Hayes et al., 1985). However, mice and rats continuously exposed to PCBs for two years or more developed preneoplastic livers (Ito et al., 1973; Nishizumi, 1976), gastric lesions (Morgan et al., 1981), and hepatocellular and gastric carcinomas. Therefore, initiation may occur with long-term exposure to PCBs.

Because PCBs are potent inducers of hepatic microsomal cytochrome P-450 isozymes, hepatocytes that were previously induced by PCBs may be more susceptible to initiation by carcinogens that require microsomal activation for genotoxicity . Promotion by PCBs is dose-dependent and there is a threshold dose below which promotion of preneoplastic liver lesions is not observed. This threshold may be above what most animals and humans encounter in the environment (Morales and Matthews, 1979).

## Human Health Effects

There are three major scenerios in which humans have been exposed to PCBs: 1. workers who produced or utilized PCBs; 2. accidental exposure; and 3. environmental exposure through contaminated food, air or water (Safe, 1994). PCBs enter the human body in an occupational setting through the body surface by direct contact (dermal exposure) or through the respiratory tract. The reported effects of PCBs on occupationally- exposed humans include dematological conditions, liver damage, induction of hepatic monoxygenase enzymes and pulmonary dysfunction (Warshaw et al., 1979). These individuals also have relatively high levels of PCBs in their serum or adipose tissues and increased serum activities of hepatic enzymes and serum concentration of lipids. Women exposed to PCBs typically have given

birth to children with low birthweights (Hara, 1985; Lawton et al., 1985; Takamatsu et al., 1985). It has also been reported that there is a correlation between serum PCB concentration and concentrations of PCBs in milk which is available to nursing infants. Many of these responses were reversible once exposure to PCBs ceased, the serum concentrations of PCBs decreased. Though no overall increases in cancer-related mortality have been correlated with occupational exposure to PCBs, increased incidences of specific cancers have been reported (Brown, 1987). It is unlikely that environmental uptake of PCBs results in significant human health effects, however, individuals who consume large amounts of fish from PCB-contaminated waters may be exposed to high levels of PCBs which are reflected in elevated serum PCB concentrations (Kreiss et al., 1981).

Although PCBs were produced in large quantities since the 1930s, it was not until 1968, when a major human poisoning (" Yusho") occurred in Japan that interest in their toxicity was aroused. In 1968, 1600 individuals in southwestern Japan suffered toxic effects after consuming rice oil contaminated with a commercial PCB industrial fluid, Kanechlor 400. Eleven years later a second large-scale human poisoning (" Yu-Cheng") occurred in Taiwan due to contaminated rice oil. PCBs were used as a heat conductor in the process of heating the rice oil. The leakage of PCB from a heating pipe to the rice oil resulted in the contamination (Safe, 1987).

Though the most characteristic symptoms of PCB intoxication in humans included dermal problems such as chloracne, patients reported a broad spectrum of effects including headaches, stomach aches, numbness of the extremeties, coughing, bronchial disorders, and joint pains. Children who were poisoned in the 1968 accident had retarded growth rates and

abnormal tooth development. Newborns were undersized and exhibited systemic pigmentation (Urabe and Koda, 1976; Urabe and Asahi, 1984). In the initial stage of intoxication, nonspecific symptoms such as fatigue and weight loss were observed. With time, prominant features such as swelling of the upper eyelids, cheeselike discharge from the eyes, temporary failing of the eyesight, acneform eruptions, and blackening of the pores, nails, and dermal pigmentation were observed ( Urabe and Koda, 1976; Urabe and Asahi, 1984). There are many non-dermal manifestations of PCB poisoning including nervous, endocrine, respiratory, hematologic, hepatic, metabolic, bone and joint disorders and effects on fetal and infant life ( Harada, 1976; Hirayama, 1976; Iwashito et al., 1977 and Ohnishi and Arakawa, 1977).

PCB-contaminated fish may be part of the human food chain. Because lakes are stocked and sport fishing is popular, people consume these fish despite guidelines on the amount that should be eaten due to contamination. Evidence suggests that mammals that eat these fish are at risk of physiological and behavioral changes attributed to these chemicals. Jacobson et al. (1984) studied the behavioral differences between children of mothers who ate Great Lakes fish versus those who did not. It was found that the offspring of mothers who ate two to three Lake Michigan fish meals per month for at least six years, had a lower birth weight, smaller head circumference, shorter gestational age, and less neuromuscular activity than offspring born to mothers who ate little to no fish from the Great Lakes (Fein et al., 1984). The exposed babies also had poorer lability states, a greater amount of startle, weak reflexes and were more worrisome than non-exposed infants. At four years of age, these babies had lower verbal and memory scale scores and refusal to cooperate was common (Jacobson et

al., 1990).

## Laboratory Animal Studies

PCBs produce a wide variety of biological effects in experimental animals. These include enzyme induction and inhibition, decreased reproductive efficiency, changes in plasma lipid concentrations, decreased immunocompetence, dermatological effects and changes in liver morphology which include hepatic porphyria, and liver tumor production in rodents (Neal, 1985).

Most species of animals administered an acute dose of PCBs will display a "wasting syndrome" characterized by progressive body weight loss followed by weakness, debilitation and death. The dramatic loss of weight is due only in part to feed refusal. The acute toxicity of PCBs in domestic mammals appears to decrease as the percent chlorination increases, whereas toxicity increases with increasing percent chlorination for mallards, pheasants, bobwhites and Japanese quail while the opposite is true for the chicken. Birds show a depressed growth rate, ruffled feathers, decreased egg production and hatchability, embryonic death, structural deformaties, weakness and death. For a given species, the female is often more susceptible than the male to the toxic effects of PCBs and this susceptibility usually decreases with age. This is because males have a higher drug-metabolizing capability (Parkinson and Safe, 1987).

There are marked differences in the sensitivity of various species of animals to the toxic efects of PCBs. Various strains of mice differ in their susceptibility to PCB intoxication. Species also differ qualitatively in their response to PCBs. Rabbits are more sensitive to

PCBs than rats in regards to fetotoxic and reproductive effects (Villeneuve et al., 1971) and mink are more sensitive than rats or birds (Aulerich et al., 1973). Rats administered PCBs develop diarrhea, diminished exploratory behavior, decreased response to pain stimuli, adipsia, oliguria, anorexia, erythema of the limbs, ataxia, coma and death. Reproductive effects include decreased number of females that give birth, decreased mating performance, reduced litter size, and increased newborn mortality (Kimbrough et al., 1978). Avian species administered PCBs displayed tremor, ataxia, ruffling and loss of feathers and fluid accumulation in the abdominal and thoracic cavities. Chickens display a specific edematous disorder called hydropericardium. Rhesus monkeys are sensitive to the acnegenic effects. However, the most consistent symptom of halogenated aromatic hydrocarbon intoxication in all species is thymic atrophy, one of the most sensitive responses to PCB exposure (Parkinson and Safe, 1987).

## The Effects of PCBs on Mink

PCBs are persistent environmental contaminants that continue to pose a potential risk to humans and wildlife even though their production has been banned since 1977. Within the last few decades, noticable declines in wild mink populations in Sweden (Gerell, 1967) and throughout the Great Lakes basin (Wren et al., 1986) have been reported. These declines have been attributed to PCB contamination of species consumed by mink.

In the mid 1960s, mink farmers in the Great Lakes region reported decreased litter sizes in mink fed fish from the Great Lakes and its tributaries (Hartsough, 1965). With the introduction of coho salmon into the Great Lakes and the eventual use of this species for

feeding mink, high incidences of reproductive failure in mink were reported by fur farmers (Aulerich and Ringer, 1977). Newborn mink kit mortality as high as 80% was observed, although the adult mink appeared unaffected. It was originally thought that the reproduction problems in the mink associated with feeding them Great Lakes fish were due to pesticide contamination of the fish. However, mink feeding studies conducted at Michigan State University revealed that the concentration of PCBs in the fish was directly related to the degree of reproductive impairment in the mink.

These and other studies (Platonow and Karstad, 1973; Bleavins et al., 1982,1984; Wren, 1991) have shown that mink are among the most sensitive mammals, if not the single most sensitive, to PCBs. Mink have also been shown to be highly sensitive to other halogenated hydrocarbon contaminants including dioxins (Hochstein et al.,1988), hexachlorobenzene (Bleavins et al., 1982,1984) and polybrominated biphenyls (Aulerich et al., 1986). Since mink are carnivores that occupy a top position in the food chain, they are exposed to higher concentrations of metabolized forms of contaminants, such as PCB, than species lower in the food chain. Thus, they have become a perferred species for studying the effects of these contaminants in animals (See Calabrese et al., 1992 for a review of the use of mink as an animal model).

The clinical signs and lesions or alterations observed in mink exposed to PCBs include, decreased feed consumption (Aulerich et al., 1985), progressive body weight loss (Bleavins et al., 1980; Aulerich et al., 1985), bloody stools (Bleavins et al., 1980; Aulerich et al., 1986) and lethargy (Bleavins et al., 1980). Clinical examination of PCB-intoxicated mink have demonstrated an increase in several organ weights (Heaton, 1992), fatty liver (Heaton, 1992),

hemorrhagic gastric ulcers (Kimbrough et al., 1978) kidney degeneration (Kimbrough et al., 1978) and induction of enzyme activity (Aulerich et al., 1985).

The effects on reproduction include a high incidence of embryo toxicity (Bleavins et al., 1980) and therefore a decrease in the number of females whelping, smaller litter sizes (Bleavins et al., 1980), decreased kit birth and four-week body weights (Aulerich and Ringer, 1980; Bleavins et al., 1980) and higher kit mortality (Aulerich et al., 1973; Heaton, 1992).

## MERCURY

### General Introduction to Mercury and the Mercury Cycle

Mercury (Hg) is a rare element in the earth's crust. In elemental form in liquid state, it is relatively nontoxic. In general, inorganic mercurials are not significant problems in environmental contamination. In nature, mercury is distributed by a complex cycle involving the atmosphere, hydrosphere and lithosphere. Mercury is released into the environment from degassing of the earth's crust through volcanic gases into the atmosphere or by evaporation from waters. Mercury vapor is converted into soluble forms of mercury and is returned to the lithosphere by sedimentation from water and precipitates from the atmosphere. Once depositied in sediments, mercury rapidly and strongly binds to those components that have sulfur-containing organic and inorganic particles or iron and manganese oxides. Very little mercury is found in the environmnet in the unbound form. Mercury from industrial discharges is mainly in the inorganic form. Once released into the environment, elemental mercury becomes available for potential methylation by certain classes of organisms present in the soil.

Inorganic mercury is methylated to mono-and dimethylmercury compounds (Williams, 1981). Dimethylmercury is highly volatile and lipophilic and decomposes to the highly toxic and stable methylmercury at acidic pH levels. Methylation is a normal biologic process which may occur in anaerobic ecosystems which are associated in an industrialized society with polluted waters.

## Methylmercury and the Environment

In regard to the environment the main concerns are with the organic mercurials. There are two major classes of organic mercurials, the aryl compounds and the alkyl compounds. It is the latter group which poses the greatest threat to animals as the alkyl compounds are highly toxic and inflict a wide range of damage from congenital mental retardation to chromosome abnormalities while the aryl mercurials are rapidly metabolized to inorganic mercury (Williams, 1981).

Methylmercury is probably the most lethal compound of mercury. Although methylmercury can be converted to inorganic mercury, the rate of decomposition is slow. It is completely reabsorbed when excreted in the bile and urinary excretion is low. The strong carbon-mercury bond is not readily dissociated and the toxic effects are attributed to the action of the intact molecule (Williams, 1981). Methylmercury is rapidly cleared from water starting with the uptake in small organisms such as plankton and reaching its greatest concentrations in large predatory fish. Methylmercury strongly binds to muscle and accumulates with increased muscle mass.

The toxicity and target organs for methylmercury vary with different animal species. In

man and other primates, the central nervous system serves as the target. The fetal brain of primates is critical if methylmercury is ingested during pregnancy, while in lower mammals the peripheral nervous system may be affected. Methylmercury is easily absorbed by the body, either in the gastrointestinal system, respiratory tract or through the skin. Methylmercury readily crosses biological membranes and thus the brain is an easy target for methylmercury poisoning as methylmercury easily passes through the blood brain barrier after being rapidly taken up by erythrocytes and distributed to all tissues and organs of the body. Methylmercury decreases the number of neurons in the cerebellum causing permanent damage due to the high affinity mercury has for the sulfur in the sulfhydryl groups in the cell membrane proteins. Mercury affects physiological functions in which proteins are involved. Neurological symptoms of methylmercury toxicosis in humans occur from one week to several months after exposure. Signs include: numbness of the lips, mouth, hands and feet, ataxia, visual disturbances and difficulty in speaking. Moderate cases have displayed difficulty in hearing, tunnel vision and partial paralysis. With increased exposure, there are mental changes, involuntary movements, loss of vision, complete paralysis , coma and death (Clarkston, 1983).

## Human Poisonings

In the 1950's and 60's, poisoning of humans and wildlife in Japan, Iraq and Sweden as well as the high concentrations of mercury found in freshwater fish in Canada, the northern U.S. and Scandinavia (Joslin, 1994) lead to much concern over mercury in the environment (Nelson et. al., 1971). A factory close to the Minamata River in Japan using inorganic

mercury salts as catalysts, released an effluent containing mercuric chloride into the river. This was transformed into methylmercury, which concentrated in the tissues of fish. Some fish contained up to 20 ppm methylmercury. Widespread fatalities were reported among the people of the nearby fishing villages that consumed the fish. Other epidemics involving mercury, including Niigata (1946-1965) and Iraq (1971-1972) also involved significant mortality and morbidity. In the latter case, humans misused methyl and ethylmercury fungicides. Farmers used the fungicide treated grain for homemade bread instead of for planting (WHO, 1976: Tsubaki and Irukayama, 1977).

## Mercury Concentrations in the Tennessee River System

In recent decades, portions of the Tennessee River System have received major industrial discharges of mercury including the Clinch River and Watts Bar Reservoir downstream from Oak Ridge, Tennessee. While mercury concentrations in fish immediately downstream from these sources have been found to be elevated, current mercury concentrations have returned to acceptable levels (<0.5 ppm; Dycus, 1986). Piscivorous fish from 26 U.S. states had mean fish flesh mercury concentrations that exceeded the widely used criterion from advisories concerning fish consumption by pregnant women (0.5 ppm; Wiener and Stokes, 1990) and the recommended level of consumption by the general public (1.0 ppm) and thus a fish consumption advisory was published (Clean Water Fund, 1992). The states with the fish containing the highest concentrations of mercury are Minnesota, Wisconsin, Michigan and New York.

## Effects of Mercury on Wildlife

Mercury has a great capacity to accumulate in organisms occupying the upper trophic level of food webs (Wren, 1987). Piscivorous mammals such as river otter (Lutra canadensis) and mink (Mustela vison) can serve as sensitive indicator species of the adverse effects of contaminants in the environment as they are top carnivores in aquatic food webs, relying on fish for a large percentage of their diet. Mercury-contaminated fish in inland waters have been reported and are often considered to be responsible for the decline in wild mink and otter populations (Wren, 1985; Mason et al., 1986). Mercury concentrations have been reported in fish from many locations including remote unpopulated areas (Johnson et al., 1986).

Mercury has been reported in the tissues of numerous piscivorous mammals and birds (Eisler, 1987). The lethality of methylmercury to wildlife has been documented numerous times (Borg et al., 1966; Aulerich et al., 1974; Wobeser et al., 1976). O'Connor and Nielson (1981) reported that 2 ppm methylmercury in the diets of river otter caused death in two out of three otters within 213 days. Mink are sensitive to dietary methylmercury, with fatalities reported at 1 ppm in the diet for two months. They are however, more tolerant of inorganic mercury, where it is documented that 10 ppm in the diet for five months caused no adverse effects. The inorganic form of mercury is more readily excreted while the organic form easily penetrates the brain which could account for the differences in their toxicity (Eyle et al., 1970). Aulerich et al. (1974) noted 5 ppm methylmercury in the diet was lethal to adult mink within one month. According to Kirk (1971), mink can be raised succussfully on diets that contain up to 0.5 ppm mercury from contaminated fish, however, 1 ppm caused fatality within two months. Total consumption of 18 mg methylmercury caused death in female mink

(Aulerich et al., 1974) which compares with results reported by Hanko et al. (1970) in which a total of 20 mg mercury caused death in female ferrets fed a diet that contained 5.0 ppm methylmercury.

