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LONG-TERM EFFECTS OF GREAT LAKES FISH DIET
ON REPRODUCTION IN MICE

presented by

Chang-Yi Lin

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of the requirements for

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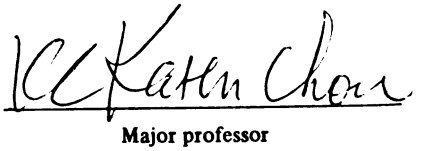

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LONG-TERM EFFECTS OF GREAT LAKES FISH DIET ON REPRODUCTION IN MICE

By

Chang-Yi Lin

A THESIS

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ABSTRACT

LONG-TERM EFFECTS OF GREAT LAKES FISH DIET ON REPRODUCTION IN MICE

By

Chang-Yi Lin

Pregnant C57BL/6J mice, bred to DBA/2J males, were randomly divided into three treatment groups: C (82% lab chow with 18% fish oil), I (60% Iowa carp with 40% lab chow) and G (60% Great Lakes carp with 40% lab chow). The treatment diets were provided from parturition through lactation. The offspring (F-1) were fed their respective diets from weaning to termination. High neonatal mortality was observed in the G treatment group. Decreases in body weights were also observed in F-1 males and females of the same treatment group. F-1 males at 1-year of age were paired with non-treated females, low fecundity was observed in the G treatment group. At 53 weeks of age, sperm concentration and *in vitro* sperm fertilizing ability of mice in the G treatment group were significantly lower than in the C and I treatment groups. F-1 female mice in the G treatment group had a delay in vaginal opening.

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LIST OF ABBREVIATIONS

ALH Dis.	amplitude of lateral head displacement
BW	body weight
p,p'-DDE	1,1-dichloro-2,2-bis-[*p*-chlorophenyl]ethylene
p,p'-DDT	1,1,1-trichloro-2,2-bis[*p*-chlorophenyl]ethane
HCG	human chorionic gonadotropin
i.p.	intraperitoneal
i.v.	intravenous
PCBs	polychlorinated biphenyls
PMSG	pregnant mare's serum gonadotropin
ppb	parts per billion
ppm	parts per million
TCB	3,3',4,4'-tetrachlorobiphenyl
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin

INTRODUCTION

Industrial contaminants in the Great Lakes have been implicated as causative agents in the decline of wildlife populations as well as in the increase of some human health effects (Mineau et al., 1984; Gilbertson, 1988; Government of Canada, 1991; Jacobson and Jacobson, 1993). The Virtual Elimination Task Force of the International Joint Commission (IJC) identified 11 persistent toxic substances in 1985 (IJC, 1993). The use of six of these major contaminants, polychlorinated biphenyls (PCBs), DDT, dieldrin, mirex, toxaphene, and alkylated lead, have been restricted or banned (Appendix I). Some of these substances are naturally occurring [mercury, benzo(a)pyrene] while others, such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and polychlorinated dibenzofurans (PCDFs), are byproducts of combustion and some industrial processes involving the use of chlorine. In spite of measures taken to reduce the input of these contaminants into the environment, these compounds and their metabolites persist in the Great Lakes ecosystem (Tanabe, 1988). Significant amounts are still present in the air, water, and soil allowing them to traverse through

the food chain to upper trophic level organisms (Weaver et al., 1965; Tanabe et al., 1984; Waid, 1987; Evans et al., 1991). Therefore, human and wildlife consumption of Great Lakes fish has become a significant health and ecological concern.

Adverse effects have been observed in laboratory animals exposed to Great Lakes carp. Offspring of adult ranch mink fed diets containing Great Lakes carp from Saginaw Bay, Michigan, have shown a significant reduction in birth weight and litter size and an increase in mortality (Hartsough, 1965; Heaton et al., 1995; Restum et al., 1997). Similar findings were reported by Summer et al. (1996) who observed a dose- and time-dependent effect on hatchability and the occurrence of teratogenic effects in chicken embryos from hens fed diets containing Great Lakes carp.

Polychlorinated biphenyls and DDT are two major contaminants in the Great Lakes (IJC, 1993). Some of the biological effects related to the Great Lakes contaminants have been observed in laboratory studies when animals are exposed to PCBs, as commercial mixtures or individual congeners, and PCB analogs; other effects have also been related to the toxicity of DDT and its metabolites. Increased embryo mortality was observed in chicken eggs injected with commercial PCB mixtures (McLaughlin et al., 1963; Carlson and Duby 1973). Hansen et al. (1992) reported that the ortho-substituted

PCBs have estrogenic activities as indicated by an increase in uterine weight in immature rats and that the non-ortho substituted PCB congener, 3,3',4,4'-tetrachlorobiphenyl (TCB), produces anti-estrogenic effects like TCDD. Male rats exposed to Aroclor 1254 during lactation were less successful in mating than control animals; non-treated females mated with treated males had a lower implantation rate and a decrease in the number of live fetuses (Sager, 1983; Sager et al., 1991). Female rats lactationally exposed to Aroclor 1254 also experienced a reduction in implantation rate (Sager and Girard 1994). Exposure of both male and female mice to PCBs has been shown to decrease the frequency of implanted ova (Kihlstrom et al., 1975). DDT has also been shown to have reproductive effects in various species. Exposure of both sexes to DDT has been shown to lower the frequency of implantation in mice (Kihlstrom et al., 1975). DDT has been shown to have estrogenic activity which is manifested by an acceleration in vaginal opening, persistent vaginal estrus, and anovulation in female rats (Gellert et al., 1972, 1974; Heinrichs et al., 1971). DDE, a major metabolite of DDT, is known to cause eggshell thinning in birds (Peakall et al., 1973). DDE has also been demonstrated to have antiandrogenic effects in exposed male rats such as decreased ventral prostate weights, a delay in the onset of puberty, and a decrease in the anogenital distance (Kelce et al., 1995).

While exposure to PCBs and DDT has been shown to cause adverse effects on reproduction, animals in the wild are rarely exposed to a single type of compound. In addition to PCBs, DDT, and their metabolites, a variety of other chemicals and ecological metabolites are present in the Great Lakes (IJC, 1993). Humans and animals, therefore, are exposed to a mixture of contaminants which may elicit different effects than individual compounds. Thus, it is important to study the effects of mixtures of contaminants as they exist in the environment.

Our laboratory has been studying the reproductive effects of these mixtures of contaminants on reproduction in mice. Great Lakes carp was used as a model carrier for mixtures of contaminants in the Great Lakes. In a previous study, in which mice were exposed to Great Lakes fish via lactation and diet from birth through 34 weeks of age, no adverse effect on reproductive performance in pubertal and mature male mice was observed (Kuo, 1994). The present study expands on our earlier study. The effects of Great Lakes contaminants on male reproduction were examined in post-maturation mice (through 15 months of age) and the effects of these contaminants on the onset of puberty in the female were also examined.

LITERATURE REVIEW

The Great Lakes

The Great Lakes (Lake Superior, Lake Michigan, Lake Huron, Lake Erie, and Lake Ontario) and their connecting channels are the largest system of fresh surface water on the earth. They contain approximately 18% of the world's fresh water (about 5,500 cubic miles of water), span 750 miles from east to west, and cover a total area of 94,000 square miles. The Great Lakes are of sizeable economic and ecological importance to both the United States and Canada. Over 35 million residents (one-tenth of the population of the United States and one-quarter of the population of Canada) live around the Great Lakes basin (EPA, 1994). The Great Lakes provide drinking water, transportation, power production, recreation, and sporting and commercial fishery.

Contaminants of The Great Lakes

Since 1940, increased commercial production and widespread use and release of agricultural and industrial compounds have contaminated the Great

Lakes ecosystem. Tens of thousands of chemicals are created, used and released into the Great Lakes each year from a variety of sources. These sources include atmospheric deposition and the dumping of polluted industrial discharge, rural sewage, urban sewage and runoff pollutants.

Sources of pollution are generally classified as two types: One type is point sources which are single sources such as industrial or municipal discharges. The other type of pollution is nonpoint, from a diffuse source which includes both urban and rural runoff from the land, as well as atmospheric deposition which originates thousands of miles from the Great Lakes. Atmospheric deposition occurs in the following manner: 1) Pollutants are released into the air from man-made or natural sources. Man-made sources include vehicle exhaust and pesticide spray. Natural sources include volcanic eruptions and particles from forest fires. 2) Pollutants are carried by continental winds away from their original source. 3) Pollutants are deposited on lakes when particles settle out of the air by gravity or are removed from the atmosphere by falling rain or snow. 4) Air pollutants can also be deposited on land and runoff into lakes. Polychlorinated biphenyls (PCBs) and dichlorodiphenyltrichlorethane (DDT) have been detected in the polar areas (Atlas et al., 1986) and in marine mammals from Antarctica (Tanabe et al., 1983). Much of the atmospheric contribution in the Great

Lakes basin is believed to result from long range transport to the region (including distances as far away as Central America). Rice (1985) reported that atmospheric loading is responsible for almost 76-98% of PCBs found in Lake Superior, 69-84% for Lake Michigan, 57-83% for Lake Huron, 45-67% for Lake Erie, and 43-63% for Lake Ontario. Nearly 100 tons/year of mercury are released into the Great Lakes from anthropogenic sources in the U.S., and another 31 tons/year from Canadian sources (IJC, 1993b).

In the past 20 years, Great Lakes' contaminants have declined consistently due to restrictions on chemical use and bans on the use of DDT, chlordane, dieldrin and PCBs (State of the Great Lakes, 1993 Annual report; Bishop and Weseloh, 1990). Numerous studies have shown that the concentrations of organochlorine pesticides and PCBs are declining in Great Lakes fish (Schmitt et al., 1985; DeVault et al., 1986; Schmitt et al., 1990; Borgmann et al., 1991). There was a rapid decrease in DDE concentrations in Lake Ontario trout from 1977 to 1980. Since this time, DDE concentrations have remained relatively constant (Borgmann et al., 1991). DeVault et al. (1986) reported that mean total DDT concentrations in coho salmon fillets from Lakes Michigan and Erie declined from 19.19 mg/kg in 1970 to 2.74 mg/kg in 1982. In a five-year human study, DDT levels of 115 Great Lakes fisheaters were compared to the levels of 95 non-fisheaters,

DDT levels were significantly decreased between 1982 and 1989 (Hovinga et al., 1992). Dieldrin concentrations in Lake Ontario trout have also declined between 1978 and 1988 (Borgmann et al., 1991). PCB concentrations have fluctuated over the years. They have decreased from 1977 to 1981, increased from 1982 until 1984, and decreased again in 1985 (Borgmann et al., 1991). Studies show that PCB concentrations in coho salmon fillets from Lakes Michigan and Erie have decreased from 22.91 mg/kg in 1974 to 5.63 mg/kg in 1982 (DeVault et al., 1986). Hovinga et al. (1992) reported that between 1982 and 1989, there was no change in serum PCB concentration in humans who consumed Great Lakes fish.

Polychlorinated biphenyls (PCBs)

Polychlorinated biphenyls are chlorinated organic compounds with biphenyl as the basic structural unit. These environmentally persistent chemicals were first described in 1881 by Schmidt and Schultz (Peakall and Lincer, 1970). PCBs have a low dielectric constant. They are stable in both acidic and basic conditions and are resistant to oxidation and reduction. PCBs have a high heat capacity and are nonflammable. In 1929, PCBs were first commercially produced and used for industrial purposes (Tanabe, 1988). PCBs were widely used in industry as hydraulic fluids, heat transfer

fluids, lubricants, capacitors, plasticizers, protective coatings, and cardboard cartons. PCBs were also used in commercial products like carbonless carbon paper, organic diluents, wax extenders, printing ink, dust-reducing agents, flame retardants, and adhesives (Safe et al., 1987).

Chlorination of the biphenyl ring can produce 209 possible congeners from monochloro- to decachloro-. The most popular brand of PCBs in North America was "Aroclor®", manufactured by Monsanto Ltd. (U.S.). Aroclor products include 1242, 1248, 1254, and 1260 which are complex mixtures of different congeners. For example, Aroclor 1254 consisted primarily of tetra, penta-, and hexachlorobiphenyls. The first two digits represent the number of carbon atoms in the biphenyl group and the last two digits describe the approximate percent chlorine content (Sawhney, 1986).

Since 1929 it is estimated that 1,200,000 tons of PCBs were produced in the world. There are an estimated 370,000 tons of PCBs in the global environment which makes up 30% of the world PCB production, 65% (783,000 tons) of the world's production is still in use in electrical equipment. In 1988, over 280,000 tons of PCBs were still in use in the U.S (IJC, 1993). In spite of precautions, accidental leaking from industrial facilities and landfills, improper disposal practices, and industrial fires (Phaneuf et al., 1995) have led to significant environmental contamination.

