This is to certify that the

thesis entitled

EFFECTS OF FEEDING CARP FROM SAGINAW BAY, MICHIGAN TO RIVER OTTER

presented by

HARRY G. DAVIS

has been accepted towards fulfillment
of the requirements for

M.S. degree in **Animal Science**

Richard J. Aulerich
Major professor

Date **February 1, 1993**

EFFECTS OF FEEDING CARP FROM SAGINAW BAY, MICHIGAN TO RIVER OTTER

By

Harry G. Davis

A THESIS

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

MASTER OF SCIENCE

Department of Animal Science

1992

ABSTRACT

EFFECTS OF FEEDING CARP FROM SAGINAW BAY, MICHIGAN TO RIVER OTTER

By

Harry G. Davis

The primary objective of this study was to determine the sensitivity of otter to environmental contaminants. Saginaw Bay carp containing 5.7 ppm polychlorinated biphenyls (PCBs) were fed to otter at 0%, 20%, 40%, and 60% of the diet for 24 weeks. Blood and tissue samples were collected from the otter for hematological, chemical, and histopathologic analyses. Consumption of the carp resulted in a marked decrease in feed intake and reduced body weights, which were inversely proportional to the amount of carp in the diet. Liver PCB concentrations ranged from less than instrument detection limit to 1.45 ppm, fat PCB concentrations ranged from 1.2 ppm to 22.8 ppm, and serum concentrations ranged from 76.4 ng/g to 489.3 ng/g. Although the otter had PCB residue concentrations in their tissues, a thiamine deficiency may have been primarily responsible for the clinical signs observed and obscured any toxicological manifestations due to PCB toxicity.

ACKNOWLEDGMENTS

I would like to express my sincere thanks and appreciation to the members of my guidance committee, Dr. Richard Aulerich, Dr. Steven Bursian, Dr. James Sikarskie, and Dr. Cal Flegal, for their useful suggestions throughout my master's program and in the preparation of this manuscript.

I would also like to thank everyone at the Michigan State University Fur Farm for their help and assistance. Thanks are extended to Dr. James Render for his histopathological assistance. Thanks are also extended to the Michigan State Agricultural Experiment Station, Federal Aid in Wildlife Restoration, the Michigan Department of Natural Resources, and Pittman-Robertson Project W-127-R, which provided funds for the project. Thanks are extended to Dr. James Jay for his assistance and friendship during my time at Michigan State.

I would especially like to thank my wife, Deena Davis, and the rest of my family for their love and support during the preparation of this manuscript.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Many questions have been raised about the effects that polychlorinated biphenyls (PCBs), polychlorinated dibenzofurans (PCDFs), and polychlorinated dibenzo-p-dioxins, collectively termed planar chlorinated hydrocarbons (PCHs), have on the environment. Of these PCHs, PCBs are of particular concern because of their relative abundance, global distribution, resistance to oxidation, physical and chemical stability, and tendency to bioaccumulate. PCBs have not been produced in the United States since the late 1970s, but they still remain in the Great Lakes ecosystem and throughout the United States and Canada. Environmental samples containing PCBs have also been collected from Central America, England, Japan, and the Antarctic (D'Itri and Kamrin, 1983; Gustafson, 1970; Koeman et al., 1969).

The Great Lakes basin is the largest body of fresh water in the world. Chemical analyses have shown the presence of hundreds of toxic chemicals in every facet of this ecosystem, including water (Nadeau and Davis, 1976), sediments (Government of Canada, 1991; Nadeau and Davis, 1976), birds (Eisler, 1986), fish (Copel and Eisenreich, 1985; D'Itri and Kamrin, 1983), and terrestrial mammals (Kalmaz and Kalmaz, 1979; Zimmerman, 1982).

The widespread contamination of the Great Lakes basin has led to concern about the effects on the overall health of the animal and human populations associated with the ecosystem. These concerns led to the establishment of the International Joint Commission (IJC), which governs the obligations and rights of the United States and Canada with regard to their boundary waters. The IJC has designated 42 areas of concern--problem areas where water pollution has caused degraded water quality and environmental conditions (see Figure 1). These concerns have led to the suggested use of wildlife species or species groups as indicator species for environmental health.

Need for the Study

The mink (Mustela vison) has been used extensively as an animal model and indicator species, but the otter (Lutra canadensis) has not been studied extensively, and data relative to its sensitivity to environmental contaminants are lacking. This study was conducted to provide information on the sensitivity of river otter to some environmental contaminants and the potential use of the otter as an indicator species for environmental contamination in the Great Lakes ecosystem. Specifically, this research focused on the effect of feeding environmentally contaminated fish from Saginaw Bay, which is one of the areas of concern, to river otter.

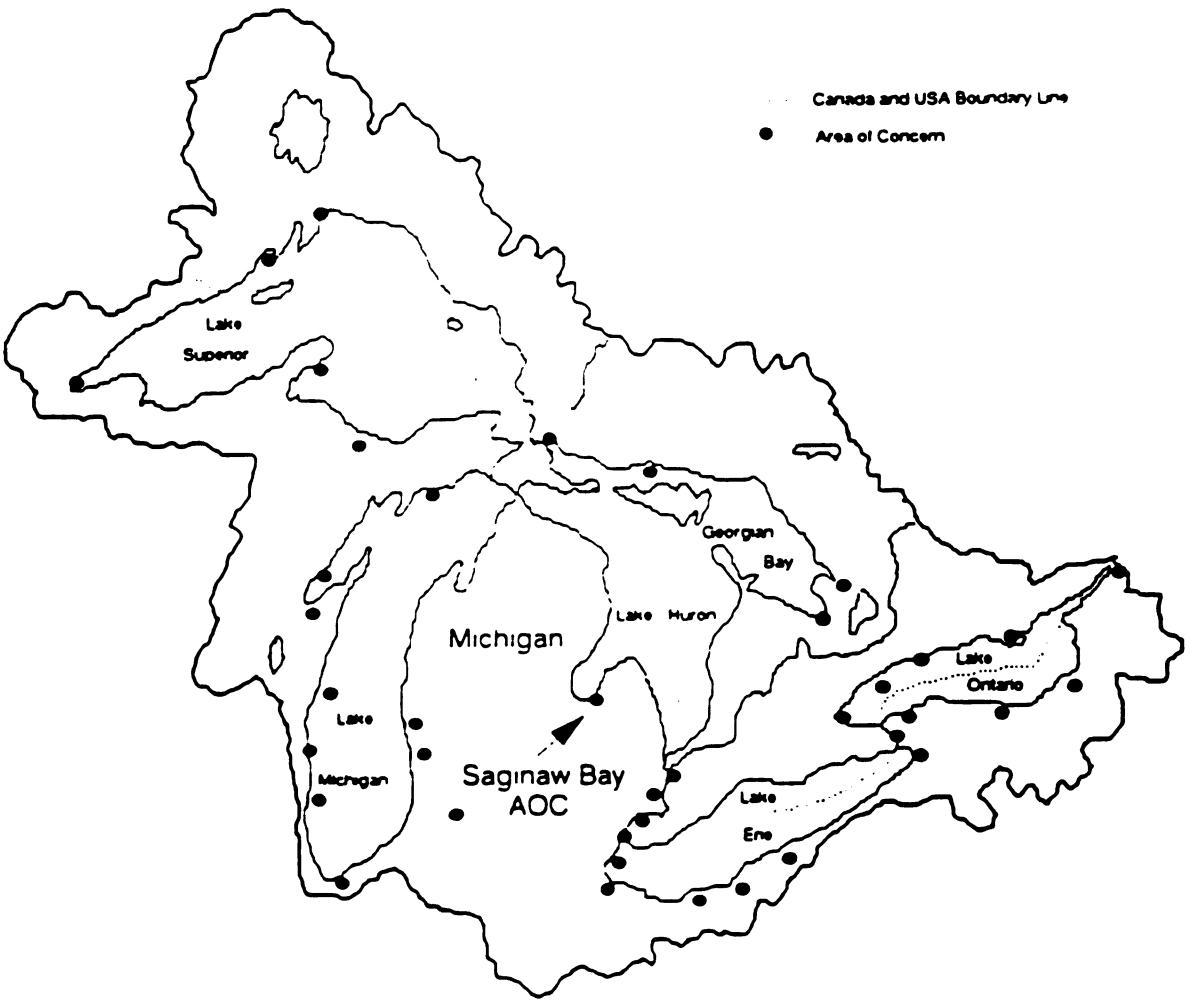


Figure 1. The 42 areas of concern identified in the Great Lakes basin (modified from the Government of Canada, 1991).

Objectives of the Study

The objectives of the study were to:

1. Determine whether environmentally contaminated fish taken from the Great Lakes are toxic when fed at known concentrations to otter.

2. Determine the sensitivity of otter to these contaminants and to characterize any toxic effects in otter.

3. Compare toxicity data obtained for otter with similar data for mink and other species, to help assess the contributions of these environmental contaminants, especially PCBs, to the decline of otter populations from certain areas of their former ranges in the United States and Canadian provinces bordering the Great Lakes.

Hypothesis

Dietary exposure to PCBs through the consumption of Great Lakes fish is known to affect experimental animals adversely. This study was designed to test the hypothesis that PCBs are responsible for the decline of northern river otter populations in the wild in certain areas of the Great Lakes basin and that long-term, low-level exposure to PCBs will have an adverse effect on the remaining otter populations in the region.

CHAPTER II

LITERATURE REVIEW

History of Polychlorinated Biphenyls (PCBs)

PCBs were first synthesized in 1881 by Schmidt and Shull (Eisler, 1986), but they were considered from a chemical standpoint to be an unattractive class of compounds. PCBs received limited attention until they were used industrially in 1929 in the United States by the Swann Corporation, which later became part of Monsanto Company (Risebrough and Brodine, 1970). In the United States, PCBs were produced under the trade name Aroclor (D'Itri and Kamrin, 1983). The Japanese produced PCBs under the trade names Aroclor, Sanotherm, and Kaneclor. In Germany, the trade name Clophen was used, whereas in France the trade names were Pyralene and Phenoclor. Fenclor and Phenoclor were trade names for PCB manufactured in Italy, and PCBs were marketed under the trade names Sovol and Delor in the USSR and Czechoslovakia, respectively (D'Itri and Kamrin, 1983). In 1977, production of commercial PCBs in the United States ceased, and the Environmental Protection Agency mandated, under the Toxic Substances Control Act, that all sales and distribution of PCBs in the United States be curtailed by July 1, 1979 (Langford, 1979).

Physical and Chemical Properties of PCBs

PCBs are comprised of 209 discrete synthetic chemical compounds called congeners, in which partial or total chlorination of biphenyl rings can occur (Table 1). One to 10 chlorine atoms can be attached to each biphenyl molecule. The empirical formula for PCBs is [$C_{12}H_{10-n}Cl_n$], where n = 1 to 10. The structural formula of the unsubstituted biphenyl molecule, with the numbering system for the carbon atoms in each ring, is shown in Figure 2.

Table 1. Composition of PCB congener groups and number of possible congeners in each group.

PCB Congener Groups	Empirical Formula	Percentage of Chlorine	Number of Congeners
Biphenyl	$C_{12}H_{10}$	0	1
Monochlorobiphenyl	$C_{12}H_9Cl$	19	3
Dichlorobiphenyl	$C_{12}H_8Cl_1$	32	12
Trichlorobiphenyl	$C_{12}H_7Cl_3$	41	24
Tetrachlorobiphenyl	$C_{12}H_6Cl_4$	49	42
Pentachlorobiphenyl	$C_{12}H_5Cl_5$	54	46
Hexachlorobiphenyl	$C_{12}H_4Cl_6$	59	42
Heptachlorobiphenyl	$C_{12}H_3Cl_7$	63	24
Octachlorobiphenyl	$C_{12}H_2Cl_8$	66	12
Nonachlorobiphenyl	$C_{12}HCl_9$	69	3
Decachlorobiphenyl	$C_{12}Cl_{10}$	71	1
Total			209

Source: Erickson (1986).

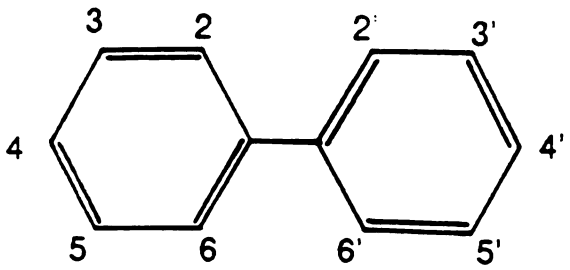
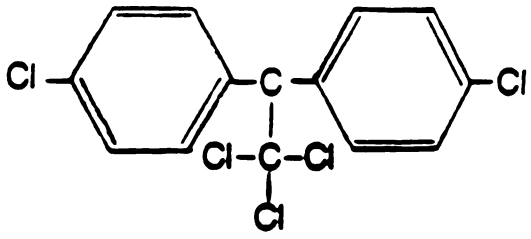


Figure 2. The structural formula of the unsubstituted biphenyl molecule, with the numbering system for the carbon atoms in each ring.

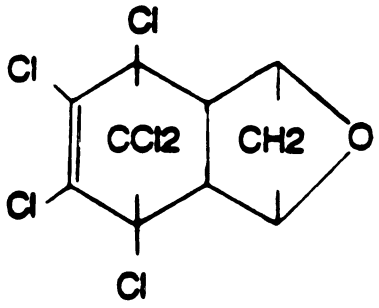
Of the 209 possible congeners, 25 account for 50% to 75% of the total PCBs in samples taken from the environment (McFarland and Clarke, 1989). Due to their chemical properties of lipid solubility and resistance to degradation, PCBs accumulate in food chains. Because of their chlorinated biphenyl ring, PCBs are similar in chemical structure to chlorinated pesticides such as dichlorodiphenyltrichloroethane (DDT) and dieldrin (Figure 3).

Most individual PCB congeners are solids at room temperature, whereas commercial mixtures are mobile oils (e.g., Aroclors 1221, 1242, and 1248), viscous liquids (e.g., Aroclor 1254), or sticky resins (e.g., Aroclor 1260 and 1262) due to the mutual depression of melting points of the components (Hutzinger et al., 1974). PCBs do not crystallize but show a "pour point" below which the material changes into a resinous state.

The physical and chemical properties of some Aroclors are presented in Table 2. From an environmental point of view, the most important physical properties are solubility and vapor pressure. PCBs are not very soluble in water; their solubility decreases as



DDT



DIELDRIN

Figure 3. Chemical structures of DDT and dieldrin, showing similarities to PCB structure (Figure 2).

the percentage of chlorine increases. The solubility of PCBs in water is complicated because these substances can be absorbed onto surfaces such as wood, plastic, and glass. They are very soluble in lipids, such as oils and fats. PCBs tend to partition out of the aquatic ecosystem in biological tissue (D'Itri and Kamrin, 1983). Similar to solubility, the vapor pressure of the Aroclors is directly related to their chlorine content. Congeners with lower chlorine content are generally more volatile than those with higher chlorine content (Hutzinger et al., 1974).

Table 2. Physical and chemical properties of some Aroclors.

Property	Aroclor 1248	Aroclor 1254	Aroclor 1260
Appearance	Clear, mobile oil	Light-yellow viscous liquid	Light-yellow soft, sticky resin
Chlorine (%)	48	54	60
Specific gravity	1.405-1.415	1.495-1.505	1.555-1.566
Acidity (mg KOH/g maximum)	0.010	0.010	0.010
Density (lb/gal 25° C)	12.04	12.82	13.50
Distillation range (°C)	340-375	365-390	385-420
Flash point (°C)	193-196	None to boiling point	None to boiling point
Pour point (°C)	-7	10	31
Fire point (°C)	None to boiling point	None to boiling point	None to boiling point

Source: Hutzinger et al. (1974).

Uses and Production of PCBs

The physical and chemical properties of PCBs that were described previously have led to numerous uses for these compounds, including dielectric fluids (capacitors and transformers), industrial fluids (hydraulic systems, gas turbines, and vacuum pumps), fire retardants, heat-transfer applications, plasticizers (adhesives, textiles, surface coatings, and sealants), and printing and copier paper (Hutzinger et al., 1974). Some PCB usage varies according to the grade of Aroclor, as shown in Table 3. Some chlorobiphenyls have been shown to have insecticidal and fungistatic activity, but they have not been used as pesticides although they have been recommended for incorporation into pesticide formulations (Hutzinger et al., 1974).

Commercial production of PCB mixtures peaked in 1970. Since 1929, 1.5 million tons of such mixtures have been produced (DeVoogt and Brinkman, 1989). Aroclor, the trade name for commercial mixtures of PCBs sold in the United States, follows a four-digit identification system. The first two digits of the Aroclor number represent the molecular type, and the last two digits represent the percentage of chlorine by weight. For example, Aroclor 1254 is a PCB as designated by molecular type and contains 54% chlorine by weight. However, there are some exceptions to this identification system; for example, Aroclor 1016 contains 41% chlorine by weight. The chlorine content of Aroclors can range from 10% to 70% by weight. Uses of commercial PCB mixtures are usually based on the amount of chlorine in each mixture.

Table 3. Uses of PCBs according to grade of Aroclor.

Use of PCB	Grade of Aroclor
Electrical capacitors	1016, 1221, 1254
Electrical transformers	1242, 1254, 1260
Vacuum pumps	1248, 1254
Gas-transmission turbines	1221, 1242
Hydraulic fluids	1232, 1242, 1248, 1254, 1260
Plasticizers in synthetic resins	1248, 1254, 1260, 1262, 1268
Adhesives	1221, 1232, 1242, 1248, 1254
Plasticizer in rubbers	1221, 1232, 1242, 1248, 1254, 1268
Heat-transfer systems	1242
Wax extenders	1242, 1254, 1268
Dedusting agents	1254, 1260
Pesticide extenders, inks, lubricants, cutting oils	1254
Carbonless reproducing paper	1242

Source: Hutzinger et al. (1974).

Environmental Fate

In 1966, Soren Jensen of the University of Stockholm discovered the widespread occurrence of PCBs in the Swedish environment. This and other events led to increased interest in these compounds. In 1967, mass spectroscopic data gave unambiguous proof of the chemical nature of these new contaminants and led to the discovery of PCBs in

ecosystems throughout the world (Holden and Mardsen, 1967; Holmes et al., 1967; Koeman et al., 1969; Risebrough et al., 1968).

Even before the above-mentioned reports were published, gas chromatographic (GC) analysis for DDT of the white tail feathers of an eagle in 1944 showed the presence of peaks of unknown compounds that interfered with the analyses of the samples (Jensen, 1966). Figure 4 shows the peaks of DDT and DDE and possibly PCB.

The characteristics that made the PCBs desirable for industrial uses also favored their bioaccumulation in the environment. PCBs enter the environment in numerous ways, but many routes of entry are difficult to trace. However, with the widespread use of PCBs, some routes of contamination can be traced. The major cause of PCB contamination appears to be poor handling of individual, industrial, and municipal waste. PCB congeners with a high chlorine content tend to bond tightly to particulate matter like soils and sediments. Congeners containing five to seven chlorine atoms per molecule, like the penta, hexa, and hepta chlorobiphenyls, tend to bioaccumulate more than those with fewer chlorine atoms. The less chlorinated congeners are readily metabolized and eliminated by organisms (Safe et al., 1982). Thus, surface waters with low particulate loads may have barely detectable levels of PCBs in the water mass, but high concentrations in bottom sediments. The effective half-life of these substances, which is estimated to be in the range of 8 to 15 years, is also an environmental concern (D'Itri and Kamrin, 1983).

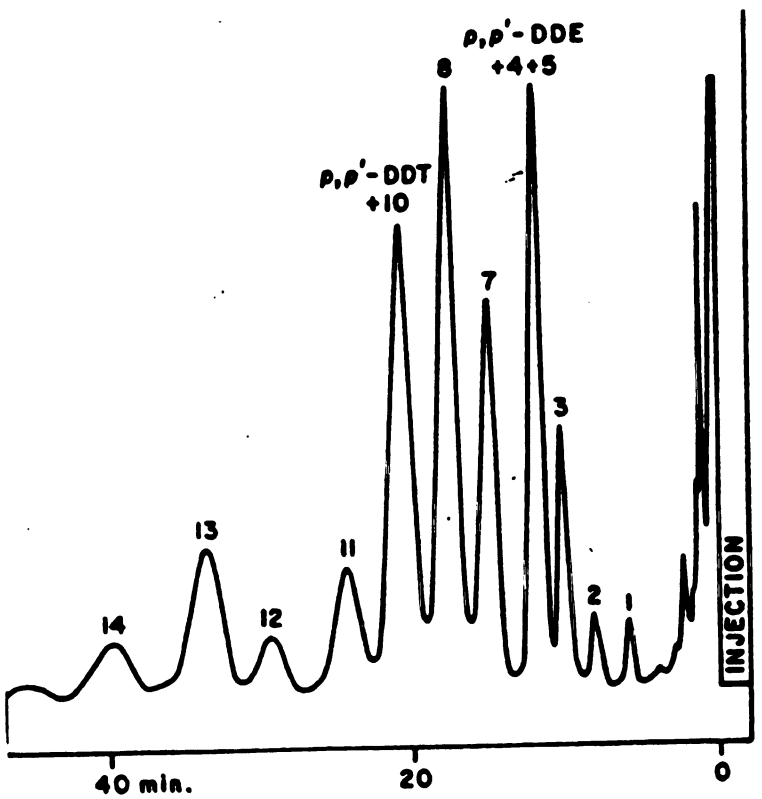


Figure 4. Gas chromatograph of the white tail feathers of an eagle in which DDT and DDE were the only peaks known. From Hutzinger et al. (1974).