The nervous system appears to be the target for methylmercury poisoning in mink. The primary action of mercury is neurotoxicity and its severity is directly related to the amount of mercury consumed. The reduction in neurological function in carnivores is thought to threaten their survival in the wild. Mercury induced behavioral and reproductive effects have been noted in mammalian and avian species in laboratory studies (Borg et al., 1969; Spyker et al., 1972; Khera, 1973). The clinical signs that have been observed in mink that have died due to mercury exposure include: incoordination, loss of balance, anorexia, loss of weight, ataxia, paralysis, tremors, convulsions, high pitched vocalizations and death (Aulerich et al., 1974). These signs are similar to those reported for rats and cats (Takeuchi, 1970), pigs (Piper et al., 1971) and ferrets (Hanko et al., 1970) exposed to mercury. Wobeser et al. (1976) observed merked posterior ataxia, shuffling gait and rear leg "splaying" in mink fed 1.8 ppm mercury in the diet. Symptoms of mercury intoxication in mink appear after a latent period which varies inversely with the mercury concentration of the diet (Hanko et al., 1970; Wobeser et al., 1976; Aulerich et. al., 1974).

## Effects of Prenatal Exposure to Methylmercury

Methylmercury is a recognized embryotoxic and teratogenic compound. Human evidence indicated fetotoxicity of methylmercury at exposure levels inducing only slight and reversible maternal toxicity (Marsh et al., 1981). Animal studies confirm that serious brain

damage could be produced by prenatal exposure to methylmercury in offspring. Mercury affects prenatal neuronal development which can lead to mental impairment, behavioral disorders, paralysis, retardation or death. Severely affected infants from the Minamata outbreak had gross impairment of motor and mental development. In studies with rodents, prenatal exposure to mercury caused cleft palate and other teratogenic malformations.

Mercury readily crosses the placenta where fetal blood concentrations are often higher than those found in maternal blood. Fetal uptake of elemental mercury in rats has been shown to be 10 to 40 times higher after exposure to inorganic mercury salts. After exposure to alkylmercuric compounds, fetal concentrations of mercury were twice those found in maternal tissues, and methylmercury concentrations in fetal red blood cells were 30% higher than in maternal red cells, which would enhance fetal exposure to mercury (Goyer, 1991).

Postmortem observations of humans that died of mercury poisoning in Japan indicated that damage was generalized throughout the brain in cases of prenatal exposure in contrast to adult exposure where focal lesions were predominant. These prenatal cases indicated a disturbance of development in the cytoarchitecture of the brain and the brain size was diminished due to neuronal damage and inhibition of cell division during the critical stages of formation of the central nervous system (Takeuchi, 1970).

There are very few laboratory investigations concerning the effects of methylmercury poisoning on the central nervous system during pregnancy. Most of what is known about the effects on fetal survival is from the Minamata outbreak involving humans in Japan. It has been observed that when the female's intake of mercury is large and she becomes ill, prenancy does not occur. When the dosage is smaller, pregnancy occurs but the fetus is aborted

spontaneously or is stillborn. An even smaller dosage permits conception and live birth, but the baby may suffer from congenital diseases involving neurological function and mental deficiency. In a study with pregnant mice, 0.1 ppm methylmercury dicyanidamide was injected into the mice on day 10 of pregnancy and produced a high frequency of resorbed litters and an increased percentage of dead fetuses (Harada, 1978).

Wren (1987) found no significant differences in the average birth weight of mink kits between treatment groups receiving 1 ppm PCB, 1 ppm methylmercury, or a combination of the PCB and mercury. At three and five weeks of age, the average weight of the kits in the group receiving 1 ppm PCB and 1 ppm methylmercury in combination, was significantly lower than the average kit weights in the other treatment groups. At a level of 0.5 ppm PCB and 0.5 ppm methylmercury in combination, there were no significant differences in the average weights of kits at three or five weeks.

Experiments have shown that methylmercury can pass the placental barrier and result in even higher concentrations in the fetus than in the mother. Infants born to mothers in Minamata Bay, Japan, showed a syndrome consisting of cerebral paresis, ataxia, mental retardation, dysarthria and hypersalivation. Effects incurred prenatally, however, may not become apparent until the nervous system has matured. High mercury concentrations have been documented in fish eating human populations in Greenland, Canada and Alaska.

## Tissue Distribution of Mercury

Studies on mustelids have shown higher concentrations of mercury in the liver than in the kidney (Wobeser and Swift, 1976; Kucera, 1983; Wren et al., 1986). Ropek and Neely

(1993) found that mercury concentrations in the liver and kidney tissues were statistically higher in males than in females. This contradicts the results of a study by Wren et al. (1986) who reported no differences in the mean tissue mercury concentration between the sexes.

Mink fed 5 ppm methylmercury had higher mercury tissue concentrations, even when receiving the diet for a significantly shorter period of time when compared to mink fed twice the concentration (10 ppm) of supplemental mercuric chloride for a longer period of time (Aulerich et al., 1974). The concentration and distribution of mercury in the tissues of the mink fed methylmercury differed considerably from those of mink fed mercuric chloride. Organic mercury tends to accumulate readily in the brain while inorganic mercury does not. Thus, the concentrations of mercury are higher in the brain than in the liver and kidney tissues in organic mercury poisoning when compared to inorganic mercury poisoning.

Wobeser et al. (1976) conducted a study with mink feeding them fish contaminated with mercury at a concentration of 0.44 ppm and found that intoxication did not occur within the experimental period. In another study, Wobeser et al. (1976) fed 1.1, 1.8, 4.8, 8.3 and 15.0 ppm dietary methylmercury to mink for 93 days. In general, the mercury concentrations in the tissues of the mink that died were similar, despite differences in the mercury content of the diet and time to death. Mercury concentrations in the brain and muscle tissue were similar and lower than those in liver and kidney of the same animal. The mean concentrations of mercury (ppm) in the tissues of mink that died were: brain 11.9, muscle 16.0, kidney 23.1, and liver 24.3 ppm. It appears that in some regions, wild mink may be exposed to far greater concentrations than 15.0 ppm in their diet. Wobeser et al. (1976) conducted postmortem analysis on wild mink from Saskatchewan, Canada and found fur, muscle and liver mercury

concentrations of 34.9, 15.2 and 58.2 ppm, respectively. Wren et al. (1986) fed mink diets containing 1.0 ppm methylmercury and mortality and clinical signs were observed on day 73 and the average liver mercury concentration of the mink that died was 44.1 ppm.

## Pathology of Mercury Poisoning

Necrospy of mink that have died from methylmercury poisoning have shown hemorrhagic and congested lungs, enlarged hearts, pale livers and kidneys, local superficial, hemorrhagic gastric ulcers and enlarged and mottled spleens. Hyperemia, fatty and hydropic degeneration of the liver and kidneys, splenic and glomerular amyloidosis and splenic giant cells were also observed (Aulerich et al., 1974). Studies with methylmercury have shown a variety of neuronal insults, including alterations in protein, DNA and RNA biosynthesis, changes in phospholipid/phosphoprotein metabolites, abnormatlities in mitochondrial function and perturbations in membrane permeability. Methylmercury also damages microtubuli and has caused prominant changes in the dorsal root ganglia and peripheral nerves (Fox, 1990).

Like histopathological results from humans who died in the Minamata outbreak, pathological changes in experimental animals are also found primarily in the cerebellum, calcarine cortec, and forsal root ganglia. Wobeser et al. (1976) fed 1.1 to 15.0 ppm methylmercury chloride to mink for 93 days and found that in all treatment groups the characteristic lesions were essentially the same, though varying in intensity, and consisted of neuronal necrosis of the occipital cortex for all the mink with axonal degeneration. An interference with protein synthesis in the nerve cells can occur as a result of methylmercury intoxication. Ultrastructure examination revealed accumulation of lysosomes, membraneous

degenerations, degranulation and destruction of the rough endosplasmic reticulum and cytoplasmic coagulation. The disintegration of the endoplasmic reticulum and degranulation of ribosomes in neurons suggests alterations in both RNA and prtoein metabolism in the nerve cells. Axonal degeneration and disintegration of myelin in peripheral nerves has also been observed (Fox, 1990).

In animals exposed in utero to methylmercury, loss of nerve cells along with cytoarchitectural abnormalities can be induced. Small hemorrhages in the cortex and white matter of the brain have been observed with methylmercury poisoning. Examination of the brain revealed neuronal destruction, disruption of neuronal migration and incomplete cerebellar granule cell layer formation. Besides non-specific cytological changes such as lysosomal accumulation, disintegration of endoplasmic reticulum, and cytoplasmic degeneration, there have been reports of incomplete myelination of axons, abnormal formation of myelin sheaths and abnormal synaptic development (Fox, 1990).

## MATERIALS AND METHODS

### FISH

Approximately 719 kg of whole Atlantic mackerel were obtained from Boston Feed Supply, (Natick, ME) for use as an uncontaminated species for the control mink diet. These fish were stored frozen in a walk-in outdoor freezer at -6.7° C. Approximately 392 kg of various species of fish were collected from the Clinch River upstream of the U.S. Department of Energy (DOE), Oak Ridge Reservation (ORR) to serve as an "uncontaminated" (control) source of fish for feeding to mink. Seven hundred fourty two kg of fish believed to contain various environmental contaminants, particularly PCBs and mercury, were collected from the Watts Bar Reservoir-Clinch River sytem downstream from the ORR for feeding to mink. All fish were collected by gill netting and kept frozen at the Oak Ridge National Laboratory until shipped to Michigan State University (MSU) by overnight Federal Express in plastic bags within "security sealed" coolers. A chain of custody form accompanying the fish was signed and dated when the fish were received at the MSU Experimental Fur Farm.

The first shipment of fish from Oak Ridge National Laboratory arrived at the Experimental Fur Farm on August 25, 1993. The final shipment of fish was received on November 30, 1993. Upon delivery, the bags of fish were weighed and the weights recorded. The fish were placed in heavy plastic bags, sealed and labelled with a tag

identifying the bag number, collection site, date, project identification number, and the researcher's initials. This procedure was repeated a second time so that all fish were sealed within two heavy plastic bags, each bag containing its own identification tag. The bags were stored in a dedicated section of a walk-in freezer at the Experimental Fur Farm at -6.7° C until needed for diet preparation. The upstream fish were kept separated from the downstream fish.

Upon acquisition of all the fish from Oak Ridge National Laboratory, both upstream and downstream fish were taken from the freezer, removed from the sealed bags, and sorted by species, making sure that the upstream fish remained in one area of the facility and the downstream fish in a different area. For each species, the fish were counted and a total weight by species was recorded. The species were identified by Dr. Thomas Koons, Professor, MSU Department of Fisheries and Wildlife (Appendix A).

## DIET PREPARATION

The mink diets were prepared using the equipment at the MSU Experimental Fur Farm. The experimental diets were formulated to meet the nutrient requirements of mink (NRC,1982). The fish portion of the two control diets contained 75% "uncontaminated" Atlantic mackerel ( Diet A) or 75% "uncontaminated" fish collected upstream from ORR (Diet B). The treatment groups diets contained 75% fish with either 25,50, or 75% "contaminated" fish from downstream ORR and the remaining portion consisting of 50, 25 or 0% Atlantic mackerel (Diets C,D,E ).

Approximately 358 kg of the frozen Atlantic mackerel were thawed and ground

through a 3/8 inch face plate and mixed in a paddle mixer for 15 minutes. The ground mackerel was then removed from the mixer and the process was repeated for a second batch of approximately 358 kg of mackerel. The two batches of mackerel were then mixed together so that a homogeneous mixture was obtained. Thirteen samples (500g each) of the mixture were placed in whirlpac bags, labelled with the diet code, date, project identification number, and researcher's initials and frozen in a chest freezer dedicated to the project. Both the upstream and downstream fish from ORR were ground separately and mixed in a paddle mixer and 13 samples (500g each) of each were frozen for subsequent analysis as described above for the Atlantic mackerel.

The diets were prepared by blending the appropriate quantity of the prescribed fish with the appropriate quantities of the other components of the mink diets (eggs, liver, vitamin and mineral premix, d-biotin, and cereal) in a paddle mixer for 15 to 20 minutes. Thirteen samples (500g each) of each diet were placed in whirlpac bags and stored frozen in a chest freezer dedicated to the study for subsequent analyses. All samples were labelled with the diet code, project identification number, date, and researcher's initials. The prepared mink diets were placed in plastic buckets lined with plastic bags. Each bucket contained a three-day supply of feed for the 10 animals on a particular treatment (approximately 6.8 kg). The bags were sealed with a twist-tie and an identification card with the diet, diet color code, date, project identification number, and researcher's initials was placed in the bucket and the buckets were sealed with plastic lids. The buckets were stored in a dedicated area of a walk-in freezer at -6.7° C until needed for feeding.

## EXPERIMENTAL DESIGN AND ANIMAL CARE

On December 2, 1993, after a two-week acclimation period, 50 standard dark mink (Mustela vison) were randomly assigned to the five treatment groups. Each treatment group consisted of two males that had previously sired litters and eight females. Care was taken so that littermates were not placed within the same treatment group in an attempt to reduce any genetic predisposition to heavy metal or PCB toxicity.

The mink were housed individually in wire cages (76 cm L×61 cm W × 47 cm H) with attached nest boxes ( 38 cm L×30.5 cm W ×30.5 cm H) and bedded with wood shavings (Pestell Agri-Products, Ontario, Canada). Prior to whelping, the female's nestbox was bedded with aspen wood shavings to prevent the kits from being exposed to terpines in the wood shavings, which are toxic to young kits, and "wood wool" excelsior (American Excelsior Company, Arlington, TX). A false floor (1/2" wire mesh) was fitted into the cage to prevent newborn kits from falling through the 1×1 1/2 inch wire mesh cage floor. A wooden nestbox divider was fitted into the nestbox to prevent the young kits from crawling out of the nestbox into the cage. Feed and water were provided ad libitum throughout the study. The mink were previously immunized against canine distemper, virus enteritis, hemorrhagic pneumonia, and botulism (Biocom-DP; United Vaccines Inc., Madison, WI)

The animals were individually identified, each having an identification card with the project identification number, mink number, and color-coded diet letter above the individual mink's cage. A color-coded feed tag identifying the diet was also fastened to the lid of each cage for ease in identifying the appropriate diet during feeding.

The animals were observed daily and any behavioral changes or clinical signs of toxicosis were recorded. Any adult mink that lost 30% of their original body weight were euthanized with carbon dioxide gas (CO2). A necropsy was performed by a veterinary pathologist (Dr. J.A. Render) on all mink that were euthanized or died before the end of the trial.

FEEDING TRIAL

All animals were acclimated over two weeks to the test facility prior to the start of the feeding trial which began on December 2, 1993 and ended in mid-June 1994. During the period of acclimation, the mink were fed a basal diet in excess of what they would consume each day and were weighed twice. After the acclimation period they were weighed at monthly intervals.

A three-day supply (1 container) of each diet was removed from the freezer and allowed to thaw overnight at room temperature. Approximately 0.23 kg of feed was placed on a wire feed grid on top of each cage. The remaining unused feed was stored in a walk-in cooler (1.7° C). If all the feed in a bucket was not used within a three-day period, it was discarded. Each morning, the previous day's feed was scraped off the feed grid and discarded before providing fresh feed.