PCBs are known to produce a spectrum of toxic responses in humans and laboratory animals including lethality, reproductive and developmental toxicity (Allen et al., 1979), porphyria, body weight loss, dermal toxicity (Tryphonas et al., 1991), immunosuppressive effects (Loose et al., 1978; Tryphonas et al., 1991), hepatotoxicity (Bruckner et al., 1974), neurotoxicity (Loose et al., 1978), thymic atrophy, carcinogenesis, and endocrine disruption (Safe, 1994).

Because of the low concentration of PCBs in the atmosphere, the majority (80-90%) of animal exposure to organochlorine compounds such as PCBs and DDT comes from the food pathway, a lesser amount (5-10%) from air, and trace amounts from water (IJC, 1993). Through oral exposure, PCBs, like other lipophilic compounds, rapidly cross the membranes of the gastrointestinal track. More than 84% of the total dose of PCBs was absorbed from the gastrointestinal tract, when a 3 mg commercial PCB mixture, containing Kanechlor-300, 400, 500, and 600 (1:1:1:1 by weight) in corn oil, was administered daily by gavage to immature male Wistar rats for 5 days (Tanabe, 1981).

After PCBs cross the membranes of the gastrointestinal tract, they are transported in the blood to the liver as well as to other organs and tissues. In the body, PCBs are associated with lipids; the highest concentrations are

found in fat-rich tissues such as adipose tissue and milk (Jensen, 1989). A study conducted in Michigan demonstrated that sport anglers who ate Great Lakes fish (especially trout and salmon) had higher blood and tissue levels of PCBs than people who never ate Great Lakes fish (Humphrey, 1988).

The toxicity of PCBs are highly dependent on the degree of chlorination of the biphenyl nucleus and the position of the chlorine atoms (Parkinson and Safe, 1987). It is generally known that the acute toxicity (LD50) of commercial PCB formulations increases with increasing chlorine content, however, the highly chlorinated Aroclors 1260, 1262, and 1268 are less toxic than Aroclor 1254 (Safe, 1984). Individual congeners are classified into 3 groups: non-*ortho*, mono-*ortho* and di-*ortho* chlorinated biphenyls. The term non-*ortho* is applied to congeners with no substitutes at the *ortho* positions. Several non-*ortho*-and mono-*ortho*-congeners may attain a coplanar conformation and have been found to induce nonneurological effects (e.g., skin lesions, thymus atrophy, a wasting syndrome, liver damage, immunotoxicity, and increased mortality) similar to those caused by TCDD. These effects of PCBs and TCDD are mediated through the same receptor, the Ah (cytosolic aryl hydrocarbon) receptor (Safe, 1990). Non-*ortho* PCBs bind to the Ah receptor and form a receptor-ligand complex which translocates into the nucleus and alters gene

expression (Spies and Gandolfi, 1991).

The toxicity of PCBs is decreased by addition of chlorine substituents at *ortho* positions which decreases the coplanarity between the phenyl rings. The reduced coplanarity of PCB decreases its capacity to bind to the Ah receptor and decreases induction of cytochrome P-450 (Parkinson and Safe, 1987).

PCBs are slowly eliminated from the body. The most important route of elimination is in tandem with lipids like milk. Lactating dams transfer most of their body burden of PCBs to their nursing pups, whereas the body burden of non-lactating animals remains essentially constant (Vodicnik and Lech, 1982). Kodama et al. (1980) reported that lactational transfer of PCBs to infants resulted in PCB blood concentration levels which exceeded the mothers' at 3 months postpartum and tended to increase until 1 year of age. However, the PCB levels in the blood of bottle-fed infants remained at a low concentration during these same time periods.

Because of their lipophilic property, PCBs require biotransformation to polar molecules before they can be excreted via feces and urine (Lutz and Dedrick, 1987). The cytochrome P-450-dependent monooxygenase enzyme systems involved in phase I metabolism of PCBs are located in the endoplasmic reticulum membranes of liver cells (Timbrell, 1991). These

enzyme systems require NADPH cytochrome P-450 reductase which consists of two flavoproteins that sequentially transfer electrons to hemoprotein pigments termed cytochrome P-450. These cytochromes catalyse, via redox-reactions, the insertion of a single oxygen atom into PCBs thereby converting them to compounds that are more polar, thus enhancing their excretion. In phase II reactions, PCBs induce epoxide hydrolases, glucuronyl transferases, and glutathione-S-transferases. These enzymes are catalyze conjugation reactions involving phase I products. The resulting metabolites are more water soluble and easily excreted (Borlakoglu and Haegele, 1991). Derivatives of gluthathione conjugates are major metabolic products of PCBs after biotransformation (Matthews and Dedrick, 1984). PCBs can also be metabolized by conjugation with cysteine and methionine, and then excreted in the bile.

Factors that affect the rate of biotransformation of PCBs include the number of chlorines on the biphenyl nucleus, the position of these chlorines, animal species, age, and sex (Parkinson and Safe, 1987; Sipes and Schnellmann, 1987).

PCBs and Reproduction

In the past 20 years, a wealth of information has become available on

the effects of PCBs on animal reproduction. The effects of PCBs on reproduction was first demonstrated by Hartsough (1965). Hartsough reported a higher mortality of kits when ranch mink were fed Great Lakes fish. Later, a series of studies by Aulerich (1971, 1973, 1977) indicated that PCBs and other environmental contaminants found in Great Lakes fish are related to the decrease in reproduction of mink.

Heaton et al. (1995) examined the reproductive performance of adult ranch mink fed diets containing 10, 20, and 40% of Great Lakes carp from Saginaw Bay, Michigan. Birth weights of the offspring were significantly reduced in the groups fed 20 and 40% carp in the diet. Female mink fed 40% carp had the fewest number of kits and the highest mortality. No offspring survived beyond 24 hours in this treatment group.

In a laboratory study, sexually mature female mice were treated with 0.025 mg of Clophen A-60 per day for 10 weeks. The oestrous cycle was 2 days longer and the frequency of implanted ova was 7.5% lower than in the control animals (Örberg and Kihlström, 1973). Kihlström et al. (1975) conducted a study where dams were injected with 50 mg/kg of Clophen A-60, on the day of parturition and once a week for 4 weeks. Non-exposed mice were bred with exposed mature offspring. No effects on frequency of implantation in both male and female offspring were observed. However

when exposed animals were bred with each other, the frequency of implantation decreased to 75% compared to 94% in control animals. Gellert (1978) reported that 24 hours after a single subcutaneous injection of 1000 μg/kg b.w. of Aroclor 1221 in 22-day-old Sprague-Dawley rats, the uterine weight was significantly increased by about 20 % when compared with the control group. This effect was not observed with treatment by Aroclor 1242, 1254 and 1260. Female rat pups were injected subcutaneously with 1 and 10 mg of Aroclor 1221 on the 2nd and 3rd days of life. There was precocious puberty, persistent vaginal estrus and anovulation by 6 months of age in the 10 mg Aroclor 1221 treatment group (Gellert, 1978).

Male Holtzman rats exposed to 8, 16, 32, or 64 mg/kg Aroclor 1254 during lactation were mated to untreated females at 120 days of age. Lower implantation rates were observed in the 16, 32 and 64 mg/kg treatment groups (Sager et al., 1991). Eight, 32 or 64 mg/kg of PCB (Aroclor 1254) were dissolved in peanut oil and administered orally to lactating dams on days 1, 3, 5, 7, and 9. Male offspring exposed to PCBs during lactation, were mated to untreated females at 130 day-of-age. Implantation rates were decreased 22, 59 and 64%, respectively (Sager et al., 1987). No effect on sperm production, morphology, or motility was observed in these two studies. Adult male white mice fed 50 and 200 ppm Aroclor 1254 for 15

days exhibited reduced testicular spermatozoa concentrations of about 13 percent (Sanders, 1977). Treating adult male white-footed mice (*Peromyscus leucopus*) with 400 ppm of Aroclor 1254 for 2 weeks reduced testicular spermatozoa numbers by 40% when compared to the control group (Sanders, 1975). Pregnant Holtzman rats were treated on day 15 of gestation with a single oral dose of TCDD (1.0 μg/kg). Pups were fostered and cross-fostered to produce the following groups: pups not exposed to TCDD (control), pups exposed to TCDD in utero (IU), pups exposed via lactation (L), and pups exposed both in utero and via lactation (IUL). Epididymal sperm concentrations from 63-day-old rats were decreased 64, 48, and 49%, in the IU, L, and IUL groups respectively, when compared to the control (Bjerke and Peterson, 1994). Thirty-one-day-old male Fisher rats were treated with 0, 0.1, 1, 10, or 25 mg/kg/day Aroclor 1254 by gavage for 5, 10, or 15 weeks. Cauda epididymal sperm storage was reduced by approximately 20% in the 25 mg/kg/day, 15 week exposure group. No effect was observed on sperm motility and daily sperm production in other treatment groups (Gray et al., 1993). In another study, dams were fed a diet of 60% Great Lakes carp, from parturition through lactation. Offspring of these dams received the same diet until termination. No alteration in sperm concentration, motility or reproductive performance

were observed at 6, 7, 8, 15, 23, 32 or 34 weeks (Kuo, 1994). Huang (1995) performed a study where dams received 0, 3, or 30 ppm TCB through mating, gestation, and lactation. Male offspring received the same diet through the entire study. No differences were observed in epididymal sperm concentration and sperm motility in any of the treatment groups at 9 and 19 weeks. However, sperm fertilizing ability decreased in the 30 ppm treatment group at 19 weeks.

Kholkute et al. (1994) reported that Aroclor 1221, 1254 and 1268, added to *in vitro* fertilization medium at various concentrations (0.01, 0.1, 1, and 10 μg/ml), inhibited fertilization and increased the incidence of degeneration of oocytes and abnormalities in the early mouse embryos at doses of 1 and 10 μg/ml. Mousa et al. (1996) examined the effects of Aroclor 1242 and 1254 on B_6D_2F1 mouse sperm fertilizing ability. In this study, 0, 10, and 20 ppm Aroclor 1242 and 1254 were added to the *in vitro* fertilization medium, BMOC-3. In the absence of PCBs, few eggs (1-7%) degenerated and most eggs (83-86%) were fertilized. At the high dose (20 ppm), Aroclor 1242 and 1254 caused all eggs to degenerate and completely eliminated fertilization. At the low dose (10 ppm) of Aroclor 1242, fertilization rates were 35% and few eggs degenerated. Exposure to 10 ppm of Aroclor 1254 resulted in 21% fertilization and 66% egg degeneration.

In Rhesus monkeys, disturbances in the menstrual cycle, reduced conception rates, diminished ability to maintain pregnancy and low birth weights were observed when adult animals consumed diets containing 2.5 and 5.0 ppm of Aroclor 1248 for 4 and 7 months. The total PCBs consumed ranged from 60 to 120 mg for the 4 months treatment group, and 105 to 210 mg for the group treated for 7 months (Barsotti et al., 1976).

In human female "Yusho" patients who inadvertently consumed PCBs through contaminated rice oil, 60% showed abnormalities of menstrual cycle length, 55% had menstrual cycles of abnormal duration, and 85% had abnormal basal body temperature patterns. Urinary estrogen and pregnanediol concentrations in these exposed women were lower than the non-exposed women (Truelove et al., 1990). During the "Yusho" epidemic, 11 women with Yusho and 2 wives whose husbands had Yusho, but who themselves did not show the disease, delivered 10 liveborn and 2 stillborn babies. Most of the fetuses born were smaller than the national standards (Kimbrough, 1974). Similar exposure to PCBs occured in the "Yu-Cheng" patients in Taiwan. One hundred and fifty-nine pregnant women were exposed to PCBs during this outbreak. Three were pregnant when the surveyed was given, 5 miscarried, 8 were aborted, 6 were stillborn, 5 were born live and later died, and 132 infants lived (Rogan et al., 1988).

Women who ate an average of 6.7 kg of fish from Lake Michigan every year for an average of 16 years are reported to have had shorter gestational periods, and gave birth to children with low birth weights and small head circumferences. The exposed infants were 4.9 days less in gestational age, 160 to 190 gm lighter than controls, and their heads were 0.6 to 0.7 cm smaller (Fein et al., 1984). These children showed discernible cognitive motor and behavioral deficits when tested at 7 months and 4 years compared to infants born to women who had not consumed Lake Michigan fish prior to or during their pregnancies (Jacobson and Jacobson, 1993; IJC, 1993).

Wassermann et al. (1982) compared 17 cases of premature delivery to 10 women with normal pregnancy. Seventeen of the premature deliveries, 8 were associated with high serum PCBs concentration (128.0 ppb versus 19.25 ppb in the control group) and 5 cases were associated with high serum DDT concentration (119.6 ppb versus 26.5 ppb). In some of these cases, high levels of heptachlor epoxide and dieldrin were also found.

Dichlorodiphenyltrichlorethane (DDT)

Dichlorodiphenyltrichlorethane (DDT) is another environmentally

persistent pollutant in the Great Lakes. It was first synthesized by Zeidler in 1874. In 1939, Paul Muller discovered the insecticidal properties of DDT. It became the most popular pesticide for agriculture and household use from the late 1940s until the early 1970s. Based on ecological considerations, DDT has been banned in the U.S. since 1972.