Because of differences in the physical and chemical properties of PCB mixtures, it is difficult to determine their effect on the fauna. This determination is further complicated by differences in metabolism and physiology between species (Hutzinger et al., 1974). PCBs found in warm-blooded animals have a tendency to resemble the mixtures from which they originated (Hansen et al., 1983). Fish have been found to be indicators of PCB contaminants in aquatic ecosystems, and their consumption may be a potential hazard to humans and wildlife (D'Itri and Kamrin, 1983).

Great Lakes Contamination

The Great Lakes comprise the largest body of fresh water on earth. Lake Superior is the second largest lake in the world, Lake Huron the fifth, Lake Michigan the sixth, Lake Erie the thirteenth, and Lake Ontario the seventeenth (Beeton, 1984). Because of the large surface areas and other characteristics, such as extreme depth and highly sensitive biota, these aquatic ecosystems are very susceptible to contamination by PCBs and other PCHs. In the early 1960s, pesticides such as DDT were found in the Great Lakes. Soon afterwards, PCBs and other organochlorine pesticides were also discovered in these lakes (Williams, 1975). These discoveries brought about scientific evidence to suggest that the atmosphere can be a major source of PCBs in the Great Lakes (D'Itri and Kamrin, 1983). It was estimated that 80% of the PCBs in Lake Michigan were from atmospheric sources (Murphy and Rzezutko, 1979). Eisenreich et

al. (1981) estimated that 60% of the PCBs in Lake Michigan came from atmospheric deposition. As a result, the first Great Lakes Water Quality Agreement (GLWQA) was established in 1988. It set guidelines for the United States and Canada with regard to the water quality of the Great Lakes (Michigan Department of Natural Resources, 1988).

When considering water quality, fish are of particular concern, especially the sport species, which can accumulate PCB concentrations from 2 to 20 ppm (D'Itri and Kamrin, 1983). Jelinik and Corneliussen (1976) reported that freshwater fish were the primary source of PCBs in the diets of humans and animals. This finding was of great concern to the sport fishermen and their families because, as a group, they consume more than three times the national average of fish per year (D'Itri and Kamrin, 1983). The Michigan Department of Public Health set the allowable concentration for PCBs in Great Lakes fish for human consumption at 2 ppm (Michigan Department of Natural Resources, 1991). This standard has led to health advisories in the Great Lakes region and other areas contaminated with PCBs. The proportions of Canadian sites in Lakes Ontario, Erie, Huron, and Superior with consumption advisories for sports fish are listed in Table 4.

Table 4. The percentage of sites that exceeded the consumption advisories for PCBs in sports fish and top predator species in the Great Lakes of Canada, 1989.

Species	Lake Ontario	Lake Erie	Lake Huron	Lake Superior
Lake trout	100 (82)[1]	0 (1)	43 (7)	60 (35)
Siscowet	--	--	--	100 (7)
Rainbow	55 (11)	0 (3)	25 (12)	0 (4)
Coho	80 (5)	0 (6)	33 (3)	--
Chinook	100 (5)	--	0 (5)	0 (3)
Walleye	75 (16)	31 (13)	87 (16)	100 (7)

Source: Ontario Ministry of the Environment (1989).

[1]Calculated as a percentage of the number of sites tested ( ) where the species exceeded the allowable concentration of PCBs.

Toxicology of PCBs

The incidence of PCBs and other halogenated biphenyls resulting in contamination of animal species including humans is both prevalent and worldwide. Exposure to PCBs has been known to cause skin lesions, tumors, thymic atrophy, decreased food consumption, body-weight loss, teratogenesis, reproductive failure, and death in sensitive species (Aulerich et al., 1970; Barsotti et al., 1976; Eisler, 1986).

The effects of PCB exposure on certain species have been the subject of numerous studies. In 1972, Vos and Nolenboom-Ram administered PCBs topically to rabbits. After 4 weeks of exposure

to Aroclor 1260 (20 applications of 120 mg PCB per treatment), microscopic examination of the liver showed degeneration of cell membranes and damaged endoplasmic reticula. Liver weights increased, and chloracne was evident. This study indicated that, at very low concentrations, PCBs were more toxic than originally thought.

Allen et al. (1973) reported that rhesus monkeys fed diets containing 300 ppm of PCBs (Aroclor 1248) or 5000 ppm of PCT (polychlorinated triphenyl, Aroclor 5460) for 90 days developed alopecia, acneform lesions of the skin, and liver hypertrophy and hyperplasia. Custer and Heinz (1980) fed 9-month-old mallards 25 ppm of Aroclor 1254 for one month (before egg laying) with no adverse effects.

Health et al. (1972) tested six mixtures of PCBs ranging from 32% to 62% chlorine on several species of birds. Bobwhite quail were most sensitive to the PCBs, followed by pheasants, mallards, and Coturnix. The affected birds became lethargic following administration (gavage) and assumed a crouching position. Although each species had different sensitivity to PCBs, increased toxicity was directly associated with an increased percentage of chlorine.

Hartsough (1965) reported that mink that were fed diets containing Great Lakes fish experienced reproductive problems. The problem was originally thought to be due to chlorinated pesticides such as DDT and dieldrin, but mink-feeding studies indicated that the adverse reproductive effects were not due to these pesticides (Aulerich and Ringer, 1970). Kit mortality was reported by mink

farmers to be as high as 80% when Lake Michigan coho salmon were used in mink diets (Aulerich and Ringer, 1977). Subsequent feeding studies using technical-grade PCBs showed that mink are very sensitive to chlorinated compounds like PCBs (Aulerich et al., 1985; Platanow and Karstad, 1973; Ringer et al., 1972).

Mink-feeding studies using several species of Great Lakes fish revealed that the PCBs in the fish caused reproductive complications and high kit mortality. The adverse effects were dependent on the species of fish and the environment from which they were collected (Aulerich and Ringer, 1977).

The northern river otter is a semi-aquatic, carnivorous mammal that is found throughout the Great Lakes basin. It feeds almost entirely on aquatic prey. The otter's diet is composed primarily of fish, although it also consumes crustaceans, reptiles, amphibians, birds, insects, and mammals, which are of less importance (Melquist and Dronkert, 1987). The river otter has few natural enemies, and man is considered to be the major cause of mortality. Many researchers have described concentrations of organochlorine contaminants in wild mink and otter populations throughout the world (Chanin and Jeffries, 1978; Frank et al., 1979; Henny et al., 1981; Hill and Dent, 1985; MacDonald, 1983). However, considerably less is known about the accumulation of acute and chronic effects of organochlorine chemicals in river otter than mink in the Great Lakes basin.

To this researcher's knowledge, there have been no controlled laboratory studies of the effects of PCBs on river otter. Without such studies, it is impossible to determine the levels of chemicals in tissues that are associated with adverse effects (Government of Canada, 1991). In a study of methyl mercury with captive river otter, O'Connor and Nielson (1980) suggested that ranch mink and otter may have very similar sensitivities to this chemical. In studies comparing mercury body burdens of mink and otter taken in the same region, the mercury body burdens of the otter exceeded those of the mink (Kucera, 1983; O'Connor and Nielson, 1980; Sheffy and St. Amant, 1982; Wren et al., 1986), reflecting the larger proportion of aquatic prey in the diet.

In a report concerning water pollution and the distribution of otter in Europe, Mason (1989b) hypothesized that PCBs were associated with the decrease in otter populations. He found that four decreasing otter populations had PCB concentrations above 2 ppm in the liver, kidneys, and muscles, whereas five stable populations had PCB concentrations of less than 2 ppm. Mason noted that 2 ppm was the minimal concentration associated with reproductive failure in PCB-dosed mink. There have been reports of tissue concentrations ranging from 2 to 10 ppm in road-killed otter and otter found dead of unknown causes (Henny et al., 1981; Mason, 1989a; Olsson et al., 1981). The information from these studies could help in assessing the role of PCBs in declining otter populations in the Great Lakes.

CHAPTER III

MATERIALS AND METHODS

Collection, Sampling, and Storage of Fish

Carp (Cypinus carpio) were collected with the cooperation of the Fisheries Division of the Michigan Department of Natural Resources in November, 1990, from the mouth of the Saginaw River (Figure 5) by electro-shocking. The fish were transported to the Michigan State University (MSU) Experimental Fur Farm, where the whole fish were ground and blended for 15 minutes in a 1200-lb-capacity paddle mixer into a homogeneous mixture. Subsamples of the ground carp were collected and placed in plastic bags for analysis of total PCBs and organochlorine pesticide residues. The remainder of the ground carp was placed in sealed plastic containers and stored at -10^{o}C until needed for incorporation into the experimental diets.

Preparation of the Diets

A control diet and three treatment diets were prepared containing 0% (control), 20%, 40%, and 60% raw Saginaw Bay carp. Each diet consisted of a total of 60% fish; the noncarp percentage of fish consisted of ocean fish scrap. The fish portion of the control diet consisted totally of the ocean fish scrap. The remaining nonfish (40%) portion of each diet consisted of the same

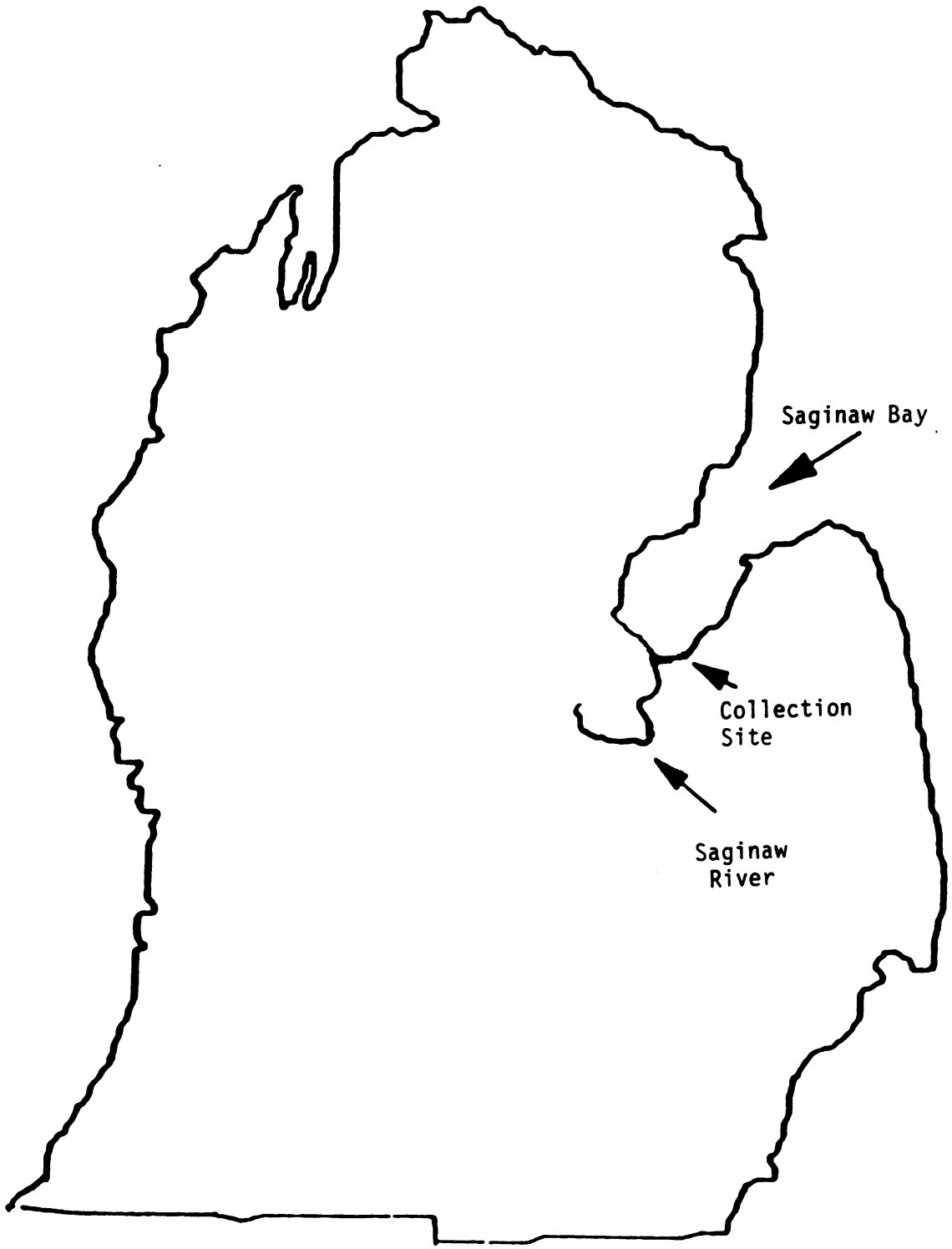


Figure 5. Site of fish collection from Saginaw Bay, Michigan.

quantities of cereal, poultry by-products, water, beef liver, and thiamine hydrochloride, as shown in Table 5.

Table 5. Composition of experimental diets.

Ingredients (%)[1]	Dietary Treatment			
	0% Carp (Control)	20% Carp	40% Carp	60% Carp
Saginaw Bay carp	0	20	40	60
Ocean fish scrap[2]	60	40	20	0
Cereal[3]	20	20	20	20
Poultry by-products[4]	10	10	10	10
Beef liver	5	5	5	5
Water	5	5	5	5
Thiamine hydrochloride (mg/kg)[5]	50	50	50	50

[1]The fish, poultry, and liver were ground through a face plate with 9.5 mm holes before mixing with the other ingredients.

[2]Cod, haddock, and flounder; Boston Feed Supply, Natick, MA 01760.

[3]XK-40 mink cereal; XK Mink Foods, Inc., Plymouth, WI 53073.

[4]Tyson Foods, Fort Smith, AK 72901.

[5]United States Biochemical Corp., Cleveland, OH 44122.

Thimaine was incorporated into all of the diets, in an attempt to override the thiaminase activity in the carp. Thiaminase is an enzyme contained in certain freshwater fish species such as carp.

It has the physiological activity to cleave the thiamine molecule and render it inactive, creating a thiamine deficiency in animals consuming these fish. Heating the fish to inactivate the thiaminase was not done in this study because it was believed that cooking the fish might affect the palatability of the diet and would not be indicative of what otter are exposed to in nature. It was thought that dietary supplementation with 50 mg of thiamine hydrochloride/kg diet would compensate for the thiamine inactivated by the thiaminase.

Analyses of the Carp and Diets

Samples of the raw carp and diets were submitted to the MSU Pesticide Research Center Aquatic Toxicology Laboratory for total PCB and organochlorine pesticide residue analyses by the methods listed in Appendix B and described in detail by Schmitt et al. (1985) and Taylor (1989). Samples of the control and 60% carp diets were submitted for nutrient analysis to National Environmental Testing, Inc., Chicago, IL 60643. These samples also were analyzed for thiamine hydrochloride concentrations.

In brief, the PCB and organochlorine pesticide analyses consisted of homogenizing a 10 g sample with sodium sulfate, extracting with dichloromethane, and cleaning with mixed solvents in florisil and silica gel columns. The florisil and silica gel fractions were analyzed by gas chromatography with an electron capture detector (GC-ECD). Organochlorines (OCs) and PCBs were confirmed by gas chromatograph/mass spectrometry (GC/MS) analysis in

10% of the samples. The average recovery for a mixture of six pesticides was 77.5%. The individual detection limits are given in Table B.1, Appendix B. The extraction and clean-up procedures were adapted from Schmitt et al. (1985). The precision of the method is within 20%, and the accuracy is greater than 90%. Total PCBs are reported as a mixture of Aroclors 1242, 1248, 1254, and 1260.

Animals and Their Care

Twelve wild-caught male northern river otter (Lutra canadensis) were obtained from the Bayou Otter Farm, Theriot, LA 70397, and transported to the MSU Experimental Fur Farm, East Lansing, MI 48823, on January 20, 1991. The otter were housed individually outdoors in wire-mesh cages (2.44 m long x 1.22 m wide x 1.22 m high) suspended above the ground, with attached wooden nest boxes (0.91 m long x 0.61 m wide x 0.51 m high). The cages were surrounded by a 5-foot-high wire-mesh fence to keep out other animals and to facilitate capturing any otter that escaped from their pens (Figure 6).

Upon arrival at MSU, the otter were netted and anesthetized with 130 mg of ketamine hydrochloride (Ketaset, Fort Dodge Labs, Fort Dodge, IA 50501) and 4 mg of xylazine (Rompun, Mobay Corp., Animal Health Division, Shawnee, KS 66201) administered intramuscularly. They were weighed and received a physical examination by a veterinarian. Fecal samples were collected and examined for evidence of internal parasites. The animals were immunized against mink virus enteritis and botulism (Biocom, United

Figure 6. Otter housing facilities at the MSU Experimental Fur Farm.

Vaccines, Madison, WI 53711) and given a booster shot for canine diseases (Vanguard 5, Smithkline Beecham Animal Health, Lincoln, NB 68501). They had been vaccinated with Galaxy 6 and Eclipse 4 (Solvan Animal Health, Inc., Mendota Heights, MN 55118) at the time of capture.

The otter were acclimated for 63 days to the facilities and the control diet. Feed and water were provided ad libitum throughout the acclimation period and during the study. The animals were observed daily for any indications of illness or abnormal behavior and were weighed every two weeks during acclimation (Figure 7).

Feeding Trial

The otter feeding trial was initiated on April 22, 1991. The 12 male river otter were divided into four groups, blocked by weight. Body weights and feed consumption (based on two consecutive days' consumption) of the otter were measured weekly. The otter were fed twice a day in excess of what they would consume each day (approximately 850 to 1000 g of feed). All orts were collected and weighed. The animals were observed daily for abnormal behavior and clinical signs of toxicity. Any otter that lost 30% of their pretrial body weight were euthanized.

Collection and Analyses of Blood Samples

Blood samples were collected for comparison of hematologic and serum chemistry values and PCB and OC (organochlorine) pesticide residue concentrations among the treatment and control groups for

Figure 7. Otter in handling device (made of fiber glass with a sliding door at one end) used for weighing the otter.

the same exposure period. Otter were chemically restrained (as previously described) and blood samples collected via the jugular vein during acclimation (April 13, 1991), after 37 days of exposure to the experimental diets (May 29, 1991), and at the termination of the feeding trial on October 7, 1991. Approximately 17 ml of blood were collected from each animal by jugular venipuncture into three vacuum tubes (5 ml in a lithium heparin-treated tube for hematology determinations, 10 ml in a clot tube for serum chemistry, and about 2.5 ml in an ethylenediaminetetraacetic acid (EDTA)-treated tube for triiodothyronine (T_3) and thyroxine (T_4) determinations. A blood smear was also made, and it was examined for the presence of parasites. Following collection, the blood samples were taken to the Michigan State University Veterinary Clinical Pathology Laboratory for hematologic and serum chemistry analyses.

A Technicon H1 system (Technicon Diagnostic Systems Division, Tarrytown, NY 10591) was used in determining the red blood cell (RBC) count, white blood cell (WBC) count, hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), spun packed cell volume (PCV), plasma total solids (plasma TS), and differential cell count.

The serum chemistry analyses and calculations were performed with an Abbott Spectrum analyzer (Abbott Laboratories, Dallas, TX 75381) to determine calcium (Ca), chloride (Cl), iron (Fe), phosphorus (P), potassium (K), magnesium (Mg), sodium (Na), carbon dioxide (CO_2), anion gap, total protein, albumin, globulin, albumin/globulin ratio (A/G ratio), creatinine, alkaline phosphatase (Alk

phos), alanine amino transferase (ALT), amylase, aspartate amino transferase (AST), creatinine kinase (CK), gamma glutamyl transpeptidase (GGTP), sorbitol dehydrogenase, cholesterol, glucose, triglycerides, blood urea nitrogen (BUN), and osmolality.

Serum electrophoresis analyses for albumin, amino acids, total protein, and alpha 1, alpha 2, beta, and gamma globulins were conducted with an EDC Electrophoresis Data Center (Helena Laboratories, Beaumont, TX 75657).

Serum element concentrations of aluminum (Al), boron (B), barium (Ba), Ca, copper (Cu), Fe, Mg, manganese (Mn), molybdenum (Mo), Na, P, and zinc (Zn) were determined by inductively coupled plasma-atomic emission spectroscopy, Jarrel-Ash, model 955, Plasma Autocomp. Direct Reading Spectrometer (Applied Chemical Corp., Waltham, MA 022154) as described by Braselton et al. (1981). Routine radioimmunoassay procedures (MSU Animal Health Diagnostic Laboratory) were used for the T_3 and T_4 determinations.

The 5 ml blood samples in lithium heparin-treated tubes were submitted to the MSU Pesticide Research Center Aquatic Toxicology Laboratory for determination of total PCBs and organochlorine pesticide residues. One ml plasma fractions were denaturized with methanol, extracting with a 1:1 mixture (v/v) of hexane-ethyl ether, and cleaning with mixed solvents in florisil and silica gel columns. The florisil and silica gel fractions were analyzed by gas chromatography with an electron capture detector (GC-ECD). OCs and PCBs were confirmed by GC/MS in 10% of the samples. Average

recovery for a mixture of six pesticides was 77.5%. A more detailed description of the analytical procedures is presented in Appendix B.