Because some species of fish contain the enzyme thiaminase which hydrolyzes thiamine resulting in Chastek's paralysis, a thiamine supplement was given to the mink. Each day, 0.4 mg of thiamine was dissolved in 50 ml of water and mixed into 1 kg of a basal farm diet (containing thiaminase-free fish). Each mink was fed approximately 20 g of the thiamine-supplemented feed daily before the treatment diet was provided.

REPRODUCTION

Mating of females to the males within their respective treatment group began March 1, 1994 and ended March 22, 1994. Females were given the opportunity to mate every fourth day until a confirmed mating (presence of motile sperm in a vaginal aspiration) was obtained. Once a confirmed mating was obtained, the female was given the opportunity for additional matings the day following the initial mating and/or eight days later.

During mating attempts, males were locked out of their nestboxes and the females were introduced into the male's cage. If no evidence of mating was observed within the first 15 minutes, the female was returned to her cage and given a check mark on her breeding records for that day. If mating appeared to be occurring, the pair was left alone until they separated. The female was then taken into the laboratory where a pipet, containing a small amount of warm saline, was inserted into her vagina. An aspiration was taken and placed on a glass slide and examined under a microscope. If motile sperm were found, the female was considered bred and the male's identification number was written on her breeding chart for that day. The male was given an "X" for the day on his chart to indicate that he had produced motile sperm in a mating. If no sperm or non-motile sperm were found in the aspiration, the female was either given the opportunity to mate with the other male in her treatment group that day or given the opportunity to mate the following day. Mating attempts were continued throughout the breeding season until at least two confirmed matings were obtained for each female.

All nestboxes were checked daily during the whelping season for newborn kits. Newborn kits were sexed, counted, and weighed at birth. Any dead (stillborn) kits were sexed,

weighed, and placed in whirlpac bags and frozen for future analysis. The mother's body weight was also recorded at whelping. The dam and all surviving kits were counted and weighed again at three and six weeks of age.

At three weeks of age, the females that whelped and their kits were fed the appropriate diet, which had been watered down, on a feed plate placed on the bottom of the cage in front of the nestbox entrance. The diet was mixed with water so that it's consistency would encourage the kits to begin consuming "solid" feed. This feed was fed over the next three weeks with the amount of added water gradually reduced until the kits were consuming the solid diet at six weeks of age. Once the kits were old enough to venture out of the nestbox and begin consuming solid feed (three weeks of age), the nestbox divider was taken out to promote easy access from the nestbox to the cage.

## BLOOD COLLECTION

Blood samples were collected from the eight adult female mink fed Diets B and E (75% upstream fish and 75% downstream fish) during the acclimation period (November, 1993) and in February and June, 1994 for measurement of various hematologic parameters. All females were anesthetized with 0.4 mg Ketaset (ketamine hydrochloride; Fort Dodge Laboratory, Inc., Fort Dodge, IA) injected intramuscularly into the hind leg. Four ml of blood were collected for a serum biochemistry profile in Serum Separation Tubes and 0.5 ml blood was collected for hematologic measurements in Microtainer Tubes coated with EDTA.

The serum biochemical analyses and calculations were performed by the MSU Veterinary Clinical Pathology Laboratory with an Abbott Spectrum Analyzer (Abbott Laboratories,

Dallas, TX) to determine calcium (Ca), chloride (Cl), iron (Fe), phosphorus (P), potassium (K), magnesium (Mg), sodium (Na), carbon dioxide (CO2), anion gap, total protein, albumin, globulin, albumin globulin ratio (A/G), creatinine, alkaline phosphatase (Alk. Phos.), aspartate amino transferase (AST), creatine kinase (CK), gamma glutamyl transpeptidase (GGTP), sorbitol dehydrogenase, cholesterol, glucose, blood urea nitrogen (BUN), and osmolality.

A Technicon H1 system (Technicon Diagnostic Systems Division, Tarrytown, NY) was used for the determination of the red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and total platelets (PLT).

## NECROPSY

Two dates for necropsy were selected so that all the kits would be at least six weeks of age and not over four days older than the youngest kit on the day of the necropsy. On June 8, 1994, 17 females and 24 kits were examined. On June 15, the remaining females, their kits, and the breeder males were examined.

The females were anesthetized with Ketaset (ketamine hydrochloride). After losing consciousness, the females were weighed on an electronic balance and their weights recorded. Their backs were shaved using electric clippers and 5g of hair were collected from each mink for mercury analysis. The hair was placed in a labelled whirlpac bag and frozen for subsequent analysis. Twenty ml of blood were collected from each adult mink via heart puncture using a 5 cc syringe and 18 gauge 1 1/4 inch needle. Ten ml of blood were placed

in each of two labelled test tubes. The serum was separated by centrifugation. Enough blood to fill two microhematocrit tubes was taken from each adult via toe clip. For the females fed Diets B and E, an additional four ml of blood were collected via heart puncture using a 5 cc syringe and 18 gauge 1 1/4 inch needle for a biochemical profile and various hematologic measurements which were conducted by the MSU Veterinary Clinical Pathology Laboratory.

After the blood samples were obtained, the females and kits were placed in a tightly sealed wooden box and euthanized by an overdose of carbon dioxide gas. The brain, liver, kidneys, spleen, lungs, heart, ovaries, thyroids, adrenals, and a sample of adipose tissue were collected from the adults at necropsy. All tissues, except for adipose tissue, were trimmed and weighed. The weights of all organs were recorded. Portions of the liver, spleen, kidneys, lungs, ovaries, and brain were placed in a 10% neutral-buffered formalin solution in labelled glass jars for subsequent histopathologic examination. The remaining portion of these tissues, along with the adipose tissue, was wrapped individually in labelled tin foil, frozen in liquid nitrogen, and stored in an ultra-cold freezer at -57° C until shipped to Oak Ridge National Laboratory for analyses. The liver, kidneys, and spleen were taken from the kits and weighed. A portion of each tissue was then frozen in liquid nitrogen and stored in the ultra-cold freezer. Additionally, a sample of each tissue was placed in formalin for histolopathologic examination as previously described for the adults. All carcasses of kits were wrapped in labelled tin foil, frozen in liquid nitrogen, and stored in the ultra-cold freezer until shipped to Oak Ridge National Laboratory for analyses. The reproductive tracts of the females that did not whelp were excised and their ovaries and uteri were examined for corpura lutea and implantation sites under a dissecting microscope.

## HISTOPATHOLOGY

After fixation, the organs collected for histolopathologic examination were trimmed, processed according to routine histologic procedures, sectioned at 5um, stained with hematoxylin and eosin and examined using a light microscope. The blood collected for analyses was centrifuged for 6 minutes at 1200 rpm. The serum was collected and placed in labelled plastic centrifuge tubes and stored in the ultra-cold freezer at -57° C for subsequent analysis by Oak Ridge National Laboratory. The microhematocrit tubes were centrifuged for 6 minutes using a microcapillary centrifuge and the percent packed cell volume determined with a microcapillary reader and recorded.

The frozen tissue samples, carcasses, hair and serum samples were released to Dr. Richard Halbrook at the termination of the trial. The tissues stored in formalin for histopathological examination were submitted to Dr. James A. Render, a veterinary pathologist in the Department of Pathology, Michigan State University.

## CHEMICAL ANALYSIS

Ten subsamples of each diet (A,B,C,D, and E) as well as subsamples of each of the homogenous mixtures of Atlantic mackerel, and fish from upstream and downstream from ORR were shipped frozen on dry ice in a sealed cooler via Federal Express overnight mail to Oak Ridge National Laboratory for total PCB and mercury analyses.

## DIET ANALYSIS

Samples of each diet (A,B,C,D, and E) were submitted to Litchfied Analytical Services, Litchfield, MI for analysis of nutritive content (proximate analysis). The diets were shipped frozen on dry ice in a styrofoam container.

## STATISTICS

Data were analyzed using statistical software (SAS Institute Inc., 1987). Statistical treatment of the data to determine the treatment means and standard errors was by the General Linear Models procedure. Treatment effect was determined by one-way analysis of variance (ANOVA). Comparisons among treatments were analyzed using Tukey or Scheffe's tests. The significant differences between means were based on $p \leq 0.05$.

# RESULTS

Major dietary components and the nutrient analysis of the mink diets are shown in Table 1. Crude protein, fiber and fat did not differ between the diets by more than 3.5 percent. Moisture, protein, ash and crude fiber were relatively constant in all diets. Fat content was highest in the Atlantic mackerel diet and decreased with increasing percentages of downstream fish. Thus, Diet C had the highest percent fat and Diet E had the lowest percent fat. The percent TDN (total digestible nutrients) also followed this pattern. The reason Diet B had 108-130 ppm more zinc than any other diet is unknown.

The raw fish used in the study contained from 0.03 to 0.35 ppm mercury and 0.05-2.13 ppm PCBs (Table 2 ). As shown in Table 3 , total dietary mercury concentrations ranged from 0.02-0.22 ppm wet weight and total dietary PCB concentrations ranged from 0.04-1.86 ppm.

## ADULT BODY WEIGHTS

Mean monthly body weights are summarized in Table 4. Adult mean body weights and body weight changes are summarized in Table 5. No statistical differences in initial or final body weights were noted among the treatment groups. Females in each treatment group lost weight, on average, as the trial progressed. Female mink fed the diets with the highest PCB concentrations (D and E) had the greatest body weight losses (5%). This trend, however,

Table 1. Composition and nutrient analysis of experimental diets

| | Diet A | Diet B | Diet C | Diet D | Diet E |
|---|---|---|---|---|---|
| Ingredients | | | | | |
| Ocean fish, %[1] | 75 | -- | 50 | 25 | -- |
| Upstream fish, %[2] | -- | 75 | -- | -- | -- |
| Downstream fish, %[3] | -- | -- | 25 | 50 | 75 |
| Cereal, %[4] | 18.5 | 18.5 | 18.5 | 18.5 | 18.5 |
| Eggs, % | 3 | 3 | 3 | 3 | 3 |
| Beef liver, % | 3 | 3 | 3 | 3 | 3 |
| d-biotin, mg/kg[5] | 0.05 | 0.05 | 0.05 | 0.05 | 0.05 |
| Vitamin/mineral premix, %[6] | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Nutrient analysis (wet weight)[7] | | | | | |
| Dry matter, % | 43.68 | 42.00 | 43.74 | 43.50 | 40.92 |
| Fat, % | 10.37 | 7.64 | 9.10 | 8.00 | 6.85 |
| Crude protein, % | 19.85 | 19.32 | 20.07 | 19.68 | 18.90 |
| Crude fiber, % | 0.90 | 1.09 | 0.92 | 1.04 | 0.96 |
| Total digestible nutrients, % | 42.25 | 36.89 | 40.82 | 38.55 | 35.36 |
| Calcium, % | 0.68 | 1.51 | 0.95 | 1.39 | 1.42 |
| Phosphorus, % | 0.54 | 0.77 | 0.64 | 0.83 | 0.80 |
| Potassium, % | 0.41 | 0.35 | 0.41 | 0.39 | 0.36 |
| Magnesium, % | 0.07 | 0.08 | 0.08 | 0.09 | 0.08 |
| Sodium, % | 0.2 | 0.204 | 0.217 | 0.21 | 0.198 |
| Iron, ppm | 148 | 189 | 169 | 188 | 175 |
| Manganese, ppm | 54 | 66 | 65 | 60 | 61 |
| Copper, ppm | 14 | 16 | 18 | 15 | 17 |
| Zinc, ppm | 104 | 234 | 119 | 126 | 113 |
| Ash, % | 3.76 | 5.42 | 4.16 | 5.39 | 5.39 |

[1] Atlantic mackerel, GMF Brand Ocean Fresh Fish Product, Boston Feed Supply, Natick, MA
[2] Fish from upstream of the U.S. Dept. of Energy Oak Ridge Reservation, Oak Ridge, TN
[3] Fish from downstream of the U.S. Dept. of Energy Oak Ridge Reservation, Oak Ridge, TN
[4] XK-40 Mink Food, XK Mink Foods Inc., Plymouth, WI
[5] Sigma Chemical Co., St. Louis, MO
[6] MSU Swine Vitamin Trace Mineral Premix, Michigan State University, East Lansing, MI
[7] Proximate analysis of diets by Litchfield Analytical Services, Litchfield, MI

Table 2. Concentrations (ppm) of mercury and PCBs in fish used in mink diets

| Contaminant | Atlantic mackerel | Upstream fish | Downstream fish |
|---|---|---|---|
| Mercury | .03 | .07 | .35 |
| PCB 1260 | .05 | 1.69 | 2.13 |

Table 3. Concentrations (ppm) of mercury and PCBs in diets fed to adult mink

| Contaminant | Diet A | Diet B | Diet C | Diet D | Diet E |
|---|---|---|---|---|---|
| Mercury | .02 | .05 | .09 | .15 | .22 |
| PCB 1260 | .04 | 1.11 | .45 | .84 | 1.86 |

Table 4. Mean monthly (December, 1993 through June, 1994) body weights (g) of adult female and male mink fed diets containing various percentages of "contaminated" and "uncontaminated" fish[1]

| | Diet A | Diet B | Diet C | Diet D | Diet E |
|---|---|---|---|---|---|
| Females | | | | | |
| December | 1269 ± 63.5[2] | 1245 ± 63.5 | 1374 ± 63.5 | 1258 ± 63.5 | 1230 ± 63.5 |
| January | 1292 ± 65.3 | 1269 ± 65.3 | 1374 ± 65.3 | 1273 ± 65.3 | 1223 ± 65.3 |
| February | 1224 ± 62.1 | 1169 ± 62.1 | 1308 ± 62.1 | 1175 ± 62.1 | 1116 ± 62.1 |
| March | 1243 ± 66.7 | 1209 ± 66.7 | 1372 ± 66.7 | 1191 ± 66.7 | 1164 ± 66.7 |
| April | 1331 ± 61.1 (7) | 1320 ± 61.1 (7) | 1499 ± 93.4 (3) | 1258 ± 66.1 (6) | 1209 ± 58.6 |
| May | 1199 ± 67.6 (6) | 1207 ± 62.7 (7) | 1342 ± 82.8 (4) | 1191 ± 74.1 (5) | 1073 ± 58.6 |
| June | 1138 ± 86.5 (7) | 1016 ± 80.9 | 1134 ± 80.9 | 1020 ± 80.9 | 935 ± 80.9 |
| Males | | | | | 2248 ± 34.4 |
| December | 2448 ± 34.4 | 2598 ± 34.4 | 2419 ± 34.4 | 2211 ± 34.4 | 2409 ± 172.8 |
| January | 2721 ± 172.8 | 2738 ± 172.8 | 2519 ± 172.8 | 2460 ± 172.8 | 2294 ± 161.9 |
| February | 2605 ± 161.9 | 2526 ± 161.9 | 2491 ± 161.9 | 2399 ± 161.9 | 2406 ± 122.0 |
| March | 2531 ± 122.0 | 2587 ± 122.0 | 2551 ± 122.0 | 2459 ± 122.0 | 2091 (1) |
| April | 2237 (1) | 1972 (1) | 2107 (1) | 2161 (1) | 1904 (1) |
| June | 2404 (1) | 2282 (1) | 2043 (1) | 2116 (1) | |

[1] No. mink = 8 females and 2 males per dietary group of number shown in parentheses
[2] Mean ± S.E.

Table 5. Mean body weights (g) at the beginning and end of the trial and body weight change (g) of female and male mink fed diets containing various percentages of "contaminated" or "uncontaminated" fish

| | Diet A | Diet B | Diet C | Diet D | Diet E |
|---|---|---|---|---|---|
| Female | | | | | |
| Beginning | 1269 ± 63.5[1] | 1245 ± 63.5 | 1374 ± 63.5 | 1258 ± 63.5 | 1230 ± 63.5 |
| End | 1243 ± 66.7 | 1209 ± 66.7 | 1372 ± 66.7 | 1191 ± 66.7 | 1164 ± 66.7 |
| Change | -26 | -36 | -2 | -67 | -66 |
| Male | | | | | |
| Beginning | 2448 ± 34.4 | 2598 ± 34.4 | 2419 ± 34.4 | 2211 ± 34.4 | 2248 ± 34.4 |
| End | 2531 ± 122 | 2587 ± 122 | 2551 ± 122 | 2459 ± 122 | 2406 ± 122 |
| Change | +83 | -11 | +132 | +248 | +158 |

[1] Mean ± S.E.

was not observed for the male mink, where mean body weights increased during the trial for all diets except Diet B. The greatest male body weight gains were observed in Diets D and E which contained the highest concentrations of PCBs.