DDT is an organochlorine insecticide. It exists as a crystalline solid and is tasteless and almost odorless. The term DDT is generally understood throughout the world and refers to *p,p'*-DDT (1,1,1-trichloro-2,2-bis[*p*-chlorophenyl]ethane). The compound structure permits different forms, such as *o,p'*- and *o,o'*- isomers (WHO, 1989). The major DDT metabolite, *p,p'*-DDE (1,1-dichloro-2,2-bis-[*p*-chlorophenyl]ethylene) is more persistent than the parent compound, and is therefore the form normally found in foods and humans tissues. Although DDT has been banned in the U.S. since 1972, DDT and its metabolites are still found in the Great Lakes. These products probably arise from the lake bottom sediments, contaminated tributary sediments, runoff from sites of historical use, leaking landfill sites, illegal use of old stocks, and long range transportation through the atmosphere.

DDT enters organisms through inhalation, ingestion, and dermal absorption. Although the dermal absorption of powder DDT is small, it is

easily absorbed by the skin when dissolved in oil or an organic solvent (Murphy, 1980; Wester and Noonan, 1980; Kaloyanova and El Batawi, 1991). Variations are observed in the acute oral toxicity of DDT in different species of animals. For example, the acute oral LD50 in rats is 250 mg/kg, mice have a value of 400 mg/kg and monkeys and humans have a value of 200 mg/kg (Hrdina et al., 1975). In mammals, DDT mainly affects the nervous system causing hyperexcitability, tremors, ataxia, seizures, and convulsions (Kaloyanova and El Batawi, 1991). Death from DDT poisoning is most often attributed to respiratory arrest (Hayes, 1971). DDT also affects the liver, by increasing P-450 enzymes or producing necrosis and tumors. In acute poisoning, liver glycogen is mobilized quickly and later exhausted resulting in an increase of circulating lactic acid and a compensated acidosis (Hayes, 1971).

In mammals, DDT is believed to be metabolized to DDE (1,1-dichloro-2,2-bis-[*p*-chlorophenyl]ethylene) without the formation of intermediary metabolites or after several stages of biotransformation to DDA (2,2-bis[4-chlorophenyl] acetic acid). Both are excreted in the urine (Kaloyanova and El Batawi, 1991).

DDT and Reproduction

DDT, or more specifically, its degradation product DDE, can lower the reproductive rate of birds by causing eggshell thinning that leads to egg breakage during incubation which causes embryo deaths. Laboratory experiments established that DDE inhibits calcium adenosine triphosphatase (Ca-ATPase) in the eggshell gland, thereby reducing the transport of calcium to the site of eggshell formation (Kolaja and Hinton, 1977). DDT also inhibits carbonic anhydrase (CA). Carbonic anhydrase is believed to be a necessary source of carbonate ions required for calcium carbonate deposition (Bitman et al., 1970). Bitman et al. (1970) observed that when CA activity was low, soft-shelled eggs or no eggs were observed.

When environmental levels of DDE decreased to less than the critical concentration for eggshell thinning, the population of some of the fish-eating water birds increased (Giesy et al., 1994; Burger et al., 1995). However, different species of birds are different in their sensitivity to these chemicals. For example, in the bald eagle (*Haliaeetus leucocephalus*) eggshells containing 4.0 and 16.0 μg/g DDE (wet weight) were associated with 10 and 15% thinning, respectively (Wiemeyer et al., 1993). Bald eagle eggshells collected from 15 states in the United States in 1980-84 showed that production of young was normal when eggs contained less than 3.6 μg/g DDE (wet weight), production decreased nearly 50 % when DDE

concentrations were between 3.6 to 6.3 μg/g, and decreased 75% when concentrations exceeded 6.3 μg/g (Wiemeyer et al., 1993). Captive black ducks (*Anas rubripes*) fed dietary DDE at 10 ppm for two breeding seasons produced eggs with approximately 20 % thinner shells than untreated controls. The mean DDE concentration in the eggshell was 64.9 ppm (Longcore and Stendell, 1977). Robson et al. (1976) did not observe any effects of feeding 100 or 300 ppm DDE for eight consecutive 28 day periods on eggshell thickness of Japanese quail.

The *o,p'*-DDT is a weakly estrogenic compound. Immature Sprague-Dawley rats intraperitoneally (ip) injected with 500 μg *o,p'*-DDT daily for 27 days had early vaginal opening and increased ovary and uterus weights (Gellert et al., 1972). When mature ovariectomized rats were injected ip with 10 mg/day of *o,p'*-DDT or *p,p'*-DDA for 7 days, increased uterine weight, cornified vaginal smears, and reduced serum LH concentrations were observed (Gellert et al., 1972). Mason and Schulte (1980) reported that treatment of ovariectomized adult rats with 10 mg *o,p'*-DDT or 1 μg estradiol for 3 days doubled the number of progesterone receptors in the uterine cytosol and increased uterine weight when compared to the control group. NMRI mice treated with 40 mg/kg DDT by intraperitoneal injection showed a significant increase in the length of the estrous cycle. The

prolongation of the estrous cycles appeared to decrease with time and the lengths returned to normal after three cycles (Orberg et al., 1972). Ireland et al. (1980) showed that *o,p'*-DDT (250 mg/kg) increased uterine DNA content and DNA synthesis in ovariectomized immature rats to the same extent as 40 μg/kg of estradiol.

Kihlström et al. (1975) injected dams with 50 mg/kg of *p,p'*-DDT, beginning on the day of parturition once a week for 4 weeks. Non-exposed offspring were bred with exposed animals. No effects on frequency of implanted ova were reported. When exposed animals were bred with each other, the frequency of implantation decreased to 79% compared with 94% in the control group.

Eight-week-old male mice received an oral dose of 2x150 mg/kg of body weight DDT over 2 days. Lethal mutations were induced in early spermatid and spermatocyte stages. An increase in spermatocyte chromosome breakage, stickiness and precocious separation of the X and Y bivalent were observed (Clark, 1974). Chronic oral doses of DDT (2x100 mg/kg/BW/week) for 10 weeks caused changes in seminiferous tubule morphology and degeneration of B-type spermatogonia in 8-week-old mice (Clark, 1974).

The major and persistent DDT metabolite, *p,p'*-DDE, has little ability

to bind to the estrogen receptor, but has been shown to have antiandrogenic effects. Weanling (21-day-old) male rats treated with 100 mg/kg/day of *p,p'*-DDE until puberty (age at preputial separation) had a significant 5-day delay in the onset of puberty compared to control rats (Kelce et al., 1995). In additional studies, 120-day-old male rats were castrated and implanted with testosterone-containing Silastic capsules to maintain constant serum androgen levels. The animals were then treated with 200 mg/kg/day *p,p'*-DDE by gavage for 4 days. Significant reductions in androgen-dependent seminal vesicle and prostate weights were observed (Kelce et al., 1995).

Mercury

Mercury is widely used within the industrial, medical, agricultural, and consumer sectors; over 2,000 application have been identified. The major source of mercury is the natural degassing of the earth's crust, including land areas, rivers and the ocean. Other sources of mercury include mining, burning of fossil fuel, smelting, paper pulp, fungicides, and electrical and industrial discharge. Global anthropogenic sources of mercury have reached about 8,000 to 10,000 tons per year since 1973. Nonanthropogenic sources are still the predominant sources and contribute

25,000 to 150,000 tons per year (WHO, 1976). Natural sources of mercury may contribute from one quarter to one half of the total load of mercury to the Great Lakes (IJC, 1993). The atmosphere is an important pathway for the mercury load to the Great Lakes. The estimated mercury load to Lake Superior, Lake Michigan, Lake Huron, Lake Erie, and Lake Ontario from the atmosphere is 2,181, 1,568, 1,584, 723, and 568 kg/year, respectively (IJC, 1993).

Mercury can exist in three forms: elemental, inorganic and organic. All three forms are toxic. Elemental mercury (Hg^0) may be absorbed by biological systems as a vapor and is relatively lipid soluble. It is readily absorbed from the lungs following inhalation and is oxidized in the red blood cells to mercuric ion (Hg^{2+}) (Timbrell, 1991). Before oxidation to Hg^{2+}, elemental mercury is rapidly transported in red blood cells, across the blood-brain barrier and into the central nervous system (CNS) and may seriously damage the CNS (Mitra, 1986). Elemental mercury also rapidly crosses the blood-placenta barrier into the fetus (Mitra, 1986). However, the elemental form of mercury is poorly absorbed from the gastrointestinal tract. Inorganic mercury exists as monovalent or divalent ions and is also poorly absorbed from the gastrointestinal tract. Absorption from food is less than 15% in mice and about 7% in humans (Goyer, 1991).

Organic mercury is readily absorbed (90-95%) from the gastrointestinal tract (Neathery and Miller, 1975.; Timbrell, 1991). Organic mercury can cross the blood-brain and blood-placenta barriers. One of the most significant effects of mercury pollution is that aquatic organisms can biotransform the inorganic mercury ions into methyl and dimethyl mercury. These forms of mercury are lipid soluble. They can enter the food chain through the lowest trophic levels to the top predators.

Methyl mercury is the most important form of mercury in terms of health effects from environmental exposures (Goyer, 1991). It is mutagenic, teratogenic, and carcinogenic (Eisler, 1987). Mercury concentrations in large carnivorous fish, such as pike and swordfish, have been found to exceed 1,000 ug/kg (E.P.A., 1984). Therefore, fish consumption is the most important source of general population exposure to mercury.

Mercury distribution in tissues is dependent upon chemical form and entry route. Metallic mercury is slowly absorbed by the gastrointestinal tract at a rate of 0.01 percent when compared to elemental Hg vapor through inhalation (Goyer, 1991). In orally dosed calves, absorption of radioactive methyl mercury ($CH_3{}^{203}HgCl$) was 100 times higher than absorption of the inorganic form ($^{203}HgCl_2$) (Ansari et al., 1973). The highest levels of methyl mercury were found in the kidneys and the second

highest levels are found in the liver. Fourteen days following oral administration of 10 μmol $^{203}HgCl_2$ to female mice, the residual body burden in the kidneys, liver carcass, and brain were 33.76%, 21.27%, 34.33%, and 1.36%, respectively (Nielsen and Andersen, 1990).

Mercury is eliminated from the body in the urine and feces, with the latter being the major route. Methyl mercury is 90% excreted in the feces via the bile as a cysteine conjugate, which undergoes extensive enterohepatic recirculation with a mean half-life in the blood of approximately 70 days.

The major human health effects of mercury are neurotoxic effects in adults (Bakir et al., 1973) and toxicity to the fetuses of mothers who are exposed to methyl mercury during pregnancy (Cox et al., 1989). Clinical manifestations of neurotoxic effects in adults include parathesia, ataxia, vision and hearing loss, tremor, and finally coma and death (Goyer, 1991).

Mercury and Reproduction

Mercury has been shown to alter reproduction in rodents. Single intraperitoneal injections of 2.0 mg/kg of mercuric chloride to female mice one-half to four days before mating, increased the incidence of dead implants (Suter, 1975). Oral treatment of 30 day old female rats with 1 ml of 100 mg/100 ml mercury as mercuric chloride ($HgCl_2$) solution daily for

12 weeks has been reported to disturb the estrus cycle. The disturbances were manifested by the prolonging of the diestrus phase up to 10 days, compared to the normal 4 to 5 days (Stadnicka, 1980). When golden hamsters (*Mesocricetus auratus*) were subcutaneously injected with 1 mg of mercuric chloride per day during one estrous cycle, 60% of the animals did not ovulate by day 1 of the third cycle when the total amount of mercuric chloride injected was 3 or 4 mg (Lamperti and Printz, 1974). Lee and Dixon (1975) observed that CDF1 male mice receiving a single intraperitoneal injection of 1 ppm mercuric chloride had decreased fertility and thymidine incorporation by spermatogonia.

Methylmercury is easily transported to the fetus via the placenta. The concentration of mercury in the fetal blood is about 20% higher than the maternal concentration (Report of an International Committee, 1969). Pregnant Swiss-Webster mice exposed to methylmercuric chloride (CH_3HgCl) 5 mg/kg/day orally as a single daily dose from days 6 to 17 of gestation showed no clinical symptoms of mercury poisoning. However, this resulted in 100 % stillbirth or neonatal death (Khera and Tabacova, 1973).

Male Wistar rats were administered single daily doses of 0, 1, 2.5 and 5 mg methylmercuric chloride/kg/day for 7 consecutive days. After dosing

was discontinued, the reproductive performance was examined by mating treated rats with nontreated adult virgin female rats. A dose-related reduction in litter size per pregnancy was observed during days 5-20 following treatment (Khera, 1973). In the same study, male Wistar rats were dosed daily with 0.5 mg/kg or 1 mg/kg methylmercuric chloride. Serial mating trials were programmed together. After dosing for 25-30 days at 1 mg/kg and after 85-90 days at 0.5 mg/kg, a reduction in average litter size due to preimplantation losses was noted.