Necropsies

All mortalities and euthanized (Fatal-plus) animals were necropsied. Final body weights, organ weights (brain, liver, spleen, kidneys, heart, thyroid, and adrenal glands), and any gross abnormalities were recorded.

Histopathology

Samples of liver, spleen, kidney, heart, and adrenal and thyroid glands were placed in a 10% neutral buffered formalin solution for processing for histopathological examination by Dr. Jim Render. Tissue sections were embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) according to routine histological procedures. The brains from several of the animals suspected of dying from Chastek's paralysis were submitted to Dr. Duke Tanaka, a neuroanatomist, for gross and histopathologic examination.

Analyses of Liver and Adipose Tissue for PCB Residues

At necropsy, samples of liver and adipose tissue were collected from each animal, frozen (-10^{o}C), and subsequently submitted to the MSU Pesticide Research Center Aquatic Toxicology Laboratory for total PCB and organochlorine pesticide residue analyses. Analyses were done according to the procedures described in Appendix B.

Statistical Analyses

The carp-fed groups and the control group in this experiment were tested for homogeneous variance using Bartlett's test. Throughout the experiment, all groups had homogeneous variance for all variables measured, including feed consumption, body and organ weights, and PCB-residue concentrations in the liver, fat, and serum. The values for these parameters in the control group were compared to those of the other groups using Dunnett's test. All statistical analyses were performed according to the procedures described by Gill (1978), with computations made by using Toxstat (Gulley et al., 1985) software. Unless stated otherwise, the $p < 0.05$ level of probability was used as the criterion for significant differences between treatment groups.

CHAPTER IV

RESULTS

PCB and Organochlorine Residues in Carp

Total PCBs in the five samples of ground and blended raw carp (Cyprinus carpio) collected from the mouth of Saginaw Bay ranged from 4.99 to 6.47 ppm, with a mean of 5.70 ppm based on reference to technical mixtures of Aroclors 1248, 1254, and 1260 (Table 6). Numerous organochlorine pesticides were found in the carp samples (Table 7) but at very low concentrations. Thus, they probably did not contribute significantly to the overall effects of the carp diets on the otter.

Nutrient Composition of the Diets

Results of the nutrient analyses of the experimental diets are presented in Table 8. Fat, iron, zinc, and total digestible nutrients increased with increasing percentages of carp in the diet. The 60% carp diet had a higher ash content than did the other diets. This finding can be explained, in part, by the lower moisture and higher fat content of the 60% carp diet. The rest of the nutrients were relatively constant through all diets.

Table 6. PCB residues in carp taken from the mouth of the Saginaw River, Michigan.

Aroclor	Extract PCB Concentration µg/ml (ppm)[1]					
	1248	1254	1260	r^2	# DF	Total
Comstar results						
Sample 1	16.05	14.83	4.09	0.97	72	34.97
Sample 2	15.95	15.75	4.96	0.97	71	36.67
Sample 3	13.42	13.65	4.14	0.98	62	31.21
Sample 4	17.51	17.38	5.50	0.98	65	40.38
Sample 5	16.17	14.41	4.16	0.99	65	34.47
Aroclor distribution						
Sample 1	0.46	0.42	0.12			
Sample 2	0.44	0.43	0.14			
Sample 3	0.43	0.44	0.13			
Sample 4	0.43	0.43	0.14			
Sample 5	0.47	0.41	0.12			
Aroclor concentration in fish mg/kg (ppm)[2] "wet wt." basis						
Sample 1	2.56	2.37	0.65			5.58
Sample 2	2.55	2.52	0.79			5.87
Sample 3	2.15	2.19	0.66			4.99
Sample 4	2.81	2.79	0.88			6.47
Sample 5	2.59	2.31	0.67			5.56

Mean total PCB concentration = 5.70 mg/kg (ppm)
CV = 9.4%

[1]PCB concentration in the extract of the fish sample.

[2]PCB concentration in the whole fish.

Table 7. Residue concentrations of selected pesticides in carp taken from the mouth of the Saginaw River, Michigan.

Pesticide	Concentration Adjusted for Gel Prematation Chromatography Loss (ppb)[1]						
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Average	CV %
Lindane	1.30	1.11	1.02	1.14	1.08	1.13	9.22
Heptachlor epoxide	3.60	2.82	1.59	1.12	1.07	2.04	54.91
Oxychlordane	6.10	4.97	1.45	1.36	1.14	3.00	78.27
Gama-chlordane	8.27	6.68	4.04	4.76	3.12	5.37	38.77
o,p'-DDE	< mdl[2]	< mdl	< mdl	< mdl	< mdl	< mdl	--
p,p'-DDD	13.30	10.83	7.84	8.83	6.29	9.42	28.93
Alpha-chlordane	7.38	4.56	4.23	4.25	3.95	4.87	29.08
t-nonachlor	32.08	25.88	20.54	24.32	17.02	23.97	23.76
o,p'-DDD	104.82	90.84	79.94	101.41	84.59	92.32	11.54
p,p'-DDT	1.31	0.87	0.60	1.15	0.68	0.92	32.58
Endosulfan I	0.16	< mdl	< mdl	0.44	0.14	0.15	120.95
Dieldrin	3.79	3.93	3.15	8.08	6.61	5.11	41.62
Endrin	0.18	< mdl	< mdl	1.06	0.22	0.29	151.12
Endosulfan II	0.18	< mdl	< mdl	< mdl	< mdl	0.04	223.61
Methoxychlor	< mdl	< mdl	< mdl	< mdl	< mdl	< mdl	--
Heptachlor	< mdl	< mdl	< mdl	< mdl	< mdl	< mdl	--
Aldrin	< mdl	< mdl	< mdl	< mdl	< mdl	< mdl	--
p,p'-DDE	41.61	39.36	40.37	51.11	37.35	41.96	12.75

[1]Wet weight basis.

[2]< mdl: Concentration is less than method detection limit. Concentrations of 5.0-10.0 ppb are near the reportable quantification limit. Concentrations < 5.0 ppb in fish are below the reportable quantification limit.

Table 8. Nutrient composition of experimental diets.

Nutrients (%)[1]	Dietary Treatment			
	0% Carp (Control)	20% Carp	40% Carp	60% Carp
Moisture	60.10	57.60	55.00	52.10
Fat	6.77	9.19	12.05	14.36
Protein (crude)	17.70	18.20	17.90	18.70
Fiber (crude)	0.94	0.87	1.10	0.96
TDN[2]	34.90	39.30	44.60	49.20
Ash	4.99	4.83	4.14	9.71
Calcium	1.40	1.29	1.14	1.02
Phosphorus	0.80	0.76	0.70	0.66
Potassium	0.35	0.34	0.34	0.39
Magnesium	0.08	0.08	0.08	0.08
Sodium	0.25	0.23	0.21	0.21
Iron (mg/kg)	86	89	95	93
Manganese (mg/kg)	18	19	18	20
Copper (mg/kg	3	3	3	3
Zinc (mg/kg)	34	51	64	79

[1]Analyses by Litchfield Analytical Services, Litchfield, MI 49252.

[2]Total digestible nutrients.

Feed Consumption

The mean daily, weekly, and cumulative feed consumptions (based on two consecutive days' consumption per week) by the river otter are shown in Table 9. Daily feed consumption is reported by two- and four-week periods because of the length of the feeding trial.

Mean feed consumption for the otter in the groups fed carp was significantly lower than that for the controls, and it decreased in a dose-dependent manner, except for the 40% carp group during weeks 1-2 and the 20% carp group during weeks 9-10. Feed consumption in the control group remained relatively constant throughout the feeding trial.

The carp-fed groups' consumption declined greatly (by 35%) by week 2 across all treatment groups, and by week 4 the otter in these groups showed significant decreases in body weights, as well as in feed consumption.

PCB Residues in Experimental Diets

Concentrations of dietary PCB ranged from 0.03 ppm in the control diet to 5.22 ppm in the 60% carp diet (Table 10). The cumulative dose measured in the diets of the treated groups (µg/otter), reported in total PCBs, differed significantly from that of the controls ($p < 0.05$) and increased with the increasing percentage of dietary Saginaw Bay carp (Table 10).

Table 9. Mean daily feed consumption by period of male river otter fed diets containing various concentrations of Saginaw Bay carp.

Period[1]	Daily Feed Consumption (g/otter/d)			
	0% Carp (Control)	20% Carp	40% Carp	60% Carp
Acclimation	987.61±76.85[2,3,4,a]	870.95± 88.773[a]	720.26± 60.60[b]	711.54±120.30[b]
Week 1-2	907.39±19.67[a]	665.68± 52.91[b]	617.33± 64.50[b]	590.81± 31.35[6b]
Week 3-4	963.98±18.88[a]	528.00± 67.61[b]	498.53± 65.15[b]	379.39± 52.25[c]
Week 5-6	861.74±63.64[5a]	200.88± 58.65[5b]	228.80± 46.92[5bc]	172.40± 54.58[8bc]
Week 7-8	919.53±27.62[a]	548.91±118.85[bc]	427.96±117.54[67bc]	
Week 9-10	779.63±66.25[a]	630.68± 36.67[6b]		
Week 11-14	823.63±30.76[a]	579.10± 30.67[b]		
Week 15-18	719.84±30.25[a]	596.17± 35.55[b]		
Week 19-22	783.22±30.02[a]	477.26± 20.81[b]		
Week 23-26	884.76±31.61[a]	605.38± 23.26[b]		
Cumulative feed consumption (kg/otter)	198.74	124.93	20.15	12.68

[1]Feed consumption based on the mean of two consecutive days' consumption per week.

[2]Mean ± SEM.

[3]N = 3 otter/group, unless noted otherwise.

[4]Means in same row with same superscript are not significantly different ($p > .05$).

[5]100 mg thiamine HCl/kg diet was added to each otter diet just before feeding for duration of the study, beginning in week 6.

[6]N = 2 otter.

[7]Based on 8 weeks' consumption.

[8]Based on 6 weeks' consumption.

Table 10. Feed and PCB consumption by male river otter fed diets containing various concentrations of Saginaw Bay carp.

	Dietary Treatment			
	0% Carp (Control)	20% Carp	40% Carp	60% Carp
Weeks on experimental diets	26	26	8	6
Cumulative feed consumption (kg/otter)	198.74[1]	124.93	20.15	12.68
Dietary PCB concentration (µg/g diet)	0.03	1.90	3.67	5.22
PCB consumption (mg/otter)	5.96	237.26	73.95	66.19
mg PCB consumed/ otter/day	0.03	1.30	1.42	1.57
mg PCB consumed/ kg body weight/day	0.003	0.15	0.17	0.19

[1]Mean.

Body Weights

By the second week of the trial, the otter began to lose body weight. By week 6, the otter fed the 60% carp diet had lost 30% of their initial body weight and were euthanized. The animals fed the 20% and 40% carp diets had lost 7.86% and 23.53% of their body weights, respectively, by week 6. The mean body weights of the otter are shown in Table 11. There was a significant loss in body weight in all

Table 11. Mean body weight, body weight change, and percentage body weight loss of male river otter.

Period[1]	Mean Body Weights (kg)			
	0% Carp (Control)	20% Carp	40% Carp	60% Carp
Initial	9.16±0.91[234a]	9.28±0.72[a]	9.14±0.546[a]	9.36±0.40[a]
Week 1 2	9.11±0.57[a]	9.17±0.49[a]	9.24±0.34	9.25±0.18[a]
Week 3 4	9.02±0.55[a]	8.66±0.53[a]	8.38±0.38[a]	7.65±0.40[b]
Week 5 6	8.84±0.635[a]	7.26±0.21[5b]	7.54±0.62[b]	6.72±0.20[568c]
Week 7 8	9.08±0.33[a]	6.92±0.25[b]	6.90±0.20[57b]	
Week 9 10	9.04±0.29[a]	8.16±0.65[6b]		
Week 11 14	8.90±0.45[a]	8.17±0.14[b]		
Week 15 18	8.98±0.47[a]	9.29±0.44[a]		
Week 19 22	8.36±0.17[a]	8.98±0.61[a]		
Week 23 26	8.63±0.92[a]	8.55±0.55[a]	6.99±0.40[b]	6.38±0.296[c]
Change	0.53	0.73	2.15	2.98
Percent loss	5.80	7.86	23.53	31.84

[1]Body weights were recorded once a week.

[2]Mean ± SEM.

[3]N = 3 otter/group, unless noted otherwise.

[4]Means in same row with same superscript are not significantly different ($p > .05$) for the duration of the study, beginning in week 6.

[5]100 mg thiamine HCl/kg diet was added to each otter diet just before feeding.

[6]N = 2 otter.

[7]Based on 8 weeks' consumption.

[8]Based on 6 weeks' consumption.

carp-groups compared to the control group by week 6, which was directly proportional to the amount of carp in each diet.

Mortality

The first indication of toxicity was observed by the second week of the feeding trial. There was a trend toward reduced feed consumption in groups fed carp, a sharp decrease in body weights, and a loss of coat luster. The otter fed 60% carp lost 30% of their body weight and were euthanized after 42 days on trial. It was originally thought that these clinical signs might be a result of PCB toxicity, as previous studies conducted with mink and other animals have shown anorexia and decreased body weights to be early clinical signs of PCB toxicity (Aulerich et al., 1986). However, in the otter fed the 40% carp diet, there were three separate observations of convulsions, loss of coordination, and spastic paralysis. During these convulsions, the otter appeared semiconscious and would suddenly rise and throw their heads over their backs as if they were gasping for air. After a few minutes, the convulsions would stop and the otter would return to a paralyzed state, as described by Green et al. (1942); Stout et al. (1963); and Okada et al. (1987) for mink and fox fed thiamine-deficient diets. In an attempt to treat the condition, one otter showing the convulsions was administered 25 mg of thiamine hydrochloride in 3 ml of saline i.p. and 0.3 cc of atropine sulfate i.m., but after 30 minutes no beneficial response was observed, and the otter was then euthanized. The other otter in the 40% carp group also were

euthanized because they failed to regain consciousness after experiencing similar convulsions. The clinical signs exhibited by the otter fed 40% carp strongly suggested a thiamine deficiency (Chastek paralysis) since these clinical signs have not been reported to be associated with PCB toxicity in other mammals. Although 50 mg of thiamine hydrochloride per kg of diet (wet weight) were added to each of the diets at mixing in an attempt to compensate for the thiaminase activity present in the raw carp, it may not have been adequate to provide for the thiamine requirement of the otter.

The brains of the two otter in the 40% carp group that were submitted to a neuroanatomist for gross and histopathologic examination revealed no hemorrhages or lesions indicative of a thiamine deficiency, as described for foxes by Okada et al. (1987). Analysis (National Environmental Testing, Inc., Chicago, IL 60643) of samples of the control diet and the 60% carp diet collected at the time they were fed to the otter showed thiamine hydrochloride concentrations of 3.69 and 0.011 mg/100 g diet, respectively.

Histopathology

Histopathological examination of the liver, kidney, heart, spleen, adrenal, and thyroid gland tissues taken during necropsy revealed numerous cellular manifestations. However, these were not associated directly with PCB toxicosis. The liver of every animal in each group, except for one of the controls, exhibited infiltration of the portal areas by lymphocytes, plasma cells, and

macrophages. These areas were characterized by a loss of hepatocytes, hemorrhages, accumulation of macrophages, and some multinucleated giant cells (Langhan's type). Two control otter and one otter from the 40% group had multifocal subcapsular inflammation. The livers of individual animals in each group also showed some degenerative cellular changes.

The spleens of individual animals in the control, 40%, and 60% dose groups had white and/or red pulp changes. The kidneys of one animal in the 60% dose group had vacuolation of the renal tubules. The adrenal glands of one otter in the control group and in the 20% and 60% carp-fed groups showed the presence of lymphocytes. The thyroid glands of one otter in the 60% group exhibited the presence of small follicles (Table 12).

Organ Weights

Mean organ weights of the otter are shown in Table 13. Otter in the 20% carp-fed group had significantly higher liver and lower adrenal gland weights than did the controls. Otter in the 60% carp-fed group had significantly higher spleen, thyroid, heart, and adrenal gland weights than did those in the other groups. However, because of differences in the length of the exposure periods between the controls and the 20% carp-fed group and the 40% and 60% carp-fed groups, it is difficult to interpret accurately the relative importance and meaning of these significant differences.

Table 12. Summary of histopathological findings in organs of male river otter fed diets containing various concentrations of Saginaw Bay carp.

Type of Histopathological Change	Dietary Treatment			
	0% Carp (Control)	20% Carp	40% Carp	60% Carp
Liver				
Portal lymphocytes/ plasma cells	2/3[1]	3/3	3/3	2/2
Multi-focal subcapsular inflammation	2/3	0/3	1/3	0/2
Portal hyalination	2/3	0/3	0/3	1/2
Multiple granulation	2/3	1/3	1/3	0/2
Irregular subcapsular surface	2/3	0/3	0/3	0/2
Diffuse subcapsular inflammation	1/3	0/3	0/3	0/2
Capsule tags	1/3	1/3	0/3	0/2
Cellular degeneration	0/3	0/3	0/3	1/2
Vacuolation	0/3	1/3	0/3	1/2
Spleen				
White pulp change (<)	2/3	0/3	1/3	1/2
Red pulp change (>)	1/3	0/3	1/3	1/2
Kidney				
Vacuolation	0/3	0/3	0/3	1/2
Adrenals				
Lymphocytes	1/2	1/3	0/3	1/2
Thyroids				
Small follicles	0/3	0/3	0/3	1/2

[1]Number of otter showing changes over the number necropsied in each group.

Table 13. Mean organ weights expressed as a percentage of brain weight of male river otter fed diets containing various concentrations of Saginaw Bay carp.

Organ (g)	Dietary Treatment			
	0% Carp (Control)	20% Carp	40% Carp	60% Carp
Brain	56.70 ± 2.90[1a]	53.57 ± 1.29[a]	52.37 ± 1.65[a]	51.50 ± 2.50[a]
Liver	479.71 ±12.17[2a] (272.00 ±35.30)[3]	517.39 ± 7.57[b] (277.17 ± 9.76)	487.24 ± 9.91[a] (255.17 ±16.35)	460.19 ±26.00[a] (237.00 ±42.90)
Spleen	92.89 ± 0.37[a] (52.67 ± 1.08)	100.34 ±10.60[a] (53.77 ±13.67)	89.17 ± 7.90[a] (46.70 ±13.02)	116.89 ± 5.64[b] (60.20 ± 9.30)
Kidneys	111.23 ± 2.74[a] (63.07 ± 7.65)	114.67 ± 1.36[a] (61.43 ± 1.76)	117.29 ± 3.53[a] (61.47 ± 5.82)	113.69 ± 1.24[a] (58.55 ± 2.05)
Heart	99.35 ± 2.08[a] (56.33 ± 6.06)	91.41 ± 2.95[a] (48.97 ± 3.80)	96.68 ± 0.325[a] (50.63 ± 0.52)	113.20 ± 5.33[b] (58.30 ± 8.80)
Thyroids	0.557± 0.055[a] (0.315± 0.16)	0.592± 0.023[a] (0.317± 0.03)	0.535± 0.012[a] (0.280± 0.02)	0.957± 0.054[b] (0.493± 0.09)
Adrenals	1.32 ± 0.031[a] (0.749± 0.09)	1.17 ± 0.062[b] (0.627± 0.08)	1.42 ± 0.030[a] (0.741± 0.05)	1.50 ± 0.012[a] (0.771± 0.02)

[1]Mean ± SEM.

[2]Means in same row with same superscript are not significantly different ($p > .05$).

[3]Absolute organ weights shown in parentheses as wet weight (grams).

Hematologic Profiles

The hematologic values for samples collected before and following 37 days' consumption of various concentrations of Saginaw Bay carp are shown in Tables 14, 15, and 16. Samples were taken at 37 days because some otter, especially those fed the higher-level carp diets, had lost considerable body weight and might not have survived to the termination of the trial. Collecting samples at 37 days on trial permitted comparison of values among treatment groups for the same exposure period.

Microfilaria, identified as Dirofilaria lutra, were detected in blood samples from all of the animals. No treatment was prescribed for the parasites.

Red blood cell (RBC) counts and hemoglobin and hematocrit values for the otter in the 40% and 60% carp-fed groups (Table 14) were slightly lower than those in the controls before the trial. However, they showed a marked increase following 37 days of consumption of the diets that contained carp.

Packed cell volume concentrations increased in the 20%, 40%, and 60% carp-fed groups following 37 days of consumption of the diets that contained carp. The white blood cell (WBC) counts of the carp-fed otter exhibited a marked decrease following consumption of the carp diets, whereas the WBC counts of the controls increased during the 37-day exposure period. Segmented neutrophil values exhibited marked decreases in the carp-fed groups following consumption of the carp diets, whereas the segmented neutrophil values for the control group increased during the treatment period.

Table 14. Hematologic values for male otter before and following 37 days' consumption of diets containing various concentrations of Saginaw Bay carp.