## ADULT MORTALITY

On February 2, 1994, two months after the feeding trial began, a male mink fed Diet A had blood in his feces which continued sporatically throughout the remainder of the trial. In mid-March, the second male fed Diet A, had blood in his feces. This was followed by vomiting and feed refusal. The male died two days later. In June, a female fed Diet C was euthanized with ketamine hydrochloride after refusing to eat for several days, being lethargic and passing tarry feces. A cyst was found on her ovary and her gallbladder was enlarged and a gallstone was also found. Another female fed diet C had dystocia. Examination of the reproductive tract showed 10 dead fetuses which had no visible abnormalities. The following day the female was euthanized after passing tarry feces and being lethargic and refusing feed.

## REPRODUCTION

Reproductive performance of the females is shown in Table 6 . Of the 40 females bred, 31 whelped. The average number of confirmed matings per female in the treatment groups ranged from 2.0-3.0. Of the nine females which did not whelp, four were fed Diet C. The only group in which all the females whelped was Diet E. The average gestation lengths across the treatment groups was 44.3-47.5 days. Females fed Diet C had the shortest mean

Table 6. Reproductive performance of female mink fed diets containing various percentages of "contaminated" and "uncontaminated" fish and body weights and survivability of their kits

| | Diet A | Diet B | Diet C | Diet D | Diet E |
|---|---|---|---|---|---|
| No. females whelped | 6/8[1] | 7/8 | 4/8 | 6/8 | 8/8 |
| Gestation length (days) | 44.6 ± 0.55[2] | 46.4 ± 1.26 | 44.3 ± 1.08 | 47.5 ± 1.77 | 44.9 ± 1.43 |
| Dam body weight at whelping (g) | 1199 ± 81.96[2] | 1207 ± 84.15 | 1342 ± 94.33 | 1191 ± 50.13 | 1073 ± 39.49 |
| No. kits/dam | | | | | |
| Alive | 5.2 | 6.9 | 6.0 | 5.7 | 3.9 |
| Dead | 1.3 | 0.4 | 1.8 | 0.3 | 0.4 |
| Average litter size | 6.5 | 7.3 | 7.8 | 6.0 | 4.3 |
| Kit body weight (g) | | | | | |
| Birth | 9.9 ± 0.32 | 10.5 ± 0.30 | 9.6 ± 0.44 | 12.5 ± 0.35 | 12.1 ± 0.37 |
| 3 weeks | 135 ± 4.29 | 115 ± 3.03 | 130 ± 6.07 | 129 ± 3.70 | 122 ± 1.13 |
| 6 weeks | 328 ± 13.8 | 311 ± 9.91 | 333 ± 19.6 | 307 ± 11.9 | 295 ± 11.3 |
| Kit mortality (%) | | | | | |
| Stillborn | 20.5 | 5.8 | 22.6 | 5.6 | 8.8 |
| 3 weeks | 48.7 | 21.6 | 61.3 | 25.0 | 8.8 |
| 6 weeks | 48.7 | 23.5 | 61.3 | 25.0 | 8.8 |

[1] One mated female died during whelping and her dead kits were not used for results in this table
[2] Mean ± S.E.

gestation period while females fed Diet D had the longest gestation period. The percentage of kits born alive ranged from 77-95%. Females fed Diets C and A had the fewest kits born alive. Average litter sizes at birth ranged from 4.3-7.8 across the treatments. Females fed Diet C had the largest litters while females fed Diet E had the smallest litters.

## MALE REPRODUCTIVE PERFORMANCE

Reproductive performance of the adult male mink is shown in Table 7 . All males were proven breeders with those males fed Diets A or D had 100% successful matings. One male fed Diet B had a lower percentage of successful matings (77%), but this percentage was not considered to be abnormal.

## KIT MORTALITY

Differences were seen in the proportion of mortality that occurred during birth, three and six weeks of age ( Table 6). Except for kits of females fed Diet E, which had no mortalities after birth, the mortality levels were highest from birth to three weeks of age. After three weeks of age the only kits to die were those of females fed Diet B.

## KIT BODY WEIGHTS

Kits whelped by females fed Diet C had the lowest birth weights, while those whelped by females fed Diets D and E had the greatest birth weights (Table 6 ). At three weeks of age, kits from Diets A and C had the greatest body weights while those kits from Diets B and E had lower average body weights. At six weeks of age, the kits from females fed Diet C had

Table 7. Reproductive performance of male mink fed diets containing various percentages of "contaminated" and "uncontaminated" fish

| Diet | Male no. | Total no. attempted matings | No. matings with motile sperm | % successful matings |
|---|---|---|---|---|
| A | 1 | 10 | 10 | 100 |
| | 2 | 12 | 12 | 100 |
| B | 3 | 10 | 9 | 90 |
| | 4 | 13 | 10 | 77 |
| C | 5 | 10 | 9 | 90 |
| | 6 | 9 | 9 | 100 |
| D | 7 | 9 | 9 | 100 |
| | 8 | 7 | 7 | 100 |
| E | 9 | 13 | 12 | 92 |
| | 10 | 13 | 12 | 92 |

the greatest body weights while the kits whelped by females fed Diet E had the lowest body weights.

## ADULT ORGAN WEIGHTS

Mean adult organ weights are summarized in Table 8 . No statistical differences were noted among the females in the treatment groups for brain, liver, kidneys, spleen, lungs, heart, ovary , thyroids, and adrenals. A significant difference was observed among adult male liver weights. The liver weights of the males ranged from 46.4-95.3g with Diet A males exhibiting the highest liver weights and Diet C males, the lowest.

## KIT ORGAN WEIGHTS

Thirty-five kits were euthanized at six weeks of age and subjected to necropsy. No necropsies were performed on kits from Diet D. Kit organ weights taken at necropsy are summarized in Table 9 . There were no statistical differences noted between the treatment groups for liver, kidney or spleen weights.

## NECROPSY

Gross examination of the kits and the adult mink at necrospy revealed no abnormalities.

## HISTOPATHOLOGY

Histopathlogical examination of the male from Diet A that died revealed that the mucosa of the urinary bladder was ulcerated with an intense neutrophil infiltration of the submucosa

Table 8. Organ weights (g) of eight adult female mink and two adult male mink fed diets containing various percentages of "contaminated" and "uncontaminated" fish

| Organ | Diet A | Diet B | Diet C | Diet D | Diet E |
|---|---|---|---|---|---|
| Females | | | | | |
| Brain | 7.88 ± 0.04[1] | 7.39 ± 0.04 | 8.37 ± 0.04 | 7.85 ± 0.04 | 7.85 ± 0.04 |
| Liver | 37.32 ± 2.95 | 38.87 ± 2.95 | 38.91 ± 2.95 | 40.99 ± 2.95 | 34.64 ± 2.95 |
| Kidneys | 6.97 ± 0.51 | 6.13 ± 0.51 | 7.16 ± 0.51 | 6.73 ± 0.57 | 5.77 ± 0.51 |
| Spleen | 4.68 ± 0.42 | 2.70 ± 0.42 | 3.58 ± 0.42 | 3.75 ± 0.42 | 2.32 ± 0.42 |
| Lungs | 7.67 ± 0.50 | 6.74 ± 0.50 | 8.45 ± 0.50 | 7.56 ± 0.50 | 6.75 ± 0.50 |
| Heart | 6.56 ± 0.33 | 6.37 ± 0.33 | 6.56 ± 0.33 | 6.51 ± 0.33 | 6.09 ± 0.33 |
| Ovaries | 0.177 ± 0.016 | 0.15 ± 0.015 | 0.20 ± 0.016 | 0.17 ± 0.015 | 0.18 ± 0.015 |
| Thyroids | 0.066 ± 0.006 | 0.065 ± 0.006 | 0.065 ± 0.006 | 0.068 ± 0.006 | 0.062 ± 0.006 |
| Adrenals | 0.08 ± 0.008 | 0.07 ± 0.008 | 0.08 ± 0.008 | 0.08 ± 0.008 | 0.07 ± 0.008 |
| Males | | | | | |
| Brain | 11.02 ± 0.36 | 9.94 ± 0.31 | 9.37 ± 0.21 | 10.78 ± 0.27 | 10.39 ± 1.29 |
| Liver | 95.29 ± 10.09[2] | 61.09 ± 5.52 | 46.37 ± 5.47 | 68.42 ± 2.7 | 60.89 ± 4.81 |
| Kidneys | 15.48 ± 4.42 | 12.36 ± 1.79 | 11.07 ± 0.89 | 12.39 ± 0.66 | 10.93 ± 1.48 |
| Spleen | 9.20 ± 0.28 | 4.75 ± 0.059 | 5.75 ± 1.07 | 6.47 ± 0.56 | 7.86 ± 1.26 |
| Lungs | 13.75 ± 1.75 | 13.19 ± 2.29 | 11.81 ± 1.43 | 12.24 ± 0.96 | 12.51 ± 1.75 |
| Heart | 12.79 ± 1.56 | 10.71 ± 0.54 | 10.40 ± 1.18 | 10.93 ± 0.58 | 12.29 ± 1.14 |
| Ovaries | 4.60 ± 3.39 | 3.13 ± 1.15 | 2.29 ± 1.28 | 1.60 ± 0.13 | 1.53 ± 0.36 |
| Thyroids | 0.45 ± 0.35 | 0.07 ± 0.007 | 0.07 ± 0.015 | 0.09 ± 0.027 | 0.07 ± 0.003 |
| Adrenals | 0.349 ± 0.19 | 0.093 ± 0.004 | 0.11 ± 0.009 | 0.87 ± 0.012 | 0.085 ± 0.002 |

[1] Mean ± S.E.
[2] Significant difference in organ weight between Diet A and Diet C

Table 9. Mean organ weights (g) of nine six-week-old kits exposed, via their dams, to various percentages of "contaminated" and "uncontaminated" fish during gestation and lactation and through consumption of the diets following weaning

| | Diet A | Diet B | Diet C | Diet E |
|---|---|---|---|---|
| Liver | 21.7 ± 1.30[1] | 26.9 ± 2.25 | 21.3 ± 2.29[2] | 22.0 ± 1.67 |
| Kidney | 5.95 ± 2.60 | 2.38 ± 0.14 | 1.78 ± 0.25[2] | 1.91 ± 0.20 |
| Spleen | 4.00 ± 0.33 | 4.34 ± 0.41 | 3.43 ± 0.28[2] | 3.91 ± 0.21 |

[1] Mean ± S.E.
[2] Eight kits fed diet C.

and fibrin exudation. A marked necrotizing vasculitis with vascular thrombosis and fibroblast proliferation was present. Numerous bacterial colonies were found on the luminal surface. The kidneys had petechial and ecchymotic hemorrhages, and the urethra had petechial hemorrhage. The cause for the clinical signs appeared to be due to a severe fibrinosuppurative and focally hemorrhagic bacterial cystitis.

The majority of the adults examined from all treatment groups had a prominence of Ito cells in the liver. Mild hepatocellular microvesicular cytoplasmic vacuolation was observed in several adults fed Diets A,B,C and D. Lymphocytes were present in the portal areas of the liver from an adult fed Diet A and one fed Diet B. Mild microvesicular vacuolation of renal tubular cells was observed in adults fed Diets C and E. Splenic amyloidosis was observed in three adult mink each fed a different diet ( Diets C,D and E). Megakaryocytes were also present in the spleen of a majority of the adults fed Diets C,D, and E. No histopathological alterations were present in the liver, kidney or spleen from kits in each treatment group.

## HEMATOLOGIC PARAMETERS

No significant effects on hematologic parameters were present between the females fed Diets B and E (Table 10 ). The white blood cell (WBC) counts for both treatment groups during the month of February increased significantly compared with the WBC count noted in November during acclimation and at the termination of the trial.

Table 10. Mean hematologic values for eight adult female mink fed Diet B or Diet E at various times during the study

| | Diet B | | | Diet E | | |
|---|---|---|---|---|---|---|
| | Nov., 1993 | Feb., 1994 | June, 1994 | Nov., 1993 | Feb., 1994 | June, 1994 |
| WBC ($X10^3$ cells/$\mu l$)[1] | 6.31 ± 0.883[2] | 17.55 ± 2.636 | 4.88 ± 0.950 | 5.67 ± 0.676 | 19.08 ± 1.61 | 4.35 ± 0.378 |
| RBC ($X10^6$ cells/$\mu l$)[1] | 9.59 ± 0.319 | 9.32 ± 0.279 | 9.39 ± 0.192 | 9.84 ± 0.225 | 9.24 ± 0.240 | 8.83 ± 0.287 |
| HGB (g/dl)[1] | 18.58 ± 0.462 | 18.19 ± 0.454 | 18.40 ± 0.316 | 18.68 ± 0.358 | 17.75 ± 0.429 | 18.11 ± 0.525 |
| HCT (%)[1] | 60.06 ± 1.601 | 58.68 ± 1.386 | 56.64 ± 1.095 | 59.65 ± 1.441 | 56.90 ± 1.429 | 54.74 ± 1.794 |
| MCV (fl)[1] | 62.79 ± 0.897 | 63.13 ± 0.656 | 60.40 ± 0.542 | 60.60 ± 0.477 | 61.60 ± 0.477 | 62.01 ± 0.684 |
| MCH (pg)[1] | 19.44 ± 0.284 | 19.55 ± 0.208 | 19.61 ± 0.180 | 18.98 ± 0.115 | 19.24 ± 0.206 | 20.54 ± 0.090 |
| MCHC (pg)[1] | 30.94 ± 0.107 | 30.96 ± 0.150 | 32.51 ± 0.184 | 31.31 ± 0.236 | 31.21 ± 0.215 | 33.10 ± 0.344 |
| PLT ($X10^3/\mu l$)[1] | 583 ± 37.42 | 590 ± 28.14 | 581 ± 45.86 | 663 ± 31.42 | 533 ± 82.78 | 603 ± 59.70 |

[1] WBC = white blood cells; RBC = red blood cells; HGB = hemoglobin; HCT = hematocrit; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; MCHC = mean corpuscular hemoglobin concentration; PLT = total platelets

[2] Mean ± S.E.

## SERUM CHEMISTRY PARAMETERS

No significant differences were noted among the females fed Diet B or Diet E for serum chemistry parameters for any of the three collections during the trial ( Table 11).

## CONCENTRATIONS OF PCBs IN ADULT LIVER AND FAT

The cummulative PCB dose ranged from 1.19 mg/mink to 55.24 mg/ mink with the dose for males ranging from 0.0024 mg/kg/day to 0.124 mg/kg/day and the dose for females ranging from 0.0047 mg/kg/day to 0.23 mg/kg/day (Table 12; Appendix B). Concentrations of total PCBs in livers of adult mink ranged from 0.02 $\mu$g PCB/g liver (wet wt.) for those fed Diets B and C to 7.25 $\mu$g PCB/g liver for those fed Diet E. Mink fed Diet A had livers with total PCB concentrations averaging 0.03 $\mu$g/g liver (wet wt.). The concentration of total PCB in the liver of mink fed the diets containing downstream fish increased in a dose-dependent manner.