An epidemiologic study was conducted with people living in Sweden who were exposed to methylmercury via the consumption of fish. All subjects in the exposed group had more than three meals a week of contaminated fish (0.5-7 mg mercury as methylmercury/kg fish) for more than 3 years. A correlation was found between the mercury levels of blood cells and the frequency of chromosome breakage in circulating lymphocytes cultured *in vitro* (Skerfving et al., 1970, Skerfving et al., 1974).

HYPOTHESIS

While a number of studies have examined the effects of PCBs, DDT, and mercury on reproduction, it is not clear what effects long-term exposure of male mice to low-doses of environmental mixtures of contaminants will have on reproduction. In a previous study, mice were exposed to Great Lakes fish via lactation and diet from birth through 34 weeks of age. No adverse effects on reproductive performance in pubertal and mature male mice were observed (Kuo, 1994). The present study expands on our earlier research. This study examines the effects of Great Lakes contaminants on post-maturation mice (through 15 months of age) and the effects of these contaminants on the onset of puberty in female mice. The hypothesis of the present study is that long-term exposure to Great Lakes contaminated fish will decrease sperm production, compromise sperm fertilizing ability, and delay female puberty.

MATERIAL AND METHODS

Animals

C57BL/6J females (F-0) and DBA/2J (F-0) male mice were mated to produce B_6D_2-F_1 offspring (F-1). The breeders were purchased from Jackson Labs, Bar Harbor, Maine. Mice were housed in the University Laboratory Animal Resources facilities at Michigan State University in groups of four or fewer per cage. Room temperature was maintained at 23°C, with 50%-70% relative humidity, and a light/dark cycle of 12 hours light and 12 hours dark.

Diet Preparation

There were three treatment diets, prepared on a wet weight basis: C (82% ground lab chow with 18% fish oil), I (60% Iowa carp with 40% ground lab chow), and G (60% Great Lakes carp with 40% ground lab chow). Both diets C and I served as control diets in this study. Lab Mouse Chow #5015 was purchased from Purina Mills Inc. (St. Louis, MO). For the fish control diet, carp (*Cypinus carpio*) fillets and fish oil, intended for

human consumption, were purchased from an Iowa fish farm (Stoller Fisheries, Spirit Lake, IA). Great Lakes carp (*Cypinus carpio*) were caught from the mouth of the Saginaw River in Michigan. Fish were cooked at 77°C for 30 minutes to inactivate thiaminase (Gnaedinger, 1963; Gnaedinger and Krzeczkowski, 1966). Bones were removed from the Great Lakes carp before diet preparation.

Forty percent ground lab chow was added to 60% Great Lakes carp or Iowa carp to make diets G and I, respectively. The addition of fish oil to diet C was intended to adjust the caloric content to match that of diets I and G (Table 1). Major nutrients and minerals were balanced to meet NRC requirements (NRC, 1978). The mineral sources of P, K, Mg, Na, Fe, Mn, Cu, and Zn were from $CaHPO_4$, KCl, $MgSO_4.7H_2O$, NaCl, $FeSO_4.7H_2O$, $MnSO_4.H_2O$, $CuSO_4.5H_2O$ and $ZnSO_4.7H_2O$. Calcium was calculated based on two sources: $CaCO_3$ and $CaHPO_4$. Vitamins A, D, E, K_1, B_6, biotin, choline, folacine, niacin, pantothenate, riboflavin, and thiamine were also used in the diet formulation. Nutrient analysis of the final diets was conducted by Litchfield Analytical Services (Litchfield, MI. Table 1). Analysis of organochlorine pesticides and PCBs in the diets was conducted by the Environmental Laboratory, Michigan Department of Natural Resources (Lansing, MI) using gas chromatography (HP5089)/ECD

(Electron capture detector), according to EPA SW-846 method 8081.

Chemicals and Culture Medium

Pregnant mare serum gonadotropin (PMSG), human chorionic gonadotropin (HCG), $CaCO_3$, $CaHPO_4$, $MgSO_4.7H_2O$, NaCl, $FeSO_4.7H_2O$, and pyridoxine hydrochloride were purchased from Sigma Chemical Co. (St. Louis, MO). Fiber (alphacel non-nutritive bulk), vitamins A, D, E, K_1, B_6, biotin, choline, folacin, niacin, pantothenate, riboflavin, and thiamine were obtained from ICN (Aurora, OH). Brinster's medium for oocyte culture (BMOC-3), sperm capacitation, and *in vitro* fertilization was purchased from Gibco Life Technologies, Inc. (Gaithersburg, MD).

Experimental Design

Female (F-0) mice were randomly assigned to one of three treatment groups upon birth of the F-1 offspring. Litter size was adjusted to eight or nine pups per litter one day after birth, before exposure to the treatment diets. Dams were fed the treatment diets from parturition through lactation. F-1 mice were fed the same treatment diet as their dams from weaning until the termination of the study. Prior to weaning, F-1 mice also had access to the dams' diet. At weaning, mice were sexed, weighed, and transferred to

cages which housed four or fewer mice per cage.

Data Collection

Body weight Body weights of lactating F-0 dams were recorded 3 weeks after parturition. Body weights of F-1 male mice was recorded at 3, 5, 6, 7, 8, 10, 15, 20, 25, 30, 35, 40 and 44 weeks of age. F-1 female body weight was recorded at 3, 5, 6, 7 and 8 weeks of age.

Feed intake Feed intake was recorded for 5 consecutive days at 4, 8, and 23 weeks of age. Actual feed consumption was calculated daily by subtracting the amount of feed left in the feed jars from the total feed provided on the day before and dividing by the number of mice per cage.

Organ weight Liver and thymus weights of lactating F-0 dams were recorded 3 weeks after parturition. Liver, testis and thymus weights of the treated F-1 males were recorded at 53 and 64 weeks of age. In F-1 females, liver and thymus weights were recorded at 4, 5, 6, and 8 weeks of age.

Vaginal opening Beginning at 27 days of age, F-1 females were examined daily for vaginal opening.

Natural breeding Fecundity, litter size, sex ratio, were recorded for both F-0 and F-1 males. Forty-eight and 53-week-old treated F-1 males

were individually paired with non-treated mature B_6D_2-F_1 females for 7 days between 7:30 PM to 7:30 AM, during which time feed was withheld to avoid exposing females to the treatment diets. After breeding, females were housed individually throughout gestation and lactation. Natural breeding data for 48 and 53 week old mice were combined and reported as one-year old natural breeding data, individual data for 48 and 53-week-old mice are reported in appendix V.

Survival Four day survival and 21-day survival were recorded for F-1 and F-2 mice. F-1 male mice survival was recorded at 10, 20, 30, and 44 weeks.

In Vitro Fertilization (IVF) Assay

After natural breeding, male mice were sacrificed by cervical dislocation for *in vitro* fertilization studies. Sperm were collected from the caudal epididymis. Both of the epididymides were placed in the inside well of a Falcon Organ Tissue culture dish (60x15 mm style with a center well, #3037, Becton Dickinson Labware, Lincoln Park, New Jersey) containing 1.0 ml Brinster's medium (BMOC-3) (Gibco Life Technologies, Gaithersburg, MD), a capacitation supporting medium. The outside well contained 3 ml BMOC-3 without bovine serum albumin (BSA) for

stabilizing moisture and temperature. The epididymides were pierced with a 25 g PrecisionGlide needle to release the sperm into the medium. The culture dish was then incubated at 37.5° C, in 5% Co_2 in air, and 100% humidity for one and a half hours before sperm were used for insemination. Oocytes were collected from non-treated female mice which were superovulated by intraperitoneal injection of 10 IU pregnant mare serum gonadotropin (PMSG) followed 48 to 50 hours later by 10 IU human chorionic gonadotropin (HCG). Twelve to fourteen hours after HCG injection, oviducts were removed from mice and, transferred to a Falcon Organ Tissue culture dish, containing 1 ml BMOC-3 medium. The ampulla portions of the oviducts were teased open to allow the cumulus mass to be released into the medium. Cumulus masses were transferred to another culture dish and placed in the center well containing 1 ml BMOC-3 medium; the outer well contains BMOC-3 without BSA. The pre-incubated sperm concentration was adjusted to the final concentration of 30,000 sperm/ml and transferred to the oocyte-containing well, incubated for 24 hours before examination of fertilization. The inseminated oocytes were then stained with 50 uM bisBenzimide for at least 30 minutes to allow for uptake and intercalating with DNA. Oocytes were transferred to glass slides for assessing fertilization using a Nikon Optiphot microscope equipped with a

100w mercury bulb, 365/10 nm excitation filter, 400 nm dichroic mirror, and 400 nm barrier filter. Oocytes at the two cell stage or at the one cell stage containing a second polar body were considered fertilized. Degenerated eggs were considered non-fertilized.

Sperm Concentration and Motion Analysis

Sperm concentrations were measured either manually or by computer. Sperm concentration was measured manually by placing 20 μl sperm suspension in a Petroff Hausser Counting Chamber (Hauser Scientific Co.,Horsham, PA) containing 16 cells. Sperm were counted in 5 randomly chosen cells and the total was used to calculate the final concentration (Appendix II). Computer assisted sperm concentration and motion analysis was performed at the same time of insemination using the CellSoft computer-assisted digital image analysis system (CRYO Resources Inc., New York, 1986). Twenty μl of the sperm suspension (approximately 2.4 x 10^5 sperm cells) were placed in a CellSoft 20μm chamber and a minimum of 100 sperm cells were analyzed to obtain measurements of concentration, motility, velocity, linearity, mean amplitude of lateral head (ALH) displacement, and beat/cross frequency (Appendix III).

Statistical Analysis

Quantitative data (body weight, organ weight, fertilization rate, feed intake, and ALH displacement) were tested with One-Way Analysis of Variance (ANOVA). Qualitative data (fecundity, survival index, and sperm motility) were evaluated with the Chi-square test. Log transformations were made for the discrete quantitative parameters and ratio data (litter size, sperm concentration, beat/cross frequency, sperm linearity and sex ratios) before ANOVA. These analyses were performed with Sigmastat® (Kuo et al., 1992). All statements regarding significance are based on $p < 0.05$.

RESULTS

Of all the contaminants analyzed for in the diets, only *p,p'*-DDE and PCBs were present at levels above the detection limits (240 and 2400 ppb, respectively). Diet G contained 300 ppb *p,p'*-DDE and 2500 ppb PCBs. These compounds were not detected in diets C or I (Appendix IV).

There were no significant differences in the body weights of F-0 lactating females (Table 2). Decreases in the body weights of F-1 females on diet G were observed at three, five, six, and eight weeks of age (Table 3, Figure 1). In F-1 males on diet G, there were significant decreases in body weights at all ages examined when compared to mice on either diet C or I (Table 4, Figure 2). Body weight differences were also observed between the two control groups; body weights of F-1 males on diet I were less than those on diet C from 10 weeks of age through 44 weeks of age (Table 4). Feed intake of F-1 males on diet G was less than that of mice on diet C at four and 23 weeks of age, but not at eight weeks of age (Table 5). F-1 males on diet G at 53 and 64 weeks of age also had lower body weights than those on diet C (Tables 6, 7). As the mice aged, the body weights of

mice on diet G decreased about 10% from 53 to 64 weeks of age, while those on diets C and I were unchanged.

There were no significant differences among treatment groups in the organ weights of F-0 lactating females (Table 2). There were, however, significant differences in the organ weights of F-1 females among treatment groups (Table 8, 9, 10, 11). Relative organ weights (percentage of body weight) are reported here because of the differences in body weights. Relative liver weights were greater in mice on diet G compared with those from mice on diet C at four weeks of age, but not when compared with those on diet I (Table 8). Relative thymus weights of mice on diet G were less than those of mice on diet I at five weeks of age (values for diet C were not available) (Table 9). At six weeks of age, relative thymus weights of mice on diet G were also less than those on diet I, although not different from those on diet C (Table 10) . In F-1 males, differences in organ weights were also observed at 53 and 64 weeks of age (Tables 6, 7). Relative liver weights of 53-week-old F-1 males on diet G were greater than those on diet I which, in turn, were greater than those on diet C (Table 6). The same trend in liver weights was also observed in 64-week-old F-1 males (Table 7). Relative testes weights of 53-week and 64-week-old F-1 mice on diet G were similar to those of mice on diet C, but 45% less than those on

diet I (Tables 6, 7).

The survival of offspring from F-0 females fed different treatment diets is shown in Table 12. The four-day survival was lower in pups on diet G when compared with pups on diet I. However, survival of pups on diet G was not different from that of pups on diet C. The 21-day survival was less for pups of dams on diet G than those on either diets C or I. After weaning, male mice in all treatment groups survived through 20 weeks of age. Between 20 and 40 weeks of age, 56, 91, and 100% survival was observed in mice on diets G, I, and C, respectively (Table 13).