Parameter	Dietary Treatment[1]			
	0% Carp (Control)	20% Carp	40% Carp	60% Carp
RBC count (10^3 cells/µl)				
4-13-91[2]	10.10±0.23	10.60±0.49	9.95±0.29	8.86±1.67
5-29-91[3]	9.11±0.46	11.80±0.35	10.32±1.10	14.11±0.93
Hemoglobin (g/dl)				
4-13-91	14.60±0.31	14.80±0.87	14.00±0.72	12.83±2.66
5-29-91	13.47±0.99	17.00±0.50	14.67±1.88	19.60±0.60
Hematocrit (%)				
4-13-91	46.20±1.51	47.87±3.00	43.83±1.61	39.70±7.86
5-29-91	45.83±2.90	58.20±1.06	48.80±6.07	66.60±2.80
Mean corpuscular volume (fl)				
4-13-91	44.17±1.76	44.73±0.76	43.50±0.87	44.67±1.18
5-29-91	50.20±0.80	49.40±1.70	47.17±1.99	47.35±1.15
Mean corpuscular hemoglobin (pg)				
4-13-91	14.47±0.55	13.83±0.24	14.00±0.32	14.40±0.45
5-29-91	14.76±0.38	14.43±0.47	14.17±0.79	13.90±0.50
Mean corpuscular hemoglobin concentration (g/dl)				
4-13-91	31.57±0.71	30.90±0.40	31.87±0.45	32.23±0.42
5-29-91	29.43±0.32	29.20±0.44	30.07±0.49	29.35±0.35
Spun packed cell volume (%)				
4-13-91	46.67±0.33	49.67±2.84	45.00±3.00	49.00±1.53
5-29-91	46.67±1.86	56.00±1.15	52.00±1.73	58.00±0.00

Table 14. Continued.

Parameter	Dietary Treatment[1]			
	0% Carp (Control)	20% Carp	40% Carp	60% Carp
Plasma total solids (g/dl)				
4-13-91	8.17±0.29	8.60±0.12	8.97±3.23	8.47±0.24
5-29-91	8.50±0.12	8.97±0.22	8.93±0.18	8.70±0.10
Leukocyte differential WBC count (10^3 cells/µl)				
4-13-91	9.46±0.65	15.29±0.61	12.40±3.23	10.91±2.43
5-29-91	12.10±1.70	7.66±1.80	5.57±1.12	7.84±3.67
Segmented neutrophils (10^3 cells/µl)				
4-13-91	7.71±0.58	9.23±1.50	9.92±3.14	5.14± 0.97
Percentage	81.67±3.84	59.67±7.42	77.00±7.51	61.00±10.54
5-29-91	9.61±1.33	5.46±1.77	3.92±0.92	4.99± 2.03
Percentage	79.60±1.20	69.33±7.22	69.33±7.51	66.00± 5.00
Nonsegmented neutrophils (10^3 cells/µl)				
4-13-91	--[4]	0.15±0.00[5]	--	--
Percentage	--	1.00±0.00	--	--
5-29-91	1.00±0.00[6]	--	--	--
Percentage	0.66±0.00	--	--	--
Lymphocytes (10^3 cells/µl)				
4-13-91	1.17±0.04	2.52±0.88	1.18± 0.19	1.91±0.90
Percentage	12.33±0.88	16.67±6.17	11.33± 4.33	19.33±2.40
5-29-91	1.30±0.45	1.23±0.25	1.17± 0.57	1.74±0.91
Percentage	10.67±1.67	16.00±1.53	23.67±10.20	21.50±1.50

Table 14. Continued.

Parameter	Dietary Treatment[1]			
	0% Carp (Control)	20% Carp	40% Carp	60% Carp
Monocytes (10^3 cells/µl)				
4-13-91	1.50±1.41	0.22±0.06	0.41±0.09	0.31±0.11
Percentage	2.00±1.00	2.33±0.88	4.00±0.58	3.33±0.88
5-29-91	0.35±0.38	0.38±0.07	0.71±0.46	0.80±0.47
Percentage	3.00±0.58	5.33±1.20	5.00±0.00	9.50±1.50
Eosinophils (10^3 cells/µl)				
4-13-91	0.45±0.22	3.37±0.81	0.82±0.24	1.78±1.34
Percentage	4.67±2.19	14.00±7.93	7.67±3.28	15.00±7.10
5-29-91	0.79±1.20	0.61±0.34	0.41±0.36	0.31±0.27
Percentage	6.00±2.00	13.50±3.50	5.50±4.50	3.00±2.00

[1]Mean ± SEM; N = 3 unless specified otherwise.

[2]Pre-exposure samples taken on 4-13-91 before start of trial on 4-22-91.

[3]Post-exposure samples taken on 5-29-91 after 37 days of exposure to experimental diets. N = 2 for 60% carp diet.

[4]Nondetected.

[5]N = 2.

[6]N = 1.

Total triiodothyroinine (T_3) concentrations decreased in the carp-fed groups, with the control group values exhibiting a slight increase following the exposure period. Total thyroxine (T_4) concentrations decreased in the carp-fed groups and controls after the exposure period. Other serum chemistry values (Table 15) such as alanine aminotransferase, gamma glutamyl transferase, glucose, and urea nitrogen decreased to varying degrees in all groups, whereas asparate aminotransferase and creatine kinase increased markedly following the exposure period. Cholesterol levels increased in the control and 20% groups while decreasing in the 40% and 60% groups. All of the carp-fed groups exhibited higher iron concentrations following the ingestion than did the control group.

Serum electrophoresis values (Table 16) such as alpha 1 increased, whereas alpha 2 and beta decreased in all groups. The other measurements remained constant before and following 37 days of exposure to the carp diets.

PCB Residues in Liver, Fat, and Serum

Total PCB concentrations in the otter liver samples ranged from less than the instrument detection limit (< IDL) in controls to 1.449 mg PCB/kg liver (wet weight) in the 60% carp-fed group (Table D.1, Appendix D). Liver PCB concentrations among the treated groups were significantly different from those in the control group and

Table 15. Serum chemistry values for male otter before and following 37 days' consumption of diets containing various concentrations of Saginaw Bay carp.

Parameter	Dietary Treatment[1]			
	0% Carp (Control)	20% Carp	40% Carp	60% Carp
Sodium (mEq/l)				
4-13-91[2]	159.00± 4.58	154.33± 2.03	156.00± 2.08	158.00± 1.73
5-29-91[3]	157.33± 2.40	156.33± 2.91	155.67± 1.76	157.50± 1.50
Potassium (mEq/l)				
4-13-91	4.60± 0.21	4.73± 0.34	4.70± 0.06	4.87± 0.08
5-29-91	4.63± 0.19	5.07± 0.13	4.93± 0.32	5.50± 0.10
Chloride (mEq/l)				
4-13-91	116.33± 0.88	116.00± 3.05	79.70±33.81	117.67± 2.60
5-29-91	119.00± 1.53	115.67± 3.84	116.00± 2.08	115.50± 1.50
Total CO_2 (mEq/l)				
4-13-91	22.63± 0.52	20.93± 1.89	23.20± 1.04	23.03± 0.15
5-29-91	21.72± 2.21	22.10± 0.97	21.90± 1.01	22.05± 0.45
Anion gap (calc)				
4-13-91	25.33± 3.33	25.00± 3.60	21.33± 0.67	22.33± 0.88
5-29-91	20.67± 3.53	24.00± 2.00	22.67± 1.20	25.50± 0.50
Total protein (gm/dl)				
4-13-91	7.63± 0.47	7.87± 0.17	8.60± 0.30	7.67± 0.03
5-29-91	7.60± 0.29	7.70± 0.10	7.77± 0.09	7.55± 0.15
Albumin (gm/dl)				
4-13-91	4.00± 0.21	3.96± 0.09	4.10± 0.17	4.00± 0.10
5-29-91	3.33± 0.15	3.60± 0.58	3.57± 0.13	3.55± 0.25
Globulin (gm/dl)				
4-13-91	3.63± 0.33	3.90± 0.12	4.47± 0.18	3.50± 0.23
5-29-91	4.27± 0.40	4.07± 0.07	4.16± 0.22	3.95± 0.05
Alb/glob ratio (calc)				
4-13-91	1.11± 0.09	1.02± 0.03	0.92± 0.04	1.09± 0.06
5-29-91	0.80± 0.11	0.89± 0.02	0.86± 0.07	0.90± 0.08
Total bilirubin (mg/dl)				
4-13-91	ND[5]	ND	0.20± 0.00[5]	ND
5-29-91	ND	0.15± 0.05	0.10± 0.00	ND
Creatinine (mg/dl)				
4-13-91	0.40± 0.00	0.55± 0.10	0.37± 0.03	0.47± 0.03
5-29-91	0.70± 0.06	0.77± 0.17	0.97± 0.67	1.00± 0.10
Alkaline phosphatase (IU/l)				
4-13-91	83.00±17.52	49.33±13.09	41.00±10.54	79.00±20.65
5-29-91	92.67±13.68	60.00±15.13	48.00± 2.65	13.50±13.50

Table 15. Continued.

Parameter	Dietary Treatment[1]			
	0% Carp (Control)	20% Carp	40% Carp	60% Carp
Alanine amino-transferase (IU/l)				
4-13-91	136.67± 6.36	205.33± 10.14	167.00± 28.58	159.67± 24.04
5-29-91	111.33± 40.28	157.33± 32.91	122.67± 22.63	121.50± 35.50
Amylase (IU/l)				
4-13-91	ND[6]	ND	ND	ND
5-29-91	ND	ND	ND	ND
Asparatate amino-transferase (IU/l)				
4-13-91	130.33± 35.71	139.00± 7.94	124.33± 48.88	109.33± 13.35
5-29-91	216.00± 24.00	213.00± 48.88	170.67± 35.37	163.50± 55.50
Calcium (mg/dl)				
4-13-91	8.93± 0.49	9.80± 0.27	9.90± 0.77	9.30± 0.53
5-29-91	8.13± 0.18	8.60± 0.06	8.20± 0.40	8.25± 0.35
Cholesterol (mg/dl)				
4-13-91	162.33± 6.76	165.33± 31.87	196.67± 59.30	216.33± 43.11
5-29-91	194.67± 30.75	183.33± 30.75	191.33± 30.07	153.00± 38.00
Creatine kinase (IU/l)				
4-13-91	1093.67±316.23	765.33±100.93	694.67±295.52	624.00±111.75
5-29-91	2453.33±662.25	2075.00±373.56	1783.67±326.41	2313.50±728.50
Gamma glutamyl trans. (IU/l)				
4-13-91	20.00± 3.00	15.00± 3.00	29.67± 6.69	27.67± 6.96
5-29-91	9.67± 4.63	9.67± 4.70	17.00± 5.51	10.50± 0.50
Glucose (mg/dl)				
4-13-91	85.33± 6.00	126.00± 38.04	76.00± 4.16	80.00± 6.25
5-29-91	49.00± 9.07	66.00± 8.08	75.33± 26.93	53.00± 7.00
Magnesium (mEq/l)				
4-13-91	2.26± 0.05	2.37± 0.27	2.28± 0.09	2.22± 0.04
5-29-91	2.11± 0.12	2.70± 0.05	2.23± 0.53	2.81± 0.12
Phosphorus (mg/dl)				
4-13-91	5.80± 0.36	7.80± 1.23	6.70± 0.60	5.93± 0.45
5-29-91	5.23± 0.49	6.30± 0.20	6.00± 0.55	6.45± 0.85
Urea nitrogen (mg/dl)				
4-13-91	39.33± 5.18	44.00± 3.06	46.67± 14.67	46.33± 2.60
5-29-91	38.33± 4.91	40.33± 3.38	32.00± 5.03	39.50± 5.50

Table 15. Continued.

Parameter	Dietary Treatment[1]			
	0% Carp (Control)	20% Carp	40% Carp	60% Carp
Sorbitol dehydrogenase (IU/l)				
4-13-91	8.60± 1.72	15.43± 4.18	7.93± 0.88	10.53± 1.28
5-29-91	16.77± 6.73	36.23±18.32	9.77± 1.24	12.95± 1.25
Serum osmolality (cal., mOsm/kg)				
4-13-91	337.33± 9.83	331.33± 6.74	332.67± 6.57	337.00± 4.36
5-29-91	330.67± 5.21	331.00± 6.43	326.67± 6.76	332.50± 0.50
Iron (µg/dl)				
4-13-91	159.33±37.40	126.00± 3.61	96.33±11.67	130.00±21.66
5-29-91	163.67± 7.67	211.67±30.12	245.00± 3.61	186.00± 6.00
Triglycerides (mg/dl)				
4-13-91	35.67±10.27	110.33±23.07	81.33±36.34	92.67±53.70
5-29-91	92.33±28.10	95.33±33.23	88.67±19.10	105.50±27.50
Triiodothyronine (T_3; nmol/l)				
4-13-91	0.64± 0.07	0.70± 0.05	0.59± 0.06	1.10± 0.11
5-29-91	0.74± 0.09	0.57± 0.10	0.58± 0.08	0.37± 0.19
Thyroxine (T_4; nmol/l)				
4-13-91	15.67± 4.09	16.00± 2.65	19.00± 4.73	22.67± 7.97
5-29-91	13.13± 2.60	15.23± 0.70	13.46± 0.53	11.92± 2.31

[1]Mean ± SE; N = 3 unless specified otherwise.

[2]Pre-exposure samples taken on 4-13-91 before start of trial on 4-22-91.

[3]Post-exposure samples taken on 5-29-91 after 37 days' exposure to the experimental diets. N = 2 for 60% carp diet.

[4]Nondetected; detectable level 0.1 mg/dl.

[5]N = 1 otter.

[6]Nondetected; detectable level 8 IU/l.

Table 16. Serum electrophoresis values for male otter before and after 37 days' consumption of diets containing various concentrations of Saginaw Bay carp (N = 3).

Parameter	Dietary Treatment[1]			
	0% Carp (Control)	20% Carp	40% Carp	60% Carp
Albumin (gm/dl)				
4-13-91[2]	3.50±0.15	3.37±0.20	3.27±0.13	3.30±0.15
Calculated %	44.27±2.17	44.40±2.90	38.13±1.28	43.23±3.09
5-29-91[3]	3.30±0.15	3.53±0.07	3.50±0.00	3.55±0.05
Calculated %	43.53±3.93	46.07±0.38	45.47±0.63	47.40±0.50
Amino acids (gm/dl)				
4-13-91	0.27±0.03	0.20±0.00	0.13±0.03	0.20±0.06
Calculated %	2.40±0.59	3.13±0.32	1.90±0.21	2.33±0.19
5-29-91	0.23±0.03	0.30±0.00	0.30±0.06	0.20±0.10
Calculated %	2.93±0.41	3.87±0.29	3.30±0.78	2.50±0.90
Alpha 1 (gm/dl)				
4-13-91	0.50±0.06	0.43±0.03	0.53±0.13	0.47±0.07
Calculated %	5.33±0.09	6.97±0.98	5.83±1.23	5.43±0.29
5-29-91	0.60±0.12	0.60±0.06	0.63±0.03	0.55±0.15
Calculated %	8.00±1.36	7.50±0.50	8.30±0.65	7.85±1.85
Alpha 2 (gm/dl)				
4-13-91	0.33±0.88	0.37±0.03	0.67±0.88	0.47±0.07
Calculated %	5.27±0.64	5.20±1.13	7.97±0.77	5.00±0.55
5-29-91	0.30±0.00	0.27±0.03	0.30±0.00	0.25±0.05
Calculated %	3.90±0.20	3.80±0.38	3.73±0.12	3.05±0.75
Beta (gm/gl)				
4-13-91	1.03±0.88	1.27±0.20	1.23±0.03	1.10±0.10
Calculated %	13.93±0.75	14.00±1.21	14.60±0.87	16.10±2.63
5-29-91	0.83±0.09	0.67±0.03	0.67±0.07	0.65±0.05
Calculated %	11.03±0.90	9.03±0.52	8.83±0.63	8.25±0.45
Gamma (gm/dl)				
4-13-91	2.27±0.23	2.17±0.17	2.67±0.03	1.93±0.18
Calculated %	28.50±2.62	26.33±1.51	31.60±0.96	27.90±1.19
5-29-91	2.33±0.32	2.30±0.06	2.37±0.03	2.30±0.20
Calculated %	30.57±3.66	29.70±0.53	30.30±0.76	30.90±3.40
Total protein (gm/dl)				
4-13-91	7.87±0.32	7.87±0.18	8.60±0.30	7.43±0.27
5-29-91	7.60±0.29	7.70±0.10	7.77±0.08	7.55±0.15
Albumin/globulin ratio (%)				
4-13-91	0.80±0.11	0.87±0.03	0.86±0.07	0.90±0.08
5-29-91	0.80±0.07	0.83±0.09	0.62±0.03	0.77±0.09

[1]Mean ± SE.

[2]Pre-exposure samples taken on 4-13-91 before start of trial on 4-22-91.

[3]Post-exposure samples collected on 5-29-91 after 37 days' exposure to experimental diets. N = 2 for 60% carp diet.

increased in a dose-dependent manner (Table 17). On a tissue and lipid basis, the mean PCB concentrations in liver tissue in the 20%, 40%, and 60% carp-fed groups were greater than those in the control group.

Table 17. Mean total PCB concentrations in the livers of male river otter following consumption of diets containing various concentrations of Saginaw Bay carp.

Liver	Dietary Treatment			
	0% Carp (Control)	20% Carp	40% Carp	60% Carp
Number	3	3	3	2
Exposure (days)	182	182	56	42
Total PCB (mg/kg)[1,2,3]	0.11±0.03[4a]	0.29±0.04[b]	0.60±0.05[c]	0.90± 0.60[d]
Total PCB (mg/kg lipid)[1,2,3]	5.40±0.04[a]	12.96±1.41[b]	25.40±6.49[c]	54.80±14.80[d]

[1]Mean ± SEM.

[2]Means within the same row with the same superscript are not significantly different.

[3]Wet weight basis.

[4]PCB concentration for one otter was less than detection limit.

Total PCB concentrations in the otter fat samples ranged from 1.2 mg PCB/kg fat in the controls to 22.8 mg PCB/kg fat (wet weight) in the 60% carp-fed group (Table D.2, Appendix D). The mean values of the total PCB concentrations in the otter fat increased in a

dose-dependent manner, similar to the liver samples, except the 20% carp-fed group had a mean total PCB concentration similar to the 40% carp-fed group (Table 18). This finding may be accounted for by the longer exposure period for the 20% group.

Table 18. Mean total PCB concentrations in the fat of male river otter following consumption of diets containing various concentrations of Saginaw Bay carp.

Fat	Dietary Treatment			
	0% Carp (Control)	20% Carp	40% Carp	60% Carp
Number	3	3	3	2
Exposure (days)	182	182	56	42
Total PCB (mg/kg)[1,2]	2.33±0.61[3a]	5.60±0.60[b]	5.23±0.23[b]	15.35± 7.45[c]
Total PCB (mg/kg lipid)[1,2]	9.40±2.41[a]	15.73±1.25[b]	15.83±0.93[b]	72.20±14.80[c]

[1]Mean ±SEM.

[2]Means within the same row with the same superscript are not significantly different.

[3]Wet weight basis.

Total PCB concentrations in the otter serum samples taken before exposure were found to be < 0.75 ng/g (instrument detection limit). Total PCB concentrations in the serum of the otter after exposure ranged from 76.40 ng PCB/g serum in controls to 539.30 ng PCB/g serum (wet weight) in the 60% carp-fed group (Table D.3,

Appendix D). The mean total PCB concentrations in the serum also increased significantly in a dose-dependent manner (Table 19).

Table 19. Mean total PCB concentrations in serum of male river otter following consumption of diets containing various concentrations of Saginaw Bay carp.

Serum	Dietary Treatment			
	0% Carp (Control)	20% Carp	40% Carp	60% Carp
Number	3	3	3	2
Exposure/ days	182	182	56	42
Total PCB (ng/g)[1,2]	94.10±12.18[3a]	205.33±68.18[b]	235.07± 29.09[c]	514.50±24.70[d]
Total PCB (by volume ng/ml)[1,2]	90.70±12.79[a]	183.23±55.97[b]	209.23±616.28[c]	457.25±44.35[d]

[1]Mean ±SEM.

[2]Means within the same row with the same superscript are not significantly different.

[3]Wet weight basis.

CHAPTER V

DISCUSSION

In the wild, northern river otter inhabit aquatic ecosystems throughout North America. As its broad geographic distribution would suggest, the river otter is able to adapt to diverse aquatic habitats. The habitats consist of riparian vegetation adjacent to lakes, streams, and other wetland areas (Government of Canada, 1991). The Great Lakes Basin is an area that is inhabited by the river otter. Recently, however, reports have shown marked declines in river otter populations in many areas of the Great Lakes that they formerly inhabited (Foley et al., 1988).

The river otter is a specialist, feeding almost entirely on aquatic prey. Although the diet varies seasonally, the river otter's diet is composed primarily of fish, while crustaceans, reptiles, amphibians, birds, insects, and mammals are of lesser importance (Government of Canada, 1991; Melquist and Dronkert, 1987).

Being a carnivorous predator, the otter is at the top of the food chain. Thus, the species is potentially exposed to high concentrations of compounds that bioconcentrate. As a carnivorous fish-eating species, mink also occupy a place at the top of the food chain, allowing for considerable exposure to environmental

contaminants. Studies involving mink fed PCB-contaminated fish diets have shown the detrimental effects of PCBs to this species (Aulerich and Ringer, 1977; Aulerich et al., 1971; Heaton, 1992; Hornshaw et al., 1983; Platanow and Karstad, 1973).