Concentrations of total PCBs in fat of mink ranged from 0.25 $\mu$g PCB/g fat (wet wt.) to 106.7 $\mu$g PCB/g fat in the mink fed Diet E. Females fed Diet B had the second highest concentration of PCBs in their fat at 56.6 ug PCB/g fat. The concentration of total PCB in the fat of mink fed the diets containing downstream fish increased in a dose-dependent manner. The fat samples of the mink contained numerically higher concentrations of PCBs than the liver samples. The concentrations of various PCB congeners detected in the liver and fat tissue of the mink are presented in Tables 13 and 14.

Table 11. Mean serum chemistry values for eight adult female mink fed Diet B or Diet E at various times during the study

| | Diet B[1] | | | Diet E[1] | | |
|---|---|---|---|---|---|---|
| | Nov., 1993 | Feb., 1994 | June, 1994 | Nov., 1993 | Feb., 1994 | June, 1994 |
| Sodium (m/mol/l) | 158.49 ± 0.248[2] | 160.73 ± 0.186 | 138.41 ± 21.02 | 157.16 ± 0.611 | 158.89 ± 0.603 | 157.30 ± 0.529 |
| Potassium (mmol/l) | 4.76 ± 0.111 | 4.59 ± 0.071 | 4.87 ± 0.199 | 4.64 ± 0.099 | 4.50 ± 0.099 | 4.75 ± 0.110 |
| Na/K (ratio)[1] | 33.50 ± 0.707 | 35.13 ± 0.656 | 32.75 ± 1.532 | 34.13 ± 0.639 | 35.63 ± 0.574 | 33.13 ± 0.601 |
| Chloride (mmol/l) | 118.63 ± 0.417 | 118.45 ± 0.321 | 123.45 ± 0.888 | 116.55 ± 0.396 | 118.49 ± 0.712 | 122.88 ± 0.926 |
| Calcium (mg/dl) | 10.28 ± 0.212 | 10.45 ± 0.246 | 9.39 ± 0.170 | 9.66 ± 0.115 | 10.19 ± 0.180 | 9.15 ± 0.368 |
| Phosphorus (mg/dl) | 5.36 ± 0.481 | 4.99 ± 0.289 | 5.44 ± 0.234 | 5.21 ± 0.305 | 5.10 ± 0.471 | 4.61 ± 1.136 |
| Iron (µg/dl) | 198.25 ± 9.735 | 195.63 ± 14.38 | 237.63 ± 21.12 | 207.38 ± 12.84 | 181.50 ± 9.519 | 202.75 ± 15.05 |
| Magnesium (MEQ/l) | 2.54 ± 0.059 | 2.27 ± 0.664 | 2.29 ± 0.576 | 2.45 ± 0.681 | 2.26 ± 0.040 | 2.43 ± 0.058 |
| Anion gap (mmol/l) | 14.75 ± 1.278 | 17.50 ± 0.964 | 14.00 ± 1.195 | 12.75 ± 1.606 | 16.63 ± 0.615 | 13.63 ± 0.609 |
| Glucose (mg/dl) | 81.38 ± 6.477 | 124.75 ± 14.48 | 112.50 ± 16.02 | 88.25 ± 7.454 | 130.75 ± 16.04 | 100.63 ± 7.658 |
| Cholesterol (mg/dl) | 292.63 ± 29.17 | 313.25 ± 32.48 | 352.13 ± 45.50 | 282.88 ± 15.13 | 255.25 ± 32.55 | 250.75 ± 17.32 |
| Urea nitrogen (mg/dl) | 26.63 ± 2.878 | 32.88 ± 3.621 | 32.88 ± 8.787 | 27.63 ± 2.718 | 33.75 ± 5.648 | 18.25 ± 16.01 |
| Total protein (g/dl) | 6.86 ± 0.189 | 7.10 ± 0.204 | 6.59 ± 0.185 | 6.40 ± 0.141 | 7.04 ± 0.363 | 6.46 ± 0.191 |
| Albumin (g/dl) | 3.89 ± 0.072 | 3.94 ± 0.115 | 3.74 ± 1.419 | 3.84 ± 0.025 | 3.76 ± 0.111 | 3.63 ± 0.039 |
| Globulin (calc.) (g/dl) | 2.96 ± 0.146 | 3.18 ± 0.152 | 2.85 ± 0.104 | 2.58 ± 0.012 | 3.29 ± 0.375 | 2.83 ± 0.135 |
| A/G (ratio)[1] | 1.34 ± 0.063 | 1.25 ± 0.077 | 1.32 ± 0.061 | 1.63 ± 0.128 | 1.34 ± 0.191 | 1.32 ± 0.042 |
| Total bilirubin (mg/dl) | 0.43 ± 0.095 | 0.25 ± 0.093 | 0.13 ± 0.016 | 0.18 ± 0.027 | 0.10 ± 0 | 0.11 ± 0.015 |
| Creatinine (mg/dl) | 0.65 ± 0.050 | 0.65 ± 0.267 | 0.75 ± 0.054 | 0.61 ± 0.039 | 0.59 ± 0.032 | 0.70 ± 0.033 |
| Alkaline phosphatase (U/l)[1] | 59.88 ± 7.317 | 67.25 ± 5.706 | 88.50 ± 6.453 | 61.75 ± 2.169 | 40.00 ± 5.813 | 70.00 ± 5.464 |
| AST (IU/l)[1] | 128.50 ± 22.92 | 94.00 ± 14.48 | 181.89 ± 38.50 | 72.88 ± 10.54 | 90.13 ± 10.75 | 163.50 ± 65.67 |
| Sorbitol dehydrogenase (U/l)[1] | 11.61 ± 2.230 | 10.39 ± 3.930 | 17.88 ± 6.290 | 16.41 ± 2.950 | 9.84 ± 2.010 | 8.31 ± 1.260 |
| CK (IU/l)[1] | 2225.3 ± 667.8 | 613.13 ± 158.8 | 1030.0 ± 124.5 | 656.75 ± 157.5 | 515.75 ± 113.7 | 655.50 ± 65.67 |
| GGTP (µ/l)[1] | 10.00 ± 0 | 10.00 ± 0 | 10.00 ± 0 | 10.00 ± 0 | 10.00 ± 0 | 10.00 ± 0 |
| $TCO_2$ (mmol/l)[1] | 29.66 ± 1.309 | 29.28 ± 1.004 | 25.29 ± 1.255 | 32.25 ± 0.077 | 29.31 ± 0.660 | 25.51 ± 0.581 |
| Osmolality (calc.) (mos/kg) | 331.13 ± 2.660 | 340.25 ± 1.850 | 334.13 ± 4.590 | 329.13 ± 2.710 | 339.13 ± 2.880 | 326.88 ± 6.530 |

[1] Na/K ratio = sodium potassium ratio; A/G = albumin globulin ratio; AST = aspartate amino transferase; CK = creatine kinase; GGTP = gamma glutamyl transpeptidase
[2] Mean ± S.E.

Table 12. PCB consumption for adult mink fed diets containing various percentages of Atlantic mackerel and fish from the Clinch River upstream and downstream from the Oak Ridge Reservation[1]

| | Dietary treatment | | | | |
|---|---|---|---|---|---|
| | Diet A | Diet B | Diet C | Diet D | Diet E |
| Dietary PCB concentration (μg/g) | 0.04 | 1.11 | 0.45 | 0.84 | 1.86 |
| Cumulative PCB dose (mg/mink) | 1.19 | 32.97 | 13.37 | 24.95 | 55.24 |
| PCB dose (mg/mink/day) | 0.006 | 0.166 | 0.067 | 0.126 | 0.279 |
| PCB dose for males (mg/kg body weight/day) | 0.0024 | 0.064 | 0.028 | 0.057 | 0.124 |
| PCB dose for females (mg/kg body weight/day) | 0.0047 | 0.134 | 0.049 | 0.1 | 0.23 |

[1] Cumulative feed consumption of 29,700 g/mink, based on average daily feed consumption of 150 g (Leonard, 1966)

Table 13. Mean concentrations (mg/kg; wet weight) of PCB congeners (identified by IUPAC number) in adipose tissue of adult mink fed diets containing various percentages of "contaminated" and "uncontaminated" fish

| Congener | Diet A | Diet B | Diet C | Diet D | Diet E |
|---|---|---|---|---|---|
| 77 | 0.0094 ± 0.0037[1] | 0.0322 ± 0.0051 | 0.0417 ± 0.0057 | 0.0755 ± 0.0151 | 0.137 ± 0.0093 |
| 81 | 0.1092 ± 0.0345 | 0.319 ± 0.0511 | 0.271 ± 0.0423 | 0.4066 ± 0.0228 | 0.767 ± 0.0781 |
| 99 | 0.0444 ± 0.0149 | 0.1563 ± 0.0245 | 0.7468 ± 0.1357 | 1.403 ± 0.1210 | 1.58 ± 0.2693 |
| 101 | 0.0135 ± 0.005 | 0.0877 ± 0.0176 | 0.1066 ± 0.0146 | 0.2377 ± 0.0029 | 0.307 ± 0.0299 |
| 118 | 0.1441 ± 0.0535 | 0.453 ± 0.1671 | 1.439 ± 0.2182 | 2.744 ± 0.3275 | 3.22 ± 0.2538 |
| 123 | 0.1175 ± 0.0299 | 0.448 ± 0.0705 | 0.0311 ± 0.0045 | 0.06 ± 0.005 | 2.46 ± 0.2769 |
| 126 | 0.0557 ± 0.0557 | 1.066 ± 0.1816 | 0.185 ± 0.0266 | 0.4266 ± 0.0453 | 1.439 ± 0.1356 |
| 128 | 0.0288 ± 0.0160 | 0.284 ± 0.0394 | 0.3402 ± 0.0472 | 0.738 ± 0.0689 | 1.108 ± 0.2076 |
| 138 | 0.23 ± 0.1096 | 2.61 ± 0.4974 | 3.371 ± 0.5089 | 8.822 ± 0.9279 | 9.64 ± 0.7 |
| 146 | 0.036 ± 0.0123 | 0.475 ± 0.0853 | 0.5749 ± 0.0786 | 1.305 ± 0.1152 | 1.717 ± 0.1460 |
| 153 | 0.343 ± 0.1865 | 5.14 ± 0.8855 | 4.946 ± 0.7967 | 14.06 ± 1.697 | 14.67 ± 1.207 |
| 156 | 0.0079 ± 0.0022 | 0.434 ± 0.0704 | 0.39 ± 0.0589 | 0.8466 ± 0.0794 | 0.96 ± 0.1633 |
| 167 | 0.0347 ± 0.0153 | 0.332 ± 0.0457 | 0.6004 ± 0.0873 | 1.278 ± 0.1079 | 1.46 ± 0.1035 |
| 170 | 0.0813 ± 0.0414 | 2.112 ± 0.3879 | 0.7741 ± 0.1251 | 1.877 ± 0.2135 | 2.93 ± 0.1693 |
| 171 | 0.0305 ± 0.0135 | 0.484 ± 0.0778 | 0.465 ± 0.0694 | 0.894 ± 0.1869 | 1.384 ± 0.0666 |
| 180 | 0.2355 ± 0.1514 | 6.59 ± 1.279 | 5.02 ± 0.8366 | 13.14 ± 1.392 | 13.81 ± 1.707 |
| 183 | 0.0159 ± 0.0095 | 0.512 ± 0.0873 | 0.35 ± 0.0563 | 0.83 ± 0.072 | 1.068 ± 0.1175 |
| 189 | 0.0045 ± 0.0007 | 0.0837 ± 0.0158 | 0.038 ± 0.0053 | 0.1155 ± 0.0149 | 0.179 ± 0.0154 |
| 194 | 0.0517 ± 0.0279 | 1.902 ± 0.3981 | 0.8646 ± 0.1417 | 2.133 ± 0.2271 | 2.42 ± 0.3299 |
| 195 | 0.0098 ± 0.0059 | 0.384 ± 0.0660 | 0.0311 ± 0.0045 | 0.06 ± 0.005 | 0.1345 ± 0.0164 |
| 196 | 0.0177 ± 0.0115 | 0.705 ± 0.1389 | 0.41 ± 0.0738 | 0.9888 ± 0.0943 | 1.566 ± 0.2394 |
| 201 | 0.0261 ± 0.0151 | 0.801 ± 0.1332 | 0.4519 ± 0.0651 | 1.087 ± 0.1212 | 1.69 ± 0.1629 |

[1] Mean ± S.E.

Table 14. Mean concentrations (mg/kg; wet weight) of PCB congeners (identified by IUPAC number) in the liver of adult mink fed diets containing various percentages of "contaminated" and "uncontaminated" fish

| Congener | Diet A | Diet B | Diet C | Diet D | Diet E |
|---|---|---|---|---|---|
| 77 | 0.0048 ± 0.0018[1] | 0.0064 ± 0.0008 | 0.0031 ± 0.0001 | 0.0059 ± 0.0020 | 0.003 ± 0 |
| 81 | 0.0048 ± 0.0018 | 0.0041 ± 0.0008 | 0.0031 ± 0.0001 | 0.0059 ± 0.0020 | 0.003 ± 0 |
| 99 | 0.0048 ± 0.0018 | 0.0098 ± 0.0015 | 0.043 ± 0.0089 | 0.0691 ± 0.0132 | 0.088 ± 0.0172 |
| 101 | 0.0048 ± 0.0018 | 0.004 ± 0.0008 | 0.0031 ± 0.0001 | 0.0099 ± 0.0014 | 0.0136 ± 0.001 |
| 118 | 0.015 ± 0.0019 | 0.0494 ± 0.0067 | 0.1275 ± 0.0479 | 0.1606 ± 0.0413 | 0.1788 ± 0.0111 |
| 123 | 0.0048 ± 0.0018 | 0.003 ± 0 | 0.0031 ± 0.0001 | 0.0059 ± 0.0020 | 0.1563 ± 0.0116 |
| 126 | 0.0048 ± 0.0018 | 0.0181 ± 0.0025 | 0.0031 ± 0.0001 | 0.0059 ± 0.0020 | 0.1536 ± 0.0239 |
| 128 | 0.0048 ± 0.0018 | 0.014 ± 0.0019 | 0.0158 ± 0.0039 | 0.0255 ± 0.0054 | 0.0623 ± 0.0093 |
| 138 | 0.0159 ± 0.0039 | 0.1701 ± 0.0373 | 0.2313 ± 0.0654 | 0.355 ± 0.0904 | 0.5075 ± 0.0373 |
| 146 | 0.0063 ± 0.0017 | 0.0514 ± 0.0059 | 0.0614 ± 0.0184 | 0.0843 ± 0.0136 | 0.0404 ± 0.0074 |
| 153 | 0.022 ± 0.0038 | 0.4113 ± 0.0468 | 0.4338 ± 0.1252 | 0.585 ± 0.1236 | 0.8075 ± 0.0749 |
| 156 | 0.0048 ± 0.0018 | 0.0188 ± 0.0069 | 0.0235 ± 0.0097 | 0.0335 ± 0.0074 | 0.058 ± 0.0098 |
| 167 | 0.0048 ± 0.0018 | 0.0104 ± 0.0014 | 0.0196 ± 0.0068 | 0.0186 ± 0.0065 | 0.0715 ± 0.0110 |
| 170 | 0.006 ± 0.0018 | 0.1803 ± 0.0203 | 0.0969 ± 0.0421 | 0.147 ± 0.0419 | 0.1713 ± 0.0204 |
| 171 | 0.0048 ± 0.0018 | 0.003 ± 0 | 0.0098 ± 0.0024 | 0.0155 ± 0.0028 | 0.0708 ± 0.0069 |
| 180 | 0.0143 ± 0.0026 | 0.5275 ± 0.0661 | 0.3713 ± 0.1335 | 0.5113 ± 0.1264 | 0.7112 ± 0.0918 |
| 183 | 0.0048 ± 0.0018 | 0.0331 ± 0.0034 | 0.0241 ± 0.0051 | 0.0349 ± 0.0088 | 0.0489 ± 0.0095 |
| 189 | 0.0048 ± 0.0018 | 0.0033 ± 0.0003 | 0.0053 ± 0.0023 | 0.009 ± 0.0021 | 0.009 ± 0.0015 |
| 194 | 0.0051 ± 0.0017 | 0.1499 ± 0.0172 | 0.0913 ± 0.0274 | 0.1255 ± 0.0199 | 0.1136 ± 0.0206 |
| 195 | 0.0048 ± 0.0018 | 0.003 ± 0 | 0.0208 ± 0.0049 | 0.0.319 ± 0.0042 | 0.0106 ± 0.0017 |
| 196 | 0.0075 ± 0.0019 | 0.24 ± 0.0273 | 0.0031 ± 0.0001 | 0.0059 ± 0.0020 | 0.1626 ± 0.0802 |
| 201 | 0.0048 ± 0.0018 | 0.1686 ± 0.0224 | 0.0373 ± 0.0233 | 0.0069 ± 0.0397 | 0.2563 ± 0.0377 |

[1] Mean ± S.E.