The age of vaginal opening in F-1 females was significantly delayed by exposure to diet G (Table 14). Vaginal opening was observed on day 28.4 $\pm$ 0.5 and 30.4 $\pm$ 1.1 in diet C and I treatment groups, respectively. In the diet G treatment group, a 16-day delay in vaginal opening was observed when compared with that on diet I, and there was an 18-day delay in vaginal opening when compared with that of diet C.

The treatment diets had a significant effect on F-1 male reproductive performance, sperm quality, and sperm fertilizing ability when assessed at 53 and 64 weeks of age. At one year of age, while there were no differences in litter size and sex ratio of the offspring, the fecundity was lower when males on diet G were paired with non-treated females (Table

15, Appendix V). Epididymal sperm concentration was significantly lower in 53- and 64-week-old F-1 mice on diet G when compared with mice on either diet I or C, assessed either manually or with the computerized image analyzer (Table 16). A trend towards decreased sperm motion parameters was observed at both 53 and 64 weeks of age, although only those at 15 months of age showed statistical significance (Tables 17, 18). In addition, the *in vitro* fertilizing ability of sperm from mice on diet G, when adjusted to the same concentration in the fertilization medium, was only about 10% of that from mice on diet C and I (Table 19).

DISCUSSION

Body Weights

While there were no differences among the treatment groups in the body weights of lactating F-0 females, there was a 10 to 40% decrease in post-weaning body weights of F-1 males and females on diet G. This decrease may, in part, be due to decreased feed intake, as observed in F-1 males at four and 23 weeks of age. In a previous study in our laboratory (Kuo, 1994), in which mice were also fed Great Lakes carp, no differences in body weights were observed despite identical experimental designs. In other rodent studies, conflicting results were also reported when either PCBs or DDT were examined for their effects on body weight. Reduced body weight gain was observed in rat offspring following daily oral maternal exposure to 30 mg Aroclor 1254/kg body weight for one month (Brezner et al., 1984), while no body weight changes were observed in F-1 mice exposed to 3 or 30 ppm TCB in the diet (Huang, 1995). Decreases in body weights were observed in the offspring of mice receiving 5 or 10 ppm DDT (Shabad et al., 1973; Ledoux et al., 1977). In contrast, increased body

weights were observed in weanling male rats gavaged daily with 100 mg/kg body weight *p,p'*-DDE until after puberty (Kelce et al., 1995). No changes in body weight were observed in either newborn or young rats injected with DDT (Gellert et al., 1972, 1974). Thus, no consistent pattern can be elicited to explain the various results in body weights.

Organ Weights

As reported in the present study, Cleland et al. (1987) observed an increase in liver weights in two strains of male mice (C57BL/6 and DBA/2) fed diets containing 33% coho salmon from Lake Michigan and Lake Ontario for four months following weaning. These mice also had elevated ethoxyresorufin O-deethylase activity compared to mice fed a control mouse chow diet and mice fed a diet containing Pacific coho salmon. Male mink fed Great Lakes carp diet also had greater liver weights than controls (Restum et al., 1997). A 25% increase in liver weight was observed in female C57BL mice given weekly ip injections of 100 mg TCB/kg body weight for four weeks beginning at five to seven weeks of age (Brouwer and van den Berg, 1984) and in female rats given a daily oral dose of 5 mg/kg body weight for three weeks (Clarke et al., 1984). Female C57BL/6J mice fed 30 ppm TCB in feed for eight weeks, two weeks pre-breeding and

during pregnancy and lactation, had a 15% increase in liver weight (Huang et al., in press [b]). Furthermore, in the same study, liver weights increased 19% in six-week-old female offspring and 27% in 19-week-old male offspring when compared to controls (Huang et al., in press [a,b]). Offspring of female rats treated orally with a single dose of 1 μg TCDD/kg on gestation day eight had greater liver weights than controls (Gray and Ostby 1995). As for DDT and its metabolites, male rats receiving 100 or 300 mg o,p'-DDD/kg body weight had greater liver to body weight ratios than controls (Straw et al., 1965) as did male monkeys given 10 or 20 mg DDT/kg/day for seven days (Juchau et al., 1966). Therefore, increases in liver weights are frequently observed in many species of animals exposed to PCBs and DDT as well as to Great Lakes contaminants.

The decrease in thymus weights in female offspring reported here was also observed when mice were exposed pre- and post-natally to 30 ppm TCB in feed (Huang, 1995). However, in the dams and male offspring, no decreases in thymus weights were observed in the present study or in mice exposed to TCB as reported by Huang (1995). PCBs have been shown in other studies to reduce thymus weights in adult female rats and mice (Brouwer and van den Berg, 1984; Clarke et al., 1984). PCB exposure has also been shown to decrease cortical lymphocytes of the thymus and

suppress cytotoxic T cell generation (McConnell and Moore, 1979; Clarke et al., 1984). Male and female mice exposed to 1.5 to 3.0 μg TCDD/kg body weight during gestation also had significant thymic atrophy (Blaylock et al., 1992). In addition, male mice (44-days-old) exposed to 15 - 60 μg TCDD/kg on gestation day 14 had decreased thymus weights when compared to the controls (Theobald and Peterson, 1997). Thymus weights have been reported to be reduced in third generation rat pups (males and females) exposed to 0.01 μg TCDD/kg/day in the diet (Murry et al., 1979). Based on these observations, it appears that for PCBs females are more susceptible than males to thymus effects, although both males and females appear to be equally affected by TCDD.

Changes in testes weights after PCB exposure have been observed in many studies. These alterations, whether an increase or decrease in testes weights appear to be dependent on the time of exposure. It has been hypothesized that the increase in testes weight resulting from PCB exposure is mediated through hypothyroidism when it is induced neonatally between four and eight days after birth (Cooke et al., 1992; Stone, 1995; Cooke et al., 1996). Rats injected with PCBs from birth to 25 days of age had increased testes weights while those injected with PCBs on days 12 to 37 of age showed no change in testes weights (Cooke et al., 1996). Increased

testes weights were also observed in six-month-old rats whose dams were gavaged with Aroclor 1260 (30 mg/kg body weight) during gestation (Gellert and Wilson, 1979) and in 23-week-old rats whose dams were dosed with Aroclor 1254 (32 and 64 mg/kg body weight) during lactation (Sager et al., 1983, 1991). Mice exposed to 20 and 40 mg/kg 2,2',4,4',5,5'-hexachlorobiphenyl on gestation day 13 through post natal day 24 had increased testes weights when examined at 10 to 12 weeks of age (Johansson, 1987). No increase in testes weights have been observed when the treatment occurs after the window of time (post-natal day four to eight) when the animals appear to be susceptible. In addition, the increase in testes weights are only observed when there is a recovery period, approximately two months or longer, between exposure and when the testes are examined. When four-week-old male rats were gavaged daily with Aroclor 1254 (25 mg/kg body weight) for five, 10, or 15 weeks and examined immediately at the end of treatment, no changes in testes weights were observed (Gray et al., 1993). Likewise, testes weights were unaffected in adult rats fed five, 50, or 500 ppm Aroclor 1254 for two, three, or five weeks (Garthoff et al., 1977), nor were there any changes in testes weights in adult mice fed diets of containing 62.5 to 4000 ppm Aroclor 1254 for two weeks (Sanders et al., 1974, 1977; Sanders and

Kirkpatrick 1975).

In the present study, when lactating dams and the offspring were treated continuously with diet G, no changes in testes weights were observed in the offspring. While these mice were exposed during the sensitive period, the continuous exposure did not allow for recovery. In addition, the mice in this study were exposed to a complex mixture of contaminants including compounds that are known to decrease testes weight. For example, DDT has been shown to decrease testes weights in laboratory animals. Eight-week-old mice orally dosed with 200 mg DDT/kg/week for 10 weeks had decreased testes weights (Clark, 1974).

Female Reproduction

Consistent with our findings, decreases in neonatal survival have been observed in several other species exposed to Great Lakes contaminants. Similar effects have also been observed after PCB or dioxin exposures. Restum et al. (1997) reported reduced survival in mink kits of dams fed Great Lakes carp containing 1.0 ppm PCBs. Decreased offspring survival was also observed in female mice fed 30 ppm TCB (Huang, 1995), in white-footed mice fed 10 ppm Aroclor 1254 (Linzey, 1987), and in rats fed 0.01 μg TCDD/kg/day (Murry et al., 1979). Continuous dietary exposure of

both sexes to 100 ppm DDT also resulted in decreased neonatal survival between day four and day 30 (Del Pup et al., 1978).

In this study, female offspring on diet G exhibited a delay in the onset of vaginal opening. This delay may, in part, be related to the lower body weights. Nonetheless, PCBs alone could delay vaginal opening without changing body weight. Inglis et al. (1997) observed a significant delay in vaginal opening in F-1 mice of dams orally gavaged with 32 mg Aroclor 1254/kg body weight when there were no significant differences in the body weights. Others have also shown that lactational exposure to 8 or 32 μg Aroclor 1254/g delays puberty in female rats (Sager and Girard, 1994). Restum et al. (1997) reported that both the parental and F-1 female mink exposed to 0.25, 0.5, or 1.0 ppm PCBs via consumption of Great Lakes carp exhibited a delay in the onset of estrous as measured by vulvar swelling scores and the time of mating. On the contrary, existing studies in laboratory rodents have shown that DDT and its metabolites cause accelerated puberty as indicated by early vaginal opening, persistent vaginal estrus, and anovulation when animals are exposed either during gestation or neonatally (Gellert et al., 1972; 1974). Therefore, the delayed female puberty observed in this study is most likely a result of the PCB components in the Great Lakes carp.

Male Reproduction and *In Vitro* Fertilizing Ability

In the two control treatment groups (diets C and I), the reproductive ability of one-year-old F-1 males was less than that of younger mature mice, based on comparisons of these data with historical breeding data from our laboratory. Exposure to Great Lakes carp further reduced the breeding ability of these post-maturation F-1 mice. Only one of 11 non-treated females gave birth when paired with one-year-old F-1 mice on diet G. This impaired breeding ability may be explained by a decrease in the epididymal sperm concentration and reduced sperm fertilizing ability. This observation seems to be age specific, since in a similar study with younger mice fed Great Lakes carp (six to 34 weeks of age) there were no changes in sperm concentration, motility, or reproductive performance (Kuo, 1994). In rats, although no effect on sperm production, morphology, or motility was observed in 120-day-old offspring when dams were orally gavaged with Aroclor 1254 (8, 16, 32, and 64 μg/g) on days one, three, five, seven, and nine of lactation, this postnatal exposure did reduce the percent of fertilized eggs after mating with non-treated females (Sager et al., 1991). Huang (1995) reported no effect on epididymal sperm concentration or sperm motility in nine or 19-week-old F-1 mice exposed to TCB from gestation through maturation. However, a decrease in sperm fertilizing ability was

observed in these 19-week-old mice, while sperm from younger mature mice had normal fertility. DDT has also been shown to alter the male reproductive function in laboratory animals. Although exposure of mice to feed containing 10 to 50 ppm DDT (11-14 weeks of age) did not induce abnormalities in sperm morphology (Wyrobeck and Bruce, 1975), eight-week-old mice given an oral dose of DDT (200 mg/kg/wk) for 10 weeks exhibited a reduction in sperm viability as determined by the eosin Y staining method (Clark, 1974).

The existing information on male reproductive function and the toxicity of Great Lakes contaminants does not provide a biological mechanism to explain the reduced reproductive function observed in post-maturation males and not in younger males. This could be due to any one or a combination of three possibilities: increased susceptibility of older mice to Great Lakes contaminants, increased body burdens of contaminants with age, and/or an effect of neonatal exposure to Great Lakes contaminants manifested with the aging process.

Table 1. Nutrient analysis of treatment diets[a]

	Treatment diet		
	C	I	G
Moisture %	4.73	53.75	50.91
Dry Matter %	95.27	46.25	49.09
Nutrients[b]			
Fat %	26.25	22.15	20.25
Crude Protein %	15.31	39.88	41.50
Crude Fiber %	2.70	1.75	2.00
Crude Carbohydrates %	51.04	28.82	28.05
Calcium %	0.86	1.46	1.42
Phosphorus %	0.52	0.75	0.86
Potassium %	0.72	0.94	1.17
Magnesium %	0.15	0.25	0.27
Sodium %	0.41	0.62	0.65
Iron ppm	217.00	232.00	229.00
Manganese ppm	132.00	188.00	191.00
Copper ppm	15.00	25.00	25.00
Zinc ppm	110.00	213.00	216.00
Ash %	4.70	7.40	8.20

[a] Analysis was performed by Litchfield Analytical Services, Litchfield, MI.

[b] Units expressed on dry matter basis.