Researchers have documented that metabolized forms of PCBs fed to mink were found to be more toxic than consumption of the same concentrations of technical-grade PCBs (Hornshaw et al., 1983; Platanow and Karstad, 1973). Aulerich et al. (1986) reported that death occurred sooner in mink that were fed Aroclor 1254-contaminated rabbit than in those that received the same concentration of technical-grade Aroclor 1254. Treatment groups that received the diets containing the metabolized form of Aroclor 1254 also had lower mean body weights and lower feed consumption than did treatment groups that were fed the same concentration of unmetabolized technical-grade Aroclor 1254. Because the otter is a close relative of the mink, it is possible that otter may have a similar high sensitivity to these PCHs.

Organochlorine pesticides have been measured in trapped and road-killed otter and those where the cause of death was undetermined (Foley et al., 1988; Mason et al., 1986; Somers et al., 1987). However, to this researcher's knowledge, there have been no controlled studies of the effects of PCBs and other organochlorine contaminants on river otter. Because the otter's diet is composed primarily of fish and because carp were available in large quantities and tend to accumulate "high" levels of PCBs, this

species was used in the fish portion of the experimental otter diets in the present study.

The average PCB concentration of 5.70 ppm in the five raw carp samples analyzed in this study equated to PCB concentrations of 1.90, 3.67, and 5.22 ppm in the 20%, 40%, and 60% carp diets, respectively. Based on the carp sample analyses, the dietary PCB concentrations were expected to have been about 1.19, 2.28, and 3.42 ppm total PCBs. The reason for this discrepancy between the targeted and analytical values is unclear but may be due to sampling procedures.

Overall, the concentrations of organochlorine pesticide residues detected in the carp samples (see Table 7) were generally low and probably biologically insignificant. For example, Aulerich and Ringer (1970) reported that DDT and DDE fed at 100 ppm to mink from weaning through furring did not produce any marked detrimental effects. In addition, Aulerich et al. (1990) studied mink that were fed diets that contained 0, 12.5, 25, 50, or 100 ppm heptachlor (technical grade) for 28 days to determine toxicity. They found that only diets that contained 25 ppm or more of heptachlor resulted in a significant decrease in feed consumption, whereas diets that contained 50 ppm or more caused decreased body weights.

Assuming that otter and mink are similar in their sensitivity to PCHs, the PCBs consumed by the otter in the present study suggest that they may have caused the severe weight loss and decreased food consumption. These results were similar to the observations reported by Aulerich et al. (1986), Hochstein et al. (1988), and

Bleavins et al. (1980), in which mink that were fed diets containing similar concentrations of halogenated hydrocarbons exhibited similar effects.

Other researchers have documented the adverse effects of PCBs on the reproductive performance of several different species. In a study by Allen and Barsotti (1976), 50% of infants born to rhesus monkeys that were fed diets containing 2.5 and 5.0 ppm of Aroclor 1254 for approximately 1.5 years died within one month after birth. These results were similar to the findings of Hornshaw et al. (1983) in that mink kits whelped by females that were fed diets containing 1.5 ppm of PCBs did not survive more than 24 hours. Oral exposure of rats to 30 ppm of Aroclor 1254 per day for approximately one month caused a decrease in their reproductive potential (Brezner et al., 1984). Aulerich et al. (1973) found that reproductive failure occurred in female mink that were fed several species of PCB-contaminated Great Lakes fish or various concentrations of commercial PCBs for 6 to 11 months. Some of the PCB levels and feeding methods reported in these studies were comparable to the PCB levels of the carp diets fed in the present study. Although some of the PCB levels reported for other studies were higher than the levels in the present study, they could be indicative of what otter may be exposed to in the wild. Unfortunately, the effects of PCBs and other organochlorines on the reproductive performance of otter have not, as yet, been determined. Based on the results in a number of different species as stated above, reproductive performance is

perhaps one of the most sensitive stages of the life cycle. It would therefore be logical to investigate the influence of PCHs on the reproductive performance of otter.

In a review of the toxicity of PCBs to adult mink, Bleavins et al. (1980) indicated that mortality of 50% or more occurred after 171, 153, and 122 days in adults fed 10, 20, and 40 ppm of Aroclor 1242, respectively. Aulerich et al. (1985) reported that no significant mortality was observed in adult female mink that were fed 2.5 or 5.0 ppm of the individual congeners 2,4,5,2',4',5'-hexachlorobiphenyl (HCB) or 2,3,6,2',3',6'-HCB. However, 100% of adult mink that were fed 0.5 ppm of 3,4,5,3',4',5'-HCB and 50% that were fed 0.1 ppm died, with mean survival times of 46 and 61 days, respectively. Aulerich et al. (1987) reported that dietary exposure of mink to 0.05 ppm of 3,4,5,3',4',5'-HCB for 135 days resulted in 50% mortality, whereas no deaths occurred in mink that were fed 0.01 ppm for 135 days.

The results from feeding these individual congeners to mink show a time-dose relationship of HCB toxicity and the extreme difference of the various congeners that may be present in commercial or environmental PCB mixtures. Poland et al. (1979) and Leece et al. (1985) reported that structure activity relationships (SARs) among halogenated aromatic hydrocarbons have shown that the most toxic PCB compounds are substituted in the 3, 3', 4, 4', 5, and 5' positions and are approximate isostereomers of 2,3,7,8-TCDD. Although congener-specific analysis of the carp fed to the otter in this study was not conducted, Heaton (1992, Table 5) reported

congener-specific data for carp taken from the same location in 1988. Although the diets fed in the present study may have produced the detrimental effects, as shown by Aulerich et al. (1987), it is possible that the otter would be affected in a similar manner as mink by specific toxic congeners.

The clinical signs observed in the otter of reduced feed consumption and body weights in the 40% and 60% carp-fed groups, which were euthanized after consumption of Saginaw Bay carp diets containing PCBs and organochlorines, were consistent with those reported for mink by Aulerich et al. (1986). In their study, mink exhibited anorexia, bloody stools, and nervousness. In addition to exhibiting the clinical signs during week 8 of the trial, the otter fed the 40% carp diet experienced epileptic seizures that varied in intensity and duration. Following a seizure, the body underwent a paralyzed state in which the otter's posture became very rigid. Gillette et al. (1987) reported similar observations in mink treated with 3,4,3',4'-tetrachlorobiphenyl (TCB); these animals also exhibited tremors and contorted body positions.

Marked reductions in body weights were observed in the otter and were inversely proportional to the quantity of PCBs consumed and also to the amount of carp fed. In the present study, the body weights of the otter that were fed 20%, 40%, and 60% carp decreased 18%, 15%, and 24% by week 6, respectively. Hochstein et al. (1988) and Aulerich et al. (1987) documented in mink the condition termed "wasting syndrome," which is commonly associated with halogenated

hydrocarbon intoxication. This condition has also been reported in the rhesus monkey (Barsotti et al., 1976).

Observations of convulsions and loss of coordination of the otter that were fed the 40% carp diet suggested that the clinical signs might be due to or confounded by a thiamine deficiency (Chastek paralysis). The seizure-type convulsions and loss of coordination closely resembled the clinical signs observed in mink and foxes that were fed thiamine-deficient diets (Green et al., 1942; Okada et al., 1987; Stout et al., 1963), although the central nervous system signs observed were inconsistent with PCB toxicity.

Green et al. (1942) reported the sequence of events leading to the appearance of the disease as follows:

> During the late fall or winter months, fresh frozen fish were added to the regular fox ration in a proportion varying from 10% to 50% of the total diet. No harmful effects from the change in diet were noted for several weeks; then the foxes began to refuse food. During the following week, the amount of food left by the animals increased. Some of the animals left part of the ration, and others left the entire ration. After a week or two of loss of appetite, the first signs of definite illness were observed. A few animals had an abnormal gait, as though their legs were somewhat stiff, and after the first nervous disturbance, the disease progressed rapidly. Within 23 to 36 hours, foxes exhibiting the early signs started to show symptoms such as spastic paralysis, inability to rise, and convulsions shortly before death. Animals seemed to have abnormal sensitivity to pain. For example, if its fur was touched, the animal winced. Although the animal was completely paralyzed, it remained conscious. The respiration commonly was rapid, with easy inspirations and forced expirations. A fox lying on its side suddenly would begin to struggle for air. After a few moments, the spasm would subside and the animal would take a deep breath, which was followed by an easy expiration. Death generally occurred within 12 hours after total paralysis in the limbs had set in. No animals recovered after they reached this stage.

Okada et al. (1987) described thiamine-deficiency encephalopathy in mink and foxes as having the following clinical signs: anorexia, weakness, and diarrhea followed by recumbence, tonic convulsions, spastic paralysis, and death after a period of 2 or 3 days. Upon necropsy, gross lesions were found in fox and mink. Bilaterally symmetrical hemorrhages occurred in the piriform, temporal, parietal, and occipital lobes of the cerebrum.

The clinical signs observed in the otter that were fed the Saginaw Bay carp were consistent with those reported by Green et al. (1942) and Okada et al. (1987) for thiamine-deficient fox and mink. It was originally thought that these were signs of PCB toxicity, as described earlier. However, there were three separate observations of convulsions, loss of coordination, spastic paralysis, and an inability to rise in the otter in the 40% carp-fed group (Figure 8). One of the otter that exhibited convulsions was injected with 25 mg of thiamine hydrochloride i.p. and 0.3 cc of atropine i.m. in an attempt to revive it, but no response was observed, and the otter was subsequently euthanized. The other two animals were also euthanized after 30 minutes because they failed to regain consciousness. These observations are consistent with the finding of Green et al. (1942) that once thiamine-deficient foxes reached the spastic paralysis state they did not recover. These authors, however, showed that thiamine deficiency is readily reversible in foxes administered thiamine i.p. during early to advanced stages of the deficiency. In the early stages, foxes showing inappetence and some neurologic symptoms should receive an injection of 3,000 to

Figure 8. Otter fed the 40% carp diet, showing partial paralysis of the hind limbs.

9,000 units of thiamine. Foxes exhibiting severe neurologic symptoms should receive injections of 6,000 to 18,000 units of thiamine immediately, since death is apt to occur suddenly.

To this researcher's knowledge, the thiamine requirement for river otter has not been determined. The thiamine requirement for young mink is 1.2 mg of thiamine hydrochloride per kg of dry feed, whereas mature foxes require 0.8 mg of thiamine hydrochloride per kg of dry feed (National Research Council, 1982).

It is suspected that, although the thiamine-supplemented diets fed to the otter originally contained more than adequate levels of thiamine, exposure of the vitamin to the enzyme for several hours following mixing, before the diets froze, and while the diets were thawing before feeding permitted sufficient biological inactivation of the thiamine due to the denaturing action of thiaminase (Table 20) to cause a deficiency. Green et al. (1942) reported that outbreaks of Chastek paralysis occurred on several mink ranches where fish containing thiaminase were fed at levels as low as 10% of the total diet.

Following the observations indicative of a thiamine deficiency in the otter, the diets fed to the remaining animals in the control and 20% groups were supplemented with 100 mg of thiamine hydrochloride/kg just before feeding. Within a week, the otter that were fed the 20% carp diet showed an increase in feed consumption and body weights (Table 9). These animals were retained on their respective diets for the remainder of the 26-week exposure period.

During that period, none of the remaining otter in the 20% carp-fed group showed signs that could be attributed to PCB toxicity. Thus, based on these results, it is thought that the adverse effects observed in the otter fed the carp diets were primarily due to a thiamine deficiency rather than PCB toxicity.

Table 20. Thiaminase activity of some common freshwater fish.

Species	Thiaminase Activity[1]
Carp (Cyprinus carpio)	2,003
Shad (Drosoma cepedianum)	112
Smelt (Osmerus mordax)	47
Shiner (Notropis hudsonius)	1,418
Bowfin (Amia calva)	206

Source: Gnaedinger and Krzeczkowski (1966).

[1]Micrograms of thiamine hydrochloride destroyed in 20 minutes per gram of protein of unheated raw fish.

To the author's knowledge, the literature contains no accounts of Chastek paralysis in river otter. Based on these findings, one could question whether thiamine deficiencies occur in wild otter from routine consumption of fish that contain thiaminase. Otter, like other predators, consume the most plentiful and easily accessible prey available. If fish containing thiaminase were readily available to otter in the wild for extended periods (3 to 4

weeks) and were consumed almost exclusively by the otter, a thiamine deficiency could easily be induced.

Aulerich et al. (1985) and Platanow and Karstad (1973) published reports of histopathological changes in mink that were fed Aroclor 1254. These changes included mild splenomegaly, with increased megakaryocytes and gastrointestinal-tract hemorrhage. Researchers have documented the liver as the primary target for high levels of PCBs orally administered to mammals (Allen and Barsotti, 1976; Hansen et al., 1975). Cellular changes occur in liver from exposure to PCBs, such as fatty infiltration, hypertrophy, hemorrhage, and necrosis (Koller and Zinkl, 1973). The liver is the largest gland in the body. Its many important functions include secretion of bile, excretion of waste products, storage of lipids, detoxification and conjugation of toxic lipid-soluble substances, and metabolism of fats (Dellmann and Brown, 1981).

The histological changes observed in the livers of the otter that were fed carp included portal lymphocytic infiltration, multifocal subcapsular inflammations, portal hyalinations, cellular degeneration, and vacuolation. The lymphocytic infiltration observed may have suggested that an inflammatory response was occurring, probably due to the halogenated compounds. However, some of these changes also occurred in the livers of the controls (Table 13) and might have been a result of a bacterial infection or may be due to a thiamine deficiency. Histological processing with acetone may result in the presence of vacuoles when lipid extraction occurs. The otter in the present study did not exhibit significantly

enlarged livers as compared to controls, which is the opposite effect reported by Hornshaw (1981). However, the otter in the present study accumulated PCBs in the liver at concentrations similar to those found in the livers of mink fed similar levels of PCBs for the same length of time. Wren et al. (1987) found that mink fed 1 ppm of PCB (Aroclor 1254) for 4 and 8 months concentrated a maximum of 1.98 and 3.1 ppm of PCBs, respectively, in the liver tissue. Barsotti et al. (1976) found that adult female rhesus monkeys that were fed diets containing 2.5 ppm and 5.0 ppm of Aroclor 1248 for 6 months had PCB residue concentrations in the liver of 5.6 ppm and 24.4 ppm, respectively. For comparison, in the present study, otter that were fed a PCB concentration of 1.90 ppm (20% carp-fed group) in the diet for 6 months had PCB concentrations in the liver that ranged from 4.9 ppm to 6.8 ppm. Thus, it would appear that, at comparable dietary concentrations, mink, monkeys, and otter accumulate similar PCB concentrations in the liver.

The brains of two of the otter suspected of dying as a result of a thiamine deficiency that were examined by a neuroanatomist revealed that there were no lesions associated with thiamine deficiency, as described for foxes and mink in which bilaterally symmetrical hemorrhages were observed in the piriform, temporal, parietal, and occipital lobes of the cerebrum (Okada et al., 1987). The absence of these lesions could possibly be due to the rapid onset of the disease as was exhibited by young nursing foxes (Okada et al., 1987).

There were no histological changes in the tissues examined that could be directly attributed to PCB toxicity or thiamine deficiency observed in the otter that were necropsied following consumption of various concentrations of Saginaw Bay carp like those described previously for mink that were fed various PCBs (Aulerich et al., 1985; Green et al., 1942; Okada et al., 1987; Platanow and Karstad, 1973).

The weights of the otter spleen, kidneys, and heart did not show any significant increases except in the 20% and 60% carp-fed groups. The 20% group exhibited a decrease in adrenal gland weight. The reason for the decrease in the adrenal gland weight is unclear, but the liver weight increase may have been due to the effect of the PCBs. In the 60% carp-fed group, there were increases in the weights of the thyroid glands. However, in a study of the toxicity of 3,4,5,3',4',5'-HCB to mink (Aulerich et al., 1987), spleen, thyroid, and lung weights did not increase, although the animals' liver, kidney, and adrenal gland weights generally increased in a dose-dependent manner. A possible reason for the increased thyroid gland weights in the 60% group is that any external chemical adversely affecting thyroid gland production or release of T_4 could possibly lead to lower levels of circulating T_4, and this, in turn, can lead to increased thyroid gland growth. The reason for the difference in organ weights between the present study and the study by Aulerich et al. (1987) may be due to the difference in the specific congeners that may affect different organs in separate species.

Hematologic values for the otter before they were fed the diets containing various concentrations of carp and after 37 days of consumption of the carp and control diets showed few notable differences in terms of hematologic parameters and serum chemistry values. One notable exception was that the RBC counts increased and WBC counts decreased in all groups fed carp, while the controls' counts exhibited opposite effects. These trends were not like those reported by Gillette et al. (1987), in which the RBCs of mink treated with 50 ppm 3,4,3',4'-TCB decreased, while WBCs increased, compared to the controls. Although higher initial serum albumin and ALT concentrations for the otter in all groups decreased after 37 days on trial to levels comparable to those reported for otter by Hoover et al. (1984, 1985), AST values did increase for the same period, possibly indicating some form of liver damage. Cholesterol values exhibited a trend toward increased concentrations in the control and 20% groups, while they decreased in the 40% and 60% groups. The values in the present study do not follow the trend of a study by Carter (1984), in which rats fed various concentrations of PCBs (Aroclor 1254) for 10 days had increased cholesterol levels with increased PCBs in the diet. T_4 concentrations exhibited a trend toward decreased levels in the 40% carp-fed group, with T_3 and T_4 concentrations also decreasing in the 60% carp-fed group. Similar observations occurred when fish from the Wadden Sea containing high (1.5 ppm) concentrations of PCBs were fed to the common seal (Phoca vitulina), which resulted in significantly lower

T_3 and T_4 concentrations when compared to seals fed north-east Atlantic fish containing low (0.22 ppm) concentrations of PCBs (Brouwer et al., 1989). Aulerich et al. (1987) also reported decreased T_4 concentrations in mink fed 0.5 ppm 3,4,5,3',4',5'-HCB. This may suggest that a build-up of T_3 and T_4 in the thyroid gland was due to the gland's lack of ability to synthesize these hormones, indicating possible effects from the PCBs. The remaining serum chemistry values before and after exposure were comparable to those reported by Hoover et al. (1984, 1985). No values for serum amino acids or for alpha, beta, or gamma globulins for otter were found in the literature.

Microfilaria identified as Dirofilaria lutra were present in the subcutaneous and muscle facia of all otter. According to Orinel (1965), this is a common parasite found in otter from the southeastern United States. Thus, it was decided that no treatment would be prescribed for the condition because the otter thrived (gained weight) during acclimation and showed no adverse effects that could be attributed to the parasites.

A secondary objective of this study was to compare the data in the present study that could be used in evaluating the risk to wild otter from exposure to environmental contaminants in the Great Lakes Basin. Determination of PCB concentrations in livers (or fat) of captive otter could be useful to compare with PCB concentrations found in livers or fat tissue of wild otter environmentally exposed to PCBs and other contaminants through the consumption of Great Lakes fish.

In a study by Foley et al. (1988), organochlorine concentrations were measured in tissues from mink and otter taken from eight areas of New York State (within 5 miles of Lake Ontario). Wet weight PCB concentrations in the adipose tissues of the mink and otter ranged as high as 67 and 114 µg/g, respectively. Mason et al. (1986) reported the findings of analyses of data from hunts of 23 European otter, in which PCBs were detected in 15 animals, 5 of which had adipose tissue concentrations exceeding 50 ppm. Three of these animals originated from areas located close to an industrial site. Other studies (Mason et al., 1986; Somers et al., 1987) showed that liver and muscle/tissue PCB residues on a lipid basis in wild otter ranged from traces to 232 ppm and from 3 ppm to 300 ppm, respectively.

In a review on water pollution and otter distribution in Britain and Europe, Mason (1989b) hypothesized that PCBs were responsible for the decline of the otter populations in Europe. Four decreasing populations had mean body-tissue levels above 2 ppm, whereas five stable or thriving populations had levels less than 2 ppm. Henny et al. (1981) found that PCB levels in the livers of all otter taken from the lower Columbia River in Oregon exceeded 2 ppm, with a mean of 9 ppm. In East Anglia, Oregon, two adult otter found dead of unknown causes had liver PCB residues of 10 ppm and 2 ppm. Researchers have reported PCB concentrations in tissues of wild otter averaging from traces to 114 ppm in fat on a wet weight basis, and from traces to 46 ppm in liver on a wet weight basis (Foley et

al., 1988; Kruuk and Conroy, 1991; Mason et al., 1986; Somers et al., 1987).

Several liver samples collected from wild otter had levels of PCBs within the range of the otter in the present study. Proulx et al. (1987) found that liver tissue and whole-body homogenates taken from wild otter in Dunn-Rainham and Marsea townships in Ontario, Canada, had concentrations of 29.2 ppm and 25 ppm on a lipid basis. These values were comparable to the PCB concentrations (on a lipid basis) in the liver tissue of otter that were fed dietary concentrations of 1.90 ppm and 3.67 ppm of PCBs from carp containing 5.70 ppm for six and two months, respectively. Fat samples collected for PCB analyses from the otter in the present study contained 2.33 to 15.35 ppm of PCB and were also within the range (0.5 to 232 ppm) of PCB residues reported in fat from wild otter. Although the present study did not determine the otter's sensitivity to PCBs, it did, however, show how quickly the otter can accumulate these compounds in their tissues. Otter in the wild are most likely exposed to higher concentrations than the ones used in the present study. Because the otter is a close relative of the mink and the mink is very reproductively sensitive to these compounds, a similar sensitivity of the otter could possibly help explain the otter's decline from the Great Lakes Basin.