## CONCENTRATIONS OF MERCURY IN ADULT KIDNEY, HEART AND LIVER TISSUE

The cumulative mercury dose ranged from 0.594 mg/ mink to 6.53 mg/mink while the mercury dose for the males ranged from 0.001 mg/kg/day to 0.015 mg/kg/day and for the females ranged from 0.002 mg/kg/day to 0.027 mg/kg/day (Table 15; Appendix C). Concentrations of total Hg in the kidneys of adult mink ranged from 0.79 $\mu$g Hg/g kidney (wet wt.) for mink fed Diet A to 4.23 $\mu$g Hg/g kidney for mink fed Diet E ( Table 16 ). Concentrations of total mercury in the heart of adult mink ranged from 3.44 $\mu$g Hg/g heart (wet wt.) for mink fed Diet A to 17.82 $\mu$g Hg/g heart for mink fed Diet E. Concentrations of total mercury in the liver of adult mink ranged from 0.38 $\mu$g Hg/g liver (wet wt.) for mink fed Diet A to 3.27 $\mu$g Hg/g liver for mink fed Diet E.

The concentrations of mercury in all tissues of those mink fed the diets containing downstream fish (Diets C,D, and E) increased in a dose-dependant manner. The mercury concentrations in tissues of mink fed Diet B were higher than those of mink fed Diet A . The concentrations of mercury in the tissues of mink fed the upstream fish were higher than those found in the tissues of mink fed the downstream fish. The heart contained numerically higher concentrations of mercury than the kidney or liver for every diet group.

Table 15. Mercury consumption for adult mink fed diets containing various percentages of Atlantic mackerel and fish from the Clinch River upstream and downstream of the Oak Ridge Reservation[1]

| | Diet A | Diet B | Diet C | Diet D | Diet E |
|---|---|---|---|---|---|
| Dietary mercury concentration (μg/g) | 0.02 | 0.05 | 0.09 | 0.15 | 0.22 |
| Cumulative mercury dose (mg/mink) | 0.594 | 1.48 | 2.67 | 4.45 | 6.53 |
| Mercury dose (mg/mink/day) | 0.003 | 0.008 | 0.014 | 0.023 | 0.033 |
| Mercury dose for males (mg/kg body weight/day) | 0.001 | 0.003 | 0.006 | 0.01 | 0.015 |
| Mercury dose for females (mg/kg body weight/day) | 0.002 | 0.006 | 0.01 | 0.02 | 0.027 |

[1] Cumulative feed consumption of 29,700 g/mink based on average daily feed consumption of 150 g (Leonard, 1966)

Table 16. Mean mercury concentrations (mg/kg; weight weight) in organs from adult mink fed a diet containing various percentages of "contaminated" or "uncontaminated" fish

| Organ | Diet A | Diet B | Diet C | Diet D | Diet E |
|---|---|---|---|---|---|
| Kidney | 0.794 ± 0.120[1] | 1.250 ± 0.140 | 2.022 ± 0.419 | 3.435 ± 0.418 | 4.233 ± 0.333 |
| Heart | 3.445 ± 0.356 | 6.845 ± 0.581 | 7.564 ± 0.510 | 12.83 ± 0.789 | 17.82 ± 0.925 |
| Liver | 0.382 ± 0.055 | 0.626 ± 0.086 | 0.997 ± 0.090 | 1.823 ± 0.141 | 3.273 ± 0.367 |

[1] Mean ± S.E.

## DISCUSSION

The primary goal of this study was to assess the effects of consumption of environmental contaminants contained in fish collected from the ORR on mink as a method of evaluating the extent and degree of adverse effects of ORR operations on wild piscivorous populations. Attempts were made to simulate the exposure of mink to an environmentally-contaminated diet. Mink are opportunistic predators. While a great proportion of their diet is comprised of fish (30%), they will also eat amphibians, reptiles, birds and other mammals (Heaton et al., 1995). Even though mink may consume a variety of prey species, concentrations of compounds like PCBs and organochlorine pesticides in fish correlate well with concentrations found in mink tissues. Thus, it can be concluded that fish comprise a significant vector of exposure of wild mink to environmental contaminants. In the present study, fish comprised an abnormally large percentage (75%) of the diet and this may in part account for some of the reproductive problems that were observed. The concentration of fish in the diets did not follow the standard operating procedure followed by the MSU Experimental Fur Farm, but was specified by the biologists at Oak Ridge National Laboratory. Commercial mink diets typically contain less than 50% fish (Aulerich, personal communication).

In addition to PCBs, the fish collected from the Oak Ridge Reservation undoubtedly contained other organochlorine contaminants and variable concentrations of mercury. The

fish were only analyzed for PCBs and mercury as these were the primary contaminants of interest to ORR and thus were the focus of this research. Therefore, it is possible that effects attributed to total PCBs and mercury in this study may be due in part to the presence of other contaminants in the fish. Because the fish collected from upstream of the ORR ( originally considered to be "uncontaminated") were found to contain PCB concentrations near that of the downstream "contaminated" fish (1.69 vs. 2.13 ppm, respectively) all the diets that contained fish from ORR were considered as treatment groups. The diet comprised of upstream fish (Diet B) was not considered as a control when calculating statistical differences. The fish collected from upstream of the ORR contained considerably less mercury than those fish collected downstream from the ORR (0.07 and 0.35 ppm respectively). Thus, operations at ORR appear to be contributing to the contamination of the river system with mercury.

Many researchers have documented a "wasting syndrome" or marked reduction in body weights of mink associated with halogenated hydrocarbon intoxication (Aulerich et al., 1987; Hochstein et al., 1988). Other species, including rhesus monkeys (Barsotti et al., 1976) and rats (Courtney et. al., 1978) have also exhibited a marked reduction in body weights when exposed to PCBs. This weight loss could be due in part to the dose-dependent decrease in food consumption observed in animals exposed to PCBs. No statistical differences in initial or final body weights were noted among the mink in the treatment groups in this study. Although feed consumption was not measured in this study, emperical observation suggested that the mink fed the higher concentrations of PCBs did not decrease their daily food intake during the study period. Heaton (1992) did not observe the "wasting syndrome" in mink fed PCB-contaminated fish, though she did observe other clinical signs of toxicity. Perhaps the

wasting syndrome was not observed because of the greater caloric value obtained from feeding the unusually high percentage of fish in the mink diets. This may also partly explain why no significant body weight losses were observed in the present study . The body weight loss observed for Diet D and E females (5%) is not unusual for lactating females because of the greater energy demand for milk production. Also, Diet E females had, on average, more kits to nurse than females in the other treatment groups, thus placing an even greater demand on the female's energy reserves that would normally allow her to gain or maintain body weight.

Previous studies of the effects of PCBs on mink by Aulerich et al. ( 1971, 1973, 1986) Bleavins et al. ( 1980), and Wren ( 1987) showed that PCBs have a detrimental effect on adult mink survivability. Numerous investigations have documented the toxicity of PCBs to adult mink. Bleavins et al. (1980) demonstrated the sensitivity of adult mink to PCBs. In general, the mean survival time of mink fed Aroclor 1242 was inversely related to the concentration of PCB. Adult mink fed 20 ppm Aroclor 1242 experienced 100% mortality within nine months while 10 ppm caused 66.7% mortality within nine months. All animals that died were subjected to necropsy which revealed emaciation characterized by almost complete absence of body fat. This "wasting syndrome" is commonly associated with halogenated hydrocarbon poisoning and has been documented for mink (Aulerich and Ringer, 1979; Aulerich et al., 1985). This body weight loss is related only in part to decreased food intake and the cause of death is unknown.

In another study by Aulerich and Ringer (1977), deaths occurred earlier and mortality was greater for mink receiving metabolized Aroclor 1254 than for those mink fed the same

concentration of a technical grade Aroclor 1254. At 75 ppm, 80% of the mink receiving the metabolized form died within 28 days, while 50% of those receiving the technical grade died within the 28 days. Aulerich et al.,(1985) found that even greater mortality occurred in adult mink fed considerably lower concentrations of congeners having chlorine atoms in three of the four lateral positions in the aromatic ring system.

Mink have been shown to be more sensitive to PCB-contaminated fish canning by-products (heads, fins, tails, viscera and belly fat) than to the whole raw fish (Aulerich and Ringer, 1970). The higher rate of mortality from consumption of comparable quantities of the canning by-products was attributed to the higher fat content of the by-products. PCBs are stored in the fatty tissue and thus the mink were exposed to higher concentraitons of PCBs in the fatty by-products.

Several studies have indicated that female mink may be more susceptible to PCB contamination than male mink. Although no differences were found in mortality rates between males and females fed diets supplemented with 10, 20 or 40 ppm Aroclor 1245, Aroclor 1242 fed at 5 ppm or Aroclor 1016 fed at 20 ppm caused mortality limited to female mink. Male mink have also been shown to survive longer than females when fed low concentrations (0.64-3.57 ppm) of Aroclor 1254 (Platonow and Karstad, 1973). Parkinson and Safe (1987) suggest that females are more susceptible to PCB toxicity than males because males have a higher drug metabolizing activity and the toxicity of PCBs to mink is inversely related to their drug metabolizing acitivity.

The clinical signs that have been observed in mink that have died during exposure to PCBs include listlesness, nervousness, bloody stools, and anorexia (Bleavins et. al., 1980;

Aulerich et al., 1986). In the present study, mortality was limited to one adult male mink fed Diet A while two female mink were euthanized that were fed Diet C. Althought two of the mink were "off feed" for two days before death and passed tarry stools, none of the mink displayed the other signs commonly observed with the "wasting syndrome" associated with PCB intoxication. In mink, tarry stools is a common clinical sign associated with anorexia. In this study, mortality from such a low level of exposure to PCBs would not be expected to occur. The male mink that died had a fibrinosuppurative and focally hemorrhagic bacterial cystitis and one female that was euthanized had complications associated with a gallstone and had a cyst on her ovary. The other female that was euthanized had complicationsdue to dystocia but no necropsy was performed.

The clinical signs that have been observed in mink that have died due to mercury exposure include incoordination, loss of balance, anorexia, loss of weight, ataxia, paralysis, tremors and high pitched vocalizations (Aulerich et al., 1974). No clinical signs of mercury exposure were observed in the current study. Mortality of mink would not be expected to occur with the low concentration of mercury present in the experimental diets when compared to those concentrations causing death in mink in other studies. For example, Aulerich et al. (1974) found that 5 ppm methylmercury was lethal to adult mink within one month and that total consumption of 18 mg methylmercury caused death in female mink, which compares to results reported by Hanko et. al. (1970) who found that consumption of 20 mg mercury caused death in female ferrets fed a diet containing 5.0 ppm methylmercury. The cummulative mercury consumption for the adult mink in the current study ranged from 0.594 mg/mink to 6.534 mg/mink (Table 15; Appendix C). Thus, the cummulative mercury

consumption for even the highest treatment group in the current study was one-third that reported by Aulerich and coworkers and death would not be expected to occur in the adult mink on this trial.

Previous studies of the effects of PCBs on mink by Aulerich et al. (1971,1973,1986); Bleavins et al. (1980) and Wren (1987) showed that PCBs have not only a detrimental effect on adult mink survivability, but also on mink reproduction.

Subchronic consumption of PCBs can cause reproductive failure in female mink, although some females exposed to PCBs ovulate and implant fertilized eggs. Dietary concentrations as low as 2 ppm Aroclor 1254 impaired mink reproduction when fed for eight months. Complete fetotoxicity for Aroclors 1242 or 1254 occurred at less than 5 ppm, with 50 percent lethality of the adult mink being 8.6 and 6.65 ppm respectively (Aulerich et al., 1981). Bleavins et al. (1980) reported that female mink fed Aroclor 1016 had fewer full term pregnancies and those that whelped, had kits with lower birth weights and lower four week body weights than the control kits. Kit mortality reaching 80% occurred when female mink were fed 15% ground, whole, raw coho salmon from Lake Michigan containing 15 ppm total PCBs and no kits survived longer than 24 hours (Aulerich et al., 1973).

In the present study, there were several reproductive parameters that were statistically significant between one or more treatment groups. Although statistically significant, differences in parameters which included kit birth weight, kit three-week body weight, and kit mortality at birth may not be relavent as they fall within a range of values considered to be"normal" for mink. Results from studies by Aulerich and Ringer (1977) and Bleavins et al. (1980) have demonstrated that mink are among the most sensitive species to PCBs.

However, in these studies, the dietary PCB concentrations were greater than those in the current study. Aulerich and Ringer (1977) found that reproductive failure occurred in female mink fed only 2 ppm Aroclor 1254 and at concentrations even as low as 5 ppm, death of the adults occurred within nine months. Another study by Aulerich and Ringer (1980) showed that levels as high as 25 ppm Aroclor 1016 fed for 18 months did not adversely affect reproduction, although growth and survival of the newborn kits was suboptimum. The concentration of PCB (Aroclor 1016) fed to mink in the latter study which did not cause reproductive impairment may have differed from those concentrations of PCBs (Aroclor 1254) fed to mink which caused reproductive impairment because of differences in absorption or greater metabolism or excretion rates between certain Aroclors or because of the different concentrations of the various congeners in the Aroclor mixtures. Platonow and Karstad (1973) and Hornshaw et al. (1983) concluded that reproductive impairment can occur in mink at lower concentrations when the PCBs have been first metabolized by another species and then fed to mink as in the current study.

It does not appear that the concentrations of PCBs or mercury in this study directly affected embryo implantation or maintenance of pregnancy since all females fed Diet E (which had the highest concentrations of both PCBs and mercury) whelped ( Table 6). A significantly higher number of females fed diet C did not whelp compared to the other dietary groups, but it cannot be concluded that PCBs or mercury were the cause of the impairment because there were only eight females per dietary treatment. One female was badly bitten by an aggressive male early in the breeding season and refused to mate, a second female was found to have a cyst on her ovary at the time of necropsy. A third female had several

implantation scars but lost the kits before whelping, while a fourth female in the group had no implantation scars at necrospy even though she had several confirmed matings. The number of kits whelped per female is important as it accounts for the proportion of females which either failed to implant or lost fetuses through early resorption. The number of kits per female that whelped between the groups in this study was not significantly different. Thus, it could be concluded that in this study the PCB and mercury concentrations were not sufficient to cause impairment of ovulation or implantation.

Studies by Wheeler (1971), and Ahamed et al. (1978) have shown that PCBs can have an adverse effects on the reproductive performance of males. Male beagle dogs fed 10 or 100 ppm of Aroclor 1254 for two years showed diffuse hyperplasia of interstitial cells of the testes and aspermatogenesis (Wheeler, 1971). Chickens fed PCBs during the maturation period showed reduced testis weights (Platonow and Funnell, 1971) and cocks fed a diet contamining PCBs exhibited decreased sperm volume and concentration (Ahamed et al., 1978). Male quail administered a C14 labelled PCB and killed 24 hours after administration showed very poor uptake of PCBs into the seminiferous epithelium (Biessman, 1981). This and other investigations by Berlin et al. (1975) and Brandt (1975) showed that there is poor uptake of PCBs into the testes which may be due in part to poor blood-flow through the testes. The reproductive performance of the male mink in this experiment was not impaired by any of the treatments ( Table 7). This agrees with the results of other studies which demonstrated that reproductive functions of female mink were much more sensitive to PCBs than of males (Bleavins et al., 1980). Because the males used in this experiment were fed PCBs after sexual maturity , the sexual organs may not have been affected by the PCBs in the

diet as might be expected if the PCBs were administered during sexual development. As stated above, the testes have been found to have poor uptake of PCBs, and with the low concentrations of PCBs in the experimental diets of this study, no detrimental effects on spermatogenesis would be expected.