Table 2. Body weight and relative organ weights of F-0[a] female mice fed diets containing lab chow (C), Iowa carp (I), or Great Lakes carp (G)

Weight[b]		Treatment		
		C	I	G
Body Wt.	(g)	26.5 ± 2.1	26.8 ± 2.7	25.2 ± 2.1
Liver Wt.	(g)	1.5 ± 0.2	1.8 ± 0.3	1.8 ± 0.2
Liver/B.W	%	5.82 ± 0.43	6.80 ± 0.59	7.05 ± 0.51
Thymus Wt.	(mg)	0.049 ± 0.006	0.036 ± 0.010	0.038 ± 0.008
Thymus/B.W	%	0.19 ± 0.03	0.13 ± 0.03	0.15 ± 0.03
Sample size		8	7	8

[a] F-0 females were provided treatment diets immediately after parturition for the subsequent 3 weeks. F-0 females was terminated on the day of weaning.

[b] Liver/B.W, Thymus/B.W represents liver weight as percentages of body weights and thymus weight as percentages of body weights respectively. Values represent mean organ weights or organ weight as percentages of body weights ± standard deviation.

Table 3. Body weights of F-1[a] female mice fed diets containing lab chow (C), Iowa carp (I), or Great Lakes carp (G)

Age	Treatment diet		
	C	I	G
3 weeks	8.4 ± 1.5[A] (44)	7.1 ± 1.1[B] (39)	6.3 ± 1.3[C] (23)
5 weeks	16.5 ± 1.1[A] (32)	15.9 ± 1.5[A] (25)	9.4 ± 2.2[B] (7)
6 weeks	17.3 ± 1.3[A] (32)	17.1 ± 0.9[A] (22)	12.3 ± 3.2[B] (7)
7 weeks	18.9 ± 1.4 (17)	18.2 ± 0.9 (11)	15.7 ± 2.6 (3)
8 weeks	19.7 ± 1.6[A] (17)	19.3 ± 1.1[A] (11)	16.7 ± 0.5[B] (3)

[a] Treatment diets were provided to the dams (F-0) immediately after parturition through lactation and were fed to the F-1 females from weaning through the end of the study. Prior to weaning, F-1 mice also had access to the dams' diet. Data presented mean ± standard deviation. Numbers in parentheses refer to sample size.

[ABC] Different superscripts represent significant difference within the same row ($p < 0.05$).

Table 4. Body weights of F-1[a] male mice fed diets containing lab chow (C), Iowa carp (I), or Great Lakes carp (G)

		C	I	G
3	weeks	8.0 ± 1.7[A] (54)	7.8 ± 1.1[A] (58)	6.4 ± 1.1[B] (37)
5	weeks	17.5 ± 1.7[A] (54)	18.1 ± 2.0[A] (58)	11.4 ± 2.6[B] (36)
6	weeks	20.3 ± 1.8[A] (53)	20.5 ± 1.7[A] (58)	15.0 ± 3.3[B] (32)
7	weeks	22.9 ± 1.5[A] (41)	22.0 ± 1.5[A] (47)	17.5 ± 3.8[B] (32)
8	weeks	23.9 ± 1.7[A] (41)	23.5 ± 1.9[A] (47)	19.5 ± 4.1[B] (32)
10	weeks	27.1 ± 2.6[A] (39)	25.3 ± 1.4[B] (47)	22.0 ± 4.1[C] (30)
15	weeks	35.6 ± 3.6[A] (35)	30.6 ± 2.0[B] (47)	24.9 ± 3.4[C] (27)
20	weeks	41.8 ± 3.9[A] (35)	34.2 ± 3.2[B] (47)	24.8 ± 3.4[C] (27)
25	weeks	47.8 ± 3.0[A] (32)	38.4 ± 3.9[B] (44)	25.0 ± 2.2[C] (25)
30	weeks	50.5 ± 3.1[A] (32)	39.9 ± 5.3[B] (42)	24.1 ± 3.1[C] (22)
35	weeks	51.8 ± 3.3[A] (32)	38.0 ± 6.9[B] (42)	24.9 ± 3.0[C] (14)
40	weeks	52.7 ± 3.6[A] (32)	35.9 ± 6.9[B] (42)	26.3 ± 1.6[C] (13)
44	weeks	51.5 ± 6.5[A] (32)	35.8 ± 7.6[B] (36)	24.9 ± 3.5[C] (13)

Table 4. (cont'd)

[a] Treatment diets were provided to the dams (F-0) immediately after parturition through lactation and were fed to the F-1 males from weaning through the end of the study. Prior to weaning, F-1 mice also had access to the dams' diet. Data presented mean $\pm$ standard deviation. Numbers in parentheses refer to sample size.

[ABC] Different superscripts represent significant differences within the same row ($p < 0.05$).

Table 5. Feed intake[a] of F-1 male mice

	C	I	G
4 weeks	1.5 ± 0.4^{A} (20)	1.3 ± 0.5^{A} (15)	$0.6 \pm .0.7^{B}$ (5)
8 weeks	1.4 ± 0.2^{A} (10)	2.0 ± 0.5^{B} (10)	1.3 ± 0.3^{A} (10)
23 weeks	3.0 ± 0.3^{A} (24)	3.0 ± 0.3^{A} (24)	2.3 ± 0.5^{B} (20)

[a] Mice were fed diets containing lab chow (C), Iowa carp (I), or Great Lakes carp (G) for 5 consecutive days at 4, 8 and 23 weeks of age. Actual feed consumption was calculated daily by subtracting the amount of feed left in the feed jars from the total feed provided on the day before and dividing by the number of mice per cage. Values represent mean ± standard deviation. Units expressed as g/mouse/day (dry weight).

[AB] Different superscripts represent significant differences within the same row ($p < 0.05$).

Table 6. Body and relative organ weights of 53-week-old F-1[a] male mice fed diets containing lab chow (C), Iowa carp (I), or Great Lakes carp (G)

	Treatments		
	C	I	G
Body Wt. (g)	48.6 ± 5.7^{A}	29.9 ± 2.4^{B}	26.1 ± 4.0^{B}
Liver Wt. (g)	1.99 ± 0.12^{A}	1.51 ± 0.30^{B}	1.51 ± 0.21^{B}
Liver/B.W %	4.14 ± 0.59^{A}	5.01 ± 0.71^{B}	5.78 ± 0.27^{C}
Thymus Wt.(mg)	25.2 ± 10.5^{A}	14.0 ± 4.7^{B}	16.5 ± 0.1^{AB}
Thymus/B.W %	0.05 ± 0.02	0.05 ± 0.01	0.06 ± 0.02
Testis Wt. (mg)	206.0 ± 30.6^{A}	214.0 ± 27.0^{A}	106.9 ± 36.9^{B}
Testis/B.W %	0.43 ± 0.06^{A}	0.71 ± 0.07^{B}	0.40 ± 0.10^{A}
Sample size	8	8	6

[a] Treatment diets were provided to the dams (F-0) immediately after parturition through lactation and were fed to the F-1 males from weaning through the end of the study. Prior to weaning, F-1 mice also had access to the dams' diet.

[b] Liver/B.W, Thymus/B.W, Testis/B.W represents liver weight as percentages of body weights, thymus weight as percentages of body weights and testis weight as percentages of body weight respectively. Values represent mean ± standard deviation.

ABC Different superscripts represent significant differences within the same row ($p<0.05$).

Table 7. Body and relative organ weights of 64-weeks-old F-1[a] male mice fed diets containing lab chow (C), Iowa carp (I), or Great Lakes carp (G)

Weight[b]		Treatments		
		C	I	G
Body Wt.	(g)	48.5 ± 8.5^{A}	30.3 ± 2.8^{AB}	23.6 ± 1.5^{B}
Liver Wt.	(g)	1.89 ± 0.37^{A}	1.34 ± 0.17^{AB}	1.23 ± 0.08^{B}
Liver/B.W	%	3.91 ± 0.27^{A}	4.99 ± 1.20^{AB}	5.23 ± 0.10^{B}
Thymus Wt.	(mg)	21.1 ± 8.5	15.4 ± 3.6	14.3 ± 10.7
Thymus/B.W	%	0.04 ± 0.02	0.05 ± 0.01	0.06 ± 0.04
Testis Wt. (mg)		213.4 ± 19.4^{A}	214.4 ± 10.0^{A}	83.1 ± 8.8^{B}
Testis/B.W	%	0.45 ± 0.08^{A}	0.71 ± 0.04^{B}	0.39 ± 0.08^{A}
Sample size		6	4	4

[a] Treatment diets were provided to the dams (F-0) immediately after parturition through lactation and were fed to the F-1 males from weaning through the end of the study. Prior to weaning, F-1 mice also had access to the dams' diet.

[b] Liver/B.W, Thymus/B.W, Testis/B.W represents liver weight as percentages of body weights, thymus weight as percentages of body weights and testis weight as percentages of body weight respectively. Values represent mean $\pm$ standard deviation.

[ABC] Different superscripts represent significant differences within the same row ($p < 0.05$).

Table 8. Body weight and relative organ weights of F-1[a] female mice fed diets containing lab chow (C), Iowa carp (I), or Great Lakes carp (G) at 4 weeks of age

Weight[b]		Treatment diet		
		C	I	G
Body Wt.	(g)	13.7 ± 2.0[A]	12.4 ± 2.0[A]	8.8 ± 2.8[B]
Liver Wt.	(g)	0.76 ± 0.14	0.74 ± 0.21	0.61 ± 0.18
Liver/B.W	%	5.5 ± 0.3[A]	5.9 ± 0.9[AB]	6.6 ± 0.4[B]
Thymus Wt.	(mg)	57.6 ± 15.8[A]	49.8 ± 8.8[A]	32.9 ± 8.4[B]
Thymus/B.W	%	0.42 ± 0.09	0.40 ± 0.02	0.31 ± 0.05
Sample size		6	6	6

[a] Treatment diets were provided to the dams (F-0) immediately after parturition through lactation and were fed to the F-1 females from weaning through 4 weeks of the age. Prior to weaning, F-1 mice also had access to the dams' diet.

[b] Liver/B.W, Thymus/B.W represents liver weight as percentages of body weights and thymus weight as percentages of body weights respectively. Values represent mean organ weights or organ weights as percentages of body weights ± standard deviation.

[AB] Different superscripts represent significant differences within the same row ($p<0.05$).

Table 9. Body weight and relative organ weights of F-1[a] female mice fed diets containing Iowa carp (I), or Great Lakes carp (G) at 5 weeks of age

Weight[b]	Treatment diet		
	C	I	G
Body Wt. (g)	---	14.7 ± 1.4	11.9 ± 2.5
Liver Wt. (g)	---	0.90 ± 0.12	0.77 ± 0.10
Liver/B.W %	---	6.04 ± 0.30	6.63 ± 0.81
Thymus Wt. (mg)	---	72.5 ± 6.0[A]	43.4 ± 18.2[B]
Thymus/B.W %	---	0.49 ± 0.01[A]	0.35 ± 0.10[B]
Sample size	---	5	5

[a] Treatment diets were provided to the dams (F-0) immediately after parturition through lactation and were fed to the F-1 females from weaning through 5 weeks of the age. Prior to weaning, F-1 mice also had access to the dams' diet.

[b] Liver/B.W, Thymus/B.W represents liver weight as percentages of body weights and thymus weight as percentages of body weights respectively. Values represent mean organ weights or organ weight as percentages of body weights ± standard deviation.

[AB] Different superscripts represent significant differences within the same row ($p < 0.05$).

Table 10. Body weight and relative organ weights of F-1[a] female mice fed diets containing lab chow (C), Iowa carp (I), or Great Lakes carp (G) at 6 weeks of age

Weight[b]		Treatment diet		
		C	I	G
Body Wt.	(g)	18.3 ± 0.8^{A}	19.1 ± 0.1^{A}	12.2 ± 3.1^{B}
Liver Wt.	(g)	1.04 ± 0.13^{AC}	1.22 ± 0.09^{A}	0.78 ± 0.25^{BC}
Liver/B.W	%	5.68 ± 0.52	6.36 ± 0.46	6.29 ± 0.51
Thymus Wt.	(mg)	57.3 ± 8.6^{A}	80.1 ± 13.2^{B}	32.9 ± 9.0^{C}
Thymus/B.W	%	0.31 ± 0.05^{A}	0.42 ± 0.07^{B}	0.27 ± 0.01^{A}
Sample size		5	3	3

[a] Treatment diets were provided to the dams (F-0) immediately after parturition through lactation and were fed to the F-1 females from weaning through 6 weeks of the age. Prior to weaning, F-1 mice also had access to the dams' diet.

[b] Liver/B.W, Thymus/B.W represents liver weight as percentages of body weights and thymus weight as percentages of body weights respectively. Values represent mean organ weights or organ weight as percentages of body weights ± standard deviation.

[ABC] Different superscripts represent significant difference within the same row ($p < 0.05$).