No data on PCB residues in the plasma of otter were found in the literature, although determination of organochlorine pesticides and PCBs in plasma of wild animals is of importance for the understanding of the relationships of the distribution of these

xenobiotics between plasma and body tissues. The plasma reaches many target organs, and PCBs and other xenobiotics are removed from the plasma as it goes through a given tissue (Monro, 1990). Being able to determine concentrations of organochlorines and PCBs in serum or plasma can accomplish many things, such as obtaining blood samples without killing and resampling the same individuals to determine seasonal variations and bioaccumulation of contaminants. In the present study, after 37 days' consumption of diets containing various concentrations of Saginaw Bay carp and ocean fish, the otter in the control group (ocean fish) showed low PCB concentrations in their serum (Tables 19 and D.3, Appendix D), probably due to some PCB contamination in the ocean-fish portion and other dietary ingredients of the control diet. At the termination of the 26-week trial, serum PCB measurements were repeated for the controls and the 20% carp-fed animals. The control animals had < IDL (0.75 ng/g). The serum PCB concentration of one of the 20% carp-fed animals increased significantly, whereas the PCB levels of the other showed a moderate increase. Continuous consumption of PCBs will increase levels to the maximum amount, and then they will level off due to the body's ability to metabolize these chemicals into excretable forms.

Results of the present study indicate that otter may not be as sensitive to environmentally contaminated Great Lakes fish as previously hypothesized. Although the otter in the present study did not exhibit many of the clinical signs commonly associated with

PCB intoxication, there were some indications that they may have been affected. For example, the 20% group exhibited increased liver weights, which are associated with long-term PCB toxicity. The 40% and 60% carp-fed groups also exhibited decreased T_3 and T_4 values, which are indicative of chemicals affecting the thyroid gland. But, at the same time, they exhibited signs of thiamine deficiency, such as decreased feed consumption and seizures, which could also be interpreted as PCB toxicity. Although the effects shown in the present study were probably due to a thiamine deficiency, the data obtained should be of assistance to biologists, veterinarians, and toxicologists interested in the species.

Recommendations

The following recommendations are made for researchers to further elucidate the effects of environmental contaminants, especially PCBs, on the status of the river otter. Based on the results of the present study, further laboratory studies should be conducted to determine more precisely the toxicity of PCBs and other organochlorines to otter. The effects of these environmental contaminants on the reproductive performance of the river otter should also be ascertained, to fully assess the influence of these contaminants on otter populations in the wild. In future laboratory studies, care should be taken to ensure that adequate thiamine levels are maintained in otter diets in which fish containing thiaminase are a dietary ingredient.

CHAPTER VI

SUMMARY

The objectives of this study were to determine whether environmentally contaminated fish taken from the Great Lakes are toxic when fed at known concentrations to otter; to determine the sensitivity of otter to these contaminants and to characterize any toxic effects in otter; and to compare toxicity data obtained for otter with similar data for mink and other species, to help assess the contribution of these environmental contaminants, especially PCBs, to the decline of otter populations from certain areas of their former range in the United States and Canadian provinces bordering the Great Lakes.

The results of this study indicate that consumption by river otter of Great Lakes fish containing PCB and other organochlorine contaminants in various concentrations over various lengths of time caused clinical signs that may be attributed, in part, to PCBs. The adverse effects observed in the otter were also probably due to a thiamine deficiency.

Otter that were fed a diet consisting of 1.90 ppm of PCB (20% carp diet) for 6 months did not show many of the clinical signs of PCB toxicity that had been previously observed in other species, although they did have slightly increased liver weights, which were

attributed to PCBs. This observation suggests that the otter may not be as sensitive to PCBs and perhaps other organochlorine contaminants as the mink. Although the otter had tissue residue concentrations of PCBs similar to those observed in other species fed comparable concentrations of PCBs for similar exposure periods, the otter did not exhibit the typical clinical signs usually associated with PCB toxicity. Thus, the present study did not provide conclusive evidence that PCBs and/or other environmental contaminants have contributed significantly to the decline in the otter populations from areas bordering the Great Lakes and Canadian provinces.

Although investigating the otter's susceptibility to thiamine deficiency was not an objective of this study, the results of this research showed that northern river otter that are fed a diet consisting of thiaminase-active carp may be susceptible to thiamine deficiency under certain conditions. Based on these findings, one could question whether thiamine deficiencies occur in wild otter in situations that involve routine consumption of fish containing thiaminase.

APPENDICES

APPENDIX A

ELEMENT CONCENTRATIONS IN SERUM FROM NORTHERN RIVER OTTER

Table A.1. Element concentrations in serum from untreated northern river otter fed a 60% fish diet (N = 12 males).

Element	Mean Concentration ± SE (ppm)
Al	ND[1] (1.0)
B	ND (1.0)
Ba	ND (0.1)
Ca	90.34 ± 1.51
Cu	1.05 ± 0.08
Fe	2.13 ± 0.26
Mg	23.33 ± 0.78
Mn	ND (0.05)
Mo	ND (0.2)
Na	3409.17 ± 16.16
P	205.08 ± 13.88
Zn	0.693 ± 0.03

[1]ND = not detected at detection limits shown in parentheses.

APPENDIX B

STANDARD OPERATING PROCEDURE: ANALYSIS OF ORGANO-CHLORINE PESTICIDES AND PCBs IN MUSCLE TISSUES OF FISH AND BIRDS

Michigan State University
Pesticide Research Center
Aquatic Toxicology Laboratory

STANDARD OPERATING PROCEDURE ANALYSIS OF ORGANOCHLORINE PESTICIDES and PCBs IN MUSCLE TISSUES OF FISH AND BIRDS

Prepared by: Miguel A. Mora
Dave Verbrugge

I. SCOPE

The scope of this method is to determine the concentrations of organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs) in muscle tissues. A list of the compounds that can be determined by this method and the individual detection limits are given in Table 1. The extraction and cleanup procedures are adapted from Schmitt et al. (1985). The method's precision is within 20% and the accuracy >90%. Total PCBs are reported as a mixture of Aroclors 1242, 1248, 1254, and 1260. The instrument detection limit (IDL), method detection limit (MDL), and method quantitation limit have been determined as described in Taylor (1989).

II. REFERENCES

Schmitt, C.J., J.L. Zajicek, and M.A. Ribick. 1985. National pesticide monitoring program: Residues of organochlorine chemicals in freshwater fish, 1980-81. Arch. Environ. Contam. Toxicol. 14:225-260.

Taylor, J.K. 1989. Quality Assurance of Chemical Measurements. Lewis Publishers, Inc., Chelsea, Michigan, 328 pp.

III. SUMMARY

This method permits the separation of organochlorine pesticides and PCBs from muscle tissues. Ten grams tissue are homogenized with sodium sulfate, extracted with dichloromethane and cleaned up with mixed solvents in Florisil and silica gel columns. The florisil and silica gel fractions are analyzed by gas chromatography with electron capture detector (GC-ECD). OCs and PCBs are confirmed by GC/MS in 10% of the samples. Average recoveries for a mixture of 6 pesticides were 77.5% (88% without dieldrin) and 90% for PCBs.

IV. SIGNIFICANCE AND USE

This method allows the analyst to determine the concentration of organochlorinated pesticides (OCs) and PCBs in a wide variety of species. The method is specific to high lipid muscle tissues. These compound will accumulate in fish and fish eating birds and to large extent is associated with the muscle tissue of the organism. By determining the concentration of OCs and PCBs in the muscle tissue, the total body burden may be directly measured for the individual. This data is integral to the modeling of toxicant distribution and migration through the aquatic environment.

V. INTERFERENCES

There are components in muscle tissues that may produce some interference. This can be avoided by following an adequate cleanup procedure. Interferences will be detected by comparing the environmental samples with periodical runs of "clean" muscle tissue blanks. Prior to initiating the studies the lab facility will be carefully cleaned to reduce contamination risks. Muscle tissue will be used as control and for recovery experiments.

VI. APPARATUS

Gas chromatograph, Perkin Elmer, model 8500, with electron capture detector (ECD) with ^{63}Ni foil at 350°C. Column: DB-5 fused silica capillary column (J & W Scientific), 30 m x 0.25 mm i.d., 0.25μm film thickness. Injector in splitless mode. Septum purge set at 3-5 ml per minute, temperature at 240°C. Carrier gas: helium at 5 psig, flow rate of @ 1 ml per minute. Makeup gas nitrogen at 45 psig. Total flow rate of @ 50 ml/min. Autosampler Perkin Elmer 8300. Data (retention times and area percentages) are transferred directly to a microcomputer.

VII. REAGENTS AND MATERIALS

A. Reagents: Dichloromethane ($MeCl_2$), hexane, diethyl ether (peroxide free), petroleum ether, isooctane, benzene, and acetone; Burdick and Jackson, Baxter, Muskegon, Michigan. All solvents used are of high purity or pesticide grade quality.

B. Sodium sulfate, anhydrous, granular and powder forms. Rinse with hexane or methylene chloride in a buchner funnel before use. Let air dry for a while, then dry in the oven at 130°C for at least 24 hr before use. May keep stored at 130°C.

C. Glass wool. Soxhlet extract glass wool with methylene chloride or hexane for at least 24 hr before use.

D. Florisil, 60/80 mesh, PR grade, Floridin Co., Pittsburgh, PA. Activate at

130°C for at least 48 hr before use. Keep stored in the oven at 130°C.

E. Silica gel 60, 70/230 mesh. Activate at 130°C for at least 24 hr before use. Store at 130°C.

F. Glassware. All glassware is washed with liquinox detergent, rinsed with tap and deionized water, then rinsed with acetone and hexane before use.

G. Reference standards.

1. Pesticide matrix spike, (3/90) catalog # 32018, Lot # A000071, Restek Corporation, Bellefonte, PA.
2. PCB matrix spike, IUPAC #14, #65, #166, obtained from Acustandards. Stock and Working solutions were prepared in our lab.
3. Certified reference material, Organochlorinated Pesticides in fish, EPA.
4. Internal standard, PCB 30 & 204, obtained from Acustandards. Stock and working solutions were prepared in our lab.
5. Aroclors 1242, 1248, 1254, and 1260, obtained from Dr. Zabik, Pesticide Research Center, MSU, originally obtained from Monsanto Co. Stock and working solutions were prepared in our lab.
6. Chlorinated hydrocarbon pesticides: Analytical reference standards obtained from U.S. EPA, Quality Assurance Division, Research Triangle Park, NC. Stock and working solutions and mixtures were prepared in our lab.

VIII. HAZARDS AND PRECAUTIONS

Some of the solvents used are flammable and explosive. Solvents should be always used under the hood and away from fire. Use of lab coats and eye protection goggles is important. In case of a spill, skin contact or inhalation problems, follow specifications in material safety data sheets (MSDS). Handling and storage precautions should follow the recommendations described in the respective MSDS. Waste should be collected and disposed properly according to MSU ORCBS indications.

IX. SAMPLING AND SAMPLE PREPARATION

The muscle tissue must be thoroughly ground and homogenized prior to subsampling. Whole fish may be ground using a double blade Hobart food processor. Fillets of fish are easily homogenized in a commercial blender. Large fish and birds (ie Double Breasted Cormorant) must be ground using a commercial meat grinder located at MSU Fur Farm (Dr. Aulerich). The intestinal track of birds must be removed in order to prevent stones from damaging the equipment. After homogenization the sample is subdivided into

manageable quantities, 50-200 grams. The original sample and sub-samples are stored in baked glass jars with teflon lined lids. The samples may be frozen at -20 °C until analyzed.
Repeated thawing and refreezing of the sample should be avoided.

X. PREPARATION OF APPARATUS

Prior to use, the instrument performance is mostly determined from previous runs. Check pressures of He at 5 psig, N_2 at 45 psig. If gases are not on, turn make-up gas on, then turn auxiliary pressure control knob to suggested psig. Percent saturation is adjusted to 0.9%. If the baseline is appropriate, then we can assume that the working conditions are optimal. Column performance should be determined from previous recent runs, and by injecting standards before the autosampler run. The autosampler is loaded and the QC run is set in the computer. Set computer to receive information from each run, then set output (GC) to external device.

XI. CALIBRATION AND STANDARDIZATION

Availability and use of appropriate standards: Our pesticide laboratory standards have been evaluated with the use of a certified pesticide matrix spike, catalog # 32018, lot # A000071, Restek Corporation, Bellefonte, PA. The relative response factors obtained for the two sets of standards were within 90%.

The performance of the GC will be monitored daily by measuring the response and retention times of several calibration mixes. The number of theoretical plates will be calculated using two compounds, C20-ATA and 2,4,6-trichlorobiphenyl (IUPAC #30). The ratio of the theoretical plates (#30/C20) will be used to monitor the condition of the column. A record of the retention times, peak responses, theoretical plates, and peak shape will be kept in the GC Log book. If the theoretical plate ratio changes by > ±25% from its mean value, or if serious column deterioration is observed, the column may be replaced if the situation cannot be corrected. If the retention time of any internal standard changes by > 0.5 min from its mean value, the system will be checked and corrected as required.

The linear range of the GC will be established for individual pesticides by using relative response factors (RRF) and for a 1:1:1:1 mix of Aroclors 1242, 1248, 1254 and 1260 by defining a performance relative response factor (PRRF).

The PRRF is defined by the equation (ex. for aroclor);

$$PRRF = AR_{\text{total area}} * ISTD_{\text{area}} / AR_{\text{area}} * ISTD_{\text{area}}$$

AR= Aroclor Mix ISTD= Internal Standard
total area= sum of peak areas for the aroclor mix
area= peak area for ISTD

conc= concentration in ng/μl

The PRRF is specific for a 1:1:1:1 mixture of Aroclors, and is used only to monitor instrument performance. The RRF and the PRRF will be constant over the linear range of the detector. Constant is defined as $\pm$3% from the mean value for the respect respose factor. This range will encompass a minimum of 1.5 orders of magnitude using a minimum of 3 concentrations. The target operating linear range will be 5, 2.5, and 0.15ng of Aroclor mix injected, and 0.25, 0.1, and 0.01 ng for OCs. Once the linear range has been established, an individual standard solution for each of the mixtures will be chromatographed. These chromatographs will be used as templates for pesticide mixtures and the Comstar PCB pattern recognition program. The integrity of the template will be checked by daily injection of pesticide mixtures and a 1:1:1:1 Aroclor performance standard. The absolute concentration of the performance standard will be adjusted to the linear range of the instrument. The calculated concentration of the mix should be $\pm$10% of the expected value.

Calibration checks will be run at the beginning and end of a sample set, where a set is approximately 10 samples. If the concentration of the standard mix is outside of the 10% range the template will be rechromatographed prior to further sample analysis. A log of the relative response factors (RRF) for the individual Aroclors will also be maintained as a check of the GC performance over the course of the study. The RRF for Aroclor analysis is defined by the equation:

$$RRF = AR_{total\ area} * ISTD_{area} / AR_{area} * ISTD_{area}$$

AR= Individual Aroclor ISTD= Internal Standard
total area= sum of peak areas for the aroclor
area= peak area for ISTD
conc= concentration in ng/μl

If the RRF for a given pesticide or aroclor changes by > 10% from its mean value, the instrument will be checked and the appropriate maintenance (i.e. bakeout, clean detector, etc.) will be completed before continuing with the analyses. The standards should be re-chromatographed and new templates prepared.

VII. PROCEDURE

A. SAMPLE extraction.

1) Transfer 10 g of homogenized tissue to a 500 ml stainless steel homogenizaton cup. Record the exact tissue weight on the sample extraction

form. Spike the sample with 50μl of PCB surrogate solution.

2) Add Na_2SO_4 at 5 X the sample weight (50g) and blend with the Omni Mixer for about 15 seconds.
3) Remove the mixing cup and blend the mixture by hand, repeat the homogenization with the Omni Mixer.
4) Place the mixing cup in a ice bath approximately 15min until the homogenate is a free flowing powder when blended by hand. The sample should be stirred periodically during this period.
5) Add the dried mixture to a 22mm id glass column that has been fitted with a plug of glass wool.
6) Elute the column with 150ml of $MeCl_2$ and collect in a 500ml round bottom flask.
7) Reduce the sample to 1ml by rotoevaporation at 32°C. Quantitatively transfer the sample to a 15ml centrifuge tube using $MeCl_2$/ hexane 1:1. Rinse the rd btm at least 3 times with 1ml of solvent. Dilute the sample to a final volume of 8ml. Note, The centrifuge tube must be calibrated at the 8ml mark.
8) Centrifuge the sample at 2000rpm for 10min. Pipette 80μl of the sample into a tared aluminum weigh boat. Record the weight on the sample extraction sheet. Calculate the number of GPC loops using equation i.
   i) $x = 100 * (W_s - W_t)/0.5$
   x: # GPC loops
   W_t: Tare weight
   W_s: Sample weight
   The sample is split into the number of centrifuge tubes indicated by x.
9) Centrifuge all centrifuge tubes for the sample at 2000rpm for 10min. Continue with gel permeation chromatography (GPC).

B. Gel Permeation Chromatography (GPC)

Refer to the GPC operators guide for information on the preparation of new columns. This equipment should only be operated by trained personnel. The GPC column used for the separation of fish lipid is packed with 60g of SX-3 Bio-Beads Gel, Bio-Rad Co.

1) Prepare the GPC column by flushing for 30min with $MeCl_2$:hexane 1:1. The column should be evenly wet over its entire surface. The pump press should be 6-10 psi, if it is not with in this range see the Supervisor.
2) Fill each sample loop with 8 ml of $MeCl_2$:hexane 1:1, and run the GPC with the collect clock set for 2min (dump and wash set to zero).
3) Load the samples onto the GPC sample loops. The injection valve must be in the load position. Thoroughly rinse the syringe between individual samples. Rinse each sample loop with $MeCl_2$:hexane prior to loading.
4) Place the injection valve in the run position. Run the GPC with the following clock settings:
   Dump: 34min

Collect: 28min
Wash: 10min

Collect the GPC eluant in 250ml rd.btm. flasks. If a sample requires two loops both loops may be collected in a single 500ml rd.btm. flask.

5) Reduce the sample to 1ml by roto-evaporation at 32°, continue with Florisil Clean-up.

C. FLORISIL cleanup and fractionation. (SEE ADDENDUM)

1) Prepare columns by placing 1 cm of granular anhydrous Na_2SO_4 on glasswool in a 1 cm x 30 cm i.d. chromatography column fitted with a 250 ml reservoir. Add five grams of 60/80 mesh Florisil and top with another 1 cm layer of sodium sulfate.
2) Wash each column by adding 20 ml of petroleum ether and draining the solvent to the top of the Na_2SO_4 (bed level). Discard the resulting effluent.
3) Add the concentrated extract (approx. 0.5 ml) and allow it to drain to the column bed level. Rinse the flask at least three times with @ 1 ml of petroleum ether each time. Transfer the rinses into the column, allowing each rinse to drain to bed level. Discard the eluent resulting from loading and rinsing.
4) Wash the column walls with 5 ml of 6:94 ratio of diethyl ether:petroleum ether and collect the eluent in a 250 ml round-bottom flask. When the solvent reaches the bed level of the Florisil add another 30 ml of the 6:94 solvent and continue collection. Set this fraction aside for silica gel fractionation.
5) Repeat the above procedure using a 25:75 ratio of diethyl ether:petroleum ether in place of the 6:94 solution and collect the eluent from the 5 ml wash + 35 ml elution in a second 250 ml flask.
6) Rotary evaporate the two resulting fractions to about 1 ml.
7) Transfer the 25% fraction (containing dieldrin, endrin, methoxychlor and o,p-DDD) to a centrifuge tube (calibrated at 1ml) with three hexane rinses. Add 0.5 ml of isooctane and then N-evap to 0.5 ml. Bring it up to 1 ml with isooctane and transfer to a 2 ml vial with teflon cap. Spike the sample with 50 μl of PCB #30 (11.4 ng/ml) before injection into the GC.

D. SILICA GEL cleanup and fractionation

1) Prepare silica gel 60 (70/230 mesh) columns in the same manner as the florisil column.
2) Wash the column with 20 ml hexane.
3) When hexane reaches the bed level of the silica gel, add the 6% florisil eluate (1-2 ml) and allow it to drain to bed level. Rinse flask three times with 3ml of hexane total, allowing each rinse to drain to the column bed level. Discard eluent.
4) Wash the column with 5 ml of a 0.5:99.5 ratio of benzene:hexane, followed by 35 ml of the solvent. Collect the eluate in a 250 ml round-bottom flask.

(This is fraction 1, silica gel).

5) Elute the columns with 40 ml of a 25:75 ratio of diethyl ether:hexane and collect the eluate in a 250 ml round-bottom flask. (This is fraction 2, silica gel).
6) Rotary evaporate both fractions to about 1 ml, then transfer to a centrifuge tube with three rinses of hexane. Add 0.5 ml of isooctane and N-evap down to @ 0.5 ml. Bring it up to 1 ml with isooctane again and transfer to 2 ml vial with teflon cap. Before GC analysis, spike the extracts with 50 μl of PCB #30 (11.4 ng/ml), then take 250 μl into an autosampler vial for GC run.