Sexual differences in whole-body clearance and tissue distribution of intestinally-absorbed methylmercury have been demonstrated in rats, mice, and humans (Magos et al., 1981). Female mice seem to retain considerably less mercury than do male mice. Therefore, the male mice were more susceptible to the toxicity of methylmercury than female mice. Although the mechanism is unknown, testosterone levels were suggested as a possible modifying factor as the sex difference observed in the adult animals (Hirayama and Yasutake, 1985).

The concentration of mercury in the present study did not impair the reproductive performance of the male mink. This agrees with the results of studies by Wren (1987) in which no effects were observed in the mating performance of male mink fed diets containing either 1.0 ppm methylmercury, a combination of 1.0 ppm PCB and 1.0 ppm methylmercury or 0.5 ppm methylmercury.

Numerous studies have shown that there is a dose-dependent decrease in body weights of offspring prenatally exposed to PCBs (Heaton, 1992). Research conducted in the early 1980s has shown that babies born to women who had eaten Lake Michigan fish containing PCBs were lighter at birth by 169-190 g and had smaller head circumferences than babies born to women who had not eaten Lake Michigan fish (Jacobson et al., 1984). Infants exposed in utero to PCBs by affected mothers in the Japanese Yusho outbreak tended to be small for their gestational age (Hirayama, 1976). Evidence of reduced birth size in humans

is consistent with experimental studies with rhesus monkeys. Ingestion of PCBs by female rhesus monkeys was associated with lower birth weights among liveborn offspring, even for those conceived more than 12 months after maternal ingestion of PCBs had ceased (Barsotti et al., 1976). Birth weights of mink kits whelped by dams fed Aroclor 1016 averaged numerically less than those of kits whelped by dams fed a control diet, however, the weights of the kits were not statistically different (Bleavins, 1980). Lower pup birth weights have also been reported when adult female rats were fed a diet containing Kanechlor 300, 500 or Aroclor 1254.

In the current study, kits born to mothers fed Diets D and E had significantly ($p \leq 0.05$) higher birth weights than those kits born to mothers fed diets having lower PCB concentrations ( Table 6). This contrasts with the results of the studies reported above. However, the average birth weights for kits in all treatment groups in the current study fell within the normal range for ranch mink (based on MSU Experimental Fur Farm records). This suggests that the PCB concentrations in the current study caused no detrimental in utero effects through placental transfer. It is possible that because, on average, fewer kits were born to those females fed Diet E, that this could account for the heavier birth weights observed in the Diet E kits.

The results of this study may support the observation that PCBs are transferred through the mother's milk. Although kits from Diet E were the heaviest at birth, by three weeks of age the kits which were exposed to the higher concentrations of PCBs were lighter than those kits from the other treatment groups. This suggests that possibly milk quality or quantitiy was adversly affected. If PCB loading had been through placental transfer, the kits

would have probably been smaller at birth. The decreased growth rate of the Diet E kits could also be because there were more kits per female for diet E females than for any other treatment group. If the mothers had poor milk quantity, the greater number of kits would only compound the problem and result in slower growth rates. Aulerich and Ringer (1977) noted impaired growth and excessive mortality in kits that were born with normal birth weights and were nursed by PCB-exposed females.

In the current study, kits born to mothers fed Diets D and E, which contained the highest concentrations of PCBs, had the greatest birth weights but gained body weight at a lesser rate to six weeks of age than did those kits born to mothers fed the lower concentrations of PCBs. Comparing kit body weights from birth to six weeks of age suggests that PCB transfer by lactation may have played a role in the reduction of body weight gains in kits from Diets D and E that were exposed to higher concentrations of PCBs during lactation. Those kits born to mothers fed Diet A (75% Atlantic mackerel) had statistically higher ($p \leq 0.05$) three week body weights than those kits born to mothers fed Diet B (75% upstream fish) that were the lightest at three weeks of age ( Table 6). This was the only statistically significant difference in kit growth from birth to six weeks of age. The reason for the greater three-week kit body weights in the Diet A kits is unknown.

Many studies of various species have documented the passage of PCBs from mother to fetus through transplacental transfer. However, most offspring, including mink, receive a considerably greater quantity of PCBs via lactation. Thus, milk is a more important source of PCB contamination to mink kits than placental transfer. Kit body weights may vary as much as 10% or more from average depending on the mother's physical condition, diet, and

the genes she inherited for milk production (Leonard, 1966). Since the genetic traits for milk production cannot be accounted for, it could be that kit growth rates may have been affected by the diet of the females during lactation. The diets contained an unusually high percentage of fish (75%), which could have resulted in marginal nutritional deficiencies that, in combination with the higher concentration of PCBs in Diets D and E could have contributed to the slower growth rates observed in diet D and E kits.

Perinatal mink mortality was studied by Martino and Villar (1990). Of 2122 kits (standard dark mink) born, 62 were stillborn and 548 of those born alive died within the first four weeks of life (25.8% mortality). Death resulted from many causes (septicemia, starvation and hypothermia were the most common). The highest percentage of kits (61.9%) died within the first week of life, with the highest mortality attributed to starvation occurring in litters of nine or more kits (46.8%) or seven to nine kits (26.6%). The number of stillborn kits was also greater in those litters having seven or more kits. Most stillborn kits had below average birth weights. Undersized newborn kits may have developmental deficits and be more susceptible to cold and less able to compete for food.

In the current study, the percentage of kits born alive (77-95%) fell within the normal range observed by Martino and Villar (1990). Although it is possible that the higher kit mortality observed in four of the five treatment groups from birth to three weeks could suggest that the kits may have obtained higher quantities of PCBs from birth to weaning than during fetal developement, all kits born alive to mothers fed Diet E survived to six weeks of age. Therefore, it is thought that the concentrations of PCBs in the diets were not high enough to cause an abnormal increase in kit mortality. The females fed Diets A and C had

significantly ($p \leq 0.05$) higher kit mortality at birth than those females fed the diets containing the highest concentrations of PCBs and mercury. It should be noted that Diet A contained only one species of fish (Atlantic mackerel) and it is possible that 75% of this single ingredient resulted in an unbalanced diet causing negative effects on kit survivability at birth.

The concentrations of mercury in the diets of the adult mink in the current study did not appear to affect pregnancy or the development of the fetal central nervous system as all females fed the diet with the highest concentration of mercury whelped and none of the kits displayed the characteristic "cerebral palsy" type symptoms indicative of mercury poisoning. The brains of the kits were not taken for histopathological examination or mercury analysis during necrospy, therefore, no comparisons could be made between treatment groups. Wren (1987) observ ed no difference in kit survival rates when mink were exposed to either 1 ppm PCB or 1 ppm methylmercury singly, but when the treatment groups received both chemicals simultaneously, a synergistic effect was noted resulting in a significant reduction (35.8%) in kit survivability. The form of mercury plays a significant role in the toxicological result. When mercury was fed in the form of mercuric chloride to adult mink at 10 ppm for five months, the number of kits whelped and alive at four weeks of age was comparable to the control (Aulerich et al., 1974).

In the current study, though there were noticeable differences in the survival rates of the kits, it could not be concluded that this was the result of in utero exposure to mercury, as kits from mothers fed Diet E containing the highest mercury concentration (0.22ppm) had the greatest survival rate when compared to the other treatment groups which contained lower concentrations of mercury.

There were also no detrimental effects on growth and body weights of mink kits observed in this study ( Table 6). When comparing mercury concentrations in the current study with previous studies, it does not appear that the concentrations of mercury in the treatment diets was high enough to cause a noticeable decrease in growth rates in kits exposed in utero as even the highest concentration of mercury in Diet E was less than half that used in the study by Wren. (1987). Although kits born to mothers fed Diet E tended to have a slower growth rate, it is believed that this may have been due to the larger average litter sizes.

Heaton (1992) observed significant increases in the weights of adult female mink liver, spleen, lungs, kidneys, thyroid glands and adrenal glands when mink had been fed PCB-contaminated fish. These results are similar to results reported by Aulerich et al. (1987) who observed increased liver, adrenal gland, and kidney weights in female mink fed a diet containing 3,4,5, 3',4',5'-hexachlorobiphenyl (HCB). However, in the latter study, no increases in the weights of spleen, lungs or thyroid glands were observed, demonstrating that mink exhibit a sensitivity and variablility between metabolized forms of PCB and individual congeners (Aulerich et al., 1987). An increase in the weight of the liver has proven to be a sensitive , but not exclusive, indicator of PCB toxicity in many species. Enlarged livers observed in mink fed Aroclor 1254 are consistent with results of other studies in which commercial PCB mixtures were fed to mink (Aulerich and Ringer, 1977), rats, mice (Orberg and Lundberg, 1974), swine (Hansen et al., 1975), monkeys (Allen et al., 1974) and rabbits (Koller and Zinkl, 1973).

In the current study, no differences were noted in organ weights among the treatment groups for the females (see Table 8). This, along with the fact that there were no differences in body weights among the females in the various groups, supports the hypothesis that the concentrations of PCBs in this study were not great enough to cause an increase in organ weights, decrease in food consumption and/or "wasting syndrome" associated with PCB intoxication. Although liver weights of males fed Diet A were twice those of males fed Diet C, (see Table 8), because there were only two males per diet, this difference was not considered significant due to the high variability in individual liver weights. In the current study, there were also no differences observed in kit organ weights between the dietary groups.

Unfortunately, very little research has been done on the effects of mercury on organ weights. However, mercury has been documented to effect organ weights in laboratory studies with mink. Five ppm methylmercury fed to mink caused a significant increase in the weights of the heart and kidneys when compared to control animals (Aulerich et al., 1974).

In the current study, no significant increases in adult or kit organ weights were observed. When compared to the concentrations of mercury in the study by Aulerich et al. (1974), the mercury concentrations in the current study were minute and therefore, would not be expected to increase organ weights.

Numerous studies have documented the harmful effects of PCBs on a variety of mammalian species. The symptoms associated with administration of a toxic dose of a commercial PCB mixture (Aroclor 1242) in rats consist of diarrhea, diminished exploratory behavior, adipsia, anorexia, erythema of limbs, ataxia, coma and death (Kimbrough et al.,

1978). In mammals, the pathology involves: follicular pyodermatitis (chloracne), liver atrophy and necrosis, and an increase in the activity of drug metabolizing enzymes. Mink fed PCBs showed depressed plasma progesterone concentrations, elevated hepatomicrosomal cytochrome P450 concentrations, increased benzo (a) pyrene hydroxylase activites and enhanced cerebral and depressed midbrain dopamine concentrations (Aulerich et al., 1985).

Symptoms of PCB poisoning in birds consist of tremors, ataxia, ruffling and loss of feathers, enlarged livers, edema of subcutaneous tissues and fluid accumulation in the abdominal and thoracic cavities. These symptoms characterize the disease designated as chick edema disease (Kimbrough et al., 1978). The physiopathology of PCB toxicosis in poultry includes: swelling and hemorrhages of the kidneys, centrilobular liver degeneration, microsplenia and depressed body growth.

In contrast to acute toxicity of PCBs, which is of lower order when the substances are administered as a single dose, subacute toxicity is of greater concern. Rhesus monkeys fed diets containing 25 ppm Aroclor 1248 for two months developed facial edema, alopecia, acne, anemia, hypoproteinemia, bone marrow atrophy and severe hypertrophic gastritis. However, no clinical signs of PCB intoxication were observed in any of the females or kits in this study. Two male mink passed blood in their feces for a short period of time, and one of these males died shortly after, but the cause of death was not related to PCB exposure.

Mink fed Aroclor 1254 have shown pathological lesions that included mild splenomegaly, increased megakaryocytes and gastrointestinal tract hemorrhage and have frequently shown ascites and pancreatomegaly ( Platonow and Karsted, 1973; Aulerich et al., 1985). The differences in the pathological effects seen with PCB intoxication may be due in part to the

interaction of the various PCB isomers which comprise technical grade Aroclors but which may not be present in those PCB mixtures found in the environment (Gillette et al., 1987). PCB induced skin lesions included hyperplasia and hyperkeratosis of epidermal and follicular epithelium in adult female New Zealand rabbits. Histopathology of the rabbit livers revealed centrolobular degeneration, liver cell atrophy, focal necrosis and cytoplasmic hyalin degeneration. PCB-induced kidney lesions included hydropic degeneration of the convoluted tubules and tubular dilation. In rats administered a single toxic dose of Aroclor 1242, all organs appeared normal except the liver and kidneys. Histopathology revealed large discrete sudanophilic vacuoles in hepatocytes and scattered foci of tubular epithelial cells present in the kidneys (Kimbrough et al., 1978). Kimbrough and coworkers also reported ulceration of gastric and duodenal mucosa in rats after a single oral dose of Aroclor 1254 or 1260.

Numerous studies have concluded that the primary target organ of orally administered PCBs is the liver in mammals (Hansen et al., 1975; Gillette et al., 1987; Heaton, 1992). Heaton (1992) reported the first mink kit to show teratogenesis that may be due to exposure to PCBs. The mother of this kit had the highest concentration of PCBs in her liver (10.6 mg/kg) of the adults on trial. Mink fed 20 or 40% Saginaw Bay carp (1.53 and 2.56 ppm PCB, respectively) had hepatic lipidosis, marked congestion and moderate lymphocytic infiltration in their livers. Rats, mice, mink, rabbits and rhesus monkeys have all demonstrated liver hypertrophy due to exposure to PCBs. Focal liver necrosis has also been observed. Sherman rats fed 100 ppm Aroclor 1242 or 100 ppm Aroclor 1016 for six months showed evidence of hepatic lipid accumulation, enlarged liver cells, inclusions in a number of livers, and hemorrhage and necrosis.

The most important hepatic effects of PCB poisoning included increased weight, fatty degeneration, hyalin degeneration, and necrosis. Increased liver weights, due to the proliferation of smooth surfaced membranes of the endoplasmic reticulum were found by Nishizumi (1970) in mice and monkeys and by Norback and Allen (1970) in rats.

Changes in the liver morphology observed using light microscopy are most commonly observed in the centrolobular zones. The distribution of the metabolizing system in the liver, resulting in a higher concentration of the ultimate toxicant in the centrolobular region, accounts for the occurrance and frequency of centrolobular toxicity. The centrolobular hepatocytes are larger, contain more smooth endoplasmic reticulum and have higher concentrations of cytochrome P450 and associated enzymes that metabolize and activate xenobiotics than perilobular hepatocytes. Following exposure to commercial PCB mixtures, mink exhibited high incidences of centrolobular fatty changes in hepatocytes, hemosiderosis of Kupffer cells and neutrophil reactions (Bergman et al., 1992). Planar PCB congeners caused centrolobular fatty changes in the livers of mink. They are most toxic due to their strong binding affinity to the cytosolic aromatic hydrocarbon (Ah) receptor protein. No cases of centrolobular fatty changes were noted when mink were treated with non-planar PCB congeners, which are considered less toxic because of their lower binding affinity to the Ah receptor protein. Occurrence of liver changes observed in mink was not due exclusively to effects caused by congeners regarded to be most toxic, but their frequency and severity were due to the combined effects of the different fractions present in the commercial PCB mixtures. The fatty changes observed in the liver due to PCB exposure suggest an excess accumulation of triglycerides within the hepatocytes which may result from disturbances in any of the events

in the sequence from fatty acid entry to lipoprotein exit.