Table 11. Body weight and relative organ weights of F-1[a] female mice fed diets containing lab chow (C), Iowa carp (I), or Great Lakes carp (G) at 8 weeks of age

Weight[b]		Treatment diet		
		C	I	G
Body Wt.	(g)	19.9 ± 0.5[A]	20.1 ± 0.9[A]	16.4 ± 1.6[B]
Liver Wt.	(g)	1.06 ± 0.08[A]	1.07 ± 0.05[A]	0.92 ± 0.07[B]
Liver/B.W	%	5.32 ± 0.29	5.34 ± 0.42	5.62 ± 0.20
Thymus Wt.	(mg)	38.5 ± 10.4	44.3 ± 8.4	46.0 ± 13.5
Thymus/B.W	%	0.19 ± 0.06	0.22 ± 0.05	0.28 ± 0.07
Sample size		5	4	4

[a] Treatment diets were provided to the dams (F-0) immediately after parturition through lactation and were fed to the F-1 females from weaning through the 8 weeks of the age. Prior to weaning, F-1 mice also had access to the dams' diet.

[b] Liver/B.W, Thymus/B.W represents liver weight as percentages of body weights and thymus weight as percentages of body weights respectively. Values represent mean organ weights or organ weight as percentages of body weights ± standard deviation.

[AB] Different superscripts represent significant difference within the same row ($p < 0.05$).

Table 12. Survival of offspring from F-0 females[a] fed diets containing lab chow (C), Iowa carp(I), or Great Lakes carp (G)

	Treatment diet		
Parameter	C	I	G
4-day survival[b]	96.0 ± 11.7^{AB}	98.7 ± 4.9^{A}	89.6 ± 12.2^{B}
21-day survival[b]	97.4 ± 5.2^{A}	84.9 ± 31.0^{A}	56.1 ± 37.2^{B}
Sample size	14	14	16

[a] F-0 female were provided treatment diets immediately after parturition for the subsequent 3 weeks.

[b] Mean ± standard deviation of the ratio of the number of live pups on day one after birth to the number of live pups on day 4 and day 21, respectively.

[AB] Different superscripts represent significant differences within the same row ($p < 0.05$).

Table 13. Survival of F-1[a] male mice fed diets containing lab chow (C), Iowa carp (I), or Great Lakes carp (G)

	Treatment diet		
Weeks	C	I	G
3	100%	100%	100%
10	100%	100%	100%
20	100%	100%	100%
30	100%	100%	87.5%
44	100%[A]	90.7%[A]	56.3%[B]
Sample size	37	43	32

[a] Treatment diets were provided to the dams (F-0) immediately after parturition through lactation and were fed to the F-1 males from weaning through the end of the study. Prior to weaning, F-1 mice also had access to the dams' diet.

[AB] Different superscripts represent significant differences within the same row ($p < 0.05$).

Table 14. Effect of treatment diets on vaginal opening in F-1[a] mice fed diets containing lab chow (C), Iowa carp (I), or Great Lakes carp (G)

	Treatment diet		
	C	I	G
Age of vaginal opening (days)[b]	28.4 ± 0.5[A]	30.4 ± 1.1[B]	46.0 ± 3.9[C]
Body weight on day of vaginal opening[b] (g)	12.8 ± 0.6	13.3 ± 1.0	11.4 ± 1.7
Number of mice	5	5	5

[a] Treatment diets were provided to the dams (F-0) immediately after parturition through lactation and were fed to the F-1 females from weaning through the end of the study. Prior to weaning, F-1 mice also had access to the dams' diet.

[b] Data represents mean ± standard deviation.

[ABC] Different superscripts represent significant differences within the same row ($p < 0.05$).

Table 15. Reproductive performance of 1-year-old F-1[a] males fed diets containing lab chow (C), Iowa carp (I), or Great Lakes carp (G)

	Treatment diet		
Parameters	C	I	G
Fecundity[b]	10/15[A]	11/15[A]	1/11[B]
Litter size[c]	8.0 ± 2.7 (10)	8.1 ± 2.3 (11)	12 ± 0 (1)
Sex ratio (male/female)	0.92 (38/41)	0.95 (42/44)	0.33 (3/9)

[a] Treatment diets were provided to the dams (F-0) immediately after parturition through lactation and were fed to the F-1 males from weaning through the end of the study. Prior to weaning, F-1 mice also had access to the dams' diet.

[b] Fecundity refers to the number of females that gave birth/number of female paired.

[c] Litter size was recorded the day after birth. Data presented as mean ± standard deviation. Numbers in parentheses refer to sample size.

[AB] Different superscripts represent significant differences within the same row ($p<0.05$).

Table 16. Epididymal sperm concentration (million/ml) in F-1[a] male mice fed diets containing lab chow (C), Iowa carp (I), or Great Lakes carp (G)

	Treatment diet		
Age(Method)	C	I	G
53-week-old(Manual)[b]	26.4 ± 6.9[A] (6)	20.9 ± 8.4[AB] (6)	6.1 ± 8.8[B] (6)
64-week-old(Manual)	27.5 ± 13.9[A] (6)	22.7 ± 10.7[A] (4)	0.01 ± 0.02[B] (4)
53-week-old(CellSoft)[c]	24.1 ± 8.8[A] (6)	21.7 ± 6.9[A] (6)	5.4 ± 6.6[B] (6)
64-week-old(CellSoft)	43.2 ± 16.0[A] (6)	36.7 ± 9.4[A] (4)	1.5 ± 0.6[B] (4)

[a] Treatment diets were provided to the dams (F-0) immediately after parturition through lactation and were fed to the F-1 males from weaning through the end of the study. Prior to weaning, F-1 mice also had access to the dams' diet.

[b] Manual sperm concentration was measured by placing 20 μl sperm suspension on a Petroff Hausser Counting Chamber (Hausser Scientific Co.,Horsham, PA) containing 16 cells. Sperm were counted in 5 randomly chosen cells and the total was used to calculate the final concentration. Data presented as mean ± standard deviation. Numbers in parentheses refer to sample size.

[c] Sperm concentration was measured by CellSoft computer-assisted digital image analysis system (CRYO Resources Inc., New York). Data presented as mean ± standard deviation. Numbers in parentheses refer to sample size.

[AB] Different superscripts represent significant differences within the same row ($p < 0.05$).

Table 17. Sperm motion analysis in 53-week-old[a] F-1 male mice fed diets containing lab chow (C), Iowa carp (I), or Great Lakes carp (G)

	Treatment diet		
Parameters	C	I	G
Percent motile (%)	55.2 ± 11.8	59.0 ± 10.5	24.6 ± 25.1
Velocity (μm/sec)	122.2 ± 7.5	124.8 ± 8.9	106.0 ± 98.5
Linearity	4.0 ± 0.6	4.3 ± 0.7	3.9 ± 3.5
A.L.H displacement (μm)[b]	5.1 ± 1.4	4.8 ± 0.8	2.1 ± 2.5
Beat/cross frequency (Hz)	11.0 ± 5.6	11.5 ± 3.2	8.2 ± 9.2
Sample size	6	6	6

[a] Treatment diets were provided to the dams (F-0) immediately after parturition through lactation and were fed to the F-1 males from weaning through the end of the study. Prior to weaning, F-1 mice also had access to the dams' diet. Data presented as mean ± standard deviation.

[b] A.L.H. displacement refers to amplitude of lateral head displacement.

Table 18. Sperm motion analysis in 64-week-old[a] F-1 male mice fed diets containing lab chow (C), Iowa carp (I), or Great Lakes carp (G)

Parameters	Treatment diet		
	C	I	G
Percent motile (%)	65.1 ± 22.2[A]	54.2 ± 17.6[A]	9.0 ± 14.3[B]
Velocity (μm/sec)	98.4 ± 13.4[A]	98.2 ± 11.5[A]	16.6 ± 33.3[B]
Linearity	5.4 ± 0.3[A]	5.5 ± 0.8[A]	1.1 ± 2.2[B]
A.L.H displacement (μm)[b]	3.8 ± 0.5[A]	3.5 ± 0.8[AB]	0.1 ± 0.2[B]
Beat/cross frequency (Hz)	12.2 ± 1.2	10.8 ± 1.3	4.4 ± 8.8
Sample size	6	4	4

[a] Treatment diets were provided to the dams (F-0) immediately after parturition through lactation and were fed to the F-1 males from weaning through the end of the study. Data presented as mean ± standard deviation.

[b] A.L.H. displacement refers to amplitude of lateral head displacement.

[AB] Different superscripts represent significant differences within the same row ($p < 0.05$).

Table 19. Fertilizing ability of epididymal sperm from 53-week-old F-1[a] males fed diets containing lab chow (C), Iowa carp (I), or Great Lakes carp (G)

	Treatment diet		
Parameters	C	I	G
Percent of oocytes fertilized[b]	74.0 ± 8.7[A]	70.4 ± 16.8[A]	7.1 ± 7.0[B]
Number of oocytes observed	158	279	255

[a] Treatment diets were provided to the dams (F-0) immediately after parturition through lactation and were fed to the F-1 males from weaning through the end of the study.

[b] Data presented as mean ± standard deviation.

[AB] Different superscripts represent significant differences within the same row ($p < 0.05$).

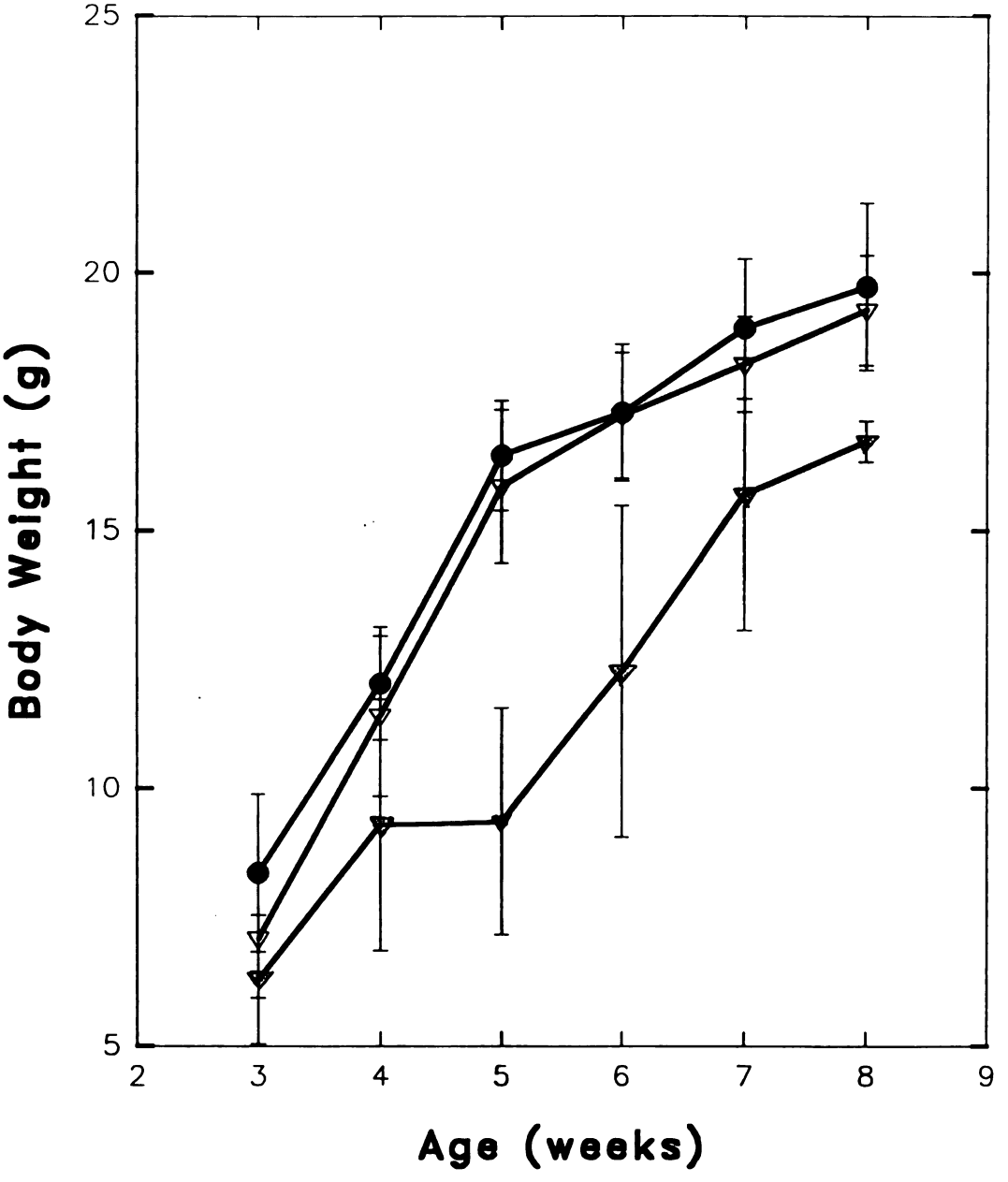

Figure 1. Body weight of F−1 female mice. Diet C (●), diet I (▽), and diet G (▼) were provided to the dams (F−0) immediately after parturition through lactation and were fed to F−1 from weaning through the end of the study.