E. GAS CHROMATOGRAPHY determination.

1. Silica Gel 25% fraction. Most pesticides come out in this fraction. use autosampler/GC program 9.
   Program 9 conditions: Injector temperature 230 °C, Detector temperature 350 °C. Gas carrier He at 5 psig, makeup gas nitrogen at 45 psig. Equilibrium time 3 min, Total run time 60 min, attenuation 8.

	Oven temperature program			
	1	2	3	4
Oven temp (°C)	120	150	225	280
Iso time (min)	3	5	10	15
Ramp rate (°C/min)	30	4	20	

2. Silica gel 0.5% fraction. PCBs and DDE come out in this fraction. Use autosampler/GC program 6.
   Program 6 conditions: Injector and detector temperatures as well as gas flow rates and everything else remains the same as in program 9, except for the oven temperature program and running time.

	Oven temperature program		
	1	2	3
Oven temp (°C)	120	260	280
Iso time (mim)	6	0	0
Ramp rate (°C/min)	2	20	

3. Florisil 20% fraction. Some pesticides come out in this fraction. Use program 9 (see above).

XIII. DEMONSTRATION OF STATISTICAL CONTROL

Statistical control of GC measurements can be demonstrated graphically by the use of control charts (Taylor 1989, p. 129). Initially, a standard of known concentration will be injected for a total of 7 independent

measurements. If the range is linear, the mean relative response factor will be used as the central line to maintain statistical control. Standards will be injected every day that a set of samples is run. If the value of the standard is within 1 standard deviation of the mean, then we can say that we have statistical control. If a known reference standard is used, then the certified concentration value can be used as the central line (Taylor 1989, p. 131). The control limits will be evaluated by the control charts.

In addition, for every set of 10 samples one sample will be run in triplicate. The calculated concentrations will be compared. If the CV (coefficient of variation is $\pm$ 20%, then we can assume that our measurements are within our established method precision. The use of standards of known concentrations will allow to construct standard reference calibration curves against which the sample runs will be compared. If an outlier is suspected, the calculations and data transfers will be rechecked. If the results are still suspect then the sample before and after suspect and the suspect sample will be reanalyzed. A value will be considered an outlier if there is an assignable cause.

XIV. CALCULATIONS

The concentration of PCBs and OCs will be determined using the internal standard method to eliminate injection variability and the need to maintain the sample at a constant final volume.

A) Organochlorine pesticides: Pesticides will be quantified based on an internal standard (PCB 30) added to the samples after the extraction step. Quantification is carried out by calculating relative response factors based on peak areas.

B) Total PCBs: PCBs will be quantified with the use of COMSTAR (see COMSTAR SOP).

XV. CONFIRMATION AND ASSIGNMENT OF UNCERTAINTY

Organochlorine pesticides will be confirmed in approximately 10% of the samples by GC/MS. This confirmation may only be possible for compounds detected at significant concentrations.

Assignment of uncertainty: A range performance chart will be constructed where the relative response factors (RRFs) at low, middle, and high concentrations will be plotted vs concentration. The upper warning limit (UWL) and lower control limit (LCL) will be the 95% CI, and the upper control limit (UCL) the 99.7% CI. Samples with values above the UCL will be diluted and reanalyzed; those with values below the LCL will be tagged as below detection limit.

TABLE 1

Retention times and limits of detection of organochlorine pesticides[1]

Compound	Retention time (min)	Method detection limit (ng/ml)	Limit of detection (ng/ml)
HCB	14.27		
gamma-HCH	14.93	2.3	0.8
Int.std PCB 30	15.41		
Heptachlor	19.37	1.1	0.4
Aldrin	21.22	1.9	0.6
Heptachlor epoxide	23.06		
Oxychlordane	23.27		
gamma-chlordane	24.17		
o,p'-DDE	24.61		
Endosulfan I	24.79		
p,p'-DDD	25.04		
α-Chlordane	25.49		
Dieldrin	26.06	1.3	0.4
p,p'-DDE	26.12		
t-Nonachlor	26.38		
Endrin	26.93	5.6	1.9
Endosulfan II	27.06		
o,p'-DDD	27.90		
p,p'-DDT	30.06	12.6	4.2
Methoxychlor	33.71		

[1] Column DB-1, Autosampler method 9; see SOP for GC conditions and procedures.

ADDENDUM

Clean-Up for PCB Analysis

Reference: T. Schwartz. 1982. Determination of Polychlorinated Biphenyls in Plant Tissue, Bull. Environ. Contam. Toxicol. 28:723-727.

Scope: This procedure may be substitued for the Florisil/Silica Gel clean-up if pesticide analysis is not required.

1. Prepare column:
   a) Place 1 cm of anhydrous Na_2SO_4 on $MeCl_2$-extracted glass wool in a 1cm x 30cm i.d. chromatography column fitted with a 75ml reservoir.
   b) Add five grams of 70-230 mesh silica gel which has been activated at 130 C for at least 12 h and then cooled to room temperature in a desiccator.
   c) Add one gram of acidic silica gel (40% conc. H_2SO_4 by weight).
   d) Add 1 cm of anhydrous Na_2SO_4.
2. Wash the column with 20ml of hexane, drain to bed level, and discard resulting effluent.
3. Load the GPC concentrate on to the column and allow it to drain to bed level.
4. Rinse the GPC concentrate flask at least twice with a total rinse volume of n-hexane of 2ml. Drain the rinses into the column and discard the eluent from loading and rinsing.
5. Wash the column walls with 5.0 ml of 0.50% benzene in n-hexane and collect the eluent in a 250ml round-bottom flask.
6. When the solvent reaches bed level, add another 45ml of the 0.5% benzene in hexane solvent and continue collection until solvent has drained from the column.
7. Concentrate the eluent to approximately 1 ml by roto-evaporation and transfer quantitatively to a 10 ml calibrated centrifuge tube using n-hexane to rinse.

APPENDIX C

STANDARD OPERATING PROCEDURE: ANALYSIS OF ORGANO-CHLORINE PESTICIDES AND PCBs IN BIRDS' PLASMA

Michigan State University
Pesticide Research Center
Aquatic Toxicology Laboratory

STANDARD OPERATING PROCEDURE
ANALYSIS OF ORGANOCHLORINE PESTICIDES and PCBs IN BIRDS' PLASMA

Prepared by: Miguel A. Mora
Dave Verbrugge

I. SCOPE

The scope of this method is to determine the concentrations of organochlorine pesticides (OCs) and polychlorinated biphenyls (PCBs) in plasma of wild birds. A list of the compounds that can be determined by this method and the individual detection limits are given in Table 1. The extraction and cleanup procedures are adapted from Sonzogni et al. (1991), Burse et al. (1990), Schmitt et al. (1985), and SOP from Michigan Department of Public Health (1987) with some modifications. The method's precision is within 10% and the accuracy >90%. Total PCBs are reported as a mixture of Aroclors 1242, 1248, 1254, and 1260. The instrument detection limit (IDL), method detection limit (MDL), and method quantitation limit have been determined as described in Taylor (1989).

II. REFERENCES

Burse,V.W., S.L. Head, M.P. Korver, P.C. McClure, J.F. Donahue, and L.L. Needham. 1990. Determination of selected organochlorine pesticides and polychlorinated biphenyls in human serum. J. Anal. Toxicol. 14:137-142.

Michigan Department of Public Health. 1987. Analysis of blood for polychlorinated and polybrominated biphenyls, and chlorinated hydrocarbon pesticides. Analytical Method No 7. Lansing, Michigan.

Monro, A.M. 1990. Interspecies comparisons in toxicology: The utility and futility of plasma concentrations of the test substance. Reg. Toxicol. Pharmacol. 12:137-160.

Sonzogni, W., L. Maack, T. Gibson, D. Degenhardt, H. Anderson, and B. Fiore. 1991. Polychlorinated biphenyl congeners in blood of Wisconsin sport fish consumers. Arch. Environ. Contam. Toxicol. 20:56-60.

III. SUMMARY

This method permits the separation of organochlorine pesticides and PCBs from small volumes of birds' plasma. One ml plasma fractions are denaturized with methanol, extracted with a 1:1 mixture (v/v) of hexane-ethyl ether and cleaned up with mixed solvents in florisil and silica gel columns. The florisil and silica gel fractions are analyzed by gas chromatography with electron capture detector (GC-ECD). OCs and PCBs are confirmed by GC/MS in 10% of the samples. Average recoveries for a mixture of 6 pesticides were 77.5% (88% without dieldrin) and 90% for PCBs.

IV. SIGNIFICANCE AND USE

The determination of organochlorine pesticides and PCBs in plasma of wild birds is of importance for the understanding of the relationships of the distribution of xenobiotics between plasma and body tissues. The plasma reaches many target organs and xenobiotics are removed from the plasma as this goes through a given tissue (Monro 1990). By being able to determine concentrations of organochlorine pesticides and PCBs in plasma we can accomplish the following: first, we can take samples of individual species without having to kill them; and second, the same individuals can be sampled over time allowing us to determine seasonal variations and bioaccumulation of contaminants in marked individuals.

V. INTERFERENCES

There are components in plasma that may produce some interference. This can be avoided by following an adequate cleanup procedure. Interferences will be detected by comparing the environmental samples with periodical runs of chicken plasma blanks. Prior to initiating the studies the lab facility will be carefully cleaned to reduce contamination risks. Chicken plasma (obtained from the chicken farm MSU) will be used as control and for recovery experiments.

VI. APPARATUS

Same as in Appendix B.

VII. REAGENTS AND MATERIALS

A. Reagents: Methanol, hexane, diethyl ether (peroxide free), petroleum ether, isooctane, benzene, and acetone; Burdick and Jackson, Baxter, Muskegon, Michigan. All solvents used are of high purity or pesticide grade quality.

B. Sodium sulfate, anhydrous, granular and powder forms. Rinse with hexane or methylene chloride in a buchner funnel before use. Let air dry for a while, then dry in the oven at 130°C for at least 24 hr before use.

May keep stored at 130°C.

C. Glass wool. Rinse glass wool with methylene chloride or hexane for at least 24 hr before use.

D. Florisil, 60/80 mesh, PR grade, Floridin Co., Pittsburgh, PA. Activate at 130°C for at least 48 hr before use. Keep stored in the oven at 130°C.

E. Silica gel 60, 70/230 mesh. Activate at 130°C for at least 24 hr before use. Store at 130°C.

F. Glassware. All glassware is washed with liquinox detergent, rinsed with tap and deionized water, then rinsed with acetone and hexane before use.

G. Reference standards.

1. Pesticide matrix spike, (3/90) catalog # 32018, Lot # A000071, Restek Corporation, Bellefonte, PA.
2. Certified reference material, PCBs (aroclor 1260) in human serum, lot # SRM1589; National Bureau of Standards (NBS), Gaithersburg, MD.
3. Internal standard, PCB 30, obtained from Acustandards. Stock and working solutions were prepared in our lab.
4. Aroclors 1242, 1248, 1254, and 1260, obtained from Dr. Zabik, Pesticide Research Center, MSU, originally obtained from Monsanto Co. Stock and working solutions were prepared in our lab.
5. Chlorinated hydrocarbon pesticides: Analytical reference standards obtained from U.S. EPA, Quality Assurance Division, Research Triangle Park, NC. Stock and working solutions and mixtures were prepared in our lab.
6. Bovine serum and plasma reference material, obtained from Department of Public Health, Lansing, Michigan (MDPH).
7. EPA human plasma for blind analysis (interlab. studies), obtained from MDPH.

VIII. HAZARDS AND PRECAUTIONS

Same as in Appendix B.

IX. SAMPLING AND SAMPLE PREPARATION

Blood from the brachial vein of fish-eating birds (caspian terns, double-crested cormorants and bald eagles) or mammals (mink and otter) was collected in heparinized tubes according to specified procedures. Each sample was marked with bird's common name, location, date and band number (if banded). The samples were either centrifuged in the field (10 min at 3000 rpm), or placed in a refrigerator (4° C) and centrifuged within 48 hours. The plasma was separated from the red blood cells and stored in the freezer until chemical analysis.

X. PREPARATION OF APPARATUS

Same as in Appendix B.

XI. CALIBRATION AND STANDARDIZATION

Availability and use of appropriate standards: Our pesticide laboratory standards have been evaluated with the use of a certified pesticide matrix spike, catalog # 32018, lot # A000071, Restek Corporation, Bellefonte, PA. The relative response factors obtained for the two sets of standards were within 90%. The performance of the GC will be monitored daily by measuring the response and retention times of several calibration mixes. The number of theoretical plates will be calculated using two compounds, C20-ATA and 2,4,6-trichlorobiphenyl (IUPAC #30). The ratio of the theoretical plates (#30/C20) will be used to monitor the condition of the column. A record of the retention times, peak responses, theoretical plates, and peak shape will be kept in the GC Log book. If the theoretical plate ratio changes by > ±25% from its mean value, or if serious column deterioration is observed, the column may be replaced if the situation cannot be corrected. If the retention time of any internal standard changes by > 0.5 min from its mean value, the system will be checked and corrected as required. The linear range of the GC will be established for pesticide mixtures and for a 1:1:1:1 mix of Aroclors 1242, 1248, 1254 and 1260 using a performance relative response factor (PRRF). The PRRF is defined by the equation (ex. for aroclor);

$$PRRF = AR_{total\ area} * ISTD_{area} / AR_{area} * ISTD_{conc}$$

AR= Aroclor Mix ISTD= Internal Standard
total area= sum of peak areas for the aroclor mix
area= peak area for ISTD
conc= concentration in ng/μl

The PRRF is specific for each OC or for a 1:1:1:1 mixture of Aroclors, and is used only to monitor instrument performance. The PRRF will be constant over the linear range of the detector. Constant is defined as ±3% from the mean value for the PRRF. This range will encompass a minimum of 1.5 orders of magnitude using a minimum of 3 concentrations. The target operating linear range will be 5, 2.5, and 0.15ng of Aroclor mix injected, and 0.25, 0.1, and 0.01 ng for OCs. Once the linear range has been established, an individual standard solution for each of the mixtures will be chromatographed. These chromatographs will be used as templates for pesticide mixtures and the Comstar PCB pattern recognition program. The integrity of the template will be checked by daily injection of pesticide mixtures and a 1:1:1:1 Aroclor performance standard. The absolute concentration of the performance standard will be adjusted to the linear range of the instrument. The calculated concentration of the mix should be ±10% of the expected value.

Calibration checks will be run at the beginning and end of a sample

set, where a set is approximately 10 samples. If the concentration of the standard mix is outside of the 10% range the template will be rechromatographed prior to further sample analysis. A log of the relative response factors (RRF) for the individual Aroclors will also be maintained as a check of the GC performance over the course of the study. The RRF is defined by the equation:

$RRF = AR_{total\ area} * ISTD_{area} / AR_{area} * ISTD_{conc}$

AR= Individual Aroclor ISTD= Internal Standard
total area= sum of peak areas for the aroclor
area= peak area for ISTD
conc= concentration in ng/μl

If the RRF for a given pesticide or aroclor changes by > 10% from its mean value, the instrument will be checked and the appropriate maintenance (ie. bakeout, clean detector, etc.) will be completed before prior continuing with the analyses. The standards should be re-chromatographed and new templates prepared.

XII. PROCEDURE

A. SAMPLE extraction.

1) Transfer 1 ml of plasma to a 10 ml test tube with teflon cap. Record also plasma weight by difference from test tube weight.
2) Add 0.5 ml methanol and vortex for about five seconds.
3) Extract with 5 ml of hexane-ethyl ether (1:1, v/v) by agitation in a burrel-wrist action shaker for 10 min.
4) Transfer extract to centrifuge tube and centrifuge at 2000 rpm for 5 min. Transfer extract to a second centrifuge tube to combine the extract volumes and further evaporation.
5. Repeat extraction procedure (steps 3 and 4) twice (three times total).
6. Add 0.5 ml of isooctane, then concentrate extract to 0.5 ml in a rotary evaporator or N-evap on a warm water bath.

B. FLORISIL cleanup and fractionation.

1) Prepare columns by placing 1 cm of granular anhydrous Na_2SO_4 on glasswool in a 1 cm x 30 cm i.d. chromatography column fitted with a 250 ml reservoir. Add five grams of 60/80 mesh Florisil and top with another 1 cm layer of sodium sulfate.
2) Wash each column with 20 ml of petroleum ether and discard resulting effluent.
3) When petroleum ether reaches the top of the Na_2SO_4 layer, add the concentrated extract (approx. 0.5 ml) and allow it to drain into the column. Rinse the flask at least three times with @ 1 ml of petroleum ether each time. Transfer the rinses into the column and discard the eluent resulting from loading and rinsing.
4) Wash the column walls with 5 ml of 6:94 ratio of diethyl ether:petroleum

ether and collect the eluent in a 250 ml round-bottom flask. When the solvent reaches the top of the Florisil add another 30 ml of the 6:94 solvent and continue collection. Set this fraction aside for silica gel fractionation.

5) Repeat the above procedure using a 20:80 ratio of diethyl ether:petroleum ether in place of the 6:94 solution and collect the eluent from the 5 ml wash + 35 ml elution in a second 250 ml flask.
6) Rotary evaporate the two resulting fractions to about 1 ml.
7) Transfer the 20% fraction (containing dieldrin, endrin, methoxychlor and o,p-DDD) to a centrifuge tube with three hexane rinses. Add 0.5 ml of isooctane and then N-evap to 0.5 ml. Bring it up to 1 ml with isooctane and transfer to a 2 ml vial with teflon cap. Rinse the vial previously with acetone, hexane and isooctane. Spike the sample with 50 μl of PCB #30 (11.4 ng/ml) before injection into the GC. Take 300 μl into an autosampler vial and load into autosampler for GC run.

C. SILICA GEL cleanup and fractionation

1) Prepare silica gel 60 (70/230 mesh) columns in the same manner as the florisil column.
2) Wash the column with 20 ml hexane.
3) When hexane reaches the top of the silica gel, add the 6% florisil eluate (1-2 ml) and allow it to drain into the column. Rinse flask with 3 ml of hexane and drain into column. Discard eluents.
4) Wash the column with 5 ml of a 0.5:99.5 ratio of benzene:hexane, followed by 35 ml of the solvent. Collect the eluates in a 250 ml round-bottom flask. (This is fraction 1, silica gel).
5) Elute the columns with 40 ml of a 25:75 ratio of diethyl ether:hexane and collect the eluate in a 250 ml round-bottom flask. (This is fraction 2, silica gel).
6) Rotary evaporate both fractions to about 1 ml, then transfer to a centrifuge tube with three rinses of hexane. Add 0.5 ml of isooctane and N-evap down to @ 0.5 ml. Bring it up to 1 ml with isooctane again and transfer to 2 ml vial with teflon cap. Before GC analysis, spike the extract with 50 μl of PCB #30 (11.4 ng/ml), then take 250 μl into an autosampler vial for GC run.

D. GAS CHROMATOGRAPHY determination.

1. Silica Gel 25% fraction. Most pesticides come out in this fraction. use autosampler/GC program 9.
   Program 9 conditions: Injector temperature 230 °C, Detector temperature 350 °C. Gas carrier He at 5 psig, makeup gas nitrogen at 45 psig. Equilibrium time 3 min, Total run time 60 min, attenuation 8.

Oven temperature program

	1	2	3	4
Oven temp (°C)	120	150	225	280
Iso time (min)	3	5	10	15

Ramp rate (°C/min)	30	4	20

2. Silica gel 0.5% fraction. PCBs and DDE come out in this fraction. Use autosampler/GC program 6.
   Program 6 conditions: Injector and detector temperatures as well as gas flow rates and everything else remains the same as in program 9, except for the oven temperature program and running time.

	Oven temperature program		
	1	2	3
Oven temp (°C)	120	260	280
Iso time (mim)	6	0	0
Ramp rate (°C/min)	2	20	

3. Florisil 20% fraction. Some pesticides come out in this fraction. Use program 9 (see above).

XIII. DEMONSTRATION OF STATISTICAL CONTROL

Same as in Appendix B.

XIV. CALCULATIONS

The concentration of PCBs and OCs will be determined using the internal standard method to eliminate injection variability and the need to maintain the sample at a constant final volume.

A) Organochlorine pesticides: Pesticides will be quantified based on an internal standard (PCB 30) added to the samples after the extraction step. Quantification is carried out by calculating relative response factors based on peak areas.

B) Total PCBs: PCBs will be quantified with the use of COMSTAR (see COMSTAR SOP).

XV. CONFIRMATION AND ASSIGNMENT OF UNCERTAINTY

Organochlorine pesticides will be confirmed in approximately 10% of the samples by GC/MS. This confirmation may only be possible for compounds detected at significant concentrations.
Assignment of uncertainty: A range performance chart will be constructed where the relative response factors (RRFs) at low, middle, and high concentrations will be plotted vs concentration. The upper warning limit (UWL) and lower control limit (LCL) will be the 95% CI, and the upper control limit (UCL) the 99.7% CI. Samples with values above the UCL will be diluted and reanalyzed; those with values below the LCL will be tagged as below detection limit. The retention times and limits of detection of organochlorine pesticides are the same as in Table 1 of Appendix B.

APPENDIX D

SUPPLEMENTARY TABLES

Table D.1. Total PCB concentrations in the livers of male river otter following consumption of diets containing various concentrations of Saginaw Bay carp.

Treatment Group	PCB Concentration[1]		
	Otter Number	Lipid Basis (mg/kg)	Tissue (mg/kg)
Control[2]	2	5.5	0.10
	4	<IDL[3]	<IDL[3]
	8	5.3	0.12
20% carp[2]	5	10.8	0.17
	9	12.5	0.30
	11	15.6	0.31
40% carp[4]	1	38.2	0.65
	10	20.8	0.55
	12	17.2	0.47
60% carp[5]	6	69.6	1.45
	7	40.0	0.34

[1]Wet-weight basis.

[2]Based on 26 weeks' consumption.

[3]Less than instrument detection limit.

[4]Based on 8 weeks' consumption.

[5]Based on 6 weeks' consumption.

Table D.2. Total PCB concentrations in the fat of male river otter following consumption of diets containing various concentrations of Saginaw Bay carp.

Treatment Group	PCB Concentration[1]		
	Otter Number	Lipid Basis (mg/kg)	Tissue (mg/kg)
Control[2]	2	12.1	3.3
	4	4.6	1.2
	8	11.5	2.5
20% carp[2]	5	15.5	5.1
	9	18.0	6.8
	11	13.7	4.9
40% carp[3]	1	14.0	5.0
	10	17.0	5.7
	12	16.5	5.0
60% carp[4]	6	116.0	22.8
	7	28.4	7.9

[1]Wet-weight basis.

[2]Based on 26 weeks' consumption.

[3]Based on 8 weeks' consumption.

[4]Based on 6 weeks' consumption.

Table D.3. Total PCB concentrations in the serum of male river otter following consumption of diets containing various concentrations of Saginaw Bay carp.

Treatment Group	PCB Concentration[1]		
	Otter Number	Total PCB (ng/g)	Total PCB by Volume (ng/ml)
Control[2]	2	117.7	116.2
	4	88.2	76.3
	8	76.4	79.6
20% carp[2]	5	79.6	75.6
	9	222.5	210.4
	11	313.9	263.7
40% carp[3]	1	293.1	241.8
	10	202.6	192.5
	12	209.5	193.4
60% carp[4]	6	539.3	501.6
	7	489.9	412.9

[1]Wet-weight basis.

[2]Based on 26 weeks' consumption.

[3]Based on 8 weeks' consumption.

[4]Based on 6 weeks' consumption.

BIBLIOGRAPHY

BIBLIOGRAPHY

Allen, J.R., L. J. Abrahamson, and D.H. Norback. 1973. Biological effects of chlorinated biphenyls and triphenyls on the subhuman primate. Env. Res. 6: 344-354.

Allen, J.R. and D.A. Barsotti. 1976. The effects of transplacental and mammary movement of PCBs on infant rhesus monkey. Toxicology. 6: 331-340.

Aulerich, R.J., S.J. Bursian, W.J. Breslin, B.A. Olson, and R.K. Ringer. 1985. Toxicological manifestations of 2,4,5,2',4',5'-, 2,3,6,2',3',6'-, and 3,4,5,3',4',5'-hexachlorobiphenyl and Aroclor 1254 in mink. J. Toxicol. Environ. Health 15: 63-79.

Aulerich, R.J., S.J. Bursian, M.G. Evans, J.R. Hochstein, K.A. Koudele, B.A. Olson, and A.C. Napolitano. 1987. Toxicity of 3,4,5,3',4',5'-hexachlorobiphenyl to mink. Arch. Environ. Contam. Toxicol. 16: 53-60.

Aulerich, R.J., S.J. Bursian, and A.C. Napolitano. 1990. Subacute toxicity of dietary heptachlor to mink. (Mustela vison). Arch. Environ. Contam. Toxicol. 19: 913-916.

Aulerich, R.J. and R.K. Ringer. 1970. Some effects of chlorinated hydrocarbon pesticides on mink. Am. Fur Breed. 43: 10-11.

Aulerich, R.J. and R.K. Ringer. 1977. Current status of PCB toxicity to mink and effect on their reproduction. Arch. Environ. Contam. Toxicol. 6: 279-292.

Aulerich, R.J., R.K. Ringer, and S. Iwamoto. 1973. Reproductive failure and mortality in mink fed on Great Lakes fish. J. Reprod. Fert. (supplement). 19: 365-376.

Aulerich, R.J., R.K. Ringer, and J. Safronoff. 1986. Assessment of primary vs. secondary toxicity of Aroclor 1254 to mink. Arch. Environ. Contam. Toxicol. 15: 393-399.

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

Aulerich, R.J., R.K. Ringer, H.L. Seagran, and W.G. Youatt. 1971. Effects of feeding coho salmon and other Great Lakes fish on mink reproduction. Canad. J. Zool. 49(5): 611-616.

Barsotti, D.A., R.J. Marlor, and J.R. Allen. 1976. Reproductive dysfunction in rhesus monkeys exposed to low levels of polychlorinated biphenyls (Aroclor 1248). Fd. Cosmet. Toxicol. 14: 99-103.

Beeton, A.M. 1984. The world's Great Lakes. J. Great Lakes Res. 10(2): 106-113.

Bleavins, M.R., R.J. Aulerich, and R.K. Ringer. 1980. Polychlorinated biphenyls (Aroclors 1016 and 1242): Effects on survival and reproduction in mink and ferrets. Arch. Environ. Contam. Toxicol. 9: 627-635.

Braselton, W.E., G.L. Meerdink, H.D. Stowe, and S.R. Tonsager. 1981. Experience with multielement analysis in diagnostic clinical toxicology and nutrition. Proc. Ann. Meet. Amer. Assn. Vet. Lab. Diagn. 24: 111-126.

Brezner, S., J. Terkel, and A.S. Perry. 1984. The effect of Aroclor 1254 (PCB) on the physiology of reproduction in the female rat--I. Comp. Biochem. Physiol. 77C(1): 65-70.

Brouwer, A., P.J.H. Reijnders, and J.H. Koeman. 1989. Polychlorinated biphenyl (PCB)-contaminated fish induces vitamin A and thyroid hormone deficiency in the common seal (Phoca vitulina). Aquat. Toxicol. 15: 99-106.

Carter, J.W. 1984. Hypercholesterolemia induced by dietary PCBs (Aroclor 1254) in Fischer rats. Bull. Environ. Contam. Toxicol. 33: 78-83.

Chanin, P.R.F. and D.J. Jeffries. 1978. The decline of the otter Lutra lutra L. in Britain: An analysis of hunting records and discussion of causes. Biol. J. Linn. Soc. 10: 305-328.

Copel, P.D. and S.J. Eisenreich. 1985. PCBs in Lake Superior, 1978-80. J. Great Lakes Res. 11: 447-461.

Custer, T.W. and G.H. Heinz. 1980. Reproductive success and nest attentiveness of mallard ducks fed Aroclor 1254. Environ. Poll. 21 (Series A): 313-319.

D'Itri, F.M. and M.A. Kamrin. 1983. PCBs: Human and Environmental Hazards. Butterworth Publishers, Woburn, MA. 443 pp.

Dellmann, H.D. and E.M. Brown. 1981. Textbook of veterinary histology, 2nd Edition. Lea and Febiger, Philadelphia, PA. 460 pp.

DeVoogt, P. and V.A. Brinkman. 1989. Production properties, and usage of polychlorinated biphenyls, Chapter 1. In: R.D. Kimbrough and A.A. Jensen (eds.). Halogenated Biphenyls, Triphenyls, Naphthalenes, Dibenzodioxins, and Related Products. Elsevier Science Publishers, New York, NY. 518 pp.

Eisenreich, S.J., B.B. Looney, and J.D. Thornton. 1981. Airborne organic contaminants in the Great Lakes ecosystem. Environ. Sci. Tech. 15: 30-38.

Eisler, R. 1986. Polychlorinated biphenyl hazards to fish, wildlife, and invertebrates: A synergistic review. U.S. Fish Wildlife Service Biological Report 85 (1.7). Washington, D.C. pp. 1-72.

Erickson, M.D. 1986. Analytical Chemistry of PCBs, Ann Arbor Science. Butterworth Publishers, Stoneham, MA. 235 pp.

Foley, R.E., S.J. Jackling, R.J. Sloan, and M.K. Brown. 1988. Organochlorine and mercury residues in wild mink and otter: Comparison with fish. Environ. Toxicol. Chem. 7: 363-374.

Frank, R., M. Van Hove Holdrinet, and P. Suda. 1979. Organochlorine and mercury residues in wild mammals in southern Ontario, Canada, 1973-74. Bull. Environ. Contam. Toxicol. 22: 500-507.

Gill, J.L. 1978. Design and Analysis of Experiments in the Animal and Medical Sciences. Vol. 1. Iowa State University Press, Ames, IA. 410 pp.

Gillette, D.M., R.D. Corey, W.G. Helferich, J.M. McFarland, L.J. Lowenstine, D.E. Moody, B.D. Hammock, and L.R. Shull. 1987. Comparative toxicology of tetrachlorobiphenyls in mink and rats. I. Changes in hepatic enzyme activity and smooth endoplasmic reticulum volume. Funda. Appl. Toxicol. 8: 5-14.

Gnaedinger, R.H. and R.A. Krzeczkowski. 1966. Heat inactivation of thiaminase in whole fish. Comm. Fisheries Rev. 28(8): 11-14.

Government of Canada. 1991. Toxic Chemicals in the Great Lakes and Associated Effects. Volume I--Contaminants, Levels and Trends. Volume II--Effects. Minister of Supply and Services, Canada. 755 pp.

Green, R.G., C.A. Evans, W.E. Carlson, and F.S. Swale. 1942. Chastek paralysis in foxes. JAVMA Vol. C (782): 394-402.

Gulley, D.D., A.M. Boelter and H.C. Bergman. 1985. Toxstat computer program. Version 2.1. University of Wyoming, Laramie, WY.

Gustafson, C. 1970. PCB's--prevalent and persistent. Environ. Sci. Tech. 4(10): 814-819.

Hansen, L.G., C.S. Byerly, R.L. Metcaff, and R.F. Bevill. 1975. Effect of polychlorinated biphenyl mixture on swine reproduction and tissue residues. Amer. J. Vet. Res. 36: 23-26.

Hansen, L.G., L.G. M.T. Tuinstra, C.A. Kan, J.J.T.W.A. Strik, and J.H. Koeman. 1983. Accumulation of chlorobiphenyls in chicken fat and liver after feeding Aroclor 1254 directly or fat from swine fed Aroclor 1254. J. Agric. Fd. Chem. 31: 254-260.

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

Heath, R.G., J.W. Spann, J.F. Kreitzer, and C. Vance. 1972. Effects of polychlorinated biphenyls on birds. Proceedings of the XVth International Ornith. Congress. (ed.) E. J. Drill. Leiden. pp. 475-485.

Heaton, S. 1992. Effects on Reproduction of Ranch Mink Fed Carp from Saginaw Bay, Michigan. M.S. thesis, Department of Animal Science, Michigan State University, East Lansing, MI. 152 pp.

Henny, C.J. L.J. Blus, S.V. Gregory, and C.J. Stafford. 1981. PCBs and organochlorine pesticides in wild mink and river otters from Oregon. In: J.A. Chapman and D. Pursley (Eds.), Worldwide Furbearer Conference Proceedings, August 3-11, 1980, Vol. III. Worldwide Furbearer Conference, Inc., Frostburg, MD. pp. 1763-1780.

Hill, E.P. and D.M. Dent. 1985. Mirex residues in seven groups of aquatic and terrestrial mammals. Arch. Environ. Contam. Toxicol. 14: 7-12.

Hochstein, J.R., R.J. Aulerich, and S.J. Bursian. 1988. Acute toxicity of 2,3,7,8-tetrachlorodibenzo-p-dioxin to mink. Arch. Environ. Contam. Toxicol. 17:33-37.

Holden, A.V. and K. Mardsen. 1967. Organochlorine pesticides in seals and porpoises. Nature 216: 1274-1276.

Holmes, D.C., J.H. Simmons, and J.O.G. Tatton. 1967. Chlorinated hydrocarbons in British wildlife. Nature 216: 227-229.

Hoover, J.P., R.J. Bahr, M.A. Nieves, R.T. Doyle, M.A. Zimmer, and S.E. Lauzon. 1985. Clinical evaluation and prerelease management of American river otters in the second year of a reintroduction study. JAVMA 18: 1154-1161.

Hoover, J.P., C.R. Root, and M.A. Zimmer. 1984. Clinical evaluation of American river otters in a reintroduction study. JAVMA 184: 1321-1326.

Hornshaw, T.C. 1981. Renewed use of Underutilized Species of Great Lakes Fish for Animal Feed. M.S. thesis, Animal Science Department, Michigan State University, East Lansing, MI. 45 pp.

Hornshaw, T.C., R.J. Aulerich, and H.E. Johnson. 1983. Feeding Great Lakes fish to mink: Effects on mink and accumulation and elimination of PCBs by mink. J. Toxicol. Environ. Health. 11: 933-946.

Hornshaw, T.C., J. Safronoff, R.K. Ringer and R.J. Aulerich. 1986. LC50 test results in polychlorinated biphenyl-fed mink: Age, season, and diet comparisons. Arch. Environ. Contam. Toxicol. 15: 717-723.

Hutzinger, O., S. Safe, and V. Zitko. 1974. The Chemistry of PCBs. CRC Press, Inc., Boca Raton, FL. 269 pp.

Jelinik, C.F. and P.E. Corneliussen. 1976. Level of PCBs in the U.S. food supply. National conference on polychlorinated biphenyls, November 19-21, 1975, Chicago, IL. NTIS PB-253-248, pp. 147-154.

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

Kalmaz, E.V. and G.D. Kalmaz. 1979. Transport, distribution and toxic effects of polychlorinated biphenyls in ecosystems. Rev. Ecol. Model. 6: 223-251.

Koeman, J.H., M.C. Ten Noever de Brauw, and R.H. de Vos. 1969. Chlorinated biphenyls in fish, mussels and birds from the River Rhine and the Netherlands coastal area. Nature 221: 1126-1128.

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

Kruuk, H., and J.W.H. Conroy. 1991. Mortality of otters (Lutra lutra) in Shetland. J. Appl. Ecol. 28: 83-94.

Kucera, E. 1983. Mink and otter as indicators of mercury in Manitoba waters. Can. J. Zool. 61: 2250-2256.

Langford, H.D. 1979. Looking at polychlorinated biphenyls as environmental object lesson. News Rept. 29(9): 1-5.

Leece, B., M.A. Denomme, R. Towner, S.M.A. Li, and S. Safe. 1985. Polychlorinated biphenyls: Correlation between in vivo and in vitro quantitative structure-activity relationships (QSARS). J. Toxic. Environ. Health. 16: 379-388.

MacDonald, S. 1983. The status of the otter (Lutra lutra) in the British Isles. Mammal. Rev. 13: 11-23.

Mason, C.F. 1989a. Relationships between organochlorine concentrations in liver and muscle of otters. Bull. Environ. Contam. Toxicol. 43: 548-549.

Mason, C.F. 1989b. Water pollution and otter distribution: A review. Lutra 32: 97-131.

Mason, C.F., T.C. Ford, and N.I. Last. 1986. Organochlorine residues in British otters. Bull. Environ. Contam. Toxicol. 36: 656-661.

Mason, C.F. and S.M. MacDonald. 1986. Otters: Ecology and Conservation. Cambridge Univ. Press, Cambridge, MA. 236 pp.

McFarland, V.A. and J.U. Clarke. 1989. Environmental occurrence, abundance and potential toxicity of polychlorinated biphenyl congeners: Considerations for a congener-specific analysis. Environ. Health Persp. 81: 225-239.

Melquist, W.E. and A.E. Dronkert. 1987. River otter. pp. 627-641. In: M. Novak, J.A. Baker, M.E. Obbard and B. Mallock (eds.). Wild Furbearer Management and Conservation in North America. Ontario Trappers Assoc. Ontario, Canada. 1150 pp.

Michigan Department of Natural Resources. 1988. Michigan Department of Natural Resources Remedial Action Plan for Saginaw River and Saginaw Bay, Area of Concern, September 1988. Mich. Dept. Nat. Res., Surface Water Quality Division. Lansing, MI. 588 pp.

Michigan Department of Natural Resources. 1991. Michigan Fish Monitoring Program, 1991 Annual Report. Surface Water Quality Division, Mich. Dept. Nat. Res. Lansing, MI. Rept. #MI/DNR/SWQ-91. 273 pp.

Murphy, J.J. and C.P. Rzezutko. 1979. Precipitation inputs of PCBs to Lake Michigan. J. Great Lakes Res. 3: 305-312.

Nadeau, R.J. and R.A. Davis. 1976. Polychlorinated biphenyls in the Hudson River/Hudson Falls-Fort Edward, New York State. Bull. Environ. Contam. Toxicol. 16(4): 436-444.

National Research Council. 1982. Nutrient requirements of mink and foxes. Nutrient requirements of domestic animals, series No. 7. National Academy Press, Washington, D.C. 72 pp.

O'Conner, D.J. and S.W. Nielson. 1980. Environmental survey of methylmercury levels in wild mink and otter from the northeastern United States and experimental pathology of methylmercurialism in otter. pp. 1728-1745. In: J.A. Chapman and P. Pursley (Eds.). Worldwide Furbearer Conference Proceedings. Frostburg, MD, Aug. 3-11, 1980.

Okada, H.M., Y. Chihaya, and K. Matsukawa. 1987. Thiamine deficiency encephalopathy in foxes and mink. Vet. Pathol. 24: 180-182.

Olsson, M., L. Reutergarth, and F. Sandergren. 1981. Var ar Uttern? Sveriges Natur. 6: 234-240.

Ontario Ministry of the Environment and Ontario Ministry of Natural Resources (MOE/MNR). 1989. Guide to Eating Ontario Sport Fish. 297 pp.

Orihel, T.C. 1965. Dirofilaria lutrae SP. N. (Nematoda Filarioidea) from otters in the southeast United States. J. Parasitol. 51(3): 409-413.

Platanow, N.S. and L.H. Karstad. 1973. Dietary effects of polychlorinated biphenyls on mink. Can. J. Comp. Med. 37(4): 391-400.

Poland, A., W.F. Greenlee, and A.S. Kende. 1979. Studies on the mechanism of action of the chlorinated dibenzo-p-dioxins and related compounds. Ann. NY Acad. Sci. 320: 214-230.

Proulx, G., D.V.C. Weseloh, J.E. Elliott, S. Teeple, P.A.M. Anghem, and P. Mineau. 1987. Organochlorine and PCB residues in Lake Erie mink populations. Bull. Environ. Contam. Toxicol. 39: 939-944.

Ringer, R.K., R.J. Aulerich, and M. Zabik. 1972. Effect of dietary polychlorinated biphenyls on growth and reproduction of mink. 164 Natl. Meeting, Amer. Chem. Soc. 12: 149-154.

Risebrough, R. and V. Brodine. 1970. Otters in the wild. Environ. 12(1): 16-26.

Risebrough, R.W., P. Rieche, and H.S. Olcutt. 1968. Polychlorinated biphenyls in the global ecosystem. Nature 220: 1098-1102.

Safe, S., L.W. Robertson, and L. Safe. 1982. Halogenated biphenyls; molecular toxicology. Canada. J. Physiol. Pharmacol. 60: 1057-1064.

Schmitt, C.J., J.L. Zajicek, and M.A. Ribick. 1985. National pesticide monitoring program: Residues of organochlorine chemicals in freshwater fish, 1980-81. Arch. Environ. Contam. Toxicol. 14: 225-260.

Sheffy, T.B. and J.R. St. Amant. 1982. Mercury burdens in furbearers in Wisconsin. J. Wildl. Manage. 46: 1117-1120.

Somers, J.D., B.C. Goski, and M.W. Barrett. 1987. Organochlorine residues in Northeastern Alberta otters. Bull. Environ. Contam. Toxicol. 39: 783-790.

Stout, F.M., J.E. Oldfield, and J. Adair. 1963. A secondary induced thiamine deficiency in mink. Nature 197: 810-811.

Taylor, J.K. 1989. Quality Assurance of Chemical Measurements. Lewis Publishers, Inc., Chelsea, MI. 328 pp.

Vos, J.G. and J. H. Koeman. 1970. Comparative toxicologic study with polychlorinated biphenyls in chickens with special reference to porphyria, edema formation, liver necrosis, and tissue residues. Toxicol. Appl. Pharmacol. 17: 656-668.

Vos, J.G. and E. Nolenboom-Ram. 1972. Dermal toxicity studies of technical polychlorinated biphenyls and reactions thereof in rabbits. Toxicol. Appl. Pharmacol. 19: 617-633.

Williams, D.J. 1975. A review of published and other data on residues of DDT, dieldrin, mercury, and PCBs in fish in the Great Lakes. Unpublished report. In: Environmental Canada, Fisheries and Marine Service, Great Lakes Biolimnology Laboratory. Burlington, Ontario.

Wren, C.D., D.B. Hunter, J.F. Leatherlard, and P.M. Stokes. 1987. The effects of PCB and methyl mercury, singly and in combination, on mink. II: Reproduction and kit development. Arch. Environ. Contam. Toxicol. 16(4): 449-454.

Wren, C.D., P.M. Stokes, and K.L. Fisher. 1986. Mercury levels in Ontario mink and otter relative to food levels and environmental acidification. Can. J. Zool. 64: 2854-2859.

Zimmerman, N. 1982. Polychlorinated biphenyls in Great Lakes fish: Toxicological justification for lowering the acceptable standard to 2 ppm. Toxic Substance Control Commission, Lansing, MI, March. 17 pp.