Hepatocellular carcinomas have been obseerved in rats fed 100 ppm Aroclor 1260 in the diet for 21 months (Kimbrough, 1973) or 500 ppm Aroclor 1254 for six months. Adenofibrosis, a focal proliferation of glandular epithelium forming ducts surrounded by extensive fibrosis, occurred with hepatocellular carcinomas found in rat livers.

Even though numerous studies have documented pathological changes in the organs of animals exposed to PCBs, no toxic changes were observed in the livers or any of the other organs collected at necropsy from either the adult mink fed the contaminated diets or their kits in this study. The low concentrations of PCBs in the current study were probably not sufficient to cause an accumulation of lipid within the hepatocytes which leads to the congestion and inflammatory response commonly observed in mink fed diets containing PCBs.

Even though numerous studies document severe pathological changes in the central nervous system of both humans and animals exposed to mercury in utero, no changes were observed at necropsy or on histopathological examination that would reveal mercury intoxication in the mink in the present study. It is assumed, therefore, that the concentrations of mercury in the current study were probably not sufficient to cause affects to the central or periopheral nervous system with in utero exposure. As stated earlier, when comparing the mercury concentration of the diets in the current study and the respective tissue concentrations with those studies of other investigators who reported pathological lesions and death, pathological alterations would not be expected with dietary mercury concentrations of only 0.02 to 0.22 ppm.

At lethal exposure levels of PCBs, hematological changes appear to be directly related to lesions in the bone marrow in all species of animals studied. In acute studies (<30 days), thrombocytopenia and lymphopenia have been reported in several species of lab animals. Increased erythrocyte counts have been reported by McConnell (1985) in acute studies, but they may have been due to dehydration. In chronic studies, mild to moderate anemias were the most consistent hematological finding. Some studies showed decreases in leukocytes while others showed leukocytosis. These differences may be due to the presence of secondary infections (McConnell, 1985).

Generalized effects on hematological parameters in mink due to PCB intoxication include increased blood concentration of thyroxine and alanine aminotransferase (ALAT) and decreases in progesterone, alkaline phosphatase (ALP), serum bile acids (BA), fructosamine, and cholesterol (Edqvist et al., 1992). Blood serum changes are very complex with PCB intoxication. Changes in serum components may reflect lesions of the hepatocytes. Since anatomic pathology varies between species, serum chemistry values vary to reflect lesions (McConnell, 1985).

Studies by Edqvist et al. (1992) revealed significantly different biochemical parameters in preganant mink fed PCBs when compared to the pregnant control females. The most frequently altered parameters were increases in serum ALAT, ALP, BA, and fructosamine. The increase in ALAT activity was due to inflammatory cell reactions in the liver due to disturbed hepatic cell integrity or hepatic necrosis. An increase in serum ALP activity is often seen with bile duct obstruction or intrahepatic cholestasis. Seasonal changes of serum ALP

activity occur in mink with the lowest activity recorded during the winter and increases occurring during the latter part of gestation reaching a ten-fold increase in the summer (Edqvist et al., 1992). Decreases in serum ALP may be related to malnourishment or stress and have been observed in anorexic humans and foxes (Edqvist et al ., 1992). Most disturbances of hepatocellular integrity are accompanied by intrahepatic cholestasis (Edqvist et al., 1992). Rhesus monkeys and mink fed PCBs have displayed decreased serum cholesterol, which can cause lowered serum BA. Elevated serum glutamate dehydrogenase (GLDH) activity has been recorded in mink fed PCBs. This enzyme is considered to be exclusively located in the mitochondria of hepatic cells. Elevated concentrations of GLDH are due to necrosis of hepatic cells (Edqvist et al., 1992).

Treatment of animals with different chemicals, including PCBs, has been shown to increase the activities of biotransformation enzymes and has been described for cytochrome P-450-dependent monooxygenases. Serum ALAT, ALP, aspartate aminotransferase (ASAT) and GLDH are not traditional induction enzymes but represent intracellular enzymes which through disturbed cell integrity, leak from the cells with an increased activity in peripheral blood (Edqvist et al., 1992).

Values of all hematologic parameters measured for the treated females fed Diets B and E in the present study were within the normal range for ranch-bred female mink (Table 10; Kennedy 1935; Kubin and Mason, 1948; Rotenberg and Jorgenson, 1971). The increase in the white blood cell (WBC) count for both treatments that occurred during the month of February could be attributed to the increased stress on the animals due to freezing temperatures.

Curley et al. ( 1971) and Weigel and Smith ( 1974) found that PCBs with a higher number of chlorines per molecule are retained in tissues for longer periods of time than those with lower percent chlorination. Goldstein et al., (1975) suggested that preferential retention of the higher chlorinated PCB congeners might explain the impairment on reproduction in mink observed with Aroclor 1254 but not with the lesser chlorinated PCBs.

Several researchers (Kimbrough, 1973; Burse et al., 1974; Biessman, 1981) have shown that PCBs tend to accumulate in higher concentrations in the liver and adipose tissue of experimental animals. Continued dietary exposure to commercial PCB mixtures in mammals results in their storage in adipose tissue and over an extended period of time, high levels may be attained (Burse et al., 1974). Rats fed 100 ppm Aroclor 1242 or Aroclor 1016 for six months had the highest PCB concentrations in the adipose tissue, where steady state was approached in two months and reached in four months after exposure was discontinued (Burse et al., 1974). After a 10 month feeding period, the concentration of both PCBs was about the same in the liver of rats that were subjected to necropsy, but in rats sampled four months after exposure ceased, almost twice as much Aroclor 1016 as Aroclor 1242 was found in the liver. The levels in the adipose tissue revealed that after two months, no appreciable increases were observed in the adipose tissue for either PCB in the rat. Following a six month recovery period, the residue levels in adipose tissue of the rats were 21.8% of those observed after the six month exposure period to Aroclor 1242. Following a five month withdrawal period, the residue concentrations in the adipose tissue were 11.8% of those observed after the six month exposure period to Aroclor 1016 (Burse et al., 1974).

Male Sherman rats fed dietary concentrations of 500 ppm Aroclor 1254 for six months had pronounced lipid accumulation in the liver which persisted for a 10 month period following the discontinuation of exposure to PCBs. At 10 months, high concentrations of the higher chlorinated biphenyl isomers were still present in adipose and liver tissue (Kimbrough., 1973). When Sherman rats were allowed to recover for 16 months following dietary exposure to 100 ppm Aroclor 1254 for six months, a concentration of 4.4 mg/kg of PCB was still present in the liver and 152 mg/kg in the adipose tissue (Kimbrough et al., 1975).

In the current study , PCB liver and adipose tissue residues were directly proportional to the dietary PCB concentration. Adult mink fed Diet B or diet E had significantly ($p \leq 0.05$) higher concentrations of PCBs in their liver and adipose tissues than mink fed the other treatment diets.

The concentrations of mercury in the tissues of kits was not analyzed in the current study. The adult mink consumed from 0.594 mg to 6.53 mg (see Table16) total mercury while on trial and the highest adult mercury tissue concentrations were found in the heart and ranged from 3.44 ppm to 17.82 ppm wet weight. Because the kits received mercury through the placenta, it is possible that they could have accumulated concentrations higher than those found in the adults at necropsy.

The adult mink fed Diets D and E had significantly ($p \leq 0.05$) higher mercury concentrations in their liver, heart and kidney tissues than mink fed the other treatment diets. This was to be expected since these mink were fed the two diets containing the highest concentrations of mercury. However, unlike Wobeser and Swift (1976), Kucera (1983) and Wren et al. (1986) who found higher mercury concentrations in the liver of mustelids

compared to the kidney, analyses in this study showed higher mercury concentrations in the kidneys than in the livers of the adult mink in all treatment groups. When comparing the concentrations of mercury in the diets of mink in the current study with previously discussed studies, it is clear that even the highest concentration of mercury in Diet E (0.22 ppm) which produced tissue concentrations in the kidney, heart and liver of 4.23, 17.8 amd 3.2 mg/kg, respectively, was very small when compared to the concentrations of mercury used in the diets and found in the tissues by other investigators who observed pathological alterations and death in mink.

## SUMMARY

Environmentally altered PCBs and mercury, as well as other contaminants in fish collected from the ORR and fed to mink three months prior to breeding did not significantly impair mink reproduction. All females consuming Diet E ( 75% downstream fish) having the highest concentrations of both PCBs (1.86 ppm) and mercury (0.22 ppm) whelped. The kits were born with above average birth weights and had below normal mortality rates and average growth rates through weaning. No teratogenic effects were observed. The normal birth weight of kits is eight to ten grams and females in all treatment groups whelped kits averaging normal to above normal birth weights. Those kits born to females fed Diet A (Atlantic mackerel) and Diet C ( 25% downstream fish) had significantly higher mortality rates at birth and three weeks of age than kits whelped by dams fed Diet E (75% downstream fish). A possible explanation could be that the diets contained too high a concentration of a single ingredient (75% Atlantic mackerel) and that this in some way affected kit survivability.

A statistically significant number of females fed Diet C did not whelp, however the reasons for the reproductive failures were not attributed to mercury or PCB concentrations. There were no apparent detrimental affects on implantation or gestation in any of the treatment groups. All females that whelped had within treatment group gestations averaging 44 to 47 days. There were also no apparent affects on male reproduction or spermatogenesis,

as all the males had viable sperm and all but one male had over 90% successful matings.

The mean mercury concentrations were highest in the adult heart and lowest in the liver. The mercury and total PCB tissue concentrations showed a positive correlation with dietary mercury and PCB concentrations. The highest PCB concentrations were found in the fat of the adult mink. Though concentrations of both PCB and mercury were found in several organ tissues of the adult mink, no histopathological alterations were observed in any organs.

Neither the adult males nor females displayed the commonly observed "wasting syndrome" associated with PCB exposure and their body weights differed only slightly between the start and termination of the trial.

No significant differences were noted in hematological or serum chemistry values for the females fed Diet B or Diet E. The increase in white blood cells in both treatment groups during February was thought to be due to increased stress due to colder winter temperatures.

## FUTURE STUDIES

Future studies which may involve feeding conatminated fish to mink should consider increasing the number of individuals per treatment group, thus compensating for unexpected losses or individuals that may refuse to mate. It may also be expedient to feed the kits on trial to 12 weeks of age in order to determine long-term effects on behavior, growth and development. For example, when mink kits from this study, which were not necropsied, were weighed two months after the trial's termination and compared to untreated mink kits, the treated kits tended to be smaller in body size and lighter in body weight. It was also noted that these kits tended to be more hyperactive than untreated kits. In the current study, the kits ate the contaminated feed for approximately two weeks, and because of their small size, they ingested very little of the feed and were thus exposed to only minute quantities of the PCBs and mercury through solid feed. If allowed to eat the experimental diets for several weeks, effects on reproductive maturity or on the developing nervous system that could not be seen in the current study might be observed which would be expected in nature if the only food source available was contaminated. Another important consideration for future studies would be to reduce the total dietary percentage of fish, as some of the detrimental effects observed in the current study may have been associated with the abnormally high percentage of one ingredient (fish) in the diet. Also, more than one species of "clean" fish in a control diet may prove to be beneficial as detrimental effects on kit survivability at birth were seen

in the control group which contained 75% Atlantic mackerel.

# APPENDICES

## APPENDIX A

Kilograms and number of species of fish collected from the Oak Ridge Reservation used in the experimental diets

| Species | Kg | Number |
|---|---|---|
| Upstream | | |
| Flathead catfish (Pylodictis olivaris) | 35.2 | 6 |
| Longnose gar (Lipisosteus osseus) | 1.4 | 2 |
| Black redhorse (Moxostoma duquesnei) | 3.36 | 4 |
| Channel catfish (Istalurus punctatus) | 17.8 | 11 |
| Freshwater drum (Aplodinotus grunniens) | 28.6 | 15 |
| Gizzard shad (Dorosoma cepedianum) | 15.6 | 149 |
| Common carp (Cyprinus carpio) | 132.9 | 127 |
| Small mouth buffalo (Ictiobus bubalus) | 145.3 | 94 |
| Downstream | | |
| Gizzard shad (Dorosoma cepedianum) | 57.8 | 556 |
| White bass (Morone chrvsops) | 10.8 | 22 |
| Skipjack herring (Alosa chrysochloris) | 23.0 | 39 |
| Freshwater drum (Aplodinotus grunniens) | 7.9 | 40 |
| Crappie (Pomoxis annularis) | 0.4 | 1 |
| Shortnose gar (Lepisosteus platostonus) | 19.1 | 9 |
| Common carp (Cyprinus carpio) | 184.0 | 88 |
| Spotted sucker (Minytrema melanops) | 95.7 | 165 |
| Blue catfish (Ictalurus furcatus) | 130.2 | 93 |
| Striped bass (Morone saxatilis) | 61.7 | 29 |
| Walleye (Stizostedion vitreum) | 11.6 | 12 |
| Small mouth bass (Micropterus dolomieu) | 0.8 | 2 |
| Small mouth buffalo (Ictiobus bubalus) | 121.9 | 61 |

# APPENDIX B

**Calculations for consumption of feed, polychlorinated biphenyls (PCBs) and TCDD-EQ.**

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| | |
|---|---|
| **A. Average daily feed consumption (g/mink/day)** | **150 g** |
| **B. Cummulative feed consumption (g/mink)** | $\Sigma \{ \text{(g/mink/day) x (days)} \}$ |
| **C. Cummulative PCB dose (mg/mink)** | **C=B x dietary PCB concentration** |
| **D. Daily average PCB dose (mg PCB/mink/day)** | **D= C / # days** |
| **E. Adjusted daily average PCB dose (mg/kg body weight/ day)** | **E= D / X weight** |

1. 150g is an average value for mink (Leonard, 1965).

# APPENDIX C

**Calculations for consumption of feed and mercury .**

| | |
|---|---|
| **A. Average daily feed consumption (g/mink/day)** | 150 g |
| **B. Cummulative feed consumption (g/mink)** | $\Sigma \{ \text{(g/mink/day) x (days)} \}$ |
| **C. Cummulative mercury dose (mg/mink)** | **C=B x dietary mercury concentration** |
| **D. Daily average mercury dose (mg mercury/mink/day)** | **D= C / # days** |
| **E. Adjusted daily average mercury dose (mg/kg body weight/ day)** | **E= D / X weight** |

1. 150g is an average value for mink (Leonard, 1965).

# APPENDIX D

## IUPAC numbers of PCB congeners found in fish from the Oak Ridge Reservation

| Number | Congener |
|---|---|
| 77 | 3,3',4,4'-Tetrachlorobiphenyl |
| 81 | 3,4,4',5-Tetrachlorobiphenyl |
| 101 | 2,2',4,5,5'-Pentachlorobiphenyl |
| 118 | 2,3',4,4',5-Pentachlorobiphenyl |
| 126 | 3,3',4,4',5-Pentachlorobiphenyl |
| 128 | 2,2',3,3',4,4'-Hexachlorobiphenyl |
| 138 | 2,2',3,'4,4',5'-Hexachlorobiphenyl |
| 153 | 2,2',4,4',5,5'-Hexachlorobiphenyl |
| 156 | 2,3,3',4,4',5-Hexachlorobiphenyl |
| 167 | 2,3',4,4',5,5'-Hexachlorobiphenyl |
| 170 | 2,2',3,3',4,4',5-Heptachlorobiphenyl |
| 171 | 2,2',3,3',4,4',6-Heptachlorobiphenyl |
| 180 | 2,2',3,4,4',5,5'-Heptachlorobiphenyl |
| 183 | 2,2',3,4,4',5',6-Heptachlorobiphenyl |
| 189 | 2,3,3',4,4',5,5'-Heptachlorobiphenyl |
| 194 | 2,2',3,3',4,4',5,5'-Octachlorobiphenyl |
| 195 | 2,2',3,3',4,4',5,6-Octachlorobiphenyl |
| 198 | 2,2',3,3',4,5,5',6-Octachlorobiphenyl |

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