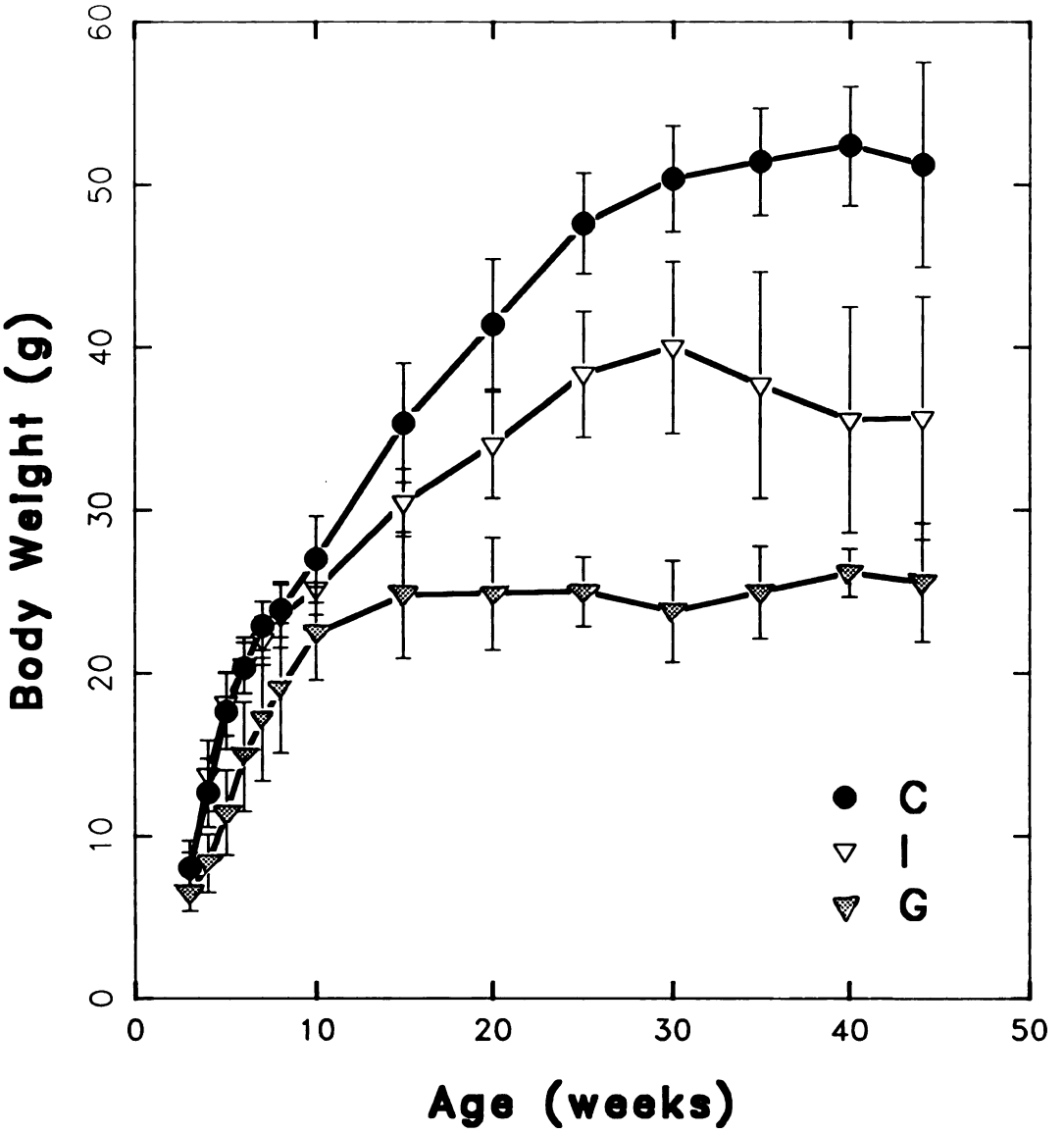

Figure 2. Body weight of F−1 male mice. Diets containing lab chow (C), Iowa carp (I), or Great Lakes carp (G) were provided to the dams (F−0) immediately after parturition through lactation and were fed to F−1 from weaning through the end of the study.

SUMMARY

Lactational and dietary exposure to the contaminants in Great Lakes carp had an adverse effect on reproduction in mice. High neonatal mortality and decreased body weight were observed in F-1 mice on the Great Lakes treatment diet. Liver enlargement was also observed in 4-week-old F-1 females and 53, 64-week-old F-1 male mice. In the Great Lakes treatment group, ninety-one percent of the one-year-old mice failed to breed with the non-treated females. Lower sperm fertilizing ability, motility, velocity, linearity, and amplitude of lateral head (ALH) displacement were also observed. Delayed vaginal opening was observed, in female mice on the Great Lakes treatment diet.

It is not clear why the Great Lakes fish diet adversely effected the reproduction of old mice in present study but not young mature male mice in a previous study. In future studies, the effects of individual PCB congeners and DDT metabolites, the major contaminants in Great Lakes, should be investigated in post-maturation male mice.

APPENDICES

APPENDIX I

Appendix I

Appendix I. Pesticides banned, suspended, or severely restricted in the U.S.A.

	Action	Year
Aldrin	All uses canceled except termite control	1974
Chlordane	Cancellation of most uses, except termite control All uses canceled	1978 1988
DDT	All agricultural use canceled Use only for public health emergencies	1972
Diazinon	Use on golf courses and sod farms canceled	1986
Dieldrin	Cancellation of most uses	1974
Ethylene dibromide (EDB)	All uses canceled	1984
Mirex	All use canceled except pineapples in Hawaii	1977
Nitrofen (TOK)	Voluntary cancellation	1983
2,4,5-T/Silvex	Emergency suspension All use canceled	1979 1985
Toxaphene	All use canceled except sheep and cattle dip, bananas and pineapple in Puerto Rico and Virgin Island	1982

APPENDIX II

APPENDIX II

Appendix II. Calculation of sperm concentration:

Concentration/ml = (Number sperm count x dilution factor x 2 x 10^7)/80

APPENDIX III

APPENDIX III

Appendix III. Definition of sperm motion parameter:

Percent motility:

The percentage of sperm that travel more than 20 μm/sec.

Velocity (μm/sec):

Average of the distance traveled by motile sperm in one second.

Linearity (1-10):

A measure of the straightness of a sperm track on a scale of 0 to 10. Ten indicates a perfectly straight line and 0 indicates a circular track. It is calculated by dividing the length of the straight line distance by the actual track distance.

Amplitude of lateral head displacement (ALH) (μm):

A measure of the displacement of the sperm head from a computer-calculated curval mean of its track.

Beat/Cross Frequency (Hz):

The beat/cross frequency is reported as the number of beats (cross) per second. Every time the sperm cell crosses the computer-calculated curval mean, the computer counts that crossing as one beat.

APPENDIX IV

Appendix IV: Analysis of organochlorine pesticides and PCBs in the diet

MICHIGAN DEPARTMENT OF NATURAL RESOURCES
ENVIRONMENTAL LABORATORY

REPORT TO: MISC
ATTEN KAREN CHOU

LABORATORY WORK ORDER # 94-07-077
WORK ID SAGINAW BAY
P.O. # MSU 7166 COST $ 800.10
RECEIVED 07/14/94 CLIENT MISC
REPORTED _____ NUMBER OF SAMPLES 3
LAB CONTACT OR MATRIX TISSUE

SEND RESULTS TO: KAREN CHOU
RM 132 ANTHONY HALL
MICHIGAN STATE UNIVERSITY
EAST LANSING, MI. 48824

Report prepared By: Lorri C. Utens

SAMPLE ID CL1071494FFB FRACTION 01A TEST CODE S_SC_3 NAME Scan 3 Soil/Sediment
Iowa fish diet (I) Date & Time Collected not specified Category

ANALYST TAIT
ANALYZED 08/04/94
DILUTION 4.8

UNITS ug/Kg ppb

CAS#	COMPOUND	RESULT	REMARK	REPORTED DETECTION LIMIT
541-73-1	1.3-Dichlorobenzene	ND		2400
106-46-7	1.4-Dichlorobenzene	ND		2400
95-50-1	1.2-Dichlorobenzene	ND		2400
67-72-1	Hexachloroethane	ND		240
120-82_1	1.2.4-Trichlorobenzene	ND		2400
87-68-3	Hexachlorobutadiene	ND		240
91-58-7	2-Chloronaphthalene	ND		7200
118-74-1	Hexachlorobenzene	ND		240
58-89-9	g-BHC (lindane)	ND		240
82-68-8	Pentachloronitrobenzene	ND		240
76-44-8	Heptachlor	ND		240
309-00-2	Aldrin	ND		240
1024-57-3	Heptachlor epoxide	ND		240
5103-74-2	g-Chlordane	ND		240
5103-71-9	a-Chlordane	ND		240
72-55-9	4.4'-DDE	36	T	240
72-54-8	4.4'-DDD	ND		240
50-29-3	4.4'-DDT	ND		240
79-34-5	Hexabromobenzene	ND		480
2385-85-5	Mirex	ND		240
53469-21-9	Aroclor 1242 (PCB)	ND		2400
11097-69-1	Aroclor 1254 (PCB)	240	T	2400
11096-82-5	Aroclor 1260 (PCB)	ND		2400
12674-11-1	*Aroclor 1016 (PCB)	ND		2400
11104-28-2	*Aroclor 1221 (PCB)	ND		2400
11141-16-5	*Aroclor 1232 (PCB)	ND		2400
12672-29-6	*Aroclor 1248 (PCB)	ND		2400
- -	*Aroclor 1262 (PCB)	ND		2400
11100-14-4	*Aroclor 1268 (PCB)	ND		2400
37324-23-5	BP-6 (PBB)	ND		1200
8001-35-2	*Toxaphene	ND		2400

COMMENTS MN-FISH SAMPLES, 16% FAT

ND = not detected at the specified detection limit.
* Results and Det. Limit reported semi-quantitatively*

MICHIGAN DEPARTMENT OF NATURAL RESOURCES	PROCEDURE ENVIRONMENTAL LABORATORY	NO: PD-13 DATE: Rev. 2/1/84

Subject: ***Laboratory Result Remark Codes***

A	value reported is the mean of two or more determinations.
C	value calculated from other independent parameters.
J	estimated value or value not accurate.
K	actual value is known to be less than the value given, i.e. substance, if present, is below detection limit.
L	actual value is known to be greater than the value given.
T	value reported is less than criteria of detection.
W	value observed is less than lowest value reportable under "T" code.
DL	sample analyzed using a dilution(s).
DM	dilution required due to matrix problems.
HT	recommended laboratory holding time was exceeded before analysis.
LH	Q. C. indicated possible low recovery. Actual level may be higher.
LL	Q. C. indicated possible high recovery. Actual level may be lower.
MM	analytical method or matrix is not within SOP of this laboratory.
NC	no confirmation by a second technique.
NH	non-homogeneous sample made analysis of a representative sample questionable.
PI	possible interference may have affected the accuracy of the laboratory result.
QC	quality control problems exists.
RB	Reagent Blank. The level of reagent blank contamination is reported in the comment column and may be subtracted from the analyte value by the user.
ST	recommended sample collection/preservation technique not used.
ACC	laboratory accident resulted in no obtainable value.
FCN	free cyanide was not analyzed due to low level of total cyanide.
INT	interference encountered during analysis resulted in no obtainable value.
IST	improper sample collection/preservation. Sample not suitable for analysis.
NAV	requested analysis not available.
QNS	quantity not sufficient to perform requested analysis.
STR	settleable residue was not analyzed due to low suspended solids.

Approved by: _____ 2/2/4
George Su, Laboratory Director — Date

APPENDIX V

Appendix V. Reproductive performance of 48- and 53-week-old F-1[a] males fed diets containing lab chow (C), Iowa carp (I), or Great Lakes carp (G)

Reproductive performance of 48-week-old F-1[a] males fed diets containing lab chow (C), Iowa carp (I), or Great Lakes carp (G)

	Treatments		
Parameters	C	I	G
Fecundity[b]	8/10	7/10	1/6
4-day survival[c]	94.4 ± 8.7	95.7 ± 7.9	100 ± 0.0
21-day survival[c]	97.9 ± 5.9	100 ± 0.0	100 ± 0.0
Sex ratio male/female	29/31(0.94)	25/29(0.86)	3/9(0.33)

[a] Treatment diets were provided to the dams (F-0) immediately after parturition through lactation and were fed to the F-1 females from weaning through 4 weeks of the age. Prior to weaning, F-1 mice also had access to the dams' diet.

[b] Fecundity refers to the number of females that gave birth/number of female paired.

[c] Mean ± standard deviation of the ratio of the number of live pups on day one after birth to the number of live pups on day 4 and day 21, respectively.